GSICA
THE ITALIAN SCIENTIFIC GROUP
OF FOOD PACKAGING

Instituto Universitario de Investigación
de Ingeniería de Aragón
Universidad Zaragoza

SLIM 2010

Shelf-life International Meeting

Zaragoza, 23 – 25 June 2010

Edited by

CRISTINA NERÍN & JESÚS SALAFRANCA

Special Issue

ITALIAN JOURNAL
OF
FOOD SCIENCE

CHIRIOTTI EDITORI

GSICA

THE ITALIAN SCIENTIFIC GROUP of
FOOD PACKAGING

In cooperation with

THE ARAGÓN INSTITUTE OF ENGINEERING RESEARCH (13A) OF THE UNIVERSITY OF ZARAGOZA

Zaragoza, June 23rd-25th 2010

Edited by

CRISTINA NERÍN & JESÚS SALAFRANCA

Special Issue

ITALIAN JOURNAL
OF
FOOD SCIENCE

CHIRIOTTI EDITORI

This Special Issue of the Italian Journal of Food Science collects the presentations given at the "SLIM 2010, Shelf Life International Meeting" organized by GSICA and the Aragón Institute of Engineering Research (I3A) of the University of Zaragoza, held at Zaragoza on June 23-25th 2010.
These papers were reviewed by the Scientific Committee of the congress before their presentation but they did not undergo the conventional reviewing system of the Italian Journal of Food Science.

Chiriotti Editori S.R.L. - Pinerolo - Italy

ISSN 1120-1770

ITALIAN JOURNAL OF FOOD SCIENCE
(RIVISTA ITALIANA DI SCIENZA DEGLI ALIMENTI) 2nd series

Founded By Paolo Fantozzi under the aeges of the University of Perugia
Official Journal of the Italian Society of Food Science and Technology
Società Italiana di Scienze e Tecnologie Alimentari (S.I.S.T.Al)
Initially supported in part by the Italian Research Council (CNR) - Rome - Italy
Recognised as a "Journal of High Cultural Level"
by the Ministry of Cultural Heritage - Rome - Italy

Publisher:
Alberto Chiriotti
Chiriotti Editori srl, Viale Rimembranza 60, I-10064 Pinerolo, Italy - Tel. +39 0121 393127 - Fax +39 0121 794480
e-mail: info@chiriottieditori.it - URL: www.chiriottieditori.it

Aim: The Italian Journal of Food Science is an international journal publishing original, basic and applied papers, reviews, short communications, surveys and opinions on food science and technology with specific reference to the Mediterranean Region. Its expanded scope includes food production, food engineering, food management, food quality, shelf-life, consumer acceptance of foodstuffs. food safety and nutrition, and environmental aspects of food processing.
Reviews and surveys on specific topics relevant to the advance of the Mediterranean food industry are particularly welcome.
Upon request and free of charge, announcements of congresses, presentations of research institutes, books and proceedings may also be published in a special "News" section.

Review Policy:
The Co-Editors with the Editor-in-Chief will select submitted manuscripts in relationship to their innovative and original content. Referees will be selected from the Advisory Board and/or qualified Italian or foreign scientists. Acceptance of a paper rests with the referees.

Frequency: Quarterly - One volume in four issues. Guide for Authors is published in each number and annual indices are published in number 4 of each volume.

Impact Factor: 5-Year Impact Factor: 0.596 published in 2009 Journal of Citation Reports, Institute for Scientific Information; Index Copernicus Journal Master List 2009 (ICV): 13.19
IJFS is abstracted/indexed in: Chemical Abstracts Service (USA); Foods Adlibra Publ. (USA); Gialine - Ensia (F); Institut Information Sci. Acad. Sciences (Russia); Institute for Scientific Information; CurrentContents®/AB&ES; SciSearch® (USA-GB); Int. Food Information Service - IFIS (D); Int. Food Information Service - IFIS (UK); EBSCO Publishing; Index Copernicus Journal Master List (PL).
IJFS has a page charge of € 25.00 each page.

Subscription Rate: IJFS is available on-line in PDF format only.
2011: Volume XXIII: Ordinary € 50.00 - Supporting € 1,000.00

SCIENTIFIC COMMITTEE

Auras R.	School of Packaging, Michigan State Univ. (US)
Barbosa-Cánovas G.V.	Washington State Univ. (US)
Buonocore G.	IMCB-CNR (I)
Cacho J.	Univ. of Zaragoza (ES)
Castle L.	DEFRA, MAFF (UK)
Dainelli D.	Analytical Dep. & Regulatory Affairs, Cryovac EMEA (I)
Del Nobile M.A.	Univ. of Foggia (I)
Ducruet V.	AgroParisTech/CNAM/INRA (FR)
Gavara R.	IATA, CSIC (ES)
Gontard N.	Univ. of Montpellier (FR)
Hotchkiss J.H.	School of Packaging, Michigan State Univ. (US)
Lee D.S.	Kyungnam Univ. (KR)
Leufven A.	N. F. R. I. Matforsk (NO)
Muratore G.	Univ. of Catania (I)
Nerín C.	Univ. of Zaragoza (ES)
Paseiro P.	Univ. of Santiago de Compostela (ES)
Pastorelli S.	Philip Morris International, Product Regulatory Compliance (CH)
Piergiovanni L.	Univ. of Milan (I)
Simoneau C.	Institute for Health and Consumer Protection of Ispra (I)
Soares N.F.	Univ. Federal of Viçosa (BR)
Yam K.L.	Rutgers Univ. (US)

Local organising committee

Nerín Cristina, Salafranca Jesús , Domeño Celia, Alfaro Pilar, Asensio Esther, Aznar Margarita , Becerril Raquel , Canellas Elena , Licciardello Fabio, Manso Sofía, Masiá Jorge , Montero Pablo, Pezo Davinson , Rodríguez Ángel , Vera Paula.

CONTENTS

SESSION II

"New Technologies for Shelf Life Extension"

Chairmen:
V. Ducruet (AgroParisTech/CNAM/INRA, FR)
C. Nerín (University of Zaragoza, ES)
M.A. Del Nobile (University of Foggia, I)
N.F. Soares (University Federal of Viçosa, BR)

SESSION III

"Shelf Life Modelling and Prediction"

Chairmen:
D.S. Lee (Kyungnam University, KR)
L. Piergiovanni (University of Milano, I)

LIST OF AUTHORS

INTRODUCTION

This volume collects the contributions to Shelf-life International Meeting (SLIM) 2010, the unique Conference dealing with shelf-life of packaged food products.

The Conference has been organized by the Italian Scientific Group of Food Packaging (GSICA) in co-operation with the Aragon Institute for Engineering Research (I3A) from the University of Zaragoza (Spain). The Fourth Shelf-life International Meeting (SLIM 2010) was held in Zaragoza (Spain), being the first time that this Conference has been organized outside Italy. It was attended by 150 participants coming from all around the world, and the researchers coming from outside Italy were this time more than 50%. Thus, we can confirm that this Conference has become a real International meeting.

The volume reports the research results presented as both 37 oral and 70 poster presentations, following the scheme of the conference sessions, addressing, respectively:

Shelf-life Testing: Non-invasive analytical techniques for testing the packaging materials and the food, sensorial techniques**,** new freshness indicators, selection and validation of reliable quality indexes and evaluation of performance of packaging materials, including migration.

New Technologies for Shelf-life Extension: New materials**,** with special emphasis on active and intelligent packaging, new packaging devices, new food processing technologies, new food preservatives alternative to the traditional ones and new techniques for risk reduction.

Shelf-life Modelling and Prediction: Prediction by means of mathematical models of shelf-life and quality decay of food and beverages, prediction of barrier and protective properties of packaging materials, study of the kinetics of food quality degradation, prediction of sensorial indexes decay and predictive microbiology.

Prediction, testing and extension of the shelf-life require a multi-disciplinary approach, which involves analytical chemistry, microbiology, food processing, food packaging, material science as well as physical chemistry. In this framework, due to the diversity of attendees, SLIM 2010 provided an excellent international forum for presenting fundamental aspects of

current developments and future directions for research and applications on the shelf-life of packaged foodstuff. In this occasion, special emphasis was dedicated to new active packaging developments, mainly due to the recent approval of the European Regulation 450/2009 dealing with active and intelligent food packaging.

Previous to the meeting, GSICA together with the I3A-University of Zaragoza organized an International Workshop and a poster competition. The Workshop **"Food Safety and Compliance"** presented the latest developments in regulation and mainly in compliance testing of food packaging materials from the leading opinions of two key figures of the food packaging world: the Food Industry that uses the packaging and the Control Authority. Thanks to workshop, scientific discussions were promoted and the need for a safer and more effective food packaging was highlighted.

The poster competition recognized the high quality of research activity in the field of Food Packaging and shelf-life. The award, consisting of a certificate, has been given to the authors of nine posters (three for each session) for their original contributions. Thanks to this competition, poster session has been enhanced and it was efficient for giving positive encouragement to young researchers as well as an excellent way for scientific exchange and cooperation.

During the gala dinner held in a traditional winery, the GSICA announced the new edition of SLIM for 2012, which will be held in Korea in cooperation with the Korean Society of Food Science and Nutrition (KFN).

Cristina Nerín & Jesús Salafranca

SESSION I

"Shelf Life Testing"

Chairmen:

A. Leufven (N. F. R. I. Matforsk, NO)
K.L. Yam (Rutgers University, US)

SHELF-LIFE STUDY OF CASE-READY MEAT IN A LOW OXYGEN SYSTEM BY MEANS OF OXYGEN SCAVENGERS AND MODIFIED ATMOSPHERE PACKAGING

S. SANTAGOSTINO[1], E. MASCHERONI[2], L. FRANZETTI[2], A. ADOBATI[2], E. UBOLDI[2] and S. LIMBO[2]

[1]Multisorb Technologies,
325 Harlem Road, Buffalo, New York I4224, Usa

[2]DISTAM, Dipartimento di Scienze
e Tecnologie Alimentari e Microbiologiche, Università di Milano,
Via Celoria 2, 20133 Milano, Italy

ABSTRACT

In this study, oxygen scavengers have been evaluated in order to establish the right atmosphere conditions inside the packages, and to verify the benefits of this new technology. Standard primary packages of PVC stretch film, closed in 30% CO_2 and 70% N_2, containing about 200 g of steaks of "Eye of round" (*Semitendinosus* muscle) from pure Italian breed "Piemontese" were put into master barrier bags with oxygen scavengers. The samples were then stored at 1±0.5°C for 21 days in the dark. After 1, 2 and 3 weeks of storage the master bags were opened and the primary packages were exposed to air and light, measuring the time for the blooming of the meat. Myoglobin forms, color parameters of the stored and bloomed meat, microbiological tests, changes in the atmosphere composition were the key indicators monitored during the storage. Results show that with this new packaging system both retailers and final consumers could have tangible advantages such as the extension of meat distribution life and the maintaining of meat quality.

Key words: Oxygen scavengers, red meat, shelf-life extension.

INTRODUCTION

Meat that is prepared at retail level is usually part of boxed meat systems where primal cuts are produced and often vacuum packaged (VP) at the packing plant and distributed to the retail centers. There, the primal cuts are removed from VP to be further processed (cut, minced, etc.) with the aim to package in consumer units that afterwards are stored in refrigerated display for few days. In the retail chain, high O_2 MAP has been readily available, but lesser used than VP. One solution consists in packaging the meat (already prepared in the final cut) in non barrier trays overwrapped with high permeable (and often micro-perforated) film, then enclosed in a larger master bags barrier film that contains multiple packages in the anoxic state. When the trays are removed from the master pack for retail display, the meat pigments become oxygenated. The absence of O_2 usually maintains myoglobin in a reduced state (i.e. in a reversible, but unfamiliar for many consumers, purplish-red color) and minimizes oxidative deteriorative reactions. The limit of this kind of packaging is that very low oxygen concentration is required (<100-500 ppm) to maintain myoglobin in a deoxygenated state (Mancini, 2005). Furthermore, at low O_2 concentration the oxydation of deoxymyoglobin to metmyoglobin is faster than the oxidation of oxymyoglobin (Venturini, 2006). The challenge is the reduction of the O_2 concentration to less than critical values inside master bags as soon as possible, using O_2 scavengers. In this way, active packaging in combination with gas flush packaging could keep the meat in an oxygen free environment (master bags) until needed, extending the distribution life at retail level and maintaining the safety and visual appeal of refrigerated meat during the display life.

MATERIALS AND METHODS

Two steaks ("Eye of round" cut) of 200 g each one and 1 cm of thickness were placed on self-absorbent expanded polystyrene trays. Each tray was overwrapped with a PVC shrink film with high O_2 transmission rate. Two trays were placed into each master bag made by a coextruded PE/EVOH/PE. Gas mixture used was 30% carbon dioxide (CO_2) and 70% nitrogen (N_2). Two ready to use O_2 scavengers (FreshPax® CR, Multisorb) were included in each master bag to scavenge residual O_2 after gas flushing. The master bags were stored in the dark at 1°±0.5°C for 7, 14 and 21 days. At each time some trays were analyzed immediately after the opening of the master bag, while other trays after the blooming that was carried out for 4 h at 3±1°C. After this period, trays were randomly placed in an illuminated retail display case at 4°±2°C for 48 h. Oxygen analysis: The O_2 concentration (%) inside master bags was analyzed by means of a non invasive oxygen measurement device (Oxisense®). Color evaluation: At each analysis time, the color evaluations of the beef steaks were carried out with an hand-held tri-stimulus colorimeter (Minolta Chroma Mether CR-210, Minolta, Osaka, Japan). Estimation of myoglobin states: The reflectance spectra (380-750 nm) were obtained from two different location of the round using a UV-visible spectrophotometer equipped with an integrating sphere (Lambda 650, Perkin Elmer, Italy). Reflectance values of the difference myoglobin oxidation states were estimated at specific wavelengths and used for quantifying the proportion (%) of Oxymyoglobin (OxyMb), Deoxymyoglobin (DeoxyMb) and Metmyoglobin (MetMb), in accordance with the AMSA guidelines (AMSA, 1991). Microbiological analyses. At each analysis time the following determinations

were performed: Mesophilic aerobic bacterial count; *Pseudomonas* and Lactic Acid Bacteria.

RESULTS AND CONCLUSIONS

The residual of O_2 after the packaging of the master bags was estimated equal to 1.1±0.3%. The presence of the O_2 absorbers allowed the decreasing of the gas up to values not detectable by the instrument that has a detection limit equal to 0.03%. From a microbiological point of view, during the first 10 days of storage in master bags, the total mesophilic bacterial count increased from 3.4 log(CFU g^{-1}) to 5 log(CFU g^{-1}), after that the count remained constant. As expected, lactic acid bacteria increased their values during storage due to the anoxic conditions created inside the master bags (fig. 1).

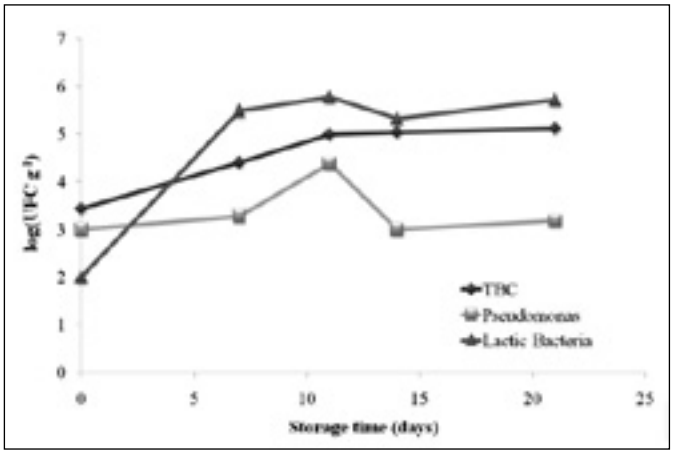


Fig. 1 - Microbial evolution in the meat during the storage in master bag.

The proportion of the three myoglobin forms on the surface of slices changed progressively over time: the OxyMb decreased but remained the prevalent form while the DeoxyMb increased (data not shown). It was possible to verify that the reduction of OxyMb on the surface of fresh meat is a two-steps reaction. In fact, at very low O_2 partial pressure OxyMb is converted to DeoxyMb proceeding through the MetMb (ferric redox state). The reduction of MetMb depends on different factors (NADH pool, reducing enzyme system, etc.) (Mancini, 2005; Bekhit, 2005). After 7, 14 and 21 days of storage inside the master bags, the overwrapped trays

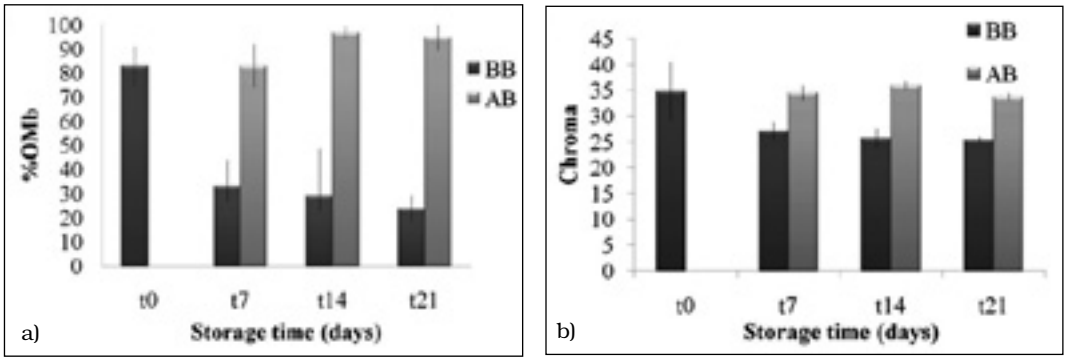


Fig. 2 - OxyMb content (fig. 2a, left) and Chroma (fig. 2b, right) of slices before and after blooming (BB and AB, respectively).

were removed. The trays were stored at 3°C in the dark in order to promote the oxygenation of the meat pigment (blooming). After blooming time (4 h at 3°C) the OxyMb reached the same values that characterized the fresh meat at time 0 (fig. 2a). Also the color (expressed as Chroma index) after the blooming regained the same value of the fresh meat (fig. 2b).

After blooming, the trays were stored in a display cabinet at 5±1.5°C for 48 h. During the display life, meat that was stored in master bag for longer period (21 days), achieved the best oxygenation (data not shown). This study was able to demonstrate the potential advantages of using an active packaging system *versus* traditional one on overall meat quality. With this new packaging system both retailers and final consumers could have tangible advantages: the distribution life at the retail level could be extended making more flexible the stocks handling and, at the same time, the quality and the safety of the meat could be guaranteed to the final consumer.

REFERENCES

Bekhit A., Faustman C. 2005. Metmyoglobin reducing activity. Meat Sci. 71: 407-439.

Mancini R.A., Hunt M.C. 2005. Current research in meat color. Meat Sci. 71: 100-121.

Venturini A.C., Contreras C.J.C., Sarantòpoulos C.I.G.I., Villanueva N.M.D. 2006. The effect of residual oxygen on the storage life of retail-ready fresh beef steaks masterpackaged under a CO_2 Atmosphere. J. Food Sci. 71: S560-S566.

SPECTROSCOPIC TECHNIQUES COUPLED WITH CHEMOMETRIC TOOLS FOR THE EVALUATION OF LIGHT-INDUCED OXIDATION IN CHEESE

N. SINELLI[1], S. LIMBO[1], E. CASIRAGHI[1] and L. TORRI[2]

[1]DiSTAM, Università degli Studi di Milano,
Via G. Celoria 2, 20133 Milan, Italy

[2]University of Gastronomic Sciences,
Piazza Vittorio Emanuele 9,
12060 Bra (CN), Italy

ABSTRACT

Aim of this study was to evaluate the application of these methods, combined with chemometric strategies, as non-destructive techniques to evaluate the light induced oxidation in cheese. Semi hard cheese (Edam cheese) was cut in slices with 1 cm thickness and wrapped with clear cling polyethylene film. Samples were stored in a refrigerated industrial display cabinet equipped with fluorescent lights. The light-induced oxidation of samples, stored in the light and in the dark, was monitored by UV-Vis (200-780 nm), FT-NIR (12,500-3,900 cm^{-1}), FT-IR (4,000-700 cm^{-1}) and fluorescence spectroscopy. The overall results have shown that spectroscopic methods are very sensitive to measure the development of light-induced oxidation in cheese products. In particular UV-VIS and fluorescence spectroscopy detected riboflavin decay that appears to be the most light absorbing constituent of cheese.

Key words: spectroscopic techniques, semi hard cheese, light-induced oxidation, chemometrics.

INTRODUCTION

Cheeses exposed to light during processing, packaging, distribution and retail undergo photo-oxidation. Light-induced oxidation causes the formation of off-flavours, discolouring, loss of nutrients (primarily riboflavin) rapidly impairing the product quality. Typically, light-induced oxidation has been evaluated by chemical determinations, which measure the formation of lipid peroxides, the development of secondary lipid oxidation products, the decrease in riboflavin and vitamin A content. These techniques are time consuming, expensive and are not suited for online analysis. Optical techniques, including UV-Visible (UV-Visible), near (NIR), mid infrared (MIR) and fluorescence spectroscopy may be used as rapid method to describe photooxidation of cheeses as their spectra could give information about the light-induced oxidation products (Mortensen *et al.*, 2004). The aim of this study was to investigate the applicability of these methods, combined with chemometric strategies, as non-destructive techniques to evaluate the light induced oxidation in cheese.

MATERIALS AND METHODS

Cheese and lighting exposition: Semi hard cheese (Edam cheese) was purchased from a local grocery store, cut in 1 cm slices and wrapped with clear cling polyethylene film. Samples were stored in a refrigerated industrial display cabinet equipped with fluorescent lights (cool white lamps, Osram Dulux 58W/840). The light intensity reaching the samples was monitored by means of a portable photoradiometer equipped with specific probes for irradiance and illuminance measurements. An equal number of samples was kept in the dark as reference. The average measured storage temperature was 8.5±0.5°C. The measurements were carried out on the same cheese slices up to 24 days of storage, at intervals of 24 to 72 hours.

Spectroscopic analyses: The light-induced oxidation of samples, stored in the light and in the dark was monitor by UV-Vis (200-780 nm), FT-NIR (12,500-3,900 cm^{-1}), FT-IR (4,000-700 cm^{-1}) and fluorescence spectroscopy. *Fluorescence measurements* were obtained as fluorescence emission spectra using a spectrofluorimeter equipped with a front-face accessory. The cheese samples with a thickness of 1 cm were placed directly on the quartz plate and measured at an angle of approximately 60°. Fluorescence emission spectra were obtained with excitation at 360 nm and emission from 500 to 560 nm (slit widths: 5 nm, scan speed: 50 nm min^{-1}). Measurements were performed during 7 days storage with intervals of 24 hours on the same slice. *UV-Vis measurements* were obtained by means of a UV-Vis spectrophotometer equipped with an integrative sphere (Perkin Elmer, Lambda 650). Spectra were acquired in the range 200-780 nm (resolution: 2 nm). *FT-NIR measurements* were obtained by means of a FT-NIR spectrometer equipped with an integrative sphere (MPA FT-NIR spectrometer, Bruker Optics). Spectra were acquired in the range 12,500-3,900 cm^{-1} (resolution: 8 cm^{-1}). *FT-IR measurements* were obtained by means of a FT-IR spectrometer equipped with ATR accessory (Vertex 70, Bruker Optics, Milano). Spectra were acquired in the range 4,000-700 cm^{-1} (resolution: 4 cm^{-1}).

Chemometric strategies: Spectroscopic data were analyzed by using principal component analysis (PCA) (The Unscrambler, version 9.7, Camo, Inondhcim, Norway).

In order to minimize the effect of baseline shifts, the spectral data were pre-processed by mathematical treatments. The NIR spectral data were transformed into the second derivative (Savitzky-Golay method, gap size=15 data points), while the MIR spectral data were standardized by Standard Normal Variate (SNV). All spectral data sets were mean-centered before performing PCA calculations.

RESULTS AND DISCUSSION

Considering the fluorescence data, during time some changes in the spectra can be observed (fig. 1a). The region from 410 to 480 nm shows an increase: the main chromophores responsible for the signal are not certain. This region typically shows fluorescence from stable oxidation products formed by aldehydes and amino acids (Andersen *et al.*, 2006). A decrease in fluorescence around 530 nm was measured during the storage time. The decrease was gradual during time and after 7 days of exposition the riboflavin peak disappeared completely. The Principal Component Analysis (PCA) applied to fluorescence data highlighted the clear separation of sample exposed to light on the basis of the storage time (fig. 1b). Samples stored in the dark had similar PC1 values, because of not significant changes in riboflavin and other photosensitizer compounds.

Also the chemometric analysis carried out on UV-VIS spectra highlighted the quality decay of the lighted samples as a function of riboflavin reduction (440-460 nm) and of secondary oxidation products formation (260-280 nm) (data not shown).

The FT-NIR and FT-MIR spectra discriminated the samples as function of storage time; samples that were exposed to light were discriminated on the basis of lipid oxidation and protein degradation, as evidenced by the score

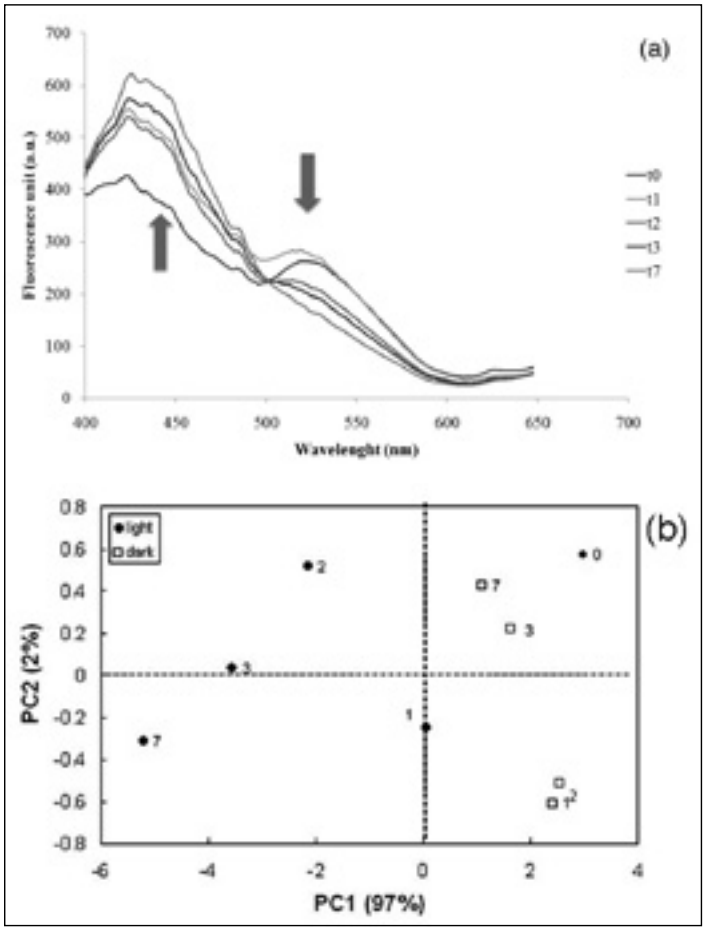


Fig. 1 - Fluorescence spectra during storage time (a) and PCA elaboration (b).

and loading plots for FT-MIR spectra shown as example in fig. 2a and 2b.

The overall results prove that spectroscopic methods are very sensitive to measure the development of light-induced oxidation in cheese products. In particular UV-VIS and fluorescence spectroscopy detect riboflavin decay that appears to be the most light absorbing constituent of cheese.

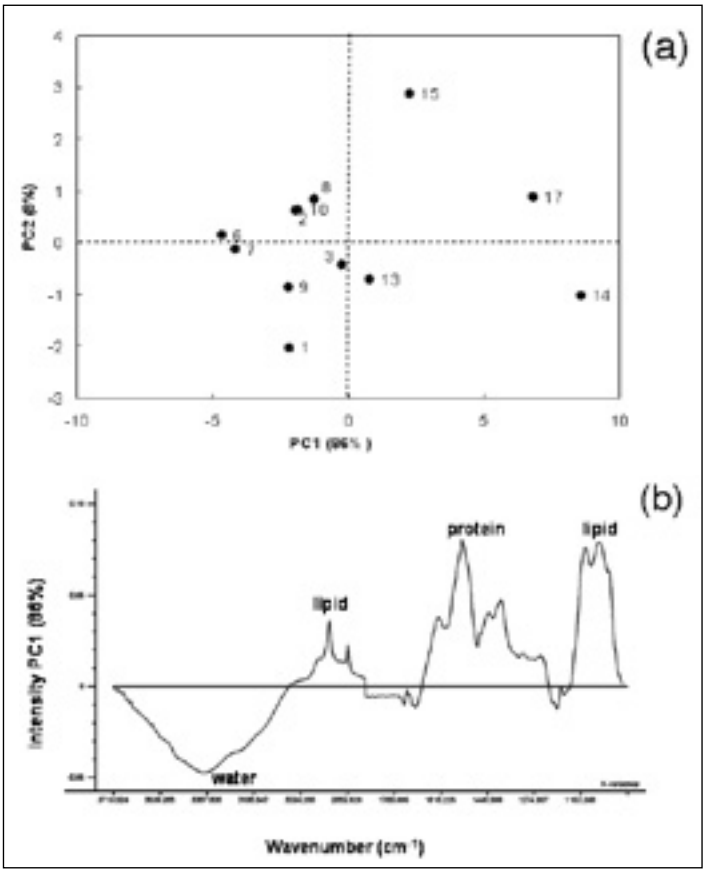


Fig. 2 - Score plot (a) and loading plot (b) of the FT-MIR spectral data.

REFERENCES

Andersen C.M., Wold J.P., Mortensen G. 2006. Light-induced changes in semi-hard cheese determined by fluorescence spectroscopy and chemometrics. Int. Dairy J. 1483-1489.

Mortensen G., Bertelsen G., Mortensen B.K., Stapelfeld H. 2004. Light-induced changes in packaged cheeses-a review. Int. Dairy J. 85-102.

PERFORMANCE OF WINE BAG-IN-BOX DURING STORAGE: LOSS OF OXYGEN BARRIER

S. FRADIQUE[1], T. HOGG[2], J. PEREIRA[2] and M.F.F. POÇAS[2]

[1]Adega Cooperativa de Murça C.R.L.,
Rua Francisco Barros Carneiro Lopes 4, 5090-134 Murça, Portugal

[2]CBQF, Escola Superior de Biotecnologia,
Universidade Católica Portuguesa,
Rua Dr. António Bernardino de Almeida,
4200-072 Porto, Portugal

ABSTRACT

Bag-in-box system is a convenient packaging system for wine. Its barrier to oxygen relies, in many cases, in an aluminium metallized polyester film laminated between two polyethylene (PE) layers as the inner bag. One on the most frequently observed system failures is the ingress of wine into the gap between the layers of the double bag. This study aimed at verifying if that influences the barrier of the system to oxygen. The results showed that the contact of wine with this barrier layer, although through a PE layer, promotes the demetallization and consequent loss of barrier properties. The type of wine, in particular its volatile acidity, and the temperatures were two variables assessed. Results showed that wine with higher acidity and stored at higher temperatures tend to yield a higher increase in oxygen transmission rate of the film.

Key words: wine packaging, bag-in-box, oxygen barrier.

INTRODUCTION

Bag-in-Box (BIB) is a convenient means of wine transportation, also referred to as “cask wines” or “box wines”. It is also well adapted to a number of other liquid or semi-liquid food applications, including milk, sauces, liquid eggs, sauces and

fruit concentrates. This system is available for wine in package volumes from 2 to 20 litres (Dufrêne *et al.*, 2007). The BIB comprises a flexible double bag, a gland (also called "spout" or "flange") welded to the film, a tap (through which the wine is poured) inserted in the gland and a box providing both mechanical protection and support for visual communication aimed at the end consumer (Dufrêne *et al.*, 2007).

The flexible bag is typically made of multilayer films with 3 or 4 layers one of which consists of an oxygen barrier material, such as ethylene-vinyl alcohol copolymer (EVOH) or metallized polyester (MET PET). The films of EVOH and MET PET are sandwiched within protective films, generally based on low density polyethylene (LDPE), that also provide good heat sealing properties (Robertson, 2006). The bag includes additionally an internal film usually also made of LDPE, which is not laminated to the external film. The structure is set together only by the seals. A typical structure of MET PET BIB used for wine is shown in fig. 1.

Oxygen is often the major issue in shelf life problems of wines in BIB. Therefore, it is important to known how oxygen actually gets inside the bag when the film is in the presence of wine on one side and air on the other (Dufrêne *et al.*, 2007). The barrier to oxygen of the system can be affected by the intrinsic permeability of the materials and by the tightness of the system, namely joins and seals. The MET PET film has a low permeability to oxygen provided by the vaporized layer of aluminium. This layer is laminated to LDPE layers that protect its integrity. However, the material is submitted to different mechanical stresses during the operations of bag manufacture, wine filling, packaging and transportation, particularly in the case of poor handling practices. This yields to mechanical damage and fatigue of the metallized layer which loses partially its barrier properties by flex cracking (Nicolini *et al.*, 2006; Doyon *et al.*, 2005; Sundell *et al.*, 1992). The damage is not enough for loss of bag integrity in terms of wine leakage, but there is an increase in the oxygen ingress, a decrease of free SO_2 and a consequent premature degradation of the wine (Anon, 2006). The films of EVOH emerged as the option to MET PET. They have a lower barrier to oxygen when compared to undamaged MET PET (Doyon *et al.*, 2005), they are less prone to flexcracking but their barrier is influenced by the relative humidity (Nicolini *et al.*, 2006; Anon, 2006).

Fig. 1 - Typical structure of MET PET films for wine BIB.

The effect of handling and transportation in the oxygen barrier of the bag (material, spout and tap) has been studied and damage of the MET PET layer, with visible demetallization, has been reported as occurring mainly near the edges and around the tap (Doyon *et al.*, 2005). This is in agreement with numerous observations made by the authors of faulty BIBs presenting evident signs of demetallization after some time of storage. Fig. 2 shows

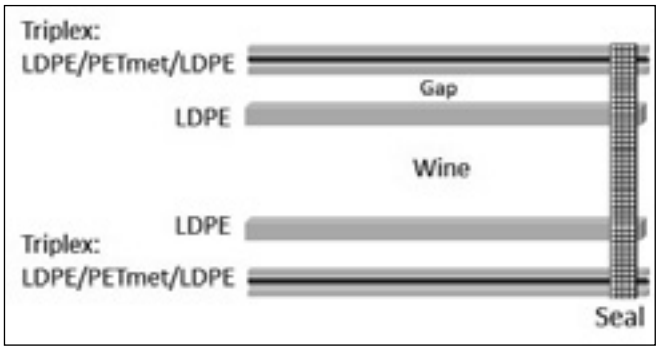


Fig. 2 - Effect of demetallization of the aluminium layer.

an example of severe demetallization around the tap. The inspection of this commonly observed defect in BIB shows that it is usually accompanied by the contact of wine with the MET PET layer when the wine passes through the seals to the gap between films. This situation is often not noticed until advanced stages and leakage occurs. Bad storage practices may also originate this situation by contact of wine in the external face of the MET PET layer if leakage in a BIB stored pilled in a pallet drops over the BIBs pilled under the faulty BIB. This situation is some times detected during storage. The apparent damage of the MET PET layer yields to a certain degree of demetallization of the aluminium layer which may have as consequence a decrease on the oxygen barrier provided. This potential loss of barrier may have an impact on the wine shelf-life depending on the extent it occurs and on the BIB surface area affected.

The objective of this work was to study how the oxygen transmission rate (OTR) of the external film of the double bag is influenced by the direct contact with wine. The influence of the parameters: temperature, time of contact and type of wine (volatile acidity) was assessed. Samples of film were immersed in wine simulating solutions and stored at different temperatures (23° and 40°C) for a period of 75 days. Periodically the samples were collected to be analysed for oxygen transmission rate.

MATERIALS AND METHODS

Films BIB

The external layer of the 10L BIB supplied by Conotainer (Madrid, Spain) was used consisting of MET PET laminated with LDPE (45 μm LDPE/12 μm MET PET/45 μm PE).

Wine simulation solutions

The solutions were prepared in order to represent average white (W solution) and red (R solution) wines in terms of the following chemical characteristics: alcohol degree (%), volatile acidity (g acetic acid/L), total acidity (g tartaric acid/L) and reducing sugars (g/L). Tab. 1 presents the characteristics of the solutions used.

Contact between film and solutions

Forty-two pieces of film were randomly cut from a 10L BIB. Ten glass jars with the W solution and ten jars with the R solution were prepared. Two pieces of film were immersed on each jar solution, half of which was stored at 23°C and the other half was stored at 40°C. Periodically one jar of each solution and storage temperature was opened, the replicates removed from the solution, dried and prepared for thickness and oxygen transmission rate measurements. The pieces edges were removed before measuring to avoid the areas where demetallization have clearly occurred.

Sampling was performed after: 14, 33, 46, 56 and 75 days of contact.

Thickness measurement

Thickness was measured with a micrometer MI20 (Adamel Lhomargy, France).

Oxygen transmission rate determination

The oxygen gas transmission rate of the samples was measured using a Coulometric Sensor OXTRAN 2/20 – MH (Mocon, Inc.) with air at 100% gradient of oxygen pressure, at 23°C and 0% relative humidity (ASTM D 3985 "Standard test methods for oxygen gas transmission rate through plastic film and sheeting using a coulometric sensor"). The oxygen transmission rate of the film without contacting the wine solutions and of samples after complete demetallization was also measured.

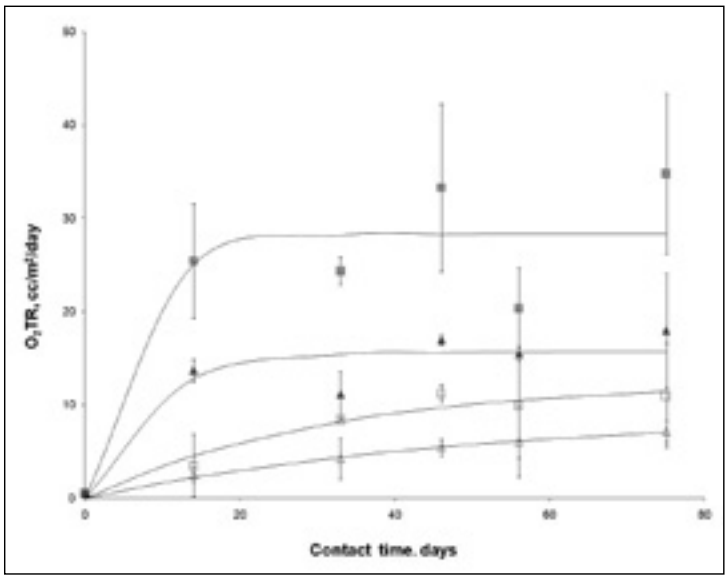


Fig. 3 - Oxygen transmission rate of film after contacting solutions simulating: red wine at 40°C (■), white wine at 40°C (▲); red wine at 23°C (□) and white wine at 23°C (Δ).

RESULTS

Fig. 3 shows the values of the oxygen transmission rates of MET PET film measured with different times of contact with the solution simulating wine. A trend line described by an exponential model was included to make the analyses more clear. In spite of a considerable variability between replicates, there was a clear increase in the oxygen transmission rate over time as a consequence of the demetallization process. Both the type of wine and temperature had an effect on the barrier's decrease: red wine solution presented an increase of 60 times at 40°C and 20 times at 23°C, whilst white wine solution presented an increase of 34 times and 12 times, respectively at 40° and 23°C. The oxygen transmission rate ranged from 0.5 cc/m^2 day (film without contacting the solution) to 103 cc/m^2 day (film completely demetallized). Red wine solution showed a greater impact on the barrier loss probably due to the higher volatile acidity.

The testing conditions followed in this work were more aggressive than the real case because the total edge of the testing pieces were immersed while in the real situation the edge comes into contact with the wine in a few spots where the seal allows for wine transfer. Additionally, in the experiment much more wine was in direct contact with the film as compared to the amount in the real situation because transfer into the gap is limited and slow. Nevertheless, the results found can

be put in perspective considering the real situation of the BIB shown in fig. 2. The total BIB surface area for oxygen transmission (A_u) is ca 2,300 cm² and the OTR of the unaffected material is $(OTR)_u$ 0.5 cc/m² day. After 30 days there is a surface area A_a of ca 100 cm² affected by demetallization that causes an decrease of the oxygen barrier of ca 10 times. The oxygen transmission rate of that area around the tap $(OTR)_a$ becomes equal to ca 5 cc/m² day. The maximum amount of oxygen Q (cc) allowed to be absorbed by the wine and still retain acceptable quality can be calculated by equation (1).

$$Q = (OTR)_u . A_u . \theta_u \quad (1)$$

Where θ_u (days) is the shelf-life time when the BIB is unaffected. Once the A_a surface area is demetallized, then the ingress of oxygen occurs in parallel by the two areas with different barriers:

$$Q = (OTR)_u . (A_u - A_a) . \theta_a + (OTR)_a . A_a . \theta_a \quad (2)$$

Where θ_a (days) is the shelf-life time when the BIB is affected. Then,

$$(OTR)_u . A_u . \theta_u = (OTR)_u . (A_u - A_a) . \theta_a + 10 . (OTR)_a . A_a . \theta_a \quad (3)$$

and

$$\theta_a = 0.72 . \theta_a \quad (4)$$

This means that the wine will present a shelf-life time 30% lower than in the case of no loss of BIB barrier.

CONCLUSIONS

Oxygen barrier properties of BIB are greatly affected by poor quality seals, allowing for wine ingress into the gap between the inner and outer film of the bag. Wine with higher volatile acidity tends to yield a higher loss of barrier. In both cases the higher the temperature the fastest and highest the demetallization. This loss of barrier may have an important impact on wine shelf-life, depending on the extent and degree of the demetallization. Therefore, even in the case of global bag integrity and absence of external leakage, the seals should avoid the contact of wine with the external film as this will cause a premature loss of wine quality.

Table 1 - Characteristics of the aqueous solutions for wine simulation.

	White wine solution (W)	Red wine solution (R)
Alcohol degree (% vol)	12.2	13.0
Volatile acidity (g acetic acid/L)	0.32	0.59
Total acidity (g tartaric acid/L)	5.51	5.72
Reduction sugars (g/L)	1.99	1.82

REFERENCES

Anonymous, 2006. Boxed wine trends drive BIB technology advances. Internet Journal of Viticulture and Enology ISSN 1826-1590; n. 12 (www.inforwine.com).

Doyon G., Clément A., Ribéreau S., Morin G., 2005. Canadian Bag-in-box Wine under Distribution Channel Abuse: Material Fatigue, Flexing Simulation and Total Closure/Spout Leakage Investigation. Packaging Technology and Science, 18, 97-106.

Dufrêne A., Shea P., Boulet J.C., 2007. Guide of Good Practices for the filling of wine in BIB. Performance BIB, França, 24 pp.

Nicolini B., 2006. Bag-in-box, Especificidades Técnicas dos Sacos. InfoWine, 1:4.

Robertson G.L., 2006. Aseptic Packaging. Chapter in Food Packaging Principles and Practice (2nd edn.). Robertson, G.L. (Ed.), Boca Raton: CRC Press, 381-408.

Sundell H.A., Holen B., Nicolaysen F., Hilton C., Lokkeberg O., 1992. Bag-in-box packaging for wine: Analysis of transport stress in barrier films. Packaging Technology and Science, 5, 321-329.

INFLUENCE OF PACKAGE SIZE FEATURE ON THE QUALITY DECAY OF SONGINO (*VALERIANELLA LOCUSTA* (L.) LATERR)

F. LOMASTRO[1], S. LIMBO[1], L. TORRI[2], L. FRANZETTI[1] and L. PIERGIOVANNI[1]

[1]DiSTAM, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, Italy

[2]University of Gastronomic Sciences, Piazza Vittorio Emanuele 9, 12060 Bra (CN), Italy

ABSTRACT

The goal of this work was to evaluate the effect of the ratio between the permeable area and the mass of produce on the quality of minimally processed Songino (*Valerianella locusta* (L.) Laterr), packaged in a polypropylene pouches under air. The preliminary hypothesis was that the ratio surface package/product mass could influence the product quality; however, for all the indexes considered, the obtained results have shown that the product mass influences the quality decay more than the permeable package surface.

Key words: minimally processed vegetables, package size, respiration rate, shelf-life.

INTRODUCTION

The need to consume fresh fruit and vegetables for a healthy diet has been emphasized recently, and the demand for more convenient fresh foods that are less processed but ready to eat has increased. The sensory appearance of the food products and the visual appearance of their packaging greatly influence the product's acceptance, as they can serve as quality cues for consumers (Dantas *et al.*, 2004). Many investigations have been performed until now to

study the effect of gas composition on produce quality and the role of different packaging materials in creating and maintaining the right gas composition inside the package (Fonseca *et al.*, 2002; Kima *et al.*, 2004). Moreover, the difficulties in matching properly product characteristics with film permeability and, at the same time, the market needs towards technological and aesthetic aspects (i.e. high transparency and strength, anti-fog properties, attractive printable surfaces etc) limit companies in their choices and in their effective strategies in prolonging shelf-life of minimally processed vegetables (MPVs). Thus, other package features could be taken into consideration in order to improve quality of MPVs during their shelf-life. For this reason, in this work, the role of packaging permeable area and the weight of product were considered in maintaining the quality of minimally processed Songino (*Valerianella locusta* (L.) Laterr).

MATERIALS AND METHODS

Minimally processed vegetable

Samples of Songino were directly supplied by a private company. The anti-fog polypropylene film had the following characteristics: O_2 permeability: 1,400 cm^3 m^{-2} $24h^{-1}$ bar^{-1} (23°C, 0%RH); CO_2 permeability: 3,780 cm^3 m^{-2} $24h^{-1}$ bar^{-1} (23°C, 0%RH); thickness: 40 μm.

For the two selected surface areas (400 and 800 cm^2), different weights of product were considered. In particular, 60, 100 and 125 g of Songino were packaged into pouches of 400 cm^2, while 120, 200 and 250 g of Songino into pouches of 800 cm^2. In this way, three different surface/mass ratios were obtained: 6.7, 4.0 and 3.2.

The Songino was packaged in air (20% of oxygen in nitrogen) directly by the company, using a vertical form-fill-seal machine and transported in refrigerated conditions to the laboratory within 2 hours. There the pouches were stored at 4÷0.5°C for 10 days.

During storage, O_2 and CO_2 levels, ethylene production, aerobic microbial growth and aroma profile by electronic nose were monitored.

Microbiological analysis

Mesophilic Aerobic Bacterial Count was done on Plate Count Agar, by pour plates, incubation at 30°C for 48 hours.

O_2 and CO_2 head space analysis

Head space analysis was carried out using a GC instrument Hewlett Packard 5890 series II (Palo Alto, CA, USA) equipped with a TCD set at 120°C and a column 2 m long stainless steel CTR I (Alltech Ass. Inc., Deerfield, IL, USA). The sampling was done in triple, injecting 50 μL of gas sampled from the packages with a 100 μL gastight syringe (Hamilton 1700).

Ethylene analysis

Ethylene analysis was carried out using a GC DANI 3800 (oven temperature 100°C and injector temperature 210°C) connected with a FID Shimadzu C-R3A Crhomatopac (detector temperature 210°C). The sampling was done in triple, injecting 1 mL of gas from each pouch using a disposable syringe.

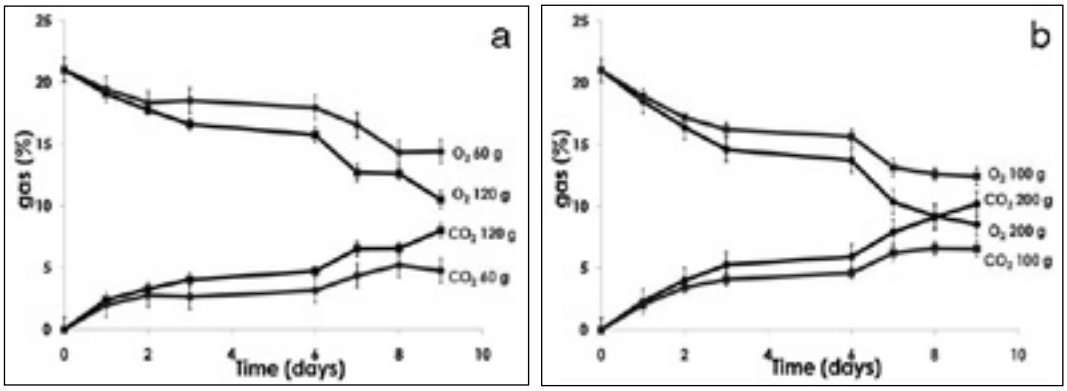


Fig. 1 - O_2 and CO_2 evolution for 6.7 (fig. 1a) and 4 surface/mass ratio (fig. 1b).

Electronic nose

A commercial portable electronic nose (PEN 2 model) from Win Muster Airsense (WMA) Analytic Inc. (Schwerim, Germany) was used. Songino was placed in 100 mL glass vials, sealed with a PTFE/silicone septum and a screw cap. For each ratio and at each storage time, five different samples were analyzed and the average of the results was used for the statistical analysis.

RESULTS AND CONCLUSIONS

Doubling the surface area of the package and the weight of the product, it would be expected a similar behaviour in terms of respiration and senescence of the vegetable. Actually, where the package mass of the product is higher, the O_2 consumption and the CO_2 production increase faster (fig. 1a and 1b).

A similar situation was monitored for the ethylene production (fig. 2a): considering the pouches with the doubled dimensions and mass of product, the quantity of ethylene accumulated over time is higher where the mass of the product is higher too.

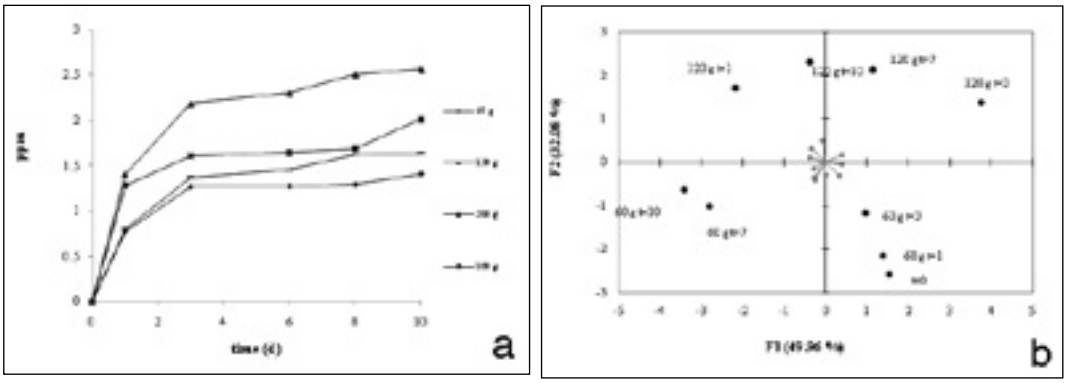


Fig. 2 - Ethylene production (fig. 2a) and PCA analysis carried out on electronic nose data (fig. 2b).

Also the Mesophilic Aerobic Bacterial Count demonstrates that lesser is the amount of product and smaller are the dimensions of the pouches, better is the microbial profile (data not shown).

Electronic nose results are described in fig. 2b; the bi-plot illustrates the mutual relationships between samples and sensors and evidences the sample separation according to the time and to the mass of the product.

Resuming, for all the indexes considered, the obtained results have shown that the product mass influences the quality decay more than the package surface. At a fixed surface/mass ratio analyzed, it has been observed that changes in quality parameters were delayed in the lowest mass of product. This is very likely due to the different head space volume that does not change proportionally to the surface change, leading to a possible higher dilution of CO_2 and ethylene.

Besides the variables which influence quality of minimally processed vegetables usually taken in account in choosing a flexible package (for example respiration of the product, microbiological growth) also the head space volume and the product mass should be adequately designed as critical features in extending produce shelf-life.

REFERENCES

Dantas, M.L.S., Minim, V.P.R., Deliza, R., Puschmann, R. 2004. The Effect of Packaging on the Perception of Minimally Processed Products. J. Intern. Food Agrib. Mark. 16, 2, 71-83.

Fonseca, S.C., Oliveira, F.A.R., Brecht, J.K. 2002. Modeling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review. J. Food Eng, 52, 2, 99-119.

Kima, J.G., Luo,Y., Gross, K.G. 2004. Effect of package film on the quality of fresh-cut salad savoy, Posth. Biol. Techn. 32, 1, 99-107.

MECHANICAL PROPERTIES OF PVC FILM AFTER CONTACT WITH FOODS SIMULANTES AND FOODS

NATHÁLIA RAMOS DE MELO[1]*, NILDA DE FÁTIMA FERREIRA SOARES[2], FILIPE SANTOS DE PAULA[2], EDIMAR APARECIDA FILOMENO[2] and WASHINGTON AZEVEDO DA SILVA[2]

[1]Federal University of Viçosa (UFV), DTA, Viçosa, MG, Brazil

[2]Fluminense Federal University, VEA, Volta Redonda, RJ, Brazil
*correspoding author: nathaliarm@vm.uff.br

ABSTRACT

Migration of packaging compounds can not only to cause toxicological problems for the consumer as well as to compromise the chemical and mechanical properties of the packaging material used. The purpose of this work was to evaluate the mechanical changes of resistance and elasticity of poly(vinyl chloride) (PVC) plasticizer film after being in contact with foods simulants and foods. Two kinds films were select from market and submitted to migration processes using simulants (95% ethanol, isooctane and water) at 5±1°C and 20±2°C and in contact with sliced mozzarella cheese and ham stored at 5±1°C. The films were removed after the first and fifteenth day of contact and submitted to mechanical analyses. The mechanical tests were realized using the Universal Materials Testing Machines. Changes have been observed in mechanicals properties of the PVC films after contacting the simulants and the foods. It showed increasing in tensile strength at break values and decreasing in elongation at break values in most of the evaluated cases. The film's mechanical properties changes were more accentuated when they were in contact with 95% ethanol and isooctane than water. The mechanical changes also were accentuated in tests with cheese than ham. Results showed that elongation decrease and the strength increase with temperature increase. The migration of packaging compounds change the mechanical properties can damage its utilization, consequently compromising the shelf-life of the products.

Key words: Migration, plasticizer, PVC, packaging, food.

INTRODUCTION

The last decades have been marked by the increasing use of polymeric materials, especially plastics, food packaging. Among these we can highlight the poly (vinyl chloride) (PVC), a very versatile polymer to be compatible with various plasticizers maintaining their physical and chemical stability for long period of time (Midio and Martins, 2000).

Improper use of polymeric material can lead to migration of compounds added to its formulation as the plasticizers that are added and among its goals to reduce hardness in the finished product, greatly altering its mechanical behavior (Rudolph *et al.*, 2002).

This migration depends on the physical and chemical characteristics of food that is in contact with the material, temperature and contact time of packaging the product, the ratio of contact surface/volume of food, the thickness of plastic packaging in addition to the various techniques used in food packing (Lau and Wong, 2000).

This migration depends on the physical and chemical characteristics of food that is in contact with the material, temperature and contact time of packaging the product, the ratio of contact surface/volume of food, the thickness of plastic packaging in addition to the various techniques used in food packing (Lau and Wong, 2000). Migration of packaging compounds can not only to cause toxicological problems for the consumer as well as to compromise the chemical and mechanical properties of the packaging material used.

The purpose of this work was to evaluate the mechanical changes of resistance (tensile strength at break, N) and elasticity (elongation at break, %) of poly(vinyl chloride) (PVC) plasticizer film after being in contact with foods simulants and mozzarella cheese and ham.

MATERIALS AND METHODS

Two PVC films (10 μm) were select from market.

The PVC films samples were cut in dimensions 5x10 cm and submitted to migration processes:

- Using foods simulants (95% ethanol, isooctane and water)

The samples were put inside tight closed bottle with 20 mL the simulants and kept at 5±1°C and 20±2°C.

- Using foods (mozzarella cheese and ham)

The sample were put in contact with sliced mozzarella cheese and ham and stored at 5±1°C.

In the between experiments the films were removed after the first and fifteenth day of contact and stored at 23±2°C and 50±5% relative humidity (RH) for at least 48 hours of time and submitted to mechanical analyses following the standards NBR 7452 (1982), ASTM E171-94 (1996) and ASTM D681-95 (1996) procedures. It was to evaluate the mechanical changes of resistance (tensile strength at break, N) and elasticity (elongation at break, %) of PVC plasticizer film.

The mechanical tests were realized using the Universal Materials Testing Machines (Instron - model 3367) with 1 kN load cell, a speed of 50 $mm.min^{-1}$ and initial distance of 50 mm between grips.

The processed data were obtained from ten different observations taken from

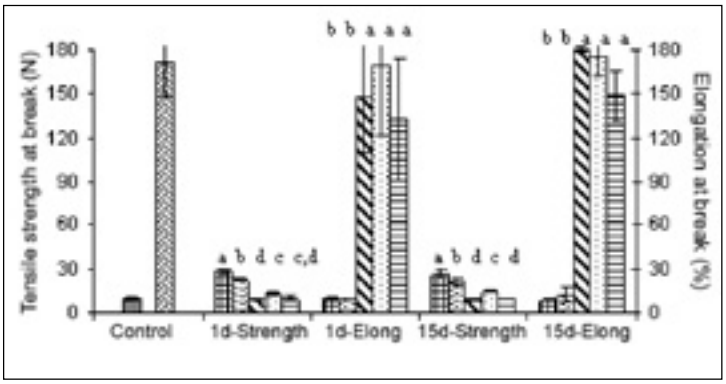


Fig. 1 - Means in the same group bearing the same letter are not significantly different (p>0,05) according to Tukey's test.
Means values to mechanicals properties of tensile strength at break (strength) and elongation at break (elong) to PVC film after contact with mozzarella cheese (▭) or ham (▭) or foods simulants (95% ethanol (▦), isooctane (▩) and water (▨)) kept at 5±1°C. In the times one (1d) and 15 days (15d). Control film: Tensile strength at break (▩) and elongation at break (▨).

three different experiments. Data were subjected to statistical analyses performed with Statistical Analysis System software package v.9.1.

RESULTS AND CONCLUSIONS

Changes have been observed in mechanicals properties of the PVC films after contacting the simulants and the foods. It showed increasing in tensile strength at break values and decreasing in elongation at break values in most of the evaluated cases. The films became more rigid. The film's mechanical properties changes were more accentuated when they were in contact with 95% ethanol and isooctane than water. The mechanical changes also were accentuated in tests with cheese than ham (fig. 1). This behavior can be explained by the possibility of a higher migration of the plasticizers from this film that presents apolar characteristic and therefore greater solubility in apolar environment. The decreasing of plasticizer's concentration increases the interactions between adjacents chains of the polymer, decreasing in that way the film mobility and flexibility. Results showed that elongation decrease with temperature (p>0,05) (fig. 2 and 3), however there was not a significant change (p<0,05) between temperature and strength, but numerically was observed a higher variation at higher temperature (increase by 2.63; 2.36 and 1.10

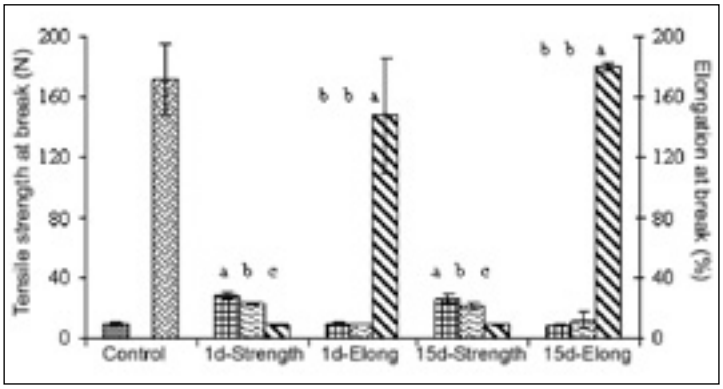


Fig. 2 - Means in the same group bearing the same letter are not significantly different (p>0,05) according to Tukey's test.
Means values to mechanicals properties of tensile strength at break (strength) and elongation at break (elong) to PVC film after contact with foods simulants (95% ethanol (▦), isooctane (▩) and water (▨)) kept at 5±1°C. In the times one (1d) and 15 days (15d). Control film: Tensile strength at break (▩) and elongation at break (▨).

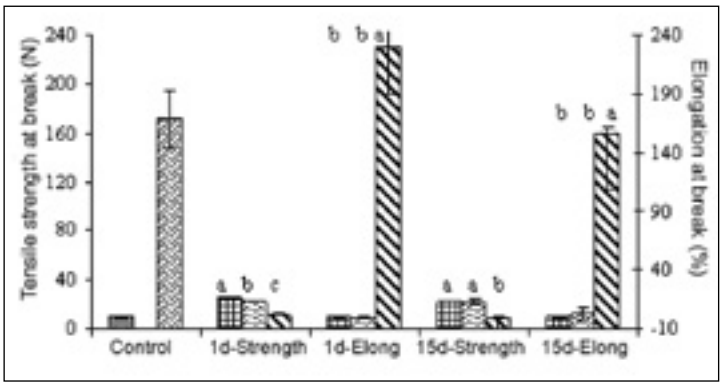


Fig. 3 - Means in the same group bearing the same letter are not significantly different (p>0,05) according to Tukey's test.
Means values to mechanicals properties of tensile strength at break (strength) and elongation at break (elong) to PVC film after contact with foods simulants (95% ethanol (▦), isooctane (▨) and water (▩)) kept at 20±1°C. In the times one (1d) and 15 days (15d). Control film: Tensile strength at break (▩) and elongation at break (▨).

times after contact with 95% ethanol, isooctane and water, respectively). Higher temperatures can lead to a higher migration of the polymer structure's components. The migration of packaging compounds change the mechanical properties can damage its utilization, consequently compromising the shelf-life of the products.

ACKNOWLEDGMENTS

CAPES, CNPq, Fapemig, FINEP, FAPERJ.

REFERENCES

Lau O.W., Wong S.K. 2000. Contamination in food from packaging material. J. Chromatography A. 882:255-70.

Midio A.F., Martins D.I. 2000. Toxicologia de Alimentos. 1st Ed. São Paulo, Varela Editora e Livraria Ltda. 295p.

Rodolfo A.Jr., Nunes L.R., Ormanji W. 2002. Tecnologia do PVC. 1st Ed. São Paulo, ProEditores/ Braskem. 399p.

American Society for Testing and Materials – ASTM. 1996. Standard practice for conditioning plastics and electrical insulating materials form testing – D618-95. Philadelphia: ASTM. 3p.

American Society for Testing and Materials – ASTM. 1996. Standard specification for standard atmosphere for conditioning and testing flexible barriers materials – E171-94. Philadelphia: ASTM. 1p.

Associação Brasileira de Normas Técnicas – ABNT. 1982. Plásticos – atmosferas padrão para condicionamento e ensaio – NBR 7452. São Paulo: ABNT. 5p.

ROLE AND FUNCTION OF FOOD PACKAGING: WHAT CONSUMERS PREFER

G. MURATORE[1] and A.S. ZARBÀ[2]
[1]DOFATA, University of Catania, Via Santa Sofia 98, 95123 Catania, Italy

[2]DISEAE, University of Catania, Via Santa Sofia 98, 95123 Catania, Italy

ABSTRACT

Today, food habits have more and more tended to prefer food according to its safety-related aspects, such as, hygienic-health conditions, the use of environmental-friendly production techniques, organic raw materials, true controls throughout the production cycle, and its ethic-related ones, that is, sustainability and protection of the environment. The packaging, then, is to be considered among the specific elements that catch consumer's needs and desires, affects the buying and consumption behavior, a sort of "means of dialog" within the market. Several are the materials used for food packaging. Some are better for processed food since they keep unaltered the taste, protect it from contamination, make the content clearly visible and have details that foster consumption. We are talking of glass packages, which have become more and more popular, especially within the large-scale retail trade. Glass packages, in fact, meet EC and national regulations concerning recyclable and eco-friendly products, on one side, and the need of increasing the added value of food, on the other. This survey has aimed at highlighting the importance of glass packaging within the distribution of processed food. Recent surveys, in fact, have pointed out a wide range of determinants of the demand for glass packages. They include more social and psychological aspects than economical ones, even if they are very much correlated to each other. However, by means of a survey among retailers, we tried to catch the reasons why consumers prefer glass containers rather than others made with different materials still widely used to package common food. The results showed that consumers prefer glass packaging because they consider glass healthier, better preserving food and its sensory and nutritional characteristics. It is also appreciated to better present the product, making it more attractive and elegant, especially that manufactured with a strong local know-how and the organic ones.

Key words: Consumer preference, glass packages, food packaging, market.

INTRODUCTION

The survey on the reasons that draw consumers towards the use of glass packages, with respect to other widely used materials employed for food products, has been based on a conceptual model which allows defining the consumer decision-making process by linking product knowledge with self-knowledge (Fabris, 2003; Ferraresi, 1999; Gutman, 1982; Olson, 1995). The basic assumption to the approach is that the consumer possesses information about the directly perceivable features of the product (attributes) and on the benefits and/or risks associated with its purchase and use (consequences), moreover, that he could recognize the general life scopes he wants to fulfill (values) in order to realize the link attributes-consequences-values (means-ends chain); it is assumed, therefore, that the consumer considers product attributes as means to achieve specific consequences or gain certain values (Dalli and Romani, 2009). The conceptual tool to which we refer, thus, represents a theoretical model which allows keeping tightly interconnected the knowledge concerning "primary containers" (intrinsic characteristics and consequences expected from their utilization) and information regarding the consumers themselves (link among expected consequences and preferred values). To sum up, consumers purchase products (package-food) not for themselves or their characteristics, but rather for the meaning they engender (Dalli and Romani, 2009). The primary package *tout court* has a potential existence until it is referred to the shop shelves and it is actualized only at the moment of purchase or successive use. The aim of the study is to identify the fundamental elements to elaborate cognitive maps or *hierarchical value maps* (HVM) to understand how the interviewed perceive hollow glass packages and the personal meaning that they attribute to it, that is the different levels of "conceptualization" of their choices (Reynolds and Gutman, 1988).

MATERIALS AND METHODS

The interviews were carried out at different points of sale of the large-scale retail trade located in the main urban areas of Sicily on a sample of 500 consumers, with the aid of a questionnaire based on the laddering interview technique, with the following purposes: 1. Indicate the "material attributes" and the "immaterial attributes" determinant in the choice; 2. Mark the motivation for choice (that is, the "functional consequences" and/or the "psycho-social consequences"); 3. Finally, associate each motivation with an "instrumental value" and with a "terminal value".

A sample of 195 subjects, on which the interview was continued, was chosen from the initial wider sample as representative of individuals whose purchase behavior differs from the habitual scheme, as in the case of brand loyalty or store loyalty, factors which bind the package preferences to the quality characteristics of the product and to preexisting cognitive schemes which play in the consumer's background (unconscious or subconscious level). In this way it was possible to set up hierarchical value maps (HVM) which reflect the wishes and behavior rules of consumers as far as the link package-product is concerned. In the setup of the HVM they were taken into account those who showed difficulties at going beyond the level of "consequences", that is towards the formulation of a final value, even if stimulated by suitable tips by the interviewer; for this reason the means-ends chain may results incomplete.

RESULTS AND CONCLUSIONS

In tab. 1 the elements of the means-ends chain for the representative sample are reported, starting from the knowledge of the interviewed on the package attributes, that is from the means to achieve certain consequences and values (aims). For space limits the hierarchical value maps have been elaborated with respect for the individual (gender, age) and socio-economic (income) characteristics most frequent in the interviewed sample. The maps include a series of "nodes" which identify the relative conceptual meanings and attributes, consequences and values (abstract) reported in tab. 1, interconnected with primary order lines (principal) and secondary order ones (secondary) from bottom to top, according to the above-mentioned means-ends order. From the observation of the principal map chain elaborated for gender (tab. 2), for both sexes prevails the association of the material attribute "ease of use" with the terminal value "tradition", in this way it will be demonstrated that up to date exists a certain binomial hollow glass container-foods, with all the

Table 1 - Distribution of the elements investigated for the components of the means-ends chain concerning the hollow glass package for foods.

Terminal values	Tradition (VT1); Environment (VT2); hédonisme (VT3).
Instrumental value	Domestic recycle (VS1); Suitable as a gift (VS2).
Psycho-social consequences	Feeling comfortable (CP1); More appreciated by others (friends, acquaintances, etc.) (CP2).
Functional consequence	Better and longer preservation of food quality (CF1); Maintains the sensory characteristics of food for longer (CF2).
Immaterial attribute	Immediate impression of quality (AI1); Makes food more appealing (AI2).
Material attribute	Transparency (AM1); Ease of use (AM2).

Table 2 - Representation of hierarchical value maps, for gender, relative to primary food packaged in Sicily (2009-2010).

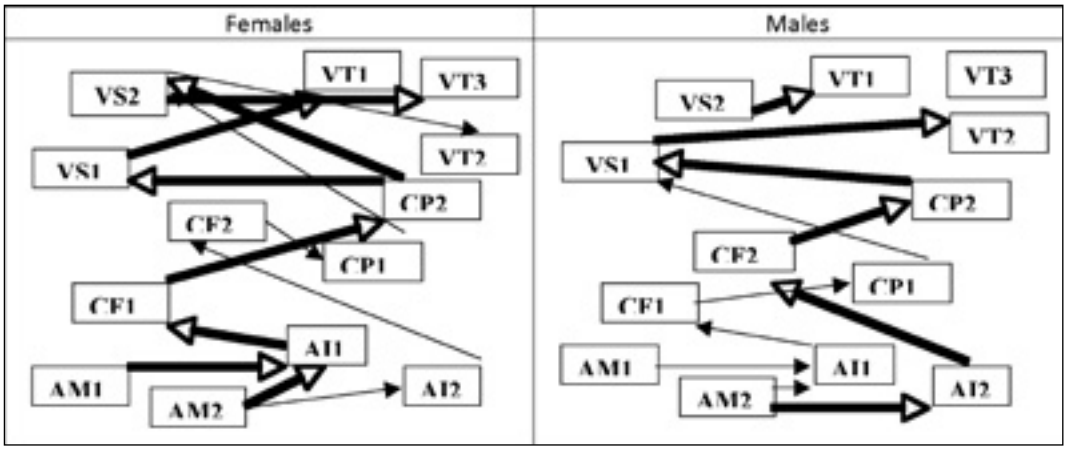
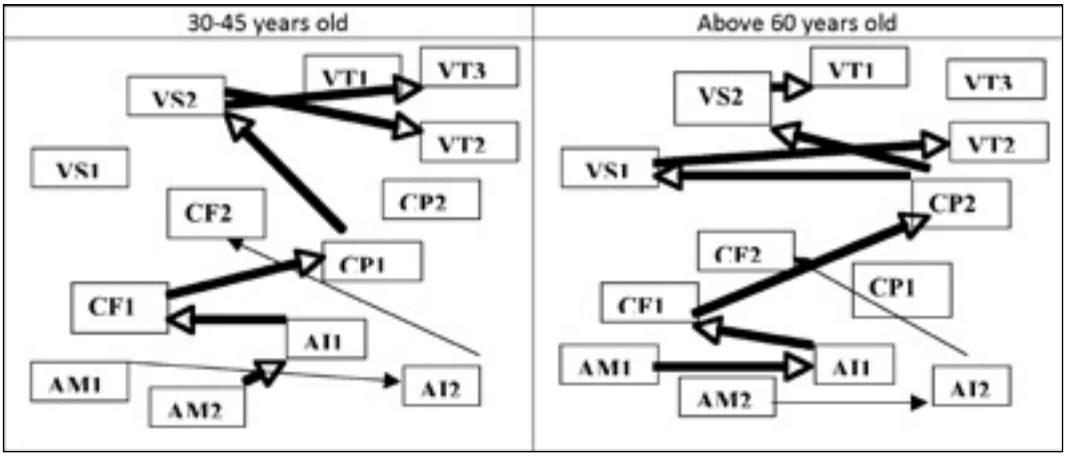
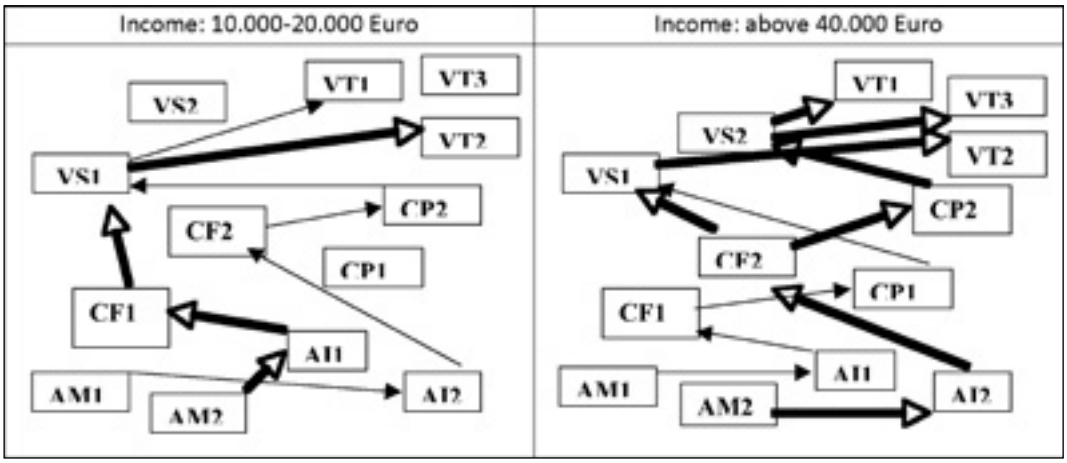


Table 3 - Representation of hierarchical value maps, for main age classes, relative to primary food packaged in Sicily (2009-2010).



relative consideration of genuineness and salubrity connected and with regards for a longer shelf-life. Other terminal values put in evidence some differences between the two sexes; in the case of female sex, the concept of "hedonism" covers some importance, while the "environmental" concept prevails for males. Moreover, shifting the point of observation on other main features of the interviewed, other differences emerge; considering the most representative age classes, it appears that in the range 30-45 years old, "tradition" do not represent a value, same as "hedonism" for the over-60 class (tab. 3). Moreover, the origin of the map is constituted, in correspondence with the former age class, by the material attribute "ease of use", while in the latter class by the "transparency" attribute, despite both classes converge towards the same immaterial attribute, "immediate impression of quality". For what concerns the income brackets examined (tab. 4), the above 40.000

Table 4 - Representation of hierarchical value maps, for main income brackets, relative to primary food packaged in Sicily (2009-2010).



Euro represents all terminal values declared by the interviewed, while in the lowest class (10.000-20.000 Euro) the "environmental" factor is the prevailing terminal value. Although consumers represent a wide and heterogeneous category, which expresses equally wide and differentiated requests, the aspects connected with tradition, environment and hedonism constitute primary needs also in the case of preferences expressed for primary packages used for foods. The citizen is now aware that the glass containers used for storing food and beverages are produced in accordance with good manufacturing practices that respect the discipline of packaging and hygiene conditions of assurance and quality control (aimed to be the basis to ensure a high level of protection of human health and consumer interests), took down in Community legislation in force, and in particular by Regulation (EC) N. 1935/2004 of the European Parliament and of the Council on materials and articles intended to come into contact with foodstuffs and Commission Regulation (EC) N. 2023/2006 on good manufacturing practice for materials and articles intended to come into contact with foodstuffs.

ACKNOWLEDGEMENTS

Authors would like to thank Valeria Allegra for the precious contribution in the phases of data collection and elaboration.

REFERENCES

Dalli D., Romani S. 2009. Il comportamento del consumatore. Acquisti e consumi in una prospettiva di marketing. F. Angeli Ed., Milano.

Fabris G. 2003. Il nuovo consumatore: verso il postmoderno. F. Angeli Ed., Milano.

Ferraresi M. 1999. Il packaging oggetto e comunicazione. F. Angeli Ed., Milano.

Gutman J. 1982. A means-end model based on consumer categorization processes. Journal of marketing, 46: 60.

Olson J.C. 1995. Introduction. Intern. J. Research in Marketing, 12: 189.

Reynolds T.J., Gutman J. 1988. Laddering theory, method, analysis, and interpretation. Journal of Advertising Research, 28: 11.

THE INFLUENCE OF LIGHTING ON SHELF-LIFE OF EXTRA VIRGIN OLIVE OIL PACKAGED IN COLOURED PET BOTTLES

V. RIZZO[1], L. TORRI[2] and G. MURATORE[1]

[1]DOFATA Dipartimento OrtoFloroArboricoltura
e Tecnologie Agroalimentari, Sezione Tecnologie Agroalimentari,
Via Santa Sofia 98, 95123 Catania, Italy

[2]University of Gastronomic Sciences,
Piazza Vittorio Emanuele 9, 12060 Bra, CN, Italy

ABSTRACT

Different storage solutions aimed to assess the shelf-life of extra virgin olive oil (EVOO) packed in PET bottles are presented. Coloured PET bottles (clear, green, orange, white and blue) were stored under different artificial lighting conditions in order to simulate the market conditions (one fluorescent cool white lamp), to perform an accelerated test (4 fluorescent cool white lamps), and in the darkness as control. Acidity, peroxide value, spectroscopic indices (K_{232}, K_{270}, K_{316}), chlorophyll fractions, β-carotene and total polyphenols were determined. Oil colour was measured according to CIE L*-a*-b* scale. Results suggested that the quality indexes most affected by lighting and useful to investigate the bottle colour influence were total peroxide value, polyphenols, chlorophyll, and carotenoid contents. Between the PET bottles stored under one fluorescent lamp, the best performances were obtained using the blue and white colour, while under accelerated conditions, the differences in the protection efficacy offered by the differently coloured bottles was reduced.

Key words: extra-virgin olive oil, light transmittance, PET bottles, photo-oxidation, shelf-life, storage time.

INTRODUCTION

Consumers perceive glass bottles as an added value to the product, assuming the pair: “glass-quality”. Olive oil is mainly packaged in glass bottles, while seed oil is already commercially available in clear or coloured PET bottles. Considering

the disadvantages of glass (price, fragility, weight, etc.), the benefits of PET bottles (mechanical resistance, lightness, etc.), and taking also into account the huge chance of tailoring their performance, it is reasonable to look at PET as a possible, valid substitute of glass bottles also for traditional products like extra virgin olive oil (EVOO). The aim of this work was to monitor the changes of qualitative parameters in EVOO packaged in PET coloured bottles stored under light and in the darkness, in order to evaluate their potential for a shelf-life extension.

MATERIALS AND METHODS

Characterization of PET bottles

Coloured PET bottles were characterized in terms of light transmission properties according to ASTM Official methods. Moreover, plastic samples were exposed to a fluorescent lamp and the radiant flux that pass through them was measured with a digital handle photo-radiometer (Delta OHM HD 2102.2) equipped with probes for the measurements of illuminance (lux) and irradiance in UVA and UVB regions ($W\ m^{-2}$) in order to evaluate the light shielding effect offered by the packaging materials, expressed as percentage of irradiance recorded in the presence of sample respect to the irradiance recorded in the absence of sample.

Sampling

Fresh EVOO was dispensed into PET bottles having different colour (clear, green, orange, white and blue). Bottles were full-fill without any headspace, then hermetically sealed, divided into three series and stored under different artificial lighting conditions: the first to simulate the market conditions (under one fluorescent cool white lamp); the second to perform an accelerated test (under 4 fluorescent cool white lamps); the last series was stored in the darkness as reference. Storage temperature was monitored periodically and EVOO was sampled every 20 days for 6 months.

Analysis

The acidity, the peroxide value and spectroscopic indices in the UV region (K_{232}, K_{270}, K_{316}) were determined according to the EU official methods. Oil colour was measured according to CIE L*, a*, b* scale. Chlorophyll fractions were studied measuring the absorbance at 670 and 470 nm respectively (Morello *et al.*, 2004). β-carotene were monitored at 449 nm (Caponio *et al.*, 2005). Total polyphenol analysis was performed as reported by Gutfinger (1981).

RESULTS AND CONCLUSIONS

The illuminance and the irradiance shielding offered by the PET bottles were studied. As reported in fig. 1 white and blue bottles had the highest shielding, in fact, as reported in our results, bottles of these two colours gave the best performance maintaining the EVOO quality. Transmittance spectra are useful to correlate chemical results with the difference between green and orange bottles. In fact while the orange bottle has a lower shielding in UV-VIS, the transmittance at the carotenoid and chlorophylls secondary components wavelengths, respectively 449 and 470 nm, is lower for orange then for the green bottles (data not shown). Results were sta-

tistically analysed using multivariate methods. The three-way analysis has shown that the quality indexes, most affected by lighting and useful to investigate the bottle colour influence, were total peroxide value, polyphenols, chlorophyll, and carotenoid contents, whereas changes in acidity, bitter index and absorption coefficients provided weak information in sample discrimination (fig. 2 and 3).

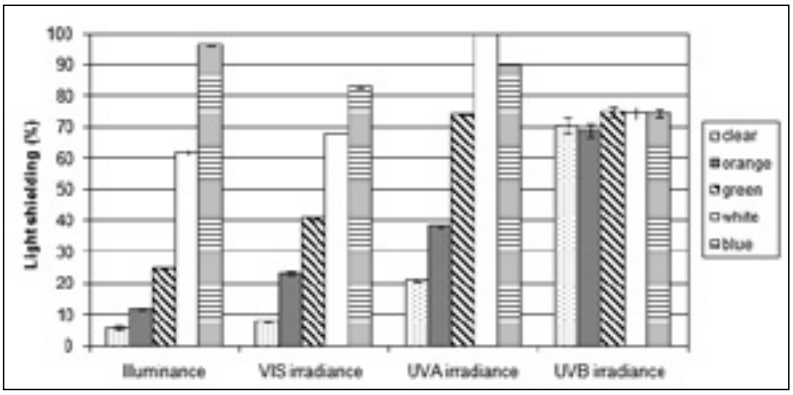


Fig. 1 - Light shielding offered by the coloured PET bottles.

Concerning the global quality of the olive oil, the best performances were obtained using the blue and white PET bottles stored under one fluorescent lamp. Under accelerated conditions, the differences in the protection efficacy offered by the differently coloured bottles was reduced. Cluster analysis results h that the parameters of EVOO stored after 80-120 days under one lamp, explained similar characteristics with those at 40-60 days under accelerated conditions (data not shown). In conclusion, obtained results seem to suggest that quadrupling the light source the EVOO degradation intensity increases twofold. Further study could be investigate and better describe the relation between the quality loss and the lumi-

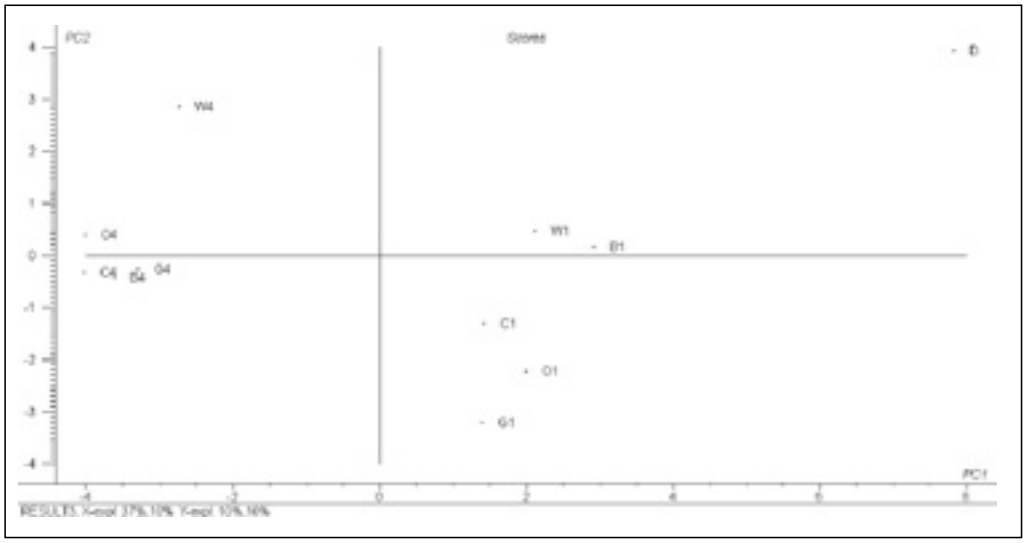


Fig. 2 - Score plot (D=darkness, B=bleu, W=white, C=colourless, O=orange, G=green; 1=one lamp, 4=four lamps).

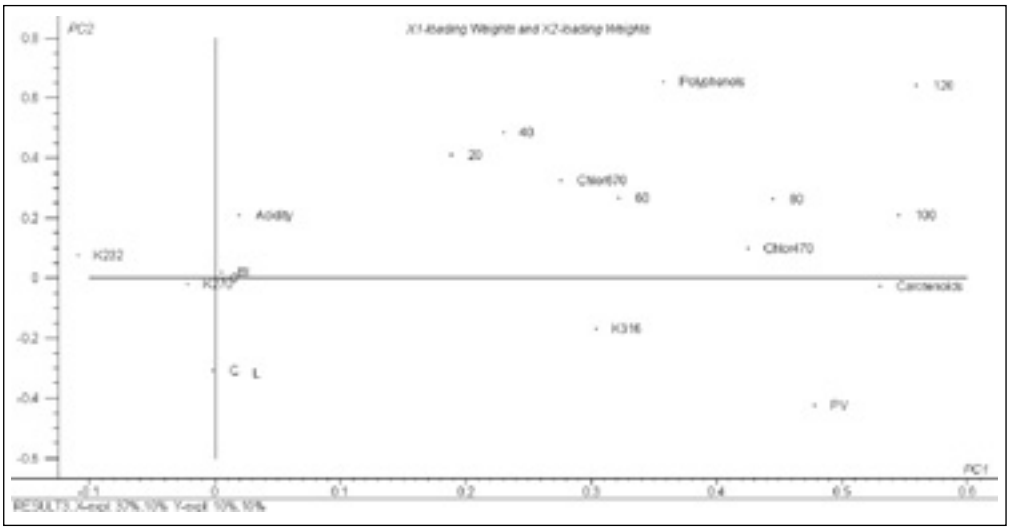


Fig. 3 - Loading plot (numbers identify the storage time in days).

nous intensity providing helpful information to design shelf-life accelerated tests or modelling studies.

REFERENCES

ASTM D1003 - 07e1 Standard Test Method for Haze and Luminous Transmittance of Transparent Plastics. Novembre 2007.

Commission Regulation (EEC) N. 2568/91 of 11 July 1991 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Off. J. Eur. Communities 1991, July 11.

Morello J.R., Motilva M.J., Tovar M.J., Romero M.P. 2004. Changes in commercial virgin olive oil (*cv Arbequina*) during storage, with special emphasis on the phenolic fraction. Food Chem. 85:357-364.

Caponio F., Bilancia M.T., Pasqualone A., Sikorska E., Gomes T. 2005. Influence of the exposure to light on extra virgin olive oil quality during storage. Euro. Food Res. Tech. 221:92-98.

Gutfinger T. 1981. Polyphenols in olive oils. J. Am. Oil Chem. Soc., 58:966-968.

ECONOMIC ANALYSIS CITRUS FRUITS DESTINED TO MARKETS

A. SCUDERI and A.S. ZARBÀ

University of Catania, Via Santa Sofia 98, 95123 Catania, Italy

ABSTRACT

The majority of Italian citrus fruit production is destined to the fresh market, with prevalence for the domestic one, while a variable amount between 5 and 8% is intended for exportation. Concerning Italian production of citrus fruit, Sicilian Red Orange started a strategy that sees internationalization as one of the strongest point to exploit it. Foreign markets for future expansion, besides European ones, could be those overseas, such as United States, Canada and Japan. In order to export to these markets, specific agreements between Italy and destination countries were signed, based on severe phytosanitary protocols (to control the healthy characteristics of products), that consider "cold treatment" over quality controls. Being compliant with such protocols and transportation logistics affects oranges to be exported a lot, with the result of final fruits of a quality that often does not meet consumers' expectations and limits shelf-life above all. This paper, retracing the main steps that have characterized the definition of the international agreements and the main stages of product treatment and transportation logistics, aims at identifying the most critical issues and solve them in order to improve quality and final shelf-life of products. This review was carried out throughout 2009, studying citrus fruit commercialization process of some Sicilian companies of the sector. From the beginning, in fact, many critical points came out either for processing procedures or transportation logistics, generating considerable costs and a limited residual shelf-life. Jointly an overall economic assessment of costs arising from exportation was made, as well as an evaluation of oranges through a home-use test, as instruments of the "consumer science" adopted for a subjective evaluation of the product.

Key words: economic, shelf-life, red orange, export, consumer.

INTRODUCTION

For Italian citrus fruits foreign markets have always been a privileged commercial route, although there has been a reduction of the amount exported up to

only 6-8% of that sent to the fresh market (equal to almost 232 thousand tons), rate also due to the significant participation of oranges produced in Sicily. In this scenario the foreign markets of reference for Sicilian citrus fruits are Central and Northern European Countries, and sometimes some overseas countries (United States, Canada) including Asia. (Japan) In such a context, in a foreign country, saving quality and improving citrus fruits of "shelf-life" is very important. Within the initiatives started in Italy, the Consortium P.G.I. Red Orange of Sicily carried out specific researches and analyses to improve the quality of the product exported to foreign markets, since it believes that the strategies and the commercial organization are the grounds for the International mission for our citrus fruits. In fact, in a market where competition is growing every day more and more, initiatives, such as technological innovations that can make products last more on the market keeping unaltered their quality characteristics, which are unique from the organoleptic point of view, especially those of pigmented oranges that last more than one month after picking, being destined to far markets, are very important because contributing to the market expansion towards foreign destinations. The work presented is intended to provide initial results of research that studies the phases of citrus fruit commercialization, including the intrinsic product characteristics, commercial processing, international phytosanitary protocols and the specialization level of export companies, which is often inadequate, where it is possible to foresee, eliminate or reduce the risks that affect quality and shelf-life of the oranges intended for exportation, in particular to Japan.

MATERIALS AND METHODS

The analysis here presented concerned operators of the orange sector. It aimed at acquiring data and information by means of a face to face interview and an ad hoc questionnaire. It aimed at pointing out the stages, operations, and sections where it is possible to intervene to prolong shelf-life of final products to be exported, in particular to Japan. Specific attention was paid to the sensory analysis on 10 samples of Tarocco oranges tested in order to link all the product's characteristics to the perception of people (Meilgaard, 1999); for this a descriptive-quantitative analysis was made (UNI 10957:2003 – Sensory analysis – Method to define food) articulated into 4 stages: 1) definition of the vocabulary used for the sensory description; 2) definition of the standards of reference for each descriptive word, corresponding to the maximum value of intensity on the scale used; 3) evaluation of the intensity of each descriptive word; 4) statistical analysis and interpretation of results.

RESULTS AND CONCLUSIONS

Specifically the following elements were considered: cultivation techniques, picking, selection, storage, post-picking treatment, packaging, National logistics, International logistics, and distribution (tab. 1). Start controls carried out by the phytosanitary authorities, to check the presence of parasites, with specific attention to *Ceratitis capitata,* and the Agecontrol for quality checks. After controls follows prerefrigeration, then transfer to self-refrigerated containers to start the in-transit cold treatment (Di Renzo, 2007). This includes cooling and ventilation by means of special devices that keep cooling goods throughout transportation up to 1°-2°C

Table 1 - Analysis of time requirement and cost of shipping to Japan (Year 2009).

Description	Time requirement (days)	Costs	
		(Euro/kg)	%
Special growing techniques	0	0,04	3,25
Picking	1	0,08	6,50
Trasportation to processing premises	0	0,02	1,63
Storage	2	0,01	0,81
Selection and packaging	1	0,30	24,39
Pre-refrigeration	1	0,02	1,63
Container preparation	1	0,03	2,44
Trasportation and logistic to Calabria	3	0,06	4,88
Trasportation by ship to Japan	28	0,42	34,15
Custom clearance	2	0,10	8,13
Internal logistic	4	0,15	12,20
Totals	**43**	**1,23**	**100,0**

for 20 days (Lanza G., 2006). Then is the turn of International transport agencies that take care of land transportation of containers up to the harbor of Gioia Tauro, in Calabria, where goods are loaded in cargo ships up to destination. Time varies from 18 days for the USA to 28 days for Japan. Then is customs clearance and phytosanitary controls carried out by local authorities, who check that the in-transit cold treatment process was regular before issuing visa to let the product in. Products are then distributed to distribution platforms for 2-5 days, and reaches final points of sale after 1-2 days. Besides the stages mentioned so far, there is shelf-life time in points of sale. It lasts from 10 to 15 days, but after 7 days already, with no cold storage, the skin starts perishing and fruits start losing compactness. On the whole, all stages of logistics last too much for the characteristics of the red orange, that is, less compactness than other oranges, and higher costs, which can meet Japanese market, but remain a big obstacle for potential expansion towards other ones, both eastern and overseas in general. Concerning the shelf-life at points of sale, it was noted that in relation to the stress that the product undergoes, it is not very satisfying from a marketing point of you, due to the fact that products stay too short a time at final destination. Out of this, we understand that quality of fruits undergoing in-transit cold treatment and logistics procedures, is very poor. Here follows a sensory analysis carried out in 2009 over 10 samples of red oranges of the Tarocco variety, which underwent the same treatment of fruits exported to Japan. The fruits were then given to 6 tasters, 3 males and 3 women, between 40 and 60 years old, who regularly eat oranges. The samples were compared to other ones taken from fruits destined to the Domestic market. All samples were randomly labeled with 3 digit-numbers in order to make them anonymous. Evaluation scale was from 0 to 100 for the following parameters: taste, smell, sweetness, external appearance and compactness. In order to avoid influence by others' comments, fruits were offered in a different order to each taster. Results of this analysis show lower values for smell, external appearance and compactness, and similar ones for taste and sweetness. From the shelf-life point of view, the three elements that got a negative result, affect it a lot, confirming that, despite the good taste, it is very difficult to have consumers choose our product for its external characteris-

tics, which is a very important parameter for a niche market as the Japanese one, where final consumers pay great attention to it (Kotler, 2002). Results reported in the previous paragraph show us some indications on how to improve the stages of commercialization of oranges to save quality and prolong shelf-life at final destinations, that is, foreign markets, especially the far ones, like Japan. So, the resistance of fruits must be strengthened over 40 days after picking. Besides, this issue deserves special attention since exportation trends have progressively dropped. This survey has provided data and information concerning the interventions necessary to better prepare oranges destined to exportation. It has not gone further so far. In fact, data emerged from the survey cannot exhaustively cover the huge range of interventions that can be made for the orange productions destined to exportation, which can be improved by supporting research and involving other field's operators who are more interested in foreign markets. However, we deem that the results obtained can draw a good picture on today's trade conditions for oranges and offer important suggestions on how to develop good strategies to maintain quality in foreign markets.

REFERENCES

Kotler P., 2002. Il marketing secondo Kotler. Come creare, sviluppare e dominare i mercati. Il sole 24 ore, Milano.

Lanza G., Strano M.C., Calandra M.R., 2006. Mantenimento della qualità dei frutti di agrume destinati all'esportazione: aspetti biologici, tecnici e fitosanitari; in Informatore Fitopatologico, 56 (11), p. 16-25.

Meilgaard M., Civille G.V., Car B.T. 1999. Sensory evaluation technique, CRC Press, 3rd ed. Boca Raton, p. 173-183.

ANALYSIS OF THE POTENTIAL USE OF ASLT FOR PREDICTING THE SHELF-LIFE OF FOOD PRODUCT

M. UCHEREK

Cracow University of Economics, Faculty of Commodity Science,
Packaging Department, Rakowicka 27 St., 31-510 Cracow, Poland
corresponding author: ucherekm@uek.krakow.pl,
Tel. ++48/+12/2935167, Fax ++48/+12/2935017

ABSTRACT

The purpose of carried out tests is to analyze the possibility of using accelerated testing to determine the sustainability of the products packed in a mixture of gases, depending on type of packaging and output composition of gas mixture in the package.

Snacks packaged in MAP using metal laminate bags as well as cans were used as material for the experiment. The alternative versions of the initial composition of gas mixture were used. In order to determine the influence of the storage on the durability of the products, the research was carried out in a different microclimate conditions.

The obtained results presented that in relation to ASLT there is not any general and universal shortening of research duration theory developed, nor a unified scheme of using microclimatic storing conditions, so they should be identified individually for each type of food and packaging system.

Key words: ASLT, microclimate storage condition, quality changes of food, shelf-life.

INTRODUCTION

In the literature, different approaches for the determination of the packaged foods sustainability can be met, among which the following methods were identified: analysis of literature data, long-term shelf-life tests in normative conditions and Accelerated Shelf-life Testing (ASLT) (Lee *et al.*, 2008).

The methods of ASLT usually include the packed product stability study in terms of intensified activity of environmental factors, and the results are extrapolated to the standard conditions of storage. This research technique requires knowledge of mathematical relationship between the durability of the product in accelerated and normative conditions. In case of accelerated testing there is neither general theory for reducing research time nor standardized set of concepts developed (Ucherek, 2009).

It should be noticed that during accelerated tests food is treated with prohibited storage term conditions, which can initiate quite unusual degradation mechanisms leading to incorrect estimates. For this reason, many authors including T. Labuza (Labuza, 2000) as well as M. Lisińska-Kuśnierz (Lisińska-Kuśnierz, 2008) recommend the verification of the results obtained from accelerated tests carried out during normative storing time.

The purpose of carried out tests is to analyze the possibility of using accelerated testing to determine the sustainability of the products packed in a mixture of gases, depending on type of packaging and output composition of gas mixture in the package.

MATERIALS AND METHODS

The research material included snacks packed in mixture of gases in two kinds of packaging (150 g), namely: bags (PETmet/EVOH-LDPE) and steel cans. For the purpose of the tests, different variants of the initial composition of modified atmosphere were applied, namely: 0-1,0% O_2; 1,1-3,0% O_2.

The research pertained to the following quality parameters of the packaged product: an index of total sensorial quality (ITSQ), according to ISO 4121, the peroxide value (PV), according to ISO 3960, the acid value (AV), according to ISO 660, the water content (X), using Electronic Weighting Equipment (Service Manual of Electronic Weighting Equipment, 2002) and the oxygen content in packaging (0_2), using an Oxygen Analyzer LC-700 F (Total Gas Control, 2005).

In order to determine the influence of the storage conditions on the durability of the products, the research was carried out in a special microclimate, as following: the normative condition (A):T=18°C±1°C, RH=75±2% (research cycle: 4 weeks, time of storage: 52 weeks) and the accelerated ageing of environment conditions (B): T=28°C±1°C, RH=75±2% (research cycle: 1 week, time of storage: 13 weeks) as well as (C): T=38°C±1°C, RH=85±2% (research cycle: 1 week, time of storage: 13 weeks) (Ucherek, 2009).

RESULTS AND CONCLUSIONS

For the calculations, the method of discriminatory function analysis using Statistica 9,0 was applied. This analysis was performed using a standard method, assuming a model in which the grouping variable was the type of packaging, output variant of the gas mixture in the packaging and storage conditions, and where classifying variables were measured product quality parameters and O_2 in the package. The canonical value of two elements, which are presented in tab. 1. suggest that product durability tests carried out in normative conditions (A) can be replaced by the stability products tests associated with storage in accelerated conditions.

However, analyzing the position of each element of observation the recommended conditions of accelerated tests can be indicated, depending on type of packaging and the composition of output gas mixture in the package, namely:

- accelerated conditions (B) in case of products in bags (1,1-3%),
- accelerated conditions (C) in case of products in bags (0-1,0% O_2), as well as in case of products in cans, regardless gas mixture output content.

Next, to determine the extent of shortening of test duration while applying the accelerated tests ASLT, the rate of shortening of research duration q was assigned.

$$q = \frac{t_{nor}}{t_{acc}}$$

where: t_{nor} - time of research in normative conditions,
t_{acc} - time of research in accelerated conditions.

The obtained q values, taking into account both the accelerated conditions (B) and (C) for the various packaging options and the output content of the gas mixture in the package, were used to estimate time of achieving the critical value of ITSQ=3,0 points. The shortening of test duration indicators are presented in tab. 2.

The obtained results presented that in relation to accelerated testing there is not any general and universal shortening of research duration theory developed, nor a unified scheme of using microclimatic storing conditions, so they should be identified individually for each type of food.

In addition, on basis of comprehensive studies of snacks packed in the mixture

Table 1 - The canonical value of two elements regarding to different types of packaging, output content of gas mixture in package and different storing.

Output content of gas mixture in package	Storage condition	Element 1	Element 2
	Bag		
0-1,0% O_2	Normative (A)	**0,39**	**-0,94**
	Accelerated conditions (B)	0,88	-1,99
	Accelerated conditions (C)	**0,22**	**-1,48**
1,1-3,0% O_2	Normative (A)	**-0,88**	**0,14**
	Accelerated conditions (B)	**-1,05**	**0,13**
	Accelerated conditions (C)	-1,02	0,38
	Can		
0-1,0% O_2	Normative (A)	**-0,87**	**0,34**
	Accelerated conditions (B)	-0,22	-0,59
	Accelerated conditions (C)	**-0,38**	**0,35**
1,1-3,0% O_2	Normative (A)	**0,94**	**1,29**
	Accelerated conditions (B)	1,08	-0,14
	Accelerated conditions (C)	**0,67**	**1,63**

Source: author's work.

Table 2 - The shortening of test duration indicators, depending on type of packaging and the initial content of oxygen in the gas mixture.

Output content of gas mixture in package	Storage condition	The shortening of test duration indicators (q)	Time in which ITSQ=3,0 point. [days]
	Bag		
0-1,0% O_2	Normative (A)	-	337
	Accelerated conditions (C)	5,69	338
1,1-3,0% O_2	Normative (A)	-	313
	Accelerated conditions (B)	4,53	312
	Can		
0-1,0% O_2	Normative (A)	-	361
	Accelerated conditions (C)	6,25	363
1,1-3,0% O_2	Normative (A)	-	343
	Accelerated conditions (C)	6,13	346
Source: author's work.			

of gases, it can be stated that for products packaged in MAP while determining accelerated tests parameters, both type of packaging and initial composition of gas mixture in the package, must be taken into account.

REFERENCES

ISO 660. 2009. Animal and vegetable oils and fats. Determination of acid value.

ISO 3960. 2007. Animal and vegetable oils and fats. Determination of peroxide value.

ISO 4121. 2003. Sensory analysis. Methodology. Evaluation of food products by methods using scales.

Labuza T. 2000. "The Search for Shelf-life", Food Testing Analysis, Texas.

Lee D., Yam K., Piergiovanni L. 2008. "Food Packaging Science and Technology", CRC Press, Boca Raton – New York.

Lisińska-Kuśnierz M. 2008. Dynamics of the changes of the sensory quality of bakery products. Italian Food & Beverage Technology. No 5: 13-21.

Service Manual of Electronic Weighting Equipment. 2002. Radwag, Radom.

Total Gas Control. 2005. PBI Dansensor A/S, Ringsted, Denmark.

Ucherek M. 2009. Testing procedure of shelf-life of product packages in a mixture of gases. Italian Journal of Food Science. Special Issues: 237-241.

NEW CONSUMPTION TRENDS OF "READY-MADE" BLUE-FISH

A.S. ZARBÀ

University of Catania, Via Santa Sofia 98, 95123 Catania, Italy

ABSTRACT

It is well-known that social and behavioral trends in the last decade have changed towards the preference of highly-processed food, seafood included. Final consumers, in fact, are more and more demanding processed products instead of fresh ones, that is, frozen and deep-frozen food, preserves and semi-ones. This consumption trend may have been affected not only by economic factors, but also by quality, convenience and health reasons. Besides, as far as blue-fish is concerned, distributors are specializing their offer preparing themselves ready-made food, also considering the growing importance of their role as supplying services and added value. Products include sliced and scaled fish, fish cut into fillets, breaded, etc. First of all the importance of preparation of ready-made fresh food, whose offer is to be attractive in terms of price, performance and service, which guarantees the product on its whole. Second, the importance of communication, because not only it transfers information, but it can also "modify" the behavior of final consumers. This essay was meant to point out on one side the importance of socio-economical interactions between ready-made bluefish offer and its consumption, and on the other, the opportunity of knowing more and start a technical-economical analysis concerning the offer of short shelf-life products. Our research, carried in Sicily. We have interviewed the consumers of the blue-fish by means of a special questionnaire. Data were elaborated, then, by means of special methodologies.

Key words: ready-made, blue-fish, quality, healt.

INTRODUCTION

The aim of this study is to make a report of the first results. This is a specific investigation of those principles of the valuation and preferences that drive the consumer to make the decisions to purchase and consume blue-fish. The results reflect behavioral patterns described in literature by three main approaches: "cognitive," "behavioural" and "experiential". Substantially we observed whether the consumer's behavior when he is buying fresh or prepared blue-fish is influenced by the elaboration of information closely linked to the consumer's social cultural level as well as

to the emotional conditions (Dalli and Romani, 2000; Fabris, 2003): of that moment (cognitive approach), by extra-personal influences induced by environmental stimuli (behavioral approach) or, finally, by emotion-based behavior driven from the overall experience (experiential approach). The study was made in Sicily (D'Amico and Zarbà, 2007), the Italian region with the highest percentage of fishing, accounting for 1/5th of the national fishing production of all fish. During the 21st century, on average production is between 250-300,000 tons per year (based on data taken from official Italian statistics - ISTAT). Sicily is among the regions where the households spend monthly for fish consumption the same as regions (for example Campania and Apulia) whose total monthly expense for import and consumption of fishing products on average at the highest is 11% of their expense for food and beverage, which, according to the cited source of statistics, in recent years, amounts to around 500 euros.

MATERIALS AND METHODS

The research was directed towards product family (Scheme 1), namely those that refer (see Ministry of Agriculture and Forestry, Italy) to the blue-fish (traditional) and those species that are similar for their colour (blue fish). The direct survey covered by data collected from 18 outlets selling fresh fish and processed fish, positioned in four provinces of Sicily on the Ionian coast, namely Messina, Catania, Siracusa and Ragusa. These are towns with a busy coast line that has developed a thriving maritime, with high rates of consumption of fish, both along the coast and inland. The inland, being distant from the sea, was unusual to have fish as part of the daily diet and also the sale of fish, in some cases, was realized to be seasonal. The interviews, conducted between late 2009 and early 2010, concerned 336 and the customers purchasing the fish. The interviews were conducted directly, face to face, using a questionnaire sheet prepared ad hoc. The latter, in particular, has been divided into three sections: the first was the acquisition of information relating to the social economic characteristics of the consumers interviewed (sex, age, total number of members of the household, education level, professional employment of respondents). The second was addressed to identify the reasons and the specific consumption of fresh fish products and those handled (types of products, frequency of consumption and places of purchase) with particular reference to oily fish (Lanari, 1996). The third related to information about the perceived quality and the reasons for any refusal to consume the fish products. The information and data was collected in a methodical framework to allow the structure of consumption of oily fish in the fore mentioned towns.

RESULTS AND CONCLUSIONS

The results revealed that in the household expenditure concerning blue-fish, great importance is given to the worked blue fish (frozen/deep-frozen). Particularly amongst the younger-aged and also in the class of middle-aged indeed the values of processed fish remain on dominant positions, although the fresh blue-fish shows higher rates (tab. 1). Other important results concern the period of purchase (tab. 2). Another element that acts to determine the choice of blue-fish, is the number of the family members. Blue-fish, frozen/deep-frozen and processed, prevails in the purchases of couples with children, while childless couples as well as single consume more fresh blue-fish. Even the profession of the consumers determines

Table 1 - Ferst type to blue-fish and to blue fish.

	Product family
Blue-fish (*)	Aguglia, Allunga, Alice or Anchovy, Horse Mackerel, Mackerel, Sandeel, Saury, Spanish Mackerel, Sardines, Sardinian, Shad, Sprat or Skullcaps
Blue fish (**)	Little tuna, Swordfish, Tuna

(*) First type of small sized fish-mainly from shallow fishing.
(**) Fish medium to large, caught from deep sea fishing or Mediterranean fishing.

certain differences. Self-employed consumers purchase mainly fresh blue-fish amongst other types (preserved, frozen/deep-frozen fish), chosen prevalently by employed and not-employed consumers.

Concerning all the results above mentioned about the consumers behaviour purchasing blue-fish, it should be noted that the results in many cases show the effects of different price levels between fresh fish and processed fish. It is also important where the fish, conserved and/or prepared, is purchased. In deed, the increase of sales is more important near the factories of fish. This seems to be due to the direct sale from "producer" to "consumer" into their producing places, often situated near tourism sea areas. This allows also to work out a system of information, incentives and marketing for tourists carrying on a real busyness. The behavioural patterns related to the consumption of fatty fish, according to the results of research conducted in the areas investigated, appear different depending on the case of fresh fish or seafood processing. The first case was mostly an approach "cognitive" that individuals bring into force the information they acquire from the environment in order to achieve its goals, especially those of a healthy and nutritional effect of omega-3 and protein in these fish. Another aspect of cognition that may be critical to determine reliably the purchase of blue fish is fresh confidence to the seller, due to a reduced shelf life. But

Table 2 - Preferences expressed by consumers by age classes and type of blue-fish.

Age classes	Fresh fish		Frozen fish		Canned fish		Total	
	N	%	N	%	N	%	N	%
Under 25 years old	22	14,8	10	18,2	20	15,2	52	15,5
%	**42,3**		**19,2**		**38,5**		**100,0**	
26 - 35 years old	31	20,8	11	20,0	29	22,0	71	21,1
%	**43,7**		**15,5**		**40,8**		**100,0**	
36 - 45 years old	21	14,1	7	12,7	20	15,2	48	14,3
%	**43,8**		**14,6**		**41,7**		**100,0**	
46 - 55 years old	25	16,8	9	16,4	20	15,2	54	16,1
%	**46,3**		**16,7**		**37,0**		**100,0**	
56 - 65 years old	30	20,1	12	21,8	25	18,9	67	19,9
%	**44,8**		**17,9**		**37,3**		**100,0**	
above 65 years old	20	13,4	6	10,9	18	13,6	44	13,1
%	**45,5**		**13,6**		**40,9**		**100,0**	
Totals	149	100,0	55	100,0	132	100,0	336	100,0
%	**44,3**		**16,4**		**39,3**		**100,0**	

Our calculations on data and information directly collected (between 2009/2010).

Table 3 - Distribution of consumption of types of blue-fish for temporal frequency and of consumers by age classes (2009/2010).

(Percentage)				
Age classes	Once a week	Several times a week	Every other week	Once a month
		Fresh fish		
Under 25 years old	36,4	22,7	9,1	31,8
26 - 35 years old	45,2	19,4	16,1	19,4
36 - 45 years old	33,3	19,0	42,9	4,8
46 - 55 years old	40,0	24,0	20,0	16,0
56 - 65 years old	30,0	33,3	26,7	10,0
above 65 years old	50,0	25,0	15,0	10,0
		Frozen fish		
Under 25 years old	20,0		40,0	40,0
26 - 35 years old	18,2		27,3	54,5
36 - 45 years old	14,3	14,3	28,6	42,9
46 - 55 years old	22,2	11,1	11,1	55,6
56 - 65 years old	41,7	16,7	25,0	16,7
above 65 years old	33,3		16,7	50,0
		Canned fish		
Under 25 years old	55,0	20,0	20,0	5,0
26 - 35 years old	27,6	24,1	17,2	31,0
36 - 45 years old	30,0	5,0	25,0	40,0
46 - 55 years old	35,0	10,0	25,0	30,0
56 - 65 years old	36,0	4,0	40,0	20,0
above 65 years old	38,9	22,2	16,7	22,2
Our calculations on data and information directly collected.				

consumer behavior can also be conducted behavioral approach, in fact, the low prices of blue fish in areas where the investigation also conducted agreement in buying shares almost automatically, that it is a sort of conditioning process to saving that has influence the food expenditure of individuals. In the field of fish preparation, however, it is easier to detect behavioral patterns according to the experiential approach, which sees the consumer to place the product in the wider context of involvement/ activities being evaluated by the consumer based on the tangible characteristics and performance techniques that induce desires related to fantasy. It's the in-store experience that takes shape; acts of purchase or consumption which, though based on the prerequisites of each item and also consider the performance objective, is namely their use-value that leads to experience and recreation and entertainment.

REFERENCES

D'Amico M., Zarbà A.S. (2007): Il mercato del tonno rosso: Tendenze attuali e flussi commerciali; in "L'allevamento del Tonno Rosso in Italia. Aspetti tecnici e valutazioni economiche" (a cura di D'Amico M., La Via G.). Bonanno Editore. Catania.

Dalli D., Romani S. (2000): Il comportamento del consumatore. Acquisti e consumi in una prospettiva di marketing. Franco Angeli Editore. Milano.

Fabris G. (2003): Il nuovo consumatore: verso il postmoderno. Franco Angeli Editore. Milano.

Lanari D. (1996): Trasformazione e conservazione dei prodotti ittici. Cnr-RAISA.

EFFECT OF GAMMA IRRADIATION ON CAPROLACTAM MIGRATION FROM MULTILAYER POLYAMIDE 6 FILMS INTO WATER FOOD SIMULANT AND VALIDATION OF THE METHOD

J.S. FÉLIX[1], J.E. MANZOLI[2,3], M. PADULA[4] and M. MONTEIRO[5]*

[1]Analytical Chemistry Department, I3A, CPS, University of Zaragoza, Zaragoza, Spain

[2]Nuclear and Energetic Research Institute (IPEN/CTR)
[3]University of São Judas Tadeu, São Paulo, Brazil

[4]Food Technology Institute/Packaging Technology Center (ITAL/CETEA), Campinas, Brazil

[5]Dept. of Food and Nutrition/School of Pharmaceutical Science/São Paulo State University (UNESP), Araraquara, Brazil
*corresponding author: monteiro@fcfar.unesp.br

ABSTRACT

A gas chromatographic method to determine caprolactam migration from non-irradiated and irradiated multilayer polyamide 6 films into water food simulant was developed and validated. Linear range was 0.96 to 642.82 μg/mL, with correlation coefficient of 0.9999. Limit of detection and quantification was 0.32 and 0.96 μg/mL, respectively. Method precision showed RSD values lower than 2.1%, while method accuracy showed recovery from 89 to 105%. Caprolactam levels reduced with the increase of radiation dose for films used for meat foodstuffs, while for films used as cheese packaging only slight differences occurred.

Key words: caprolactam, migration, multilayer polyamide 6 films, gamma irradiation, validation of analytical method.

INTRODUCTION

Caprolactam, the monomer used to produce polyamide 6 (PA-6), remains in the resin after polymerization and may migrate into food. PA-6 is widely used as food packaging, especially as mono and multilayer films for meat foodstuffs and cheese. PA-6 is approved for contact with prepackaged food during irradiation (ANVISA, 2001; FDA, 2009).

Irradiation is used to sterilize and extend the shelf-life of foods. Besides the benefits conferred to foods, ionizing radiation of plastic packaging can affect the migration behavior of the compounds.

The effect of irradiation on migration of compounds from plastic packaging into food is an important issue concerning packaging producers, food industry and researchers due to the possible reduction of the migration levels. The reduction of additive migration was described (Ito *et al.*, 2005; Jeon *et al.*, 2007; Stoffers *et al.*, 2004), while the effect of irradiation on caprolactam level from multilayer PA-6 films and its migration from the packaging into simulants were studied (Araújo *et al.*, 2008; Félix *et al.*, 2008; 2010).

The aim of this work was to develop and validate an analytical method to determine caprolactam in water, as well as to quantify caprolactam migration from multilayer PA-6 films, used for meat foodstuffs and cheese, into water food simulant.

EXPERIMENTAL

Samples

Virgin commercial multilayer PA-6 films, used for meat foodstuffs (brands 1-4) and cheese (brands 5-9), supplied by the Brazilian producing companies, were studied.

Validation

Calibration and linearity, limits of detection and quantification, precision and accuracy were evaluated (IUPAC, 2002). Stock standard solutions containing caprolactam and 2-azacyclononanone (internal standard), both Sigma-Aldrich, were prepared in methanol and then diluted, as necessary, in distilled water. These water standard solutions were injected in 17-A Shimadzu Gas Chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionization detector (GC-FID) using a DB-1701 (30 m x 0.25 mm x 0.25 μm) capillary column operated at 130°C for 1 min, programmed at 10°C/min up to 170°C for 1 min, then heated to 200°C at 10°C/min and held for 2 min. Hydrogen was the carrier gas (1.0 mL/min) and injections (1 μL) were made at 240°C in split mode (1:20). Detector temperature was 250°C.

Irradiation

The irradiation was performed at the Radiation Technology Center (CTR) of the Nuclear and Energetic Research Institute (IPEN), Brazil, using Gamacell 60 cobalt irradiator of 12 kCi. Multilayer PA-6 films (2x3 cm^2) were disposed in glass vials (20 mL) hermetically closed and submitted to gamma radiation in doses of 3 and 7 (meat foodstuffs) and 12 kGy (cheese) (IAEA, 2002).

Migration

Pieces of non-irradiated and irradiated films (2x3 cm^2) were, independently, placed in contact with water food simulant (10 mL) and exposed at 40°C during 10 days

(total immersion). Triplicate samples of each brand of films were analyzed for each irradiation dose as well as a blank prepared only with simulant, used as reference. After contact, film samples were removed, the internal standard solution was added, and then an aliquot was injected in the GC-FID. Two injections for each replicate of the migration test were made. Caprolactam levels obtained were submitted to the ANOVA and Tukey test was used to compare differences among averages at $p \leq 0.05$.

RESULTS AND DISCUSSION

Validation

Calibration curve for caprolactam in water food simulant was linear over the concentration range of 0.96 to 642.82 μg/mL, with correlation coefficient of 0.9999. Linear regression equation was y=-0.00739+0.90054x. Linearity was studied using the area/concentration ratio of caprolactam/2-azacyclononanone *versus* the concentration of caprolactam/2-azacyclononanone used in the calibration curve, expressed in logarithmic scale. It was verified that the concentrations used in the calibration curve were within the confidence interval of 95%. Limit of detection of the method was 0.32 μg/mL and limit of quantification of the method was 0.96 μg/mL, with 99% of accuracy and RSD values lower than 7.2%. The precision of the method was evaluated using repeatability (intra-day) and intermediary precision (inter-day) at three concentration levels. The accuracy of the method was studied during the intermediary precision, via recovery, using different spiking levels and was expressed as a percentage for the true value of the analyte in the sample and the value obtained by analysis. Method precision showed RSD lower than 2.1%, while accuracy showed recovery from 89 to 105% (tab. 1).

Migration

Once the method was validated, it was used to quantify caprolactam migration from multilayer PA-6 films into water. Typical chromatograms of caprolactam migration from the films used for meat foodstuffs into water are in fig. 1. The chromatogram of the blank did not present any interference in the retention time band of the analyte of interest or the internal standard.

The highest level of caprolactam (7.2 mg/kg) migrated from non-irradiated films for meat foodstuffs of brand 2, which did not differ ($p>0.05$) from brand 3. In general, a decrease of 6-19% between 0 and 3 kGy and 37-45% between 0 and 7 kGy occurred in caprolactam migration. For films used for cheese, the highest level migrated

Table 1 - Precision and accuracy of the method for determination of caprolactam in water food simulant.

Water Food Simulant	Repeatability (intra-day precision) (n=3)			Intermediary precision (inter-day precision) (n=10)		
Spiked level (μg/mL)	1.61	32.14	401.76	1.61	32.14	401.76
Found (mean±SD) (μg/mL)	1.41±0.02	31.57±0.28	418.09±2.65	1.43±0.03	31.40±0.32	420.06±8.92
RSD[a]	1.10	0.90	0.63	2.02	1.01	2.12
Accuracy[b]				89	98	105

[a] RSD, relative standard deviation (%).
[b] Accuracy (%).

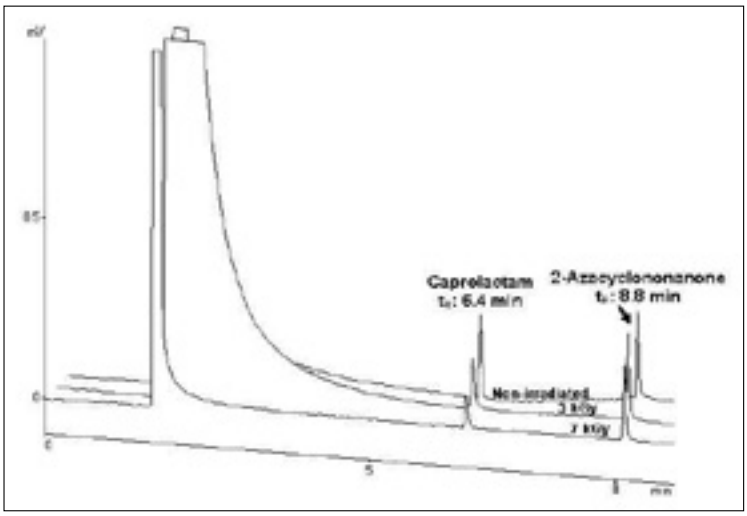


Fig. 1 - Typical chromatograms for caprolactam migration from non-irradiated and irradiated multilayer PA-6 films into water. Chromatographic conditions are in Experimental section.

from irradiated films of brand 6, which differed ($p \leq 0.05$) from all the other brands. In contrast to the films used as meat foodstuff packaging, multilayer PA-6 films used for cheese showed an increase of 1-24% in caprolactam migration when the films were irradiated at 12 kGy. Caprolactam migration from brand 8, irradiated and non-irradiated, and brand 9 (irradiated) into water was not detected (tab. 2).

Félix *et al.* (2008) described that caprolactam migration from the same PA-6 films into water and into 95% ethanol showed higher levels (7.5-13.0 mg/kg and 7.7-10.0 mg/kg, respectively), although a different analytical technique had been used. Pogorzelska and Mielniczuk (2001) described caprolactam migration from PA films into water ranging from 6.6-17.0 mg/kg, which included the caprolactam range obtained in our study. Concerning caprolactam migration from PA-6 films into water, the levels ranged from 0.3-1.8 mg/cm^2 (Barkby and Lawson, 1993) and from 0.3-2.5 mg/dm^2 for mono and multilayer films containing PA-6 (Stoffers *et al.*, 2005), pretty similar to ours.

All the samples showed caprolactam migration below the specific migration limit of 15 mg/kg, and therefore, they are in accordance to the limits established by Brazilian and European Legislation (ANVISA, 1999; EC, 2002).

Table 2 - Migration levels (mg/kg) of caprolactam from irradiated and non-irradiated multilayer PA-6 films, used for meat foodstuffs (1-4) and for cheese (5-9) into water (10 days at 40°C).

Brand	Dose (kGy)		
	0	3	7
1	6.1±0.3 (4.8)bA	5.5±0.2 (4.4)bB	3.9±0.0 (0.7)bC
2	7.2±0.4 (5.7)aA	5.8±0.4 (6.2)bB	3.9±0.1 (1.9)bC
3	6.9±0.4 (5.4)aA	6.5±0.5 (7.7)aA	4.4±0.3 (5.8)aB
4	5.7±0.4 (6.5)bA	4.8±0.3 (6.5)cB	3.6±0.1 (2.2)cC
Brand	Dose (kGy)		
	0	12	
5	4.8±0.1 (1.6)bA	4.8±0.1 (2.7)cA	
6	5.4±0.4 (6.8)aB	6.8±0.1 (0.8)aA	
7	4.8±0.3 (5.5)bB	5.9±0.1 (2.1) bA	
8	1.0±0.1 (7.4)c	ND	
9	ND	ND	

Mean of six replicates±SD; Relative standard deviation (%) between parenthesis. Values in vertical columns and in horizontal lines (capital letter) followed by different letters are signicantly different ($p \leq 0.05$). ND, not detected.

CONCLUSIONS

The method was considered effective to determine caprolactam in water, showing low limit of detection, good precision and accuracy, and a maximum time of analysis of 11 min. The effect of irradiation on caprolactam migration from multilayer PA-6 films into water showed a reduction in caprolactam levels with the increase of the irradiation dose for films used as meat foodstuffs packaging, while films used for cheese almost did not differ.

ACKNOWLEDGMENTS

CAPES, FAPESP and PADC/FCF/UNESP for the financial support.

This work was carried out during Doctorate studies at Dept. of Food and Nutrition/School of Pharmaceutical Science/UNESP.

REFERENCES

ANVISA. 1999. Res. N. 105. Regulamentos técnicos sobre uso de embalagens e equipamentos plásticos em contato com alimentos e seus anexos. http://e-legis.anvisa.gov.br/leisref/public/showAct.php?id=19772&word=.

ANVISA. 2001. Res. N. 21. Regulamento técnico para irradiação de alimentos. http://e-legis.anvisa.gov.br/leisref/public/showAct.php?id=161&wor d=.

Araújo H.P, Félix J.S., Manzoli J.E., Padula M., Monteiro M. 2008. Effects of γ-irradiation on caprolactam level from multilayer PA-6 films for food packaging: Development and validation of a gas chromatographic method. Rad. Phys. Chem. 77: 913-917.

Barkby C.T., Lawson G. 1993. Analysis of migrants from nylon 6 packaging films into boiling water. Food Addit. Contam. 10: 541-553.

EC. 2002. Commission Directive 2002/72/EC relating to plastics materials and articles intended to come into contact with foodstuffs. Off. J. Eur.Com., L220, 18-58.

FDA. 2009. Code of Federal Regulations. Packaging materials for use during the irradiation of prepackaged food. 21CFR 179.45. http://www.accessdata.fda.gov/scripts/c drh/cfdocs/cfcfr/CFRSearch.cfm?fr=179.45&SearchTerm=irradiation.

Félix J.S., Monteiro M., Manzoli J.E., Padula M., Pezo D., Romero J., Nerín C. 2008. Identification and migration of degradation compounds from irradiation of multilayer polyamide 6 films for meat foodstuffs and cheese. Anal. Bioanal. Chem. 391: 847-857.

Félix J.S., Manzoli J.E., Padula M., Monteiro M. 2010. The effect of gamma irradiation on caprolactam migration from multilayer polyamide 6 films into food simulants. Development and validation of a gas chromatographic method. J. AOAC Int. In Press.

IAEA. 2002. TRS-409: Dosimetry for food irradiation. International Atomic Energy Agency, Vienna. Technical report series, ISSN 0074-1914; n. 409.

Ito R., Seshimo F., Haishima Y., Hasegawa C., *et al.* 2005. Reducing the migration of di-2-ethylhexyl phthalate from polyvinyl chloride medical devices. Int. J. Pharm. 3003: 104-112.

IUPAC. 2002. "Harmonized guidelines for single-laboratory validation of methods of analysis" (IUPAC Technical Report). Pure Appl. Chem. 74: 835-855.

Jeon D.H., Park G.Y., Kwak I.S., Lee K.H., Park H.J. 2007. Antioxidants and their migration into food simulants on irradiated LLDPE film. LWT - Food Sci. Technol. 40: 151-156.

Pogorzelska Z., Mielniczuk Z. 2001. Determination of ε-caprolactam migration from polyamide plastics: A new approach. Packag. Technol. Sci. 14: 31-35.

Stoffers N.H., Linssen J.P.H., Franz R., Welle F. 2004. Migration and sensory evaluation of irradiated polymers. Rad. Phys. Chem. 71: 203-206.

Stoffers N.H., Dekker M., Linssen J.P.H., Störmer A., Franz R., Van Boekel M.A.J.S. 2005. Modelling of simultaneous two-sided migration into water and olive oil from nylon food packaging. Eur. Food Res. Technol. 220: 156-162.

ANALYSIS OF HISTAMINE AS INDICATOR OF SHELF-LIFE IN SEAFOOD

R. PASEIRO-CERRATO[1], A. RODRÍGUEZ-BERNALDO DE QUIRÓS[1], R. SENDÓN[1], J.M. CRUZ[2] and P. PASEIRO-LOSADA[1]

[1]University of Santiago de Compostela,
Department of Analytical Chemistry, Nutrition and Food Science,
Faculty of Pharmacy, 15782 Santiago de Compostela, Spain

[2]E.T.S.E.I. University of Vigo,
Department of Chemical Engineering, 36200 Vigo, Spain

ABSTRACT

In the present study, a reversed high performance liquid chromatographic method with diode array detection was developed to analyze histamine in sea food samples. The separation was performed on a Kromasil ODS (C18) (150x3.20 mm, 5 μm) column thermostated at 25°C and using a gradient of Milli-Q water and methanol as mobile phase. The biogenic amine was extracted from seafood samples by using 0.1 N HCl and derivatized with dansyl chloride. Histamine contents ranged from 1.0 to 2.5 mg/kg.

Key words: chromatographic analysis, derivatization, food deterioration, histamine, shelf-life.

INTRODUCTION

Histamine is a biogenic amine resulting of the enzymatic decarboxylation of the amino acid histidine. It is considered an indicator of deterioration in foods and frequently used as a biomarker for food quality control. The European regulation (CE) N. 1441/2007 establishes a caution level of 100-200 mg/kg in seafood.

Several undesirable effects on the human health such as, hypotension, nausea, headache, rash, cardiac palpitation, emesis and even intracerebral anaphylactic shock, haemorrhage and death in very severe cases caused by high levels of histamine have been reported in the literature (Chiacchierini *et al.*, 2006; Innocente *et al.*, 2007).

Numerous analytical procedures have been developed for the determination of histamine including, enzyme-linked-immunoassays (ELISA), colorimetric methods and chromatographic methods. Among them, high performance liquid chromatography appears as the most suitable technique to analyze the biogenic amine. Due to these compounds do not have a suitable fluorophore or chromophore group a derivatization is essential in order to enhance the sensitivity. Several derivatizing agents have been employed; the most commonly used are *o*-phthaldialdehyde, dansyl chloride and fluorescamine (Dugo *et al.*, 2006; Saito *et al.*, 1992).

The aim of this paper is to determine the histamine content in sea food samples by high-performance liquid chromatographic with diode array detection after derivatization with dansyl chloride.

MATERIALS AND METHODS

Reagents and standard solutions

All reagents were of analytical grade. Methanol, acetone and hydrochloric acid were from Merck (Darmstadt, Germany); sodium bicarbonate from Vorquímica (Vigo, Spain) and dansyl chloride (Dns-cl) from Fluka (Steinheim, Germany). Water used for all solutions was obtained from a Milli-Q water purification system (Millipore; Bedford, MA). Standard of histamine was purchased from Aldrich. Stock standard solution of histamine was prepared in 0.1 N HCl and stored at 4°C in the darkness. Working solutions were prepared by dilution.

Instrumentation

HPLC-analysis were performed on a HP1100 system (Hewlett-Packard Waldbronn, Germany) equipped with quaternary pump, a degassing device, an autosampler, a column thermostatting system, a diode-array detector (DAD), a fluorescence detector and Agilent Chem-Station for LC and LC/MS systems software.

Samples and extraction procedure

Surimi was selected as representative seafood and was purchased in a local supermarket. Histamine was extracted as follow; 25 mL of 0.1 N HCl were added to 5 g of sample, the mixture was homogenized by magnetic stirring for 10 min. The supernatant was removed and the residue was re-extracted with 25 mL of 0.1 N HCL. The supernatants were combined and made up to 50 mL. The solution was stored at 4°C overnight in order to precipitate lipids and proteins. Then, an aliquot of the solution

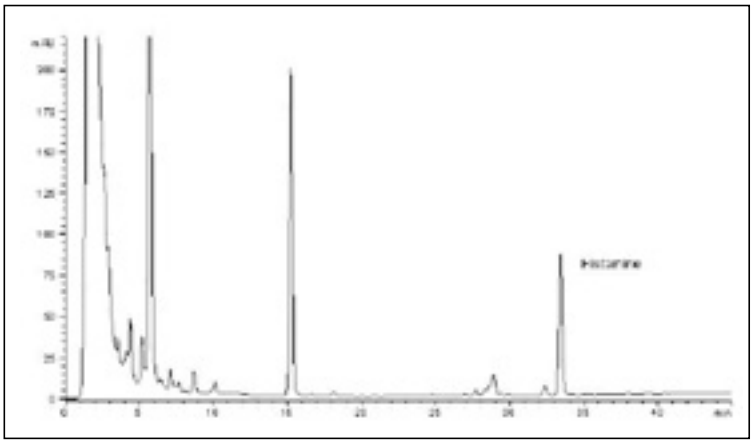


Fig 1 - HPLC chromatogram of a surimi sample.

was filtered and derivatized. The dansylated derivative of histamine was formed by adding to 1 mL of sample 1 mL of dansyl chloride solution (5 mg/mL) and 300 µL of saturated $NaHCO_3$ solution; then the mixture was incubated at 80°C 60 min. Detailed information regarding derivatization procedure is reported in a paper submitted for publication.

Chromatographic conditions

The chromatographic separation was performed on a Kromasil ODS (C18) (150x3.20 mm, 5 µm) column thermostated at 25°C.The mobile phases consisted of A (Milli-Q water) and B (Methanol). The gradient elution program was as follows: 0 min (50% A/50% B); 45 min (10% A/90% B) 50 min (50% A/50% B). The flow rate was 0.8 mL/min and the injection volume 20 µL. The diode array detector was set at 254 nm.

RESULTS AND CONCLUSIONS

Since histamine has neither suitable absorption in the UV-Vis region nor fluorescence characteristics a derivatization step is essential in order to improve the sensitivity. According to the literature dansyl chloride is one of the most suitable derivatizing agents for biogenic amines. A method that involves the derivatization of amine with dansyl chloride followed by a reversed phase high performance liquid chromatography with detection at 254 nm was used to analyze the histamine content in surimi samples.

A typical chromatogram of the dansylated derivative of the amine is shown in fig. 1.

Identification of histamine was made by comparison of the retention time and UV spectra with that of pure standard. A spectrum of the dansylated derivative is presented in fig. 2.

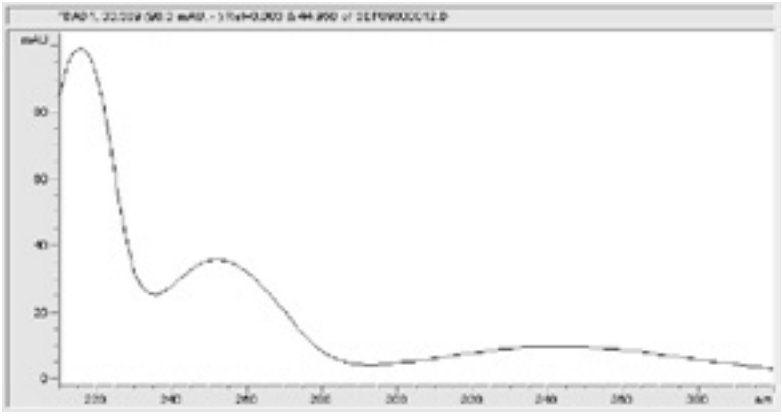


Fig 2 - UV spectra of the dansylated derivative.

Quantification was carried out with the external standard method. Calibration line was constructed based on four concentration levels of standard solutions within 1-23 mg/L range and it was fitted to a linear equation y = 132.67 x - 45.102 (R^2 0.9995).

The recovery of the method was estimated on the basis of determination after spiking the samples with known amounts of histamine (10 mg/L). Satisfactory recoveries were achieved (102.8±2.8%). Once the analytical conditions were established the method was applied to determine histamine in surimi samples. The concentrations found in the samples analyzed ranged from 1.0 to 2.5 mg/kg.

ACKNOWLEDGEMENTS

The study was financially supported by the Ministerio de Ciencia e Innovación, ref. N. AGL/2008-04146 "MIGRAMIN". Authors are grateful to "Ministerio de Ciencia e Innovación" for the Predoctoral fellowship FPI (ref. BES-2009-023016) awarded to Rafael Paseiro-Cerrato.

REFERENCES

Chiacchierini E., Restuccia D., Vinci G. 2006. Evaluation of two different extraction methods for chromatographic determination of bioactive amines in tomato products. Talanta, 69:548.

Commission Regulation (EC) N. 1441/2007 of 5 December 2007 amending Regulation (EC) N. 2073/2005 on microbiological criteria for foodstuffs.

Dugo G., Vilasi F., La Torre G.L., Pellicanò T.M. 2006. Reverse phase HPLC/DAD determination of biogenic amines as dansyl derivatives in experimental red wines. Food Chem. 95:672.

Innocente N., Biasutti M., Padovese M., Moret S. 2007. Determination of biogenic amines in cheese using HPLC technique and direct derivatization of acid extract. Food Chem. 101:1285.

Saito K., Horie M., Nose N., Nakagomi K., Nakazawa H. 1992. Determination of polyamines in foods by liquid chromatography with on-column fluorescence derivatization. Anal. Sci. 8:675.

ALTERNATIVES FOR AVOIDING DEGRADATION IN ECOLOGICAL WINE

T. BALBOA-LAGUNERO[1]*, M. AZNAR[2] and A.C. SILVA FERREIRA[3]

[1]Food and Agricultural Science Department,
IMIDRA, Finca "El Encín", A2 Km 38.2, Alcalá de Henares,
28800 Madrid, Spain

[2]Analytical Chemistry Department, GUIA Group, I3A, CPS,
University of Zaragoza, Mª de Luna 3, 50018 Zaragoza, Spain

[3]Escola Superior de Biotecnologia, Universidade Católica Portuguesa,
R. Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal
*corresponding author: tania.balboa@madrid.org

ABSTRACT

Organic wines are prone to suffer from oxidative degradation due to the restrictions in the winemaking. This work studies the influence of different chemical routes, due to thermal and oxidative processes, in the wine aromatic profile. The resistance to these processes after the addition of compounds with a presumably antioxidant capacity was evaluated. Ascorbic acid, resveratrol, calcium disodium ethylenediaminetetraacetate (EDTA), sulphur dioxide (SO_2) and the polyphenols: gallic acid, epicatechin and caffeic acid, were tested. The oxidative estate by cyclic voltammetry, aromatic composition and general profiles and sensorial analysis were performed; obtaining interesting differences depending on the applied treatment. All samples gave a characteristic voltammetry signal; showing a remarkable decrease in the current intensity on those samples that suffer a deeper degradation. Regarding the chemical analysis results, the application of the metal chelator EDTA, apart from the SO_2 treatment, might be of interest to partly reduce the oxidative effect. Sensory differences were only found in samples treated with SO_2 and those that had the addition of polyphenols, due to the appearance in these last samples of a strong vegetable aroma not perceptible in the other treatments.

Key words: antioxidant capacity, EDTA, oxidative degradation, polyphenols, SO_2, voltammetry.

INTRODUCTION

Oxidative degradation affects the final colour and aroma of wine, producing negative effects on its quality. The susceptibility for the development of the oxidative spoilage is related to three factors: the wine's redox potential, the dissolved oxygen concentration and the type and concentration of intrinsic and added antioxidants (Silva Ferreira *et al.*, 2003a). Most of the research about wine oxidation has been performed on white wines, as they are more prone to experience oxidation reactions. In the case of organic wines, this susceptibility to oxidation is more significant, as there are important restrictions during the wine making, such as the level of SO_2 that can be employed, broadening the problem to both, white and red wines.

The main aim of this work was to study the evolution of an organic red wine during spoilage conditions and to determine if the addition of specific compounds with a presumably antioxidant capacity could influence in the final oxidative level, aromatic profiles and sensorial characteristics. Obtaining alternatives to the use of sulphur dioxide could improve organic wines aromatic quality and thus encourage the market for them. Compounds such as ascorbic acid, SO_2, resveratrol, EDTA and some polyphenols were tested. The oxidative estate was evaluated in all samples by cyclic voltammetry. This technique has been recently incorporated for studies of resistance to oxidation of white wines (Oliveira *et al.*, 2002; Kilmartin *at al.*, 2001); considering its future application for monitoring aging processes and characterizing wines for their antioxidant protection. Volatile analysis and sensorial tests were as well performed for all the wine samples taken at different times of the degradation process.

MATERIALS AND METHODS

Chemicals: All the reagents used were analytical quality.

Wine: Spanish ecological young red wine: *Viña Bosquera* 2007, D.O. Madrid.

Forced age wine protocol and preparation of wine samples: Wine was divided into 1.5 L portions and prepared as follows: 1. "T° Control" and 2. "T°&O_2 Control" were left without the addition of any chemical; 3. "Ascorbic acid": 0.5 mM ascorbic acid; 4. "Resveratrol": 7.5 mg/L resveratrol; 5. "Ascorbic acid + Resveratrol"; 6. "SO_2": 50 mg/L SO_2; 7. "EDTA": 500 mg/L EDTA and 8. "Polyphenols": 475, 50 and 400 mg/L gallic acid, caffeic acid and epicatechin, respectively. Sampling times were at 0, 1, 4 and 8 days and a gradient T° program from 60° to 40°C was followed. Samples were oxygenated till saturation level at the beginning of the experiment and resaturated at each sampling time, except the "T° Control".

Cyclic voltammetry: A potentiostat (Autolab type PGSTAT30, Ecochemie) controlled by the GPES 4.9 software provided by Ecochemie was used. Voltammograms were obtained in the oxidation range of potentials (between ca. 0.2 and 1.2 V) at a scan rate of 100 mV/s using a 3 mm glassy carbon disk (BAS M-2012) working electrode.

Volatile analysis: Liquid-liquid extraction – GC-MS analysis was performed based on the methodology described in the literature (Silva Ferreira *et al.*, 2003b).

Sensorial analysis: Samples were divided in the 3 time groups. For each group, the level of similarity in a 0-10 continuous scale was asked to established; always referring the similarity to the same "T°&O_2 Control" of the specific time group. In a second session the same methodology was followed but the comparison was established between a 10-year-old Porto wine and all the samples.

RESULTS AND CONCLUSIONS

Voltammetry analysis. There was a decrease in the voltammetry intensity while the wine was suffering degradation, mostly due to oxidation. This gradual decrease indicates a reduction in the concentration of the species oxidized at these potential ranges. When all the samples were compared, the most remarkable observation was that all that have the addition of polyphenols, independently of the time that were taken during the spoilage period, had a very distinct voltammogram; showing significantly higher intensities from 0.42 V onwards. This was expected, as the voltage interval between 0.4 and 0.6 represents the most powerful reducing agents of wine; which are, apart from ascorbic acid and SO_2, that were present to some extent in all samples, polyphenols with a triphenol group on the flavonid B-ring (Martins *et al.*, 2008).

Volatiles analysis. The most remarkable volatiles, due to their behaviour along the experimental time, were methional, phenylacetaldehyde, furfural, 5-methyl-2-furfural, benzaldehyde and guaiacol. Except this last compound, the rest have previously been reported as typically present in aged and oxidized white wines and are responsible for the development of off-flavors characteristic of these type of wines, such as "honey-like", "boiled potato", "cooked vegetable", "liquorice" or "farm feed" (Escudero *et al.*, 2002; Silva Ferreira *et al.*, 2003b). All the mentioned compounds increased along the experiment due to thermal reactions; but methional, benzaldehyde and phenylacetaldehyde were highly sensible as well to the aeration action. It was observed that the combination of ascorbic acid with resveratrol and EDTA partly limited the increase with time of methional and that the SO_2, EDTA and polyphenols treatments could avoid as well part of the concentration increase of benzaldehyde. In both cases, the EDTA treatment was the most powerful one.

Levels of 1,3-dioxanes and 1,3-dioxolanes (characteristic of Porto wines) increase greatly along the spoilage protocol; mainly due to the oxygenation effect. They were partly limited with SO_2, but also, to a lesser extent, by EDTA and the polyphenols treatments.

Sensorial analysis. Sensorial data could be correlated with the main volatile analysis results. SO_2, EDTA and the polyphenols treatments were those ones with less similarity to the non supplemented control spoiled samples. It was confirmed that the "cooked" red wine has a very similar general odour with Porto wine and this can be due to the increase of compounds characteristic of oxidized wines, such as methional or benzaldehyde and the 1,3-dioxanes and 1,3-dioxolanes. The "SO_2" and the "Polyphenols" samples were the most dissimilar with respect to the Porto wine; showing lower concentrations of these types of compounds.

ACKNOWLEDGMENTS

This work has been funded by the Spanish INIA project RTA2005-00172.

REFERENCES

Escudero A., Asensio E., Cacho J., Ferreira V. 2002. Sensory and chemical changes of young white wines stored under oxygen. An assessment of the role played by aldehydes and some other important odorants. Food Chem. 77: 325.

Kilmartin P.A., Zou H., Waterhouse L. 2001. A cyclic voltammetry method suitable for characterizing antioxidant properties of wine and wine phenolics. J. Agr. Food Chem. 49: 1957.

Martins R.C., Oliveira R., Bento F., Geraldo D., Lopes V.V., Guedes de Pinho P., Oliveira C.M., Silva Ferreira A.C. 2008. Oxidation management of white wines using cyclic viltammetry and multivariate process monitoring. J. Agric. Food Chem. 56: 12092.

Oliveira C.M., Silva Ferreira A.C., Guedes de Pinho P., Hogg T.A. 2002. Development of a potentiometric method to measure the resistance to oxidation of white wines and the antioxidant power of their constituents. J. Agr. Food Chem. 50: 2121.

Silva Ferreira A.C., Oliveira C., Hogg T., Guedes de Pinho P. 2003. Relationship between potentiometric measurements, sensorial analysis and some substances responsible for aroma degradation of white wines. J. Agr. Food Chem. 51: 4668.

Silva Ferreira A.C., Hogg T., Guedes de Pinho P. 2003. Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. J. Agr. Food Chem. 51: 1377.

BARRIER PROPERTIES OF PLA TOWARDS OXYGEN AND AROMA COMPOUNDS

VIOLETTE DUCRUET[1]*, SANDRA DOMENEK[1], ALAIN GUINAULT[2], CÉCILE COURGNEAU[1], MARTA BERNASCONI[1] and CÉDRIC PLESSIS[1]

[1]UMR 1145 Ingéniérie Procédés Aliments (AgroParisTech/INRA), 1 avenue des Olympiades, 91300 Massy, France

[2]CNAM, Laboratoire des Matériaux Industriels Polymères, 292 rue Saint Martin, 75141 Paris, France

*corresponding author: violette.ducruet@agroparistech.fr

ABSTRACT

PLA samples having different degree of crystallinity and plasticized with acetyl butyl citrate were prepared by extrusion and thermo-moulding. In order to determine the solubility and diffusion coefficients of ethyl esters vapours into the polymer samples, two approaches were assessed, i) the first one by a microgravimetric method, ii) the second one by a permeability approach using a permeation system including a purge-and-trap injector coupled to GC. The structural change of PLA samples before and after aroma sorption were investigated by differential scanning calorimetry (DSC). In parallel, the oxygen barrier properties were determined. Data were compared to conventional packaging materials.

Increasing crystallinity of PLA enhances the barrier properties against ethyl acetate but has no effect on the oxygen permeability. A plasticizing effect was noticed which depends on the hydrophobicity of the ethyl ester. The more hydrophilic compound ethyl acetate is absorbed in high quantities and causes higher plasticization. PLA has, in comparison to polyethylene widely used in food packaging, promising barrier properties especially towards hydrophobic aroma compounds.

Key words: aroma, barrier properties, permeability, PLA, sorption.

INTRODUCTION

Due to their environmental merits, biomass-based polymers have been widely studied and particularly poly(lactic acid). Its moderate mechanical properties and its high transparency, make it a promising material for food packaging. However its high brittleness and its barrier properties restrict its large application. The latter property is required to preserve the organoleptic food quality during shelf-life. The transport of oxygen through the packaging could lead to the lipid oxidation and then to the appearance of off-flavour. Moreover the deterioration of food quality may also be caused by the transfer of volatile molecules, such as aroma compounds into the polymer structure (Dury-Brun *et al.,* 2007). The objective of our work is to assess the capability of PLA to preserve the quality of foodstuff during storage by protecting the aroma formulation and limiting the oxygen transfer.

MATERIALS AND METHODS

Three PLA materials were studied: PLA Biophan 121 (Treofan) is provided in film form (thickness 30 μm); PLA Biomer L9000 100% in L conformation and P(D,L)LA from Natureworks named neat PLA. Ethyl acetate (EA), ethyl butanoate (EB) and ethyl hexanoate (EH) were provided by Sigma (purity 99.5%).

Preparation of PLA films. Extruded PLA Biomer films (120 μm thickness) with different degree of crystallinity were prepared using the procedure described by Colomines *et al.*, 2010. Plasticized P(D,L)LA were prepared by direct melt mixing of acetyl tributyl citrate (5 wt%). with neat PLA in an internal mixer (Haake Rheocord 9000) at 160°C and 60 rpm for 15 min. The plasticized PLA was then thermo-moulded by compression at 185°C and 150 bar to obtain a film of approx. 200 μm thickness before being quenched at ambient.

Oxygen permeability. The oxygen transmission rate was monitored at 23°C and 0% RH with a Systech 8001 apparatus.

Sorption isotherm method. The kinetic of sorption of ethyl esters at different activities were measured at 25°C and 0% RH using an electronic microbalance, IGA-002 (Hiden, Warrington (UK) with a sensitivity of 0.2 μg; sample weight: 30-40 mg).

Permeability set-up. The permeation of ethyl esters through the PLA films were assessed at 25°C by a gas chromatographic set-up including a gas chromatograph (GC Fisons 8000) coupled to a purge and trap injector (PTI, Varian) developed by Hirata *et al.*, 2006. The ethyl ester vapour in contact with one face of the film was generated using a solution of the three ethyl esters in hexadecane. Films of PS (23μm) and LDPE (48 μm) were also tested in the same conditions.

Differential scanning calorimetry (DSC). The crystallinity of the PLA samples was measured with the help of a Pyris 1 (Perkin Elmer, France) DSC. Tests were performed at 10°C/min from 0° to 200°C.

RESULTS AND CONCLUSIONS

Oxygen transmission values (OTR), are given in tab. 1. The degree of crystallinity of the tested PLA seems to have no effect on gas barrier properties.

The kinetic of sorption by gravimetry made it possible to calculate the solubility coefficients (S) of EA in the PLA films (tab. 1). Crystalline PLA showed the

Table 1 - Oxygen permeability of PLA samples. n=2, cristallinity degree and coefficient of solubility (S) of EA (0.5 activity).

Material	Oxygen transmission rate $x10^{18}$ $m^3.m/(m^2.s.Pa)$	Crystallinity degree (%)	S $x10^3(m^{-3}.Pa^{-1})$
Extruded PLA	1.94±0.03	2	11.3
Neat PDLLA	2.30±0.1	<5	n.d
Plasticized PDLLA	5.0±0.3	<5	n.d
Amorphous PLA	2.5±0.18	6	7
PDLLA Biophan	2.2±0.08	19	8.6
Crystalline PLA	2.86±0.08	39	0.104

n.d.: not determined.

lowest coefficient of solubility at 0.5 EA activity. This behavior can be related to its higher degree of crystallinity in comparision with the others PLA.

Measurement of the glass transition temperature (Tg) of the PLA samples was carried out before and after contact with ethyl esters at different activities from 0.1 to 0.9. (fig. 1A). Ethyl esters have a plasticizing effect on PLA which depends on their hydrophilic character. EA is the most efficient. This plasticization effect is linked to the increase of the mass uptake (fig. 1B) which is the highest for EA. The sorption of EA leads to the Tg decrease towards room temperature which can be detrimental for the keeping of the barrier properties of PLA during shelf-life and thus to the quality of the food product in contact.

The kinetics of permeation allows us to calculate the diffusion coefficient of each ethyl esters according to the lag time approach. Diffusion coefficient of ethyl esters in PLA are from two to three orders of magnitude lower than in LDPE and PS, respectively for both EA and EB, and for EH. For each polymer, few differences are noticed between the coefficients of diffusion of the homologous ethyl esters. However for the plasticized PLA, the coefficients of diffusion notably increase and reach around those obtained for PS (tab. 2).

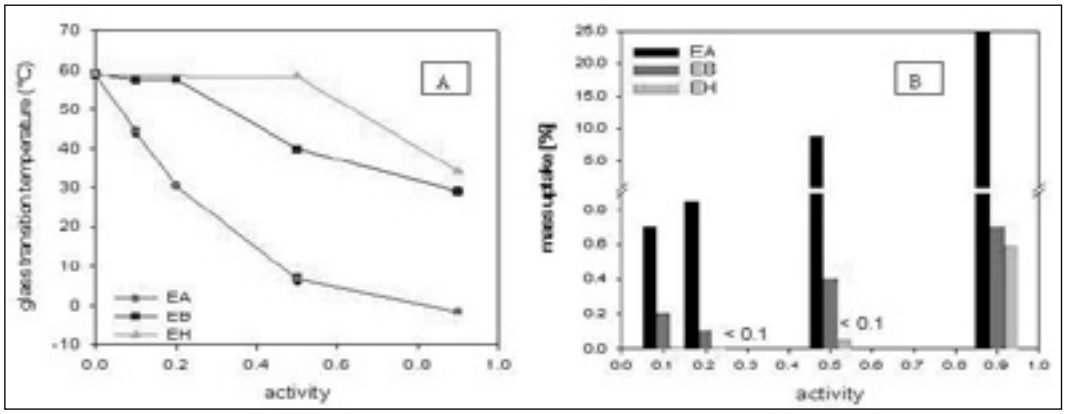


Fig. 1 - Amorphous PLLA after contact with ethyl esters at different activities A) glass transition temperature B) mass uptake (%).

Table 2 - Diffusion coefficient of ethyl esters through PLA films in comparison with PS and LDPE.

Material	Ethyl acetate $x10^{11}$ (m^2/s).	Ethyl butanoate $x10^{12}$ (m^2/s)	Ethyl hexanoate $x10^{13}$ (m^2/s)
LDPE	1.34	1.66	5.49
PS	1.05	0.99	2.51
PDLLA Biophan	$9.75x10^{-4}$	$8.2x10^{-3}$	$6.7x10^{-2}$
Plasticized PDLLA	n.d	0.33	1.46

n.d.: not determined.

CONCLUSION

Two major results are revealed in our work which can have an influence on the preservation of the quality of food product packed in PLA:

In one hand, the more hydrophilic polyester PLA has better aroma barrier properties towards hydrophobic compounds than LDPE and PS. In the opposite the plasticizing effect due to ethyl ester sorption could be detrimental for the food quality by lowering the Tg of PLA packaging material. But this effect decreases with increasing hydrophobicity of the ethyl ester. So since most aroma compounds are hydrophobic, PLA appears as a good barrier polymer for aroma compounds. Plasticization of PLA in order to improve its mechanical properties leads to a decrease in the barrier properties of ethyl esters by increasing theirs diffusion coefficients.

In the other hand, the barrier property towards ethyl acetate could be increased by increasing crystallinity of PLA but increasing crystallinity has no influence on oxygen barrier property of the tested PLA.

REFERENCES

Colomines G., Ducruet V., Courgneau C., Guinault A., Domenek S. 2010. Barrier properties of poly(lactic acid) an its morphological changes induced by aroma sorption. Polymer International: 59, 818.

Dury-Brun C., Chalier P., Desobry S., Voilley A., 2007. Multiple mass transfer of small molecules through flexible food packaging. Food Rev. Int. 23: 199.

Hirata Y., Massey M., Relkin P., Nunes P., Ducruet V. 2006. Influence of proteins on the release of aroma compounds into polymer film. In Developments in Food Science 43: Flavour Science Recent advances and trends. WLP Bredie and MA Petersen (Eds.) p. 473, Roskilde.

IDENTIFICATION OF POTENTIAL MIGRANTS IN POLY (LACTIC ACID) PACKAGINGS

ALEXIS LALANNE, ETZAEL ESPINO, RÓMULO SALAZAR, SANDRA DOMENEK* and VIOLETTE DUCRUET

UMR 1145 Ingénierie Procédés Aliments (AgroParisTech/INRA), 1 avenue des Olympiades, 91300 Massy, France

*corresponding author: sandra.domenek@agroparistech.fr

ABSTRACT

The objective of this work is to identify additives present in five commercial grades of PLA (GA, GB, GC, GD and F1) and to monitor their migrations into aqueous medium. Dissolution-precipitation was used to extract additives and was optimized using dichloromethane as solvent for PLA and ethanol as solvent for additives. Additives were identified by GC-MS and LC-MS. NMR spectroscopy was used to confirm some additives found by chromatography. Migration tests were made by cells and by immersion during 10 days at 40°C in water and acetic acid 3%.

Key words: food safety, packaging, PLA, migration.

INTRODUCTION

Biodegradable materials have received much attention and commercial products using these innovative polymers are coming to the market. Poly (lactic acid) (PLA) is a promising biodegradable material for food packaging, used in high value films or rigid thermoforms (Siracusa *et al.*, 2008). The most important function of food packaging is the protection of the product against external contamination. However, it is necessary to know if additives and other substances present in the polymeric packaging could migrate into the food in contact (Gupta and Kumar, 2007). The objective of our work is to develop an analytical strategy to characterize different grades of PLA and their migrations in contact with aqueous media.

MATERIAL AND METHODS

Optimisation of extraction: 10 g of the different grades of PLA, provided by industrial manufacturers, were dissolved in 200 mL analytical CH_2Cl_2 under reflux for 90 min and precipitated in 1,000 mL analytical ethanol. The precipitate was separated by filtration over a Sartorius 45 µm pore size filter and the supernatant was concentrated to 25 mL by evaporation. The recovery of this method was calculated with GC-FID and LC-UV using three additives absent in PLA supernatant: BHT, Tinuvin P and Irganox 1076. Samples were injected in the on-column mode in a GC 8000Top (CE Instrument) equipped with a fused-silica capillary column (DB1-1HT, 30 m x 0.32 mm I.D., 0.1 µm, J&W Scientific) and detected by FID heated at 350°C.

The samples were separated by LC on a Varian C_{18} column (250x4.6 mm, 5 µm) at a 0.8 mL.min^{-1} flow rate, delivered by a gradient pump (Gilson 321), and detected by an UV-DAD (Waters PDA 996). The mobile phase A was H_2SO_4 (0.1% v/v in water) and the gradient was increase of phase B (100% HPLC grade acetonitrile) from 30 to 100% in 30 min, maintaining 100% phase B during 20 min and returning in 10 min.

Identification of additives: First GC-FID and LC-UV were used to pre-identify some additives in the supernatants by comparison with standards diluted in ethanol (1 g/L).

Then GC-MS and LC-MS systems were used to identify the additives. Samples were injected in LC-MS (Thermo TSP) via a C_{18} column, 150x4,6 mm, 5 µm (Thermo Electron) and the detection was done by UV-DAD between 190 and 600 nm and an ESI-MS (Thermo-Finnigan), in negative effect with a m/z between 120 and 2,000 amu (atomic mass unit). The flow rate and program used were identical to that of LC-UV but sulfuric acid solution was replaced by acetic acid (0.1% v/v in water). Supernatants were injected in a GC system (Agilent 6890) coupled with a mass spectrometry (Agilent 5975) in splitless mode. Column used was DB-5 (J&W Scientific), 30 m x 0.32 mm I.D, 0.5 µm), and the temperature program was 60°C/5 min, ramp 5°C/min, to 340°C/10 min. Identifications were done by comparison with both databases Wiley7 and NIST05.

NMR spectrometer (300 MHz Bruker) was used (number of scans: 32 and relaxation time of 2 sec) to confirm additives found in LC and GC.

Migration: Migration tests were carried out on PLA sheets made by thermomoulding the pellets, in water and acetic acid 3% in water according to Norm NF 1186-4 with monoface contact and immersion contact. Analyses were made in triple with a blank for each simulant and are set on a stove at 40°C. After ten

Table 1 - Recovery rate of additives by dissolution precipitation of PLA.

Additive	Recovery rate by GC-FID	Recovery rate by HPLC-UV
BHT	54%±8%	63%±11%
Tinuvin P	101%±22%	58%±7%
Irganox 1076	40%±10%	Not calculated

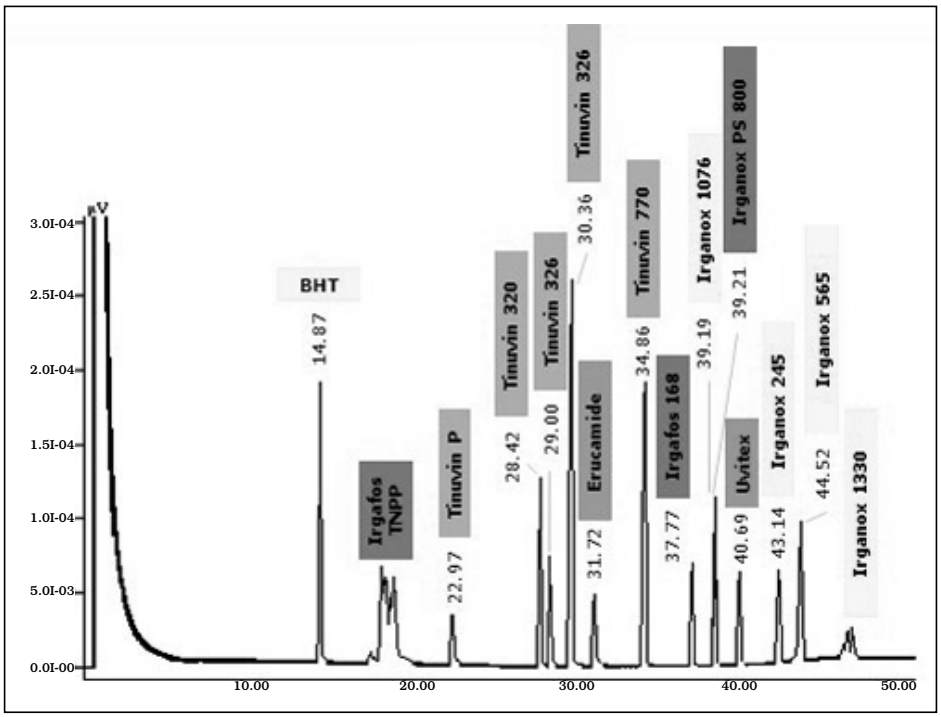


Fig. 1 - Analysis of standard additives at 1 g/L in ethanol by GC-FID.

days, the simulant was evaporated under vacuum in a rotavapor in a previously dried and tarred balloon flask, dried to stove at 105°C in an hour and weighted.

RESULTS AND CONCLUSION

Optimisation of extraction and identification of additives: The best value of recovery rate was obtained with BHT with a good relative standard deviation (tab. 1).

By GC-FID, among standard additives analysed (fig. 1), only Erucamide (t=31.72 min) seemed to match with one peak present in four PLA supernatants (tab. 2).

Many peaks were observed in PLA supernatants by GC-MS and identified as plasticizers (PEG and adipate derivatives), slip agents (Erucamide) and other lubricants (fig. 2). By HPLC-UV, no standard additive matched with PLA extracts. Oligomers

Table 2 - Retention time of GC- FID main peaks in supernatants of different samples of PLA (in bold, retention time of erucamide).

Sample	Retention time (min)
PLA GA	6.13; 7.81; 31.61
PLA GB	7.82; 13.12; 27.72
PLA GC	6.12; 7.16; 27.83; 31.73
PLA GD	6.17; 7.80; 31.61; 32.42
PLA F1	6.07; 28.88; 31.16; 31.67; 43.13; 44.49

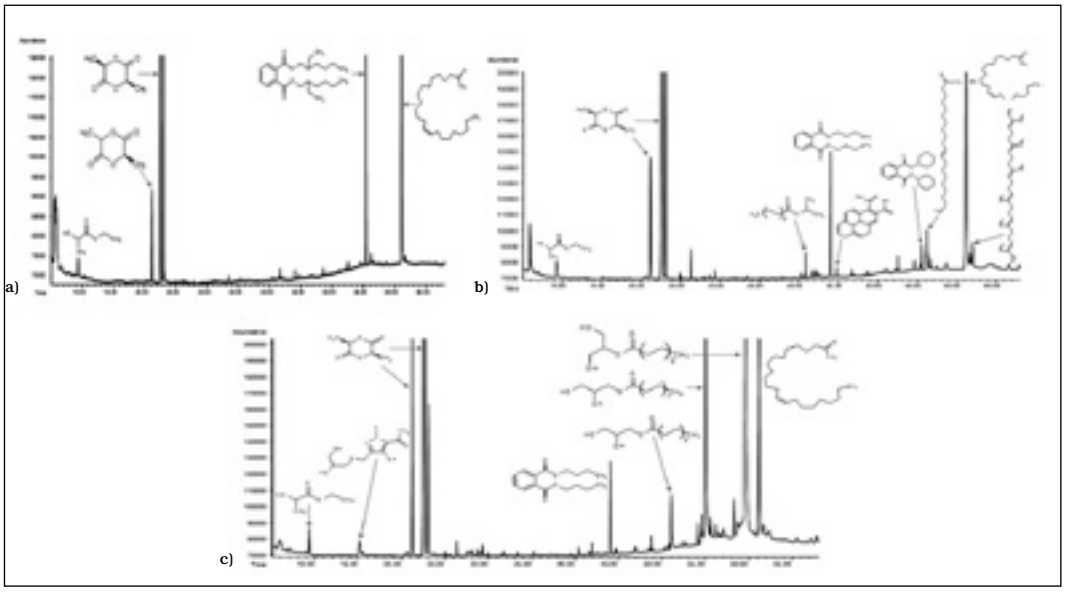


Fig. 2 - GC-MS Spectra of surfactants from pellets PLA GA (a), GD (b) and GE(c).

of lactic acid, coming from degradation of PLA, were observed in large number in LC-MS and they can trouble and mask the identification of some additives.

To confirm presence of some peaks detected by GC-MS, PLA supernatants were compared by ^{1}H NMR Spectroscopy with three standard additives: Erucamide, PEG 300 and Bis (2-ethylhexyl) adipate (fig. 3). It appeared that Erucamide was present in PLA supernatants and especially in PLA GD grade.

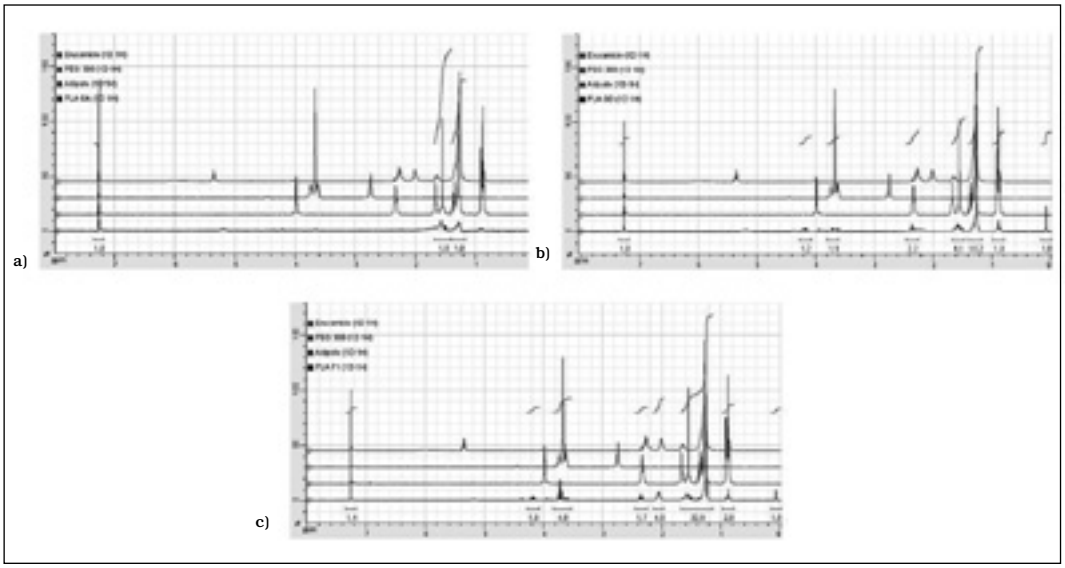


Fig. 3 - Comparison of supernatant of PLA GA (a), GD (b) and F1 (c) with standard additives (erucamide, polyethylene glycol PEG 300 and bis (2-ethylhexyl) adipate) by NMR.

Migration: Values obtained for overall migration with both methods and with two different aqueous media were under legislation value (10 mg per dm^2). The specific migration of Erucamide wasn't studied because it is 10 mg per dm^2.

In conclusion, several components, such as plasticizers, slip agents and other lubricants, have been evidenced by GC-MS, and the presence of Erucamide was confirmed by ^1H NMR. With the help of LC-MS, the presence of oligomers of PLA was confirmed in the supernatant. Overall migration tests of PLA in aqueous media confirmed that the migration of all samples is under the limit authorized by the European legislation for food contact materials and no component with an associated toxicological risk requiring a specific migration test was identified. PLA can thus be considered as a very low additivated polymer complying with European legislation. Further works are going on to improve extraction method to get a better recovery rate and to extract other potential migrants, in particular by eliminating oligomers of PLA.

REFERENCES

Gupta A.P., Kumar V., 2007. New emerging trends in synthetic biodegradable polymers - Polylactide: a critique, European Polymer Journal, 43 (10):4053.

Siracusa V., Rocculi P., Romani S., Dalla Rosa M. *et al.*, 2008. Biodegradable polymers for food packaging: a review, Trends in Food Science & Technology, 19:634.

INVESTIGATION OF OPTICAL AND GEOMETRICAL PACKAGE FEATURES IN PHOTO-OXIDATION DECAY OF A SEMI-HARD CHEESE

S. LIMBO[1], L. TORRI[2], A. ADOBATI[1] and L. PIERGIOVANNI[1]

[1]DiSTAM, Università degli Studi di Milano,
Via Celoria 2, 20133 Milano, Italy

[2]University of Gastronomic Sciences,
Piazza Vittorio Emanuele 9, 12060 Bra, CN, Italy

ABSTRACT

The aim of this work was to investigate the role of optical and geometrical features of a semi-rigid packaging system in the photo-oxidation decay of a semi-hard cheese (*Edam Holland*) during a short storage. The packaging solution chosen for this study was a polystyrene tray sealed in air with transparent plastic film, largely used in the market for MAP products. A new approach based on the interpretation of integrating sphere signal output using a double beam spectrophotometer was developed to characterize and discriminate the transmitted spectral components of the films. To fully investigate the role of optical and geometrical package features in photo-oxidation decay of the cheese, a screening design was selected. The factors considered in the design were: a) the diffused component of the total transmitted light; b) the distance between the surface of the cheese and the lid; c) the colour of the tray. Reflectance spectra in the UV-visible range were converted into colour parameters (CIE L*a*b*) and the variation of the yellow-blue index (Db*) was used as response of the experimental design.

Key words: cheese, light transmittance, package geometry, photo-oxidation, semi-rigid packaging.

INTRODUCTION

Many food products are commercialized in packaging solutions that involve the use of transparent plastic films. Although the exposure is commonly short, the

lighting environments of retail stores provide energy inducing photo-degradation, particularly evident in presence of oxygen (Bosset *et al.*, 1994). The interplay among product, packaging, light and oxygen is very complex and the degree of food photo-oxidation depends also on the ability of the package in avoiding the radiations reaching the product. In fact, when the incident light is transmitted through the material into the package headspace, a part of the radiation is reflected or absorbed by food, whereas the remaining part can be transmitted, absorbed, or reflected by the internal side of the package (Mortensen *et al.*, 2004). The aim of this work was verify if some optical characteristics of the lid and tray (i.e. spectral transmission in the UV-visible range and colour) and the geometry of the package (i.e., the distance from the product and the lid) could contribute to photo-oxidation in a real packaging system.

MATERIALS AND METHODS

Cheese: semi hard cheese (Edam Holland cheese) was purchased from a local grocery store, cut in slices of different thickness and packaged in air.

Packaging materials: the trays made by polystyrene (18*25*2.5 cm) were kindly supplied by Sirap Gema Group (Verolanuova, BS, Italy). Three different colour were considered: black, white and yellow.

Three plastic films were selected as lid closure on the basis of their different light transmission properties.

Storage: samples were stored at 4°C for 7 days, under lighting conditions obtained using a cool white fluorescent lamp (Philips 58W.33.640).

Light transmission properties of the plastic films: a new approach based on the interpretation of integrating sphere signal output in the UV-visible range (200-780 nm) using a double beam spectrophotometer (Lambda 650, Perkin Elmer, Italy) equipped with a 150 mm integrating sphere was developed to characterize and discriminate (Roos et Ribbing, 1988):

- the spectra of the total transmitted radiation;
- the spectra of the diffused radiation that passes through the material with an angle > 2.5°.

Photo-oxidation index: colour parameter (b*, yellow index) of the upper 0.5 cm layer of the cheese slices was calculated by the reflectance spectra in the visible range (acquired with the UV-visible spectrophotometer equipped with the integrating sphere) and analysed by the Color Software (version 5.0, Perkin Elemer).

Design of Experiment: For each colour tray a screening factorial design 2^3 (Modde version 7, Umetrics) was created considering three levels for each variable (tab. 1).

RESULTS AND CONCLUSIONS

The light that passed through the film (the total transmitted radiation) was divided into two main different components (specular and largely diffused) each resulting from their separate contribution to the detector signal. Tested plastic films showed similar total transmission spectra in the visible region but different spectra for the diffused component (fig. 1a and 1b). Because we hypothesized that this component could differently contribute to photo-oxidation in a tray packaging solution, the diffused value at 400 nm (wavelength of riboflavin absorption) was used as a factor

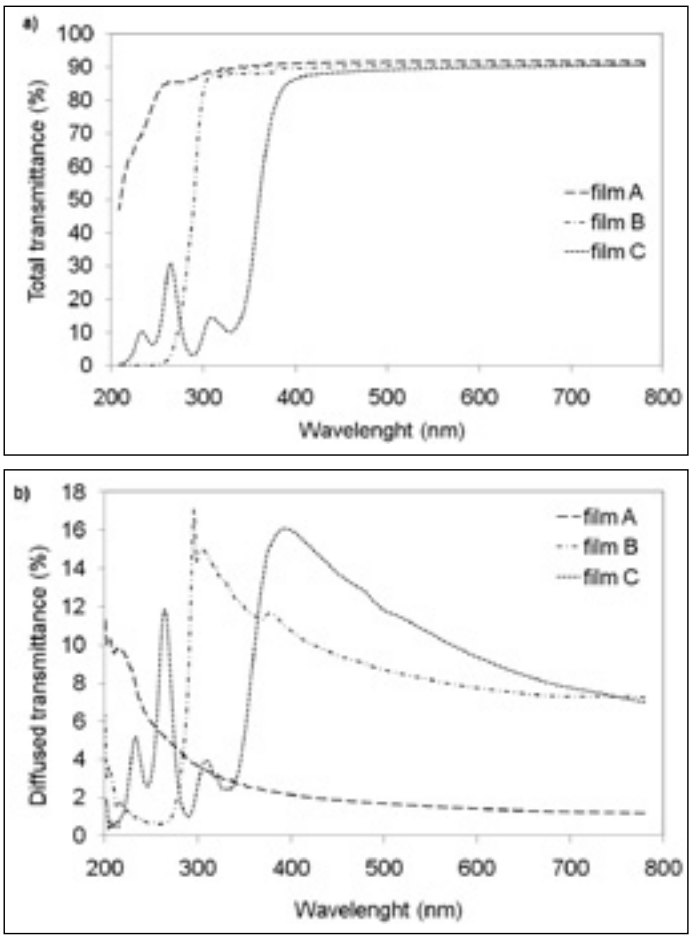


Fig. 1 - Total (a) and largely diffused (b) transmittance of the plastic films.

in the factorial design (2, 10 e 16%) (tab. 1).

At a fixed cheese-lid distance, lower b* index changes over storage time were observed using film with higher diffused transmission component. The influence of the diffused transmission component was low using the black tray but high with the white tray (black colour absorbs the diffused light and avoids its reflection on cheese, as occurs with the white tray). For each tested film and for each tray colour, the colour changes increased as the cheese-distance decreased (data not shown).

At a fixed storage time (4 days), lower b* index changes were observed with higher cheese-film distances (fig. 2a and 2b). The best protection against photo-oxidation was offered by the following combination: high diffused transmission component and high cheese-film distance. The iso-response surface of the best performance was influenced by the tray colour: the black tray offered the highest protection against photo-oxidation decay, the yellow tray the medium protection and the white tray the lowest protection.

In conclusion, the only measure of the total transmission radiation is not sufficient to obtain information about the film performance over food storage but it is important to determine also the diffused component. In designing a semi-rigid

Table 1 - Factors and their levels considered in the factorial design.

Factor	Level		
	-1	0	1
Cheese-lid distance (cm)	0	1.25	2.50
Diffused transmittance at 400 nm (%)	2	10	16
Storage time (days)	1	4	7

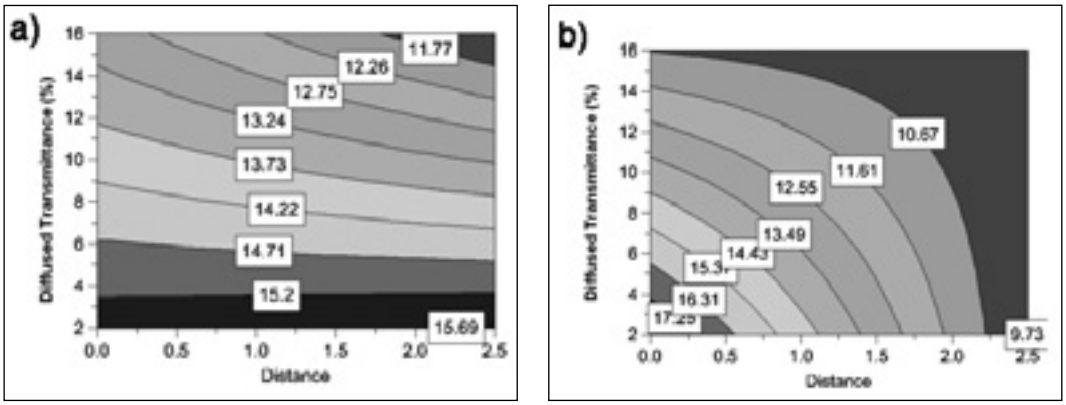


Fig. 2 - Iso-response curves of the diffused transmittance vs. distance after 4 days of storage (a: white tray; b: black tray).

food package is essential to take into account some critical geometrical and optical properties in order to optimize the food package effectiveness.

REFERENCES

Bosset J.O., Gallman P.U., Sieber R. 1994. Influence of light transmittance of packaging materials on the shelf-life of milk and dairy products - a review. Ch. 13. In "Food packaging and preservation". M. Mathlouthi (Ed.), p. 222, Blackie Academic & Professional, Glasgow.

Mortensen G., Bertelsen G., Mortensen B.K., Stapelfeldt H. 2004. Light-induced changes in packaged cheeses - a review. Int. Dairy J. 14(2):85.

Roos A., Ribbing C. 1988. Interpretation of integrating sphere signal output for non-Lambertian samples. Appl. Optics 27(18):3833.

APPLICATION OF THE OXITEST METHOD TO ESTIMATE THE KINETIC PARAMETERS IN SOYBEAN OIL UNDER ACCELERATED STORAGE CONDITIONS

L. MORA[1], S. LIMBO[1] and P. MAIOCCHI[2]*

[1]Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche (DISTAM), Università degli Studi di Milano, Via G. Celoria 2, 20133 Milan, Italy

[2]Velp Scientifica srl, Via Stazione 16, 20040 Usmate, MI, Italy
*corresponding author: analyticalsupport@velp.it

ABSTRACT

Lipid oxidation is one of the most serious problems occurring during storage of fatty foods, causing a shortage of their shelf-life. In order to predict the shelf life, food industry is very interested in easy, quick and reliable solutions able to estimate the oxygen sensitivity of a product. The degree of lipid oxidation can be measured by physical or chemical methods (i.e. peroxide value -PV) as well as stability tests, which measure the stability of a fatty food under conditions that attempt to accelerate the normal oxidation process. In the field of accelerated shelf-life testing, Oxitest permits food stability to be investigated: it is based on the absolute pressure change in a closed and thermostatted chamber, assumed as the oxygen uptake by reactive substances.

The aim of the present study was firstly the assessment of the Oxitest ability to discriminate the growing oxidation degree of soybean oil at different temperatures and secondly to verify the existence of correlation between a common method such as PV and a more innovative accelerated technique like Oxitest.

Soybean oil samples were stored at growing temperatures: the evolution of the oxidation was monitored during time by analyzing the samples by both Oxitest and PV. The increase of PV and decrease of the Induction Period (IP) values followed a zero order kinetic at all the different temperatures tested. On consequence the reaction rate constant (k) for each temperature, the Arrhenius parameters and the Q10 index were estimated. The degree of correlation between the two techniques was also evaluated pointing to the Oxitest method as a reliable alternative to classic methods.

Key words: oxidation, soybean oil, shelf-life, peroxide value, Oxitest.

INTRODUCTION

A number of accelerated methods have been developed to test the resistance of edible fats and oils to oxidation (Farhoosh *et al.*, 2008). All these accelerated methods involve the use of elevated temperatures because the rate of the oxidative reaction is exponentially related to the temperature (Hyung, 1997). Oxitest reactor (Velp Scientifica, Usmate - Italy) has been successfully used to measure the resistance to oxidation of vegetable oils and fatty foods (Mora *et al.*, 2009). In spite of that, the measure of the oxidative degradation kinetics of food during shelf life represents the main goal for the complete development of the reactor. In order to assess this instrumental capacity a preliminary study was carried out comparing the Oxitest response with the traditional measure of the Peroxide Value (PV).

MATERIALS AND METHODS

Soybean oil, bought nearby from a store of the large-scale retail trade, was used as lipid matrix due to its high susceptibility to oxidation (high polyunsaturated fatty acid content) and sampled inside opened glass bottles. The bottles were stored in thermostatted dark chambers at 25°, 50°, 70° and 90°C. After, soybean oil was analyzed by both PV and Oxitest reactor. The PV analysis was carried out on the basis of EC 2472/97 Regulation while the Oxitest working conditions were temperature: 90°C, oxygen pressure: 6 bar and amount of sample: 10 g. The Oxitest response was the Induction Period (IP) expressed as a "lag time" before the fat oxidation; it was calculated by the aid of a graphical method.

RESULTS AND DISCUSSION

For each temperature, the PV numbers of the vegetable oil and the IP values obtained from the Oxitest were recorded and plotted against the storage time. The increase of the PVs and the decrease of IP values followed a zero order kinetic: therefore the reaction rate constant (k) for each temperature was simply estimated (tab. 1).

Fig. 1 and 2 show the ability of both PV and Oxitest (IP) to measure the oxidative degree of soybean oil exposed at growing temperatures.

The Arrhenius equation ($\ln k = \ln k0 - Ea/RT$) was used to determine the activation energy (Ea) of the oxidation reaction for each index. Also, a temperature acceleration factor, (Q_{10}) based on the increase in oxidation rate from a 10°C increase in temperature, was calculated from the slopes of the lines (tab. 2).

Table 1 - Constant rates for IP and PV variations at different temperatures.

Index	25°C	50°C	70°C	90°C
k (IP) bar h^{-1}	0.060	0.092	0.676	1.817
k (PV) meq$kg^{-1}h^{-1}$	0.026	0.281	2.693	7.683

The results summarized in tab. 2 show a similarity between IP and PV responses. A good correlation between the two methods at 70°C was found (R=0,994). Similar results were obtained also for the others storage temperatures (data not shown).

CONCLUSIONS

The results show that the Oxitest could be a helpful solution for monitoring the vegetable oil oxidation. It could be used alternatively to traditional and time-consuming methods with several advantages: analytical rapidity, easiness of use, reproducibility and reduced production of chemical wastes. Oxitest is also a promising instrument in order to study oil shelf-life in accelerated conditions. A good level of correlation with a traditional method like Peroxide Value was found.

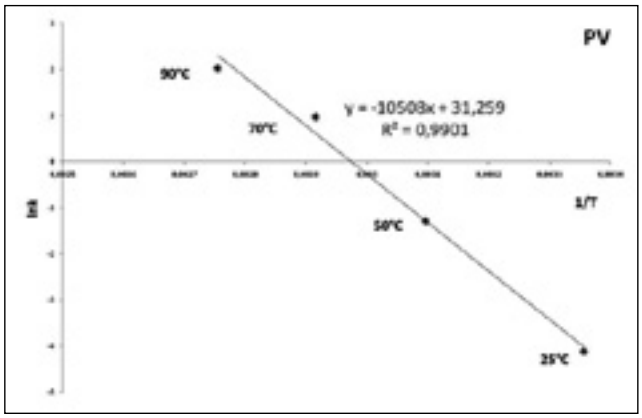


Fig. 1 - Change of the rate constant (k) at different temperatures (PV).

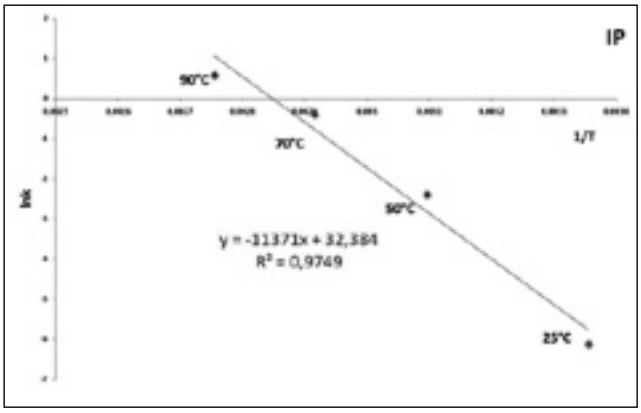


Fig. 2 - Change of the rate constant (k) at different temperatures (IP).

Table 2 - Arrhenius parameters and Q10 for lipid oxidation described by the two indexes.

Index	R2	Ea	Q10 (30°-40°C)
IP	0,97	94 kJ mol^{-1}	3,3
PV	0,99	88 kJ mol^{-1}	3,1

REFERENCES

Farhoosh R., Niazmand R., Rezaei M., Sarabi M. (2008). Kinetic parameter determination of vegetable oil oxidation under Rancimat test conditions. Eur. J. Lipid Sci. Technol. 110: 587-592.

Hyung Y.C. (1997). Reaction Mechanisms and Kinetics of Antioxidant Using Arrhenius Equation in Soybean Oil Oxidation, J. Food Sci. Nutr. 2: 6-10.

Mora L., Piergiovanni L,. Limbo S., Maiocchi P. (2009). Evaluation of vegetable oils oxidative stability through the Oxitest reactor. Industrie Alimentari, 48: 51-56.

SESSION II

"New Technologies for Shelf Life Extension"

Chairmen:

V. Ducruet (AgroParisTech/CNAM/INRA, FR)
C. Nerín (University of Zaragoza, ES)
M.A. Del Nobile (University of Foggia, I)
N.F. Soares (University Federal of Viçosa, BR)

NANOCOMPOSITE PACKAGING MATERIALS FROM POLYSACCHARIDES AND MONTMORILLONITE

J. VARTIAINEN[1], T. TAMMELIN[1], M. TUOMINEN[2], J. PERE[1], U. TAPPER[1], K. NÄTTINEN[1] and A. HARLIN[1]

[1]VTT Technical Research Centre of Finland,
P.O. Box 1000, FI-02044 VTT, Finland

[2]Paper Converting and Packaging Technology,
Tampere University of Technology,
P.O. Box 541, FI-33101 Tampere, Finland

ABSTRACT

In recent years a lot of effort has been aimed at developing new bio-hybrid nanocomposite barrier packaging materials for foods. Nanocomposite films and coatings with improved properties were produced from nanoclay and polysaccharides such as ultrasonic dispersed chitosan and high pressure fluidized pectin. The intercalation of chitosan in the silicate layers was confirmed by the decrease of diffraction angles while the chitosan/nanoclay ratio increased. Nanocomposite films and multilayer coatings had improved barrier properties against oxygen, water vapor, grease and UV-light transmission. Oxygen transmission was significantly reduced under all humidity conditions. In dry conditions, over 99% reduction and at 80% relative humidity almost 75% reduction in oxygen transmission rates was obtained. All chitosan coating raw materials were "generally recognized as safe" (GRAS) and the calculated total migration was in all cases ≤6 mg/dm^2 thus the coatings met the requirements set by the packaging legislation. Processing of the developed bio-hybrid nanocomposite coated materials was safe as the amounts of released particles under rubbing conditions were comparable to the particle concentrations in a normal office environment. Nanoclay-pectin hybrid film formation and high shear induced orientation of nanoclay platelets were investigated by means of model surfaces which were prepared using high shear spincoating. After fluidization, the nanoclay formed uniform and laterally oriented stacks consisting of approximately 15 individual nanoclay layers. Pectin films with different nanoclay concentrations were prepared by casting. Nanocomposite films made of pectin and nanoclay showed improved barrier properties against oxygen and water vapor. Films were also totally impermeable to grease. The developed bio-hybrid nanocomposite packaging materials can be potentially exploited as a safe and environmentally sound alternative for synthetic barrier packaging materials.

Key words: barrier, chitosan, coating, film, nanoclay, pectin.

INTRODUCTION

There is a growing interest in utilization of by-products of agriculture and food industry in order to develop biodegradable materials to replace petroleum based polymers in packaging applications. In addition, nanotechnology in food packaging is expected to grow strongly over the next five years as the increased globalization sets demands for shelf-life enhancing packaging (Harrington, 2009). Recently, a lot of effort has been aimed at developing new biobased polymer containing films and nanocomposites which can act as e.g. barriers in packaging materials (Aurora and Padua, 2010; Lagaron and Fendler, 2009; Vartiainen, Tuominen and Nättinen, 2010; Vartiainen, Tammelin, Pere, Tapper and Harlin, 2010). Unlike synthetic plastics, in dry conditions, the films and coatings from natural polymers exhibit good barrier properties against oxygen and grease due to the high amount of hydrogen bonds in their structure. However, natural polymers are hydrophilic in nature, thus films and coatings produced from these materials are often hygroscopic, resulting in partial loss of their barrier properties at high humidity (Hansen and Placket, 2008). A major challenge for the packaging developers is to overcome the inherent hydrophilic behaviour of biomaterials. The main goal of this work was to study the effects of nanosized montmorillonite on the barrier properties of polysaccharides as a function of relative humidity.

MATERIALS AND METHODS

Chitosan and sugar beet pulp pectin were used as continuous natural polymeric matrixes in which an inorganic nanosized material, montmorillonite, was dispersed. In order to ensure the sufficiently defoliated and nanosized structure of the nanoclay platelets, the ultrasonication and high pressure fluidization were used for homogenization polysaccharide-nanoclay dispersions. Films and coatings were prepared by solvent casting of pectin (films) and wet coating of chitosan onto plasma-activated LDPE coated paper (coatings).

RESULTS AND CONCLUSIONS

Nanoclay was delivered as dry powder with particle size of 2-15 μm. Nanoclays typically tend to be agglomerated when mixed into water. The agglomerates are held together by attraction forces of various physical and chemical nature, including van der Waals forces and water surface tension. These attraction forces must be overcome in order to deagglomerate and disperse the clays into water. Ultrasonication and high pressure fluidization were used to create alternating pressure cycles, which overcome the bonding forces and break the agglomerates. As can be seen in fig. 1a, dry nanoclay powder consisted of round particles with coarse and platelety surface. By ultrasonic dispersing (fig. 1b), and high pressure fluidization (fig. 1c) the nanoclay platelets were effectively ripped off and distributed on the surface. The diameter of the intercalated nanoplatelets varied between 100 and 500 nm.

Nanocomposite chitosan coatings effectively decreased the oxygen transmission of LDPE coated paper under all humidity conditions. In dry conditions, over 99% reduction and, at 80% relative humidity, almost 75% reduction in oxygen transmission rates were obtained. Highest concentration of nanoclay (67 wt%) offered the best barrier against oxygen, whereas the 17 wt% concentration of nanoclay

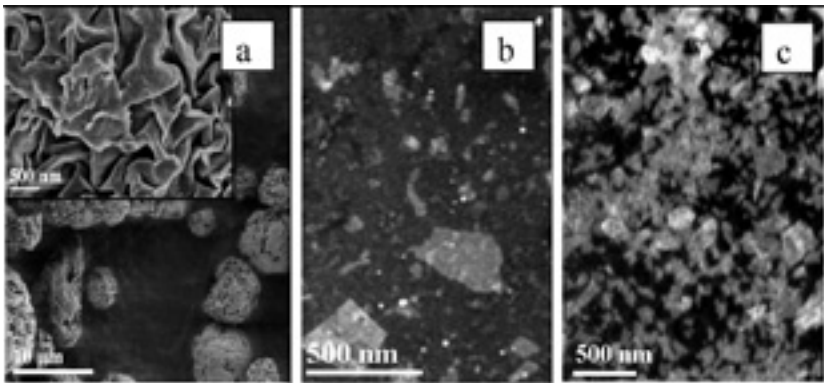


Fig. 1 - SEM images of (a) large undispersed nanoclay aggregates. The excerpt shows the surface structural features: laminar fine structure can be seen on the aggregate surfaces, (b) spincoated nanoclay platelets after dispersing with the ultrasonic microtip, and (c) spincoated nanoclay platelets after high pressure fluidizer treatment.

performed almost as good as 50 wt% of nanoclay. Barrier effects of nanoclay became less evident in dry conditions. Presumably higher nanoclay concentrations were partly agglomerated, which hindered the crystallization and hydrogen bonding formation between chitosan chains, especially in dry conditions.

Nanoclay addition also clearly improved the oxygen barrier properties of pectin films in high humidity conditions. Oxygen transmission rate was reduced by 80% with pectin films containing 30 wt% of nanoclay as compared with the pectin film without nanoclay. Water vapor transmission results indicated the improved barrier properties as well. However, the water soluble pectin was lacking the capability of fully preventing the transmission of water vapor, and thus, total barrier effect of films with 30 wt% nanoclay was not more than 23%. Pectin itself formed an excellent barrier property against grease and nanoclay addition did not improve this barrier property anyhow. All films were totally impermeable to grease under the conditions tested. Barrier improvements are explained using tortuous path theory which relates to alignment of the nanoclay platelets. As a result of the sufficient defoliation, the effective path length for molecular diffusion increases and the path becomes highly tortuous to reduce the effect of gas transmission.

As a conclusion, montmorillonite nanoclay was successfully dispersed in aqueous polysaccharide solutions using ultrasonication and high pressure fluidization. Nanocomposite coatings and films showed improved barrier properties against oxygen, water vapor and UV-light transmission. Materials were also totally impermeable to grease. The developed biohybrid nanocomposite materials can be potentially exploited as safe and environmentally sound alternative for synthetic barrier packaging materials.

REFERENCES

Arora A., Padua G.W. 2010. Review: Nanocomposites in food packaging. Journal of Food Science, 75, 43.

Hansen N., Plackett D. 2008. Sustainable films and coatings from hemicelluloses: A review. Biomacromolecules, 9, 1493.

Harrington R. 2009. http://www.foodproductiondaily.com/Packaging/Strong-growth-in- nano-packing-forecast-but-Europe-still-cautious.

Lagarón J.M., Fendler A. 2009. High water barrier nanobiocomposites of methyl cellulose and chitosan for film and coating applications. Journal of Plastic Film and Sheeting, 25, 47.

Vartiainen J., Tuominen M., Nättinen K. 2010. Biohybrid nanocomposite from sonicated chitosan and nanoclay. Journal of Applied Polymer Science, 116, 3638.

Vartiainen J., Tammelin T., Pere J., Tapper U., Harlin A. 2010. Biohybrid barrier films from fluidized pectin and nanoclay. Carbohydrate Polymers, in press.

EFFECT OF HIGH PRESSURE TREATMENT ON BOVINE WHEY PROTEINS

C. MAZRI[1], S.J. RAMOS[2], L. SÁNCHEZ[1], M. MONTSERRAT[1], M. CALVO[1] and M.D. PÉREZ[1]*

[1]Tecnología de los Alimentos, Facultad de Veterinaria, Universidad de Zaragoza, Miguel Servet, 177, Zaragoza, Spain

[2]Centro Nacional de Tecnología y Seguridad Alimentaria, Laboratorio del Ebro, Carretera NA-134, Km 50. San Adrian, Navarra, Spain
*corresponding author: dperez@unizar.es

ABSTRACT

The effect of high pressure (HP) treatment on bovine whey proteins has been studied and compared to the effect of thermal treatment. The concentration of β-lactoglobulin, α-lactalbumin, lactoferrin and IgG was determined by immunochemical techniques. The enzymatic activity of lactoperoxidase was determined by a spectrophotometric technique.

Skimmed milk was subjected to pressure at 500 MPa for different times at 20°C. Results obtained indicated that the effect of HP treatment on whey proteins depends on the protein. D-value (time for 90% denaturation at constant pressure) calculated for β-lactoglobulin was $24x10^2$ s, whereas higher values were obtained for lactoferrin, IgG and α-lactalbumin, $65x10^2$, $74x10^2$, and $194x10^2$ s, respectively. For lactoperoxidase, no loss of enzymatic activity was observed after 30 min of treatment.

Pasteurization treatment of milk at 75°C for 15 s had no effect on whey proteins unless for lactoperoxidase which decreased its activity by 35%. High pasteurization treatment at 90°C for 15 s caused high levels of denaturation of lactoferrin, lactoperoxidase and IgG, whereas β-lactoglobulin and α-lactalbumin showed small change in their concentration. These results indicate that thermal and pressure treatments have a different effect on denaturation of the individual whey proteins.

Key words: bovine milk, high pressure, thermal treatment, whey proteins.

INTRODUCTION

Thermal treatments of milk such as pasteurization or Ultra-High-Temperature treatment (UHT) are widely used to inactivate pathogenic and spoilage microorganisms and also enzymes. However, these thermal treatments can modify the nutritive value and the sensorial properties of milk, depending on their intensity.

High pressure (HP) is being investigated as an alternative to pasteurization of milk because it has the capacity to inactivate vegetative microorganisms at lower temperatures than those normally used in conventional heat treatments (Patterson, 2005). Most of the HP research in milk has been performed to evaluate its effect on microbial and enzyme inactivation, however, few studies have been performed concerning the effects of HP treatment on whey proteins. β-Lactoglobulin (β-lg) and α-lactalbumin (α-la) are the predominant proteins in bovine whey and are largely responsible for the physicochemical characteristics of whey, such as gelation, foaming and emulsification properties. Other whey proteins, like lactoferrin, IgG and lactoperoxidase, have attracted special interest because of their antimicrobial activity. The maintenance of the antimicrobial properties of these proteins gives them the potential to be used as supplements for special foods or nutraceutical products (Farrell *et al.*, 2004).

The aim of this work was to study the effect of HP treatment on denaturation of bovine whey proteins and to compare it with that produced by pasteurization treatments.

MATERIALS AND METHODS

Bovine milk was kindly donated by Quesos Villacorona (El Burgo de Ebro, Zaragoza) and lactoferrin and lactoperoxidase by Fina research (Seneffe, Belgium).

Samples of skimmed milk, obtained by centrifugation, were introduced into eppendorf tubes without headspace and treated in a high pressure food processor (Stansted Fluid Power Ltd, Stansted, Essex, UK) at 500 MPa for different times at 20°C.

Heat treatment of skimmed milk was performed in glass capillary tubes. Samples were heated at 75°C for 15 s (High Temperature Short Time, HTST) and at 90°C for 15 s by immersion of the capillaries in a water bath (±0.1°C) and immediately cooled.

The concentration of β-lg, α-la and IgG was determined by radial immunodiffusion using specific antisera (Wehbi *et al.*, 2005). Standard curves were made by plotting the square of the diameter values of the precipitating rings versus the concentration of protein standards. The concentration of lactoferrin was determined using a sandwich ELISA technique previously developed. Standard curves were made by plotting the absorbance values versus the concentration of protein standards. Enzymatic activity of lactoperoxidase was measured by a spectrophotometric method using ABTS as substrate (Marín *et al.*, 2003). Absorbance increase at 412 nm was recorded versus time and the activity calculated from the slope of the curve.

D-values (time required for 90% protein denaturation at constant pressure) were calculated from the graphical representation of the logarithm of protein concentration as a function of time. D-values were calculated as the reciprocal of the slope obtained by regression analysis.

RESULTS AND CONCLUSIONS

The degree of denaturation of β-lg, α-la, IgG and lactoferrin was determined by measuring the loss of reactivity of each protein with specific antibodies using immunochemical techniques. The degree of denaturation of lactoperoxidase was determined by its loss of enzymatic activity using a spectrophotometric technique.

The process of denaturation was studied analyzing the concentration of individual proteins after pressure treatment at different times by kinetic analysis. The degree of loss of immunoreactivity of β-lg, α-la, IgG and lactoferrin increased with time of treatment (fig. 1). Results obtained indicated that the effect of HP treatment on whey proteins depends on the protein. When milk was treated at 500 MPa, D-value calculated for β-lg was $22x10^2$ s whereas higher values were obtained for lactoferrin, IgG and α-la ($65x10^2$, $74x10^2$, and $194x10^2$ s, respectively). The higher stability of α-la to pressure, compared to β-lg, has been related to the more rigid structure of the former, caused partially by the higher number of intra-molecular disulphide bonds in α-la (4) than in β-lg (2) and to the presence of a free sulphydryl group in β-lg (Huppertz *et al.*, 2004). Results obtained for IgG are similar to those reported by Indyk *et al.* (2008) who found about 25% loss in IgG immunoreactivity after treatment a 500 MPa for 15 min (tab. 1). For lactoperoxidase, no loss of activity was observed after 30 min of treatment (results not shown), as previously reported (Ludikhuyze *et al.*, 2001).

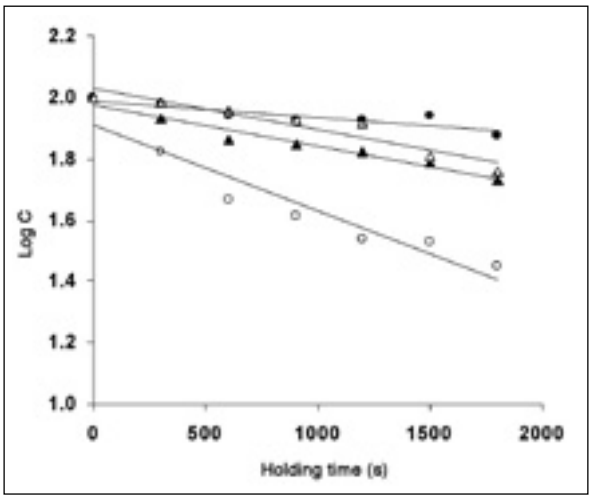


Fig. 1 - Effect of high pressure treatment at 500 MPa at 20°C on denaturation of bovine whey proteins. Concentration of β-lactoglobulin, α-lactalbumin and IgG were determined by radial immunodiffusion and that of lactoferrin by a sandwich ELISA technique. C is the concentration of immunoreactive protein at each holding time expressed as percentage of protein concentration in untreated sample. β-lactoglobulin (○), α-lactalbumin (•), IgG (Δ), lactoferrin (▲).

The effect of thermal processing was also studied (tab. 1). HTST pasteurization treatment of milk showed no significant effect on β-lg, α-la, IgG and lactoferrin as it

Table 1 - Effect of heat treatments on denaturation of bovine whey proteins. The concentration of β-lactoglobulin, α-lactalbumin and IgG were determined by radial immunodiffusion and that of lactoferrin by a sandwich ELISA technique. Enzymatic activity of lactoperoxidase was determined by a spectrophotometric technique using ABTS as substrate. Values are expressed as percentage of protein concentration or enzymatic activity in untreated milk. ND, not detected.

	75°C/15 s	90°C/15 s	500 MPa/15 min
β-Lactoglobulin	97.8	81.6	40.7
α-Lactalbumin	100.8	97.2	84.3
IgG	92.0	47.7	75.1
Lactoferrin	92.7	65.2	80.6
Lactoperoxidase	67.6	ND	100.2

has been previously reported (Mainer *et al.,* 1997; Wehbi *et al.,* 2005). However, this treatment produced a considerable effect on lactoperoxidase, decreasing its enzymatic activity by 35% (Marín *et al.*, 2003). High pasteurization treatment at 90°C for 15 s produced a slight decrease of the immunoreactive concentration, less than 20%, of β-lg and α-la, whereas it produced a marked decrease of IgG and lactoferrin concentrations, 47 and 65% respectively. No lactoperoxidase activity was detected after treatment at 90°C for 15 s.

Results obtained in this work indicate that whey proteins have a different sensitivity to denaturation depending on the type of treatment applied: high pressure or thermal treatment. The higher resistance of whey proteins with antibacterial activity to HP treatment compared to heating, should be considered in the design of treatments in order to preserve their biological function when they are added to special food products.

ACKNOWLEDGEMENTS

This work was supported by grants UZ 2009-CIE-5 from Zaragoza University, 3rd Plan Tecnológico de Navarra (Navarra Government). Chafiaa Mazri from the Institut National de la Recherche Agronomique d'Algerie is recipient of a fellowship from AECID.

REFERENCES

Farrell H.M., Jimenez-Flores R., Bleck G.T. *et al.* 2004. Nomenclature of the Proteins of Cows' Milk. Sixth Revision. J. Dairy Sci. 87: 1641.

Huppertz T., Fox P.F., Kelly A.L. 2004. High pressure-induced denaturation of α-lactalbumin and β-lactoglobulin in bovine milk and whey: A possible mechanism. J. Dairy Res. 71, 489.

Indyk H.E., Willians J.W., Patel H.A. 2008. Analysis of denaturation of bovine IgG by heat and high pressure using an optical biosensor. Int. Dairy J. 18: 359.

Ludikhuyze L.R., Claeys W.L., Hendrickx M.E. 2001. Effect of temperature and/or pressure on lactoperoxidase activity in bovine milk and acid whey. J. Dairy Res. 68: 625.

Mainer G., Sánchez L., Ena J.M., Calvo M. 1997. Kinetic and thermodynamic parameters for heat denaturation of bovine milk IgG, IgA and IgM. J. Food Sci. 62: 1034.

Marín E., Sánchez L., Pérez M.D., Puyol P., Calvo M. 2003. Effect of heat treatment on bovine lactoperoxidase activity in skim milk: kinetic and thermodynamic anlysis. J. Food Sci. 68: 89.

Patterson M.F. 2005. Microbiology of pressure-treated foods. J. Appl. Microbiol. 98: 1400.

Wehbi Z., Pérez M.D., Sanchez L., Pocovi C., Barbana C., Calvo M. 2005. Effect of heat treatment on denaturation of bovine α-lactalbumin: Determination of kinetic and thermodynamic parameters. J. Agr. Food. Chem. 53: 9730.

BIOLOGICAL ACTIVITY OF PROCESSED RECOMBINANT LYSOZYME

E. CASTILLO, M.D. PÉREZ, I. FRANCO, F.N. NAVARRO, M. CALVO and L. SÁNCHEZ

Tecnología de los Alimentos, Facultad de Veterinaria,
Universidad de Zaragoza, Miguel Servet, 177,
50013 Zaragoza, Spain

ABSTRACT

Lysozyme is an enzyme with antibacterial activity often used as food preservative and as active ingredient in pharmaceutical preparations. Lysozyme is quantitatively important in human milk and has been proposed to be used in special products to improve infant health. The production of recombinant human lysozyme (rhLz) in rice is very valuable for its application in food and pharmaceutical industry. In this work, the effect of heat treatment and high pressure (HP) on the enzymatic activity of rhLz and on its antibacterial activity against *Listeria monocytogenes* was studied. No loss of rhLz enzymatic activity was found after Low Temperature Long Time (63°C for 30 min) and High Temperature Short Time (75°C for 15 s) pasteurization treatments. Likewise, HP-treatment at 600 MPa for 15 min at 20°C of rhLz in milk, whey or phosphate buffered saline (PBS) did not cause a decrease in its enzymatic activity. HTST treatment did not affect the antibacterial activity of rhLz with respect to the non-treated rhLz. However, LTLT treatment and treatments at 72°C for 10 min and 85°C for 15 s enhanced the antibacterial activity of rhLz, causing a reduction of bacterial CFU of about 4 log cycles, compared with the reduction of 2 cycles caused by non-treated rhLz. However, the antibacterial activity of rhLz treated at 85°C for 10 min was lower than that of non-treated protein. No reduction in the number of bacteria was observed when incubating them with non-treated or HP-treated rhLz in milk or whey, whereas a reduction of 2 log cycles was found for HP-treated rhLz in PBS, similar to that obtained for the non-treated sample.

INTRODUCTION

Lysozyme is a hydrolytic enzyme that cleaves the β-(1,4) glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine in peptidoglycan, the major bacterial cell wall polymer (Ellison and Giehl, 1991). Lysozyme has been applied as a food preservative and as an active ingredient in the pharmaceutical industry due to its antibacterial activity (Proctor and Cunningham, 1988). Lysozyme is one of the most abundant proteins in human milk, being its concentration about 300 mg/L which is 3,000 times higher than in cow's milk (Lönnerdal, 1985). In addition, the lytic activity of human lysozyme is 3 and 10 times higher than that of lysozyme from egg and cow´s milk, respectively (Li *et al.*, 2006). It has been shown that lysozyme among other components of breast milk, such as lactoferrin and IgA, contributes to the well-being and health of newborn (Lönnerdal, 1985), so it would be interesting to supplement infant formulas with these components. However, the source of human lysozyme is limited. An alternative could be the use of recombinant lysozyme expressed in rice (rhLz) which is a way for the production of large quantities of protein with low cost. However, the use of rhLz as food additive or ingredient requires the evaluation of the effect that different technological processes might have on its biological properties.

In this work we have determined the effect that heat and high pressure treatments have on the enzymatic activity of rhLZ with the *Micrococcus lysodeikticus* test as well as on its antibacterial activity against *Listeria monocytogenes.*

MATERIALS AND METHODS

rhLz isolated from rice was kindly provided by Ventria Bioscience (Sacramento, CA) as a white powder containing 88% protein and an enzymatic activity of 157,000 u/mg.

Heat and HP treatment of the samples

Heat treatment of rhLz (2 mg/mL) was done in 15 mM potassium phosphate, 150 mM NaCl, pH 7.4 buffer (PBS) in a temperature-controlled water bath (±0.1°C) at different temperatures and times: 63°C for 30 min (Low Temperature Long Time, LTLT), 72°C for 15 s (High Temperature Short Time, HTST), 72°C for 10 min, 85°C for 15 s and 85°C for 10 min. HP treatment of rhLz (2 mg/mL) at 600 MPa for 15 min and at 20°C in milk, whey and PBS was carried out in a high pressure food processor (Stansted Fluid Power Ltd., Stansted, Essex, UK) in the Centro Nacional de Tecnología y Seguridad Alimentaria (San Adrián, Navarra, Spain).

Enzymatic activity assay

Lysozyme activity was determined by a turbidimetric technique, measuring the decrease in absorbance at 450 nm versus time of a *M. lysodeikticus* suspension. A fresh suspension of *M. lysodeikticus* (0.15 mg/mL) in 100 mM phosphate buffer, pH 6.2, was used as substrate. For each sample, 0.5 mL of substrate was placed in a cuvette and 0.5 mL of rhLz (final concentration of 3.3 μg/mL). Absorbance decrease was recorded versus time and the activity of each sample calculated from the slope of the curve.

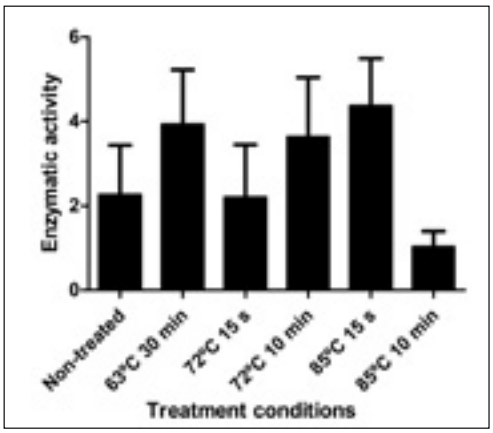


Fig. 1 - Effect of different heat treatments on the enzymatic activity of recombinant human lysozyme (rhLz). The enzymatic activity was determined by a turbidimetric method based on the lysis of *M. lysodeikticus* and is expressed as percentage of the activity of non-treated lysozyme (NT-rhLz).

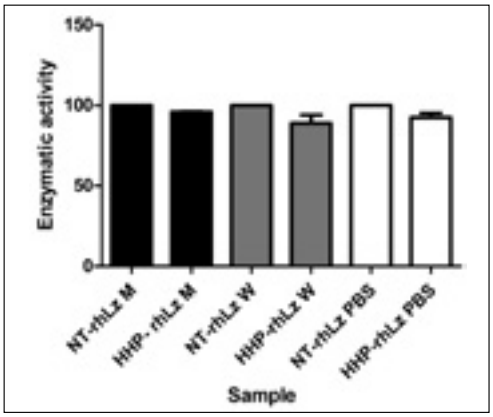


Fig. 2 - Effect of high pressure treatment (HP) at 600 MPa for 15 min at 20°C on the enzymatic activity of recombinant human lysozyme in three different media (milk, M; whey, W, and phosphate buffered saline, PBS). The enzymatic activity was determined by a turbidimetric method based on the lysis of *M. lysodeikticus* and is expressed as percentage of the activity of non-treated lysozyme (NT-rhLz).

Antibacterial activity assay

The antibacterial activity of rhLz was tested on *Listeria monocytogenes* serotype 4b, strain CECT 935 provided by the Spanish Type Culture Collection. One single colony of *L. monocytogenes* isolated in tryptic soy agar supplemented with 0.1% of yeast extract (TSA-YE) was grown in tryptic soy broth overnight at 37°C. The bacterial suspension was diluted in peptone-yeast-glucose medium to achieve 10^4-10^5 CFU/mL. Aliquots of 100 µL of the suspension were added to the wells of a microtitre plate together with 100 µL of rhLz (2 mg/mL). Control wells contained bacterial suspension without rhLz. After 24 h at 37°C the bacterial suspensions were plated onto TSA-YE plates and the number of CFU was counted after incubating the plates at 37°C for 24h. Inactivation is expressed as the logarithmic reduction of bacterial CFU expressed as log (N_0/N), where N are the CFU of samples incubated with non-treated rhLz or samples incubated with heat or HP-treated rhLz and N_0 the CFU of the control wells.

RESULTS AND CONCLUSIONS

Results of the residual enzymatic activity of rhLz on *M. lysodeikticus* after pasteurization treatments of different intensity are shown in fig. 1. The enzymatic activity of rhLz did not decrease after LTLT or HTST treatments, as was reported for lysozyme from human milk (Björkstén *et al.*, 1980). After treatments at 72°C for 10 min and 85°C for 15 s, rhLz retained at least 75% of its enzymatic activity whereas it decreased greatly, by 60% after treatment at 85°C for 10 min. When rhLz was subjected in milk, whey or PBS to HP-treatment at 600 MPa for 15 min at 20°C, the loss of enzymatic activity was lower than 10% (fig. 2). These results are in accordance with those previously reported for hen egg-white lysozyme (Michiels *et al.*, 2001).

Results of the antibacterial activity of rhLz on *L. monocytogenes* after pasteurization treatments are shown in fig. 3. Non-treated rhLz caused a reduction of 2 logarithmic cycles in the bacterial population, as was for rhLz subjected to HTST.

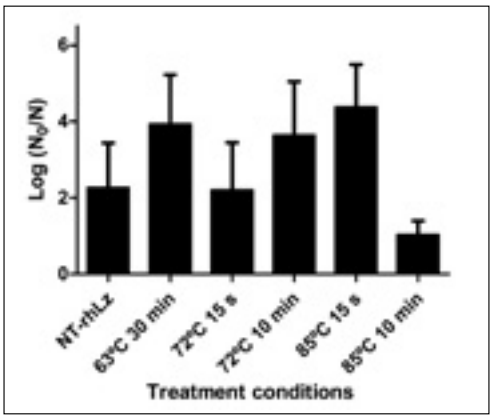


Fig. 3 - Effect of different heat treatments on the antibacterial activity of recombinant human lysozyme (rhLz) against *L. monocytogenes*. Bacteria (10^5 CFU/mL) were incubated at 37°C for 24 h with a final concentration of 1 mg/mL of non-treated (NT-rhLz) or heat treated rhLz before determining the CFU of viable cells on agar plates. The results are shown as logarithmic cycles of reduction (N_0/N) where N is the CFU of bacterial samples incubated with NT-rhLz or heat treated rhLz and N_0 the CFU of the control samples. Values are the mean ± SD of at least four independent experiments.

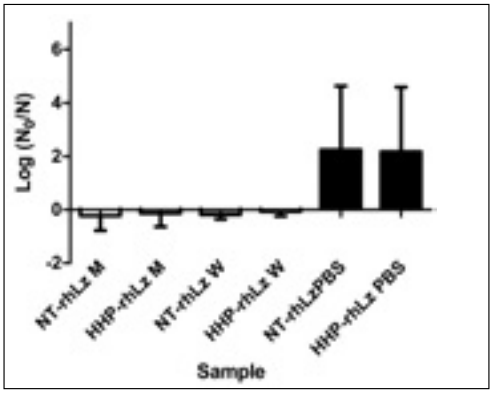


Fig. 4 - Effect of high pressure treatment (HP) at 600 MPa for 15 min at 20°C on the antibacterial activity of recombinant human lysozyme (rhLz) against *L. monocytogenes* in three different media (milk, M; whey, W, and phosphate buffered saline, PBS). Bacteria (10^5 CFU/mL) were incubated at 37°C for 24 h with a final concentration of 1 mg/mL of non-treated (NT-rhLz) or HP treated rhLz before determining the CFU of viable cells on agar plates. The results are shown as logarithmic cycles of reduction (N_0/N) where N is the CFU of bacterial samples incubated with NT-rhLz or HP treated rhLz and N_0 the CFU of the control samples. Values are the mean ± SD of at least four independent experiments.

LTLT and treatments at 72°C for 10 min, and 85°C for 15 s enhanced the antibacterial activity, causing a reduction of about 4 logarithmic cycles. Some authors have also reported an enhancement of antibacterial activity of egg lysozyme after being subjected to 80°C for 20 min (Ibrahim *et al.*, 1996). However, in our work the antibacterial activity of rhLz subjected to treatment at 85°C for 10 min was lower than that of non-treated rhLz.

When *L. momocytogenes* was incubated with non-treated or HP-treated rhLz in milk or whey, no reduction in the number of bacteria was observed (fig. 4). This fact is probably due to the complexity of the medium composition. However, when bacteria were incubated with HP-treated rhLz in PBS, a reduction of 2 logarithmic cycles were obtained, similar to that obtained for non-treated rhLz, indicating that HP-treatment applied did not reduce the antibacterial activity of the protein.

BIBLIOGRAPHY

Björkstén B., Burman L.G., De Château P., Fredrikzon B., Gothefors L., Hernell O. 1980. Collecting and banking human milk: To heat or not to heat? Brit. Med. J. 281: 765.

Ellison R.T., Giehl T.J. 1991. Killing of Gram-negative bacteria by lactoferrin and lysozyme. J. Clin. Invest. 88: 1080.

Ibrahim H.R., Higashiguchi S., Koketsu M., Juneja L.R., Kim M., Yamamoto T., Sugimoto Y., Aoki T. 1996. Partially unfolded lysozyme at neutral pH agglutinates and kills Gram-negative and Gram-positive bacteria through membrane damage mechanism. J. Agric. Food Chem. 44: 3799.

Li N., Yu Z., Meng Q., Yu H., Fan B., Yu S., Fei J., Wang L., Dai Y. 2006. Expression and bioactivity of recombinant human lysozyme in the milk of transgenic mice. J. Dairy Sci. 89: 2911.

Lönnerdal B. 1985. Biochemistry and physiological function of human milk proteins. Am. J. Clin. Nutr. 42: 1299.

Michiels C.W., Masschalck B., Van Houdt R., Van Haver E.G.R. 2001. Inactivation of Gram-negative bacteria by lysozyme, denatured lysozyme, and lysozyme-derived peptides under high hydrostatic pressure. Appl. Environ. Microbiol. 67: 339.

Proctor V.A., Cunningham F.E. 1988. The chemistry of lysozyme and its use as a food preservative and a pharmaceutical. Crit. Rev. Food. Sci. 26: 359.

ACTIVE PACKAGING: NEW TECHNOLOGIES FOR SHELF-LIFE EXTENSION

N.F.F. SOARES*, W.A. SILVA, E.A.A. MEDEIROS, R.T. CARELLI and P.J.P. ESPITIA

Food packaging laboratory, Food Technology Department, Viçosa Federal University, Av. P. H. Rolfs, 36570-000 Viçosa, MG, Brazil
*corresponding author: nfsoares@ufv.br

ABSTRACT

The food production with quality and safety is the main goal for the food industry. In this context, the convergence of several areas of knowledge has allowed researches and development of active packaging for foods. The aim of this work is to present the results of researches accomplished in active packaging area such as active films, edible coating and antimicrobial, antioxidants and aromatized sachets. These works were developed in the Food Packaging Laboratory at the Viçosa Federal University, Minas Gerais, Brazil. The use of chitosan as antimicrobial coating for minimally processed garlic showed that the fungi growth was less than 10^5 CFU·g^{-1} after 15 days of storage at 10°C. Cellulose base films incorporated with sorbic acid (6%) were effective in the conservation of pastry dough stored at (8±1°C). The pastry dough layered with the active film showed a significantly smaller microbial growth than the control pastry dough since the filamentous fungi and yeast count in the food was $<10^2$ CFU·g^{-1}. The enzymatic browning of minimally processed apple cv. "Fuji" was effectively controlled during 8 days at 8°C by the starch coating incorporated with ascorbic acid (1%). Minimally processed carrots were coated with starch + chitosan (1.5%) and showed a reduction in the aerobic mesophilic, filamentous fungi and yeast and psychrotrophic count of 1.34, 2.5 and 1.3 log cycles, respectively in relation to the control treatment. Ham slices were inoculated with 10^6 CFU·mL^{-1} of *Listeria innocua* or *Salmonella* sp. and layered with antimicrobial film (cellulose acetate incorporated with pediocin – 25 and 50%). Antifungal sachets, made of high absorption resin, incorporated with fennel 5% of essential oil (EO) were effective in the fungic growth. Papaya (*Carica papaya*) were packaged in paper bag containing antifungal sachets incorporated with lemon grass and cinnamon OE and the filamentous fungi and yeast count in the 6th day (4.2 Log CFU·g^{-1} for both EO treatments) and in the 9th day of storage (4.9 and 4.3 Log CFU·g^{-1}, respectively) were significantly smaller than the control. Active films added with iron nanoparticles (zeolite) were developed by casting method. Therefore, these results indicate that active packaging have a great potential application in the food industry to increase the shelf-life and safety food.

Key words: Active packaging, antimicrobial agents, nanocompounds.

INTRODUCTION

The food production with quality and safety is the main goal for the food industry. Food packaging is of utmost importance to protect the food from microbial and chemical contamination, oxygen, water vapor and light, promotion of convenience and providing product information. The type of packaging used therefore has an important role in determining the shelf-life of a food. Active' packaging does more than simply provide a barrier to outside influences. It can control, and even react to, events taking place inside the package. Chitosan, sorbic acid, nisin, pediocin and other polyamines have antimicrobial activity (Rodríguez *et al.*, 2003; Geraldine *et al.*, 2008; Jofré *et al.*, 2008). Sintetic antimicrobial has been used with active agent in active food packaging. Sozer and Kokini (2009) cited nanoscience and nanotechnology are new frontiers this century and their applications to the agriculture and food sector are relatively recent. The aim of this work is to present the results of researches accomplished with active packaging such as active films, edible coating and antimicrobial, antioxidants and aromatized sachets in the food packaging area. These works was developed in the Food Packaging Laboratory at the Viçosa Federal University, Minas Gerais, Brazil.

RESULTS AND CONCLUSIONS

Botrel *et al.* (2007) evaluated the effects of the use of chitosan coating on minimally processed garlic, in relation to microbiological characteristics. The use of chitosan as antimicrobial coating for minimally processed garlic showed that the fungi growth was less than 10^5 CFU·g^{-1} after 15 days of storage at 10°C. Silveira *et al.* (2007) demonstrated that pastry dough layered with the active film showed a significantly smaller microbial growth than the control pastry dough since the filamentous fungi and yeast count in the food was $<10^2$ CFU·g^{-1}. Silva *et al.* (2008) evaluated starch coating incorporated with ascorbic acid on minimally processed apple cv. "Fuji". The enzymatic browning of minimally processed apple cv. "Fuji" was effectively controlled during 8 days at 8°C by the starch coating incorporated with ascorbic acid (1%). Minimally processed carrots were coated with starch + chitosan (1.5%) and showed a reduction in the aerobic mesophilic, filamentous fungi and yeast and psychrotrophic count of 1.34, 2.5 and 1.3 log cycles, respectively in relation to the control treatment (Durango *et al.* 2006). Santiago-Silva *et al.* (2009) evaluated antimicrobial efficiency of cellulose acetate film incorporated with pediocin. Ham slices were inoculated with 10^6 CFU·mL^{-1} of *Listeria innocua* or *Salmonella* sp. and layered with antimicrobial film (cellulose acetate incorporated with pediocin – 25 and 50%). The experiment was set up overlapping the slices of ham with the films, and then these systems were packaged under vacuum and stored at 12°C. After 15 days of storage the antimicrobial films were more effective inhibiting the *L. innocua* growth since the 50% pediocin-film presented a reduction of 2 log cycles in relation to control treatment. Soares *et al.* (2007) evaluated antifungal sachets, made of high absorption resin, incorporated with fennel 5% of essential oil (EO) were effective in the growth inhibition of *Aspergillus flavus* (fig. 1a) and *Penicillium* (fig. 1b) sp. at 25°C during 15 days. Papaya (*Carica papaya*) were packaged in paper bag containing antifungal sachets incorporated with lemon grass and cinnamon OE and the filamentous fungi and yeast count in the 6th day (4.2 Log CFU·g^{-1} for both EO treatments) and in the 9th day of storage (4.9 and 4.3 Log CFU·g^{-1}, respectively) were significantly smaller than the control (Espitia, 2009). Active films added with iron nanoparticles (zeolite) were developed by casting method and after 9 days of storage at 25°C and 60% RH,

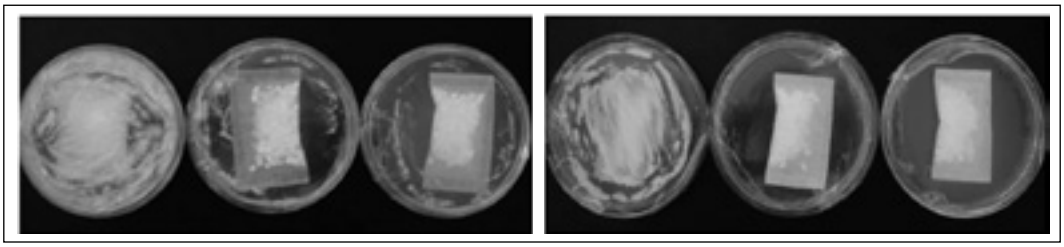


Fig. 1 - Antimicrobial effects of sachet on *Aspergillus flavus* (a) and *Penicillium* sp. (b).

the remaining amount of oxygen in flasks reduced from 20.9% to 13.2, 6.5 and 4.3% when films were used with different superficial area such as 25, 50 and 75 cm^2, respectively, being the average of the oxygen absorption rate 90.74 $mLO_2 \cdot m^{-2} \cdot day^{-1}$ (Botrel, 2008).

These results indicate that active packaging and nanotechnology have a great potential application in the food industry to increase the shelf-life and safety food.

List of units
Microbiology unit: $CFU \cdot g^{-1}$;
Temperature: °C;
Time: day;
Percents (%): w/w;
Area: cm^2;
Gas permeability: $mLO_2 \cdot m^{-2} \cdot day^{-1}$.

ACKNOWLEDGMENTS

For CNPq, FAPEMIG, CAPES and FINEP for financial support.

REFERENCES

Botrel D.A., Soares N.F.F., Geraldine R.M., Pereira R.M., Fontes E.A.F. 2007. Qualidade de alho (*Allium sativum*) minimamente processado envolvido com revestimento comestível antimicrobiano. Ciência e Tecnologia de Alimentos. 27(1): 32.

Botrel D.A. 2008. Desenvolvimento e caracterização de filme ativo adicionado de nanopartículas de ferro e avaliação da sua eficiência na absorção de oxigênio e na inibição do crescimento de fungos em amendoim M. Sc. Dissertation, Viçosa Federal University, Viçosa.

Durango, A.M., Soares N.F.F., Andrade N.J. 2006. Microbiological evaluation of an edible antimicrobial coating on minimally processed carrots. Food Control. 17: 336.

Espitia P.J.P. 2009. Desenvolvimento e avaliação de embalagens ativas incorporadas com óleos essenciais para a conservação pós-colheita de mamão M. Sc. Dissertation, Viçosa Federal University, Viçosa.

Geraldine R.M., Soares N.F.F., Botrel, D.A., Gonçalves L.A. 2008. Characterization and effect of edible coatings on minimally processed garlic quality. Food Control. 72: 403.

Jofré A., Aymerich T., Garriga M. 2008. Assessment of the effectiveness of antimicrobial packaging combined with high pressure to control *Salmonella* sp. in cooked ham. Food Control. 19: 634.

Rodríguez M.S., Ramos V., Agulló E. 2003. Antimicrobial action of chitosan against spoilage organisms in precooked pizza. J. Food. Sci. 68(1): 271.

Santiago-Silva P., Soares N.F.F., Nobrega J., Junior M., Barbosa K., Volp A.C.P., Zerdas E., Wurlitzer N.J. 2009. Antimicrobial efficiency of film incorporated with pediocin (ALTA 2351) on preservation of sliced ham. Food Control. 20: 85.

Silveira M.F.A., Soares N.F.F., Geraldine R.M., Andrade N.J., Goncalves M.P.J. 2007. Antimicrobial efficiency and sorbic acid migration from active films into pastry dough. Pack. Tech. Sc. 20: 287.

Silva W.A., Soares N.F.F., Pires A.C.S., Medeiros E.A.A. 2008. Conservação de maçã minimamente processada através de revestimento comestível incorporado com ácido ascóbico. Presented at V Encontro Nacional Sobre Processamento Mínimo de Frutas e Hortaliças, Lavras, MG, April, 2008.

Soares N.F.F., Santiago-Silva P., Souza V.G.L. 2007. Atividade antimicrobiana "in vitro" de embalagens incorporadas com óleo essencial de funcho (*Foeniculum vulgare* Mill.). presented at 7th Simpósio Latino Americano de Ciência dos Alimentos, Campinas, SP, November.

Sozer N., Kokini J.L. 2009. Nanotechnology and its applications in the food sector. T. Biotech. 27(2): 82.

FUNCTIONALIZATION OF PAPER BASED ACTIVE PACKAGING BY ADSORPTION OF ANTIMICROBIAL PROTEINS

M. ROLLINI[1]*, E. MASCHERONI[1], L. MORA[1], A. MORLEO[2], A. BARBIROLI[2], M. MANZONI[1] and G. CAPRETTI[3]

[1]DiSTAM, University of Milan, Via Celoria 2, 20133 Milano, Italy
[2]DISMA, University of Milan, Via Celoria 2, 20133 Milano, Italy
[3]SSCCP, P.zza Leonardo da Vinci 16, 20133 Milano, Italy
*corresponding author: manuela.rollini@unimi.it

ABSTRACT

Active cellulose-based packaging materials were prepared by binding lactoferrin to paper modified with anionic polyelectrolytes. About 63% of the protein added in the slurry was present in the finished paper, corresponding to about 0.28 mg protein per cm^2 of the glassine paper produced. The efficacy of the activated materials was assessed by microbiological trials, employing targets bacteria considered as spoilage and/or pathogens in food products. Lactoferrin-activated paper evidenced its efficacy against growing cells of *E. coli* and *L. innocua*, with a maximum of 0.3 and 1.3 log cycles reduction of the final population, respectively. These results can be considered a promising starting point and will be the focus of further research, aimed at developing innovative cellulose-based active food packaging.

Key words: active packaging, lactoferrin, natural antimicrobials, paper.

INTRODUCTION

Antimicrobial packaging is defined as "a type of packaging that changes the condition of the packaging to extend shelf-life or improve safety and sensory properties, while maintaining the quality of foods". Antimicrobial or active packaging can control microbial spoilage by reducing growth rate, maximum growth population and/or extending microbial *lag*-phase, functions that do not exist in conventional packaging systems (Appendini and Hotchkiss, 2002).

Paper is a simple but promising biomaterial built up of both oriented macro-

molecules (cellulose) and disoriented polymers (mainly hemicelluloses and lignin) embedded in its fibrous structure. Some of the hemicellulosic constituents carry carboxylic groups, which are potential sites for establishing ionic interaction with antimicrobial positively charged proteins, although this capacity may be increased and/or modulated by incorporating suitable polyelectrolytes in the paper itself (Mascheroni *et al.*, 2010).

The antimicrobial protein selected in this project was lactoferrin, a positively charged protein known for its antimicrobial activity, mostly due to a double mechanism. The protein acts through iron sequestration in sites infection, which deprives the microorganism of this nutrient, thus creating a bacteriostatic effect. Direct interaction of the lactoferrin molecule with the infectious agent also may occur. The positive amminoacids in LF can interact with anionic molecules on some bacterial, viral, fungal and parasite surfaces, causing cell lysis (Gonzales-Chavez *et al.*, 2009).

In previous studies performed with lysozyme, addition of carboxymethylcellulose (CMC) to paper was shown not to impair the structural and functional properties of the antimicrobial protein, and the physical and chemical properties of the paper itself, making it possible to increase and modulate the charge density of paper almost at will.

The aim of this study was to prepare a cellulose-based active packaging material by binding lactoferrin to paper modified with anionic polyelectrolytes. The efficacy of the activated material was also assessed by microbiological trials, employing targets bacteria considered as spoilage and/or pathogens in food products.

MATERIALS AND METHODS

Materials

Paper sheets were prepared according to the international standard ISO 5269-2:2004, as reported elsewhere (Mascheroni *et al.*, 2010). Sodium carboxymethylcellulose (CMC, WALOCEL CRT 60000 PPA07, DS 0.7-0.8) was from Wolff Cellulosic, Schkopau, Germany. Bovine lactoferrin was purchased by Fonterra (New Zealand).

Microorganisms and culture conditions

E. coli DSMZ 50902 (DSMZ: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany), *L. innocua* DSMZ 20649 and *S. aureus* MIM 10 (MIM: Microbiologia Industriale Milano) were employed in the research. Strains were grown on TSA medium (Tryptic Soy Agar, Micropoli, Milan), incubated at 37°C for 24 h, and then stored at 5°C.

Lactoferrin binding and stability

Protein binding and release from paper sheets were monitored through spectrophotometric methods, as reported elsewhere (Mascheroni *et al.*, 2010). The iron binding ability of transferrin was monitored by spectrophotometric titration with ferric ammonium citrate (Carbonaro *et al.*, 2004).

Antimicrobial activity

Assays were carried out in liquid conditions in sterile tubes containing 30 mL of TSB medium (Tryptic Soy Broth), inoculated (1% v/v) with a microbial suspension (OD_{600}: 0.200). In assays performed with free lactoferrin, the protein was added to cultures at a final concentration up to 30 g/L. In assays performed with activated

paper, sheet samples of up to 9 cm^2 were added to the culture. A control sample was also set-up, without paper. Cultures were incubated at 30°C and samples taken at appropriate intervals. Microbial growth (log cfu/mL) was determined employing the plate count technique.

RESULTS AND CONCLUSIONS

Lactoferrin antimicrobial activity

Lactoferrin was found to be active on growing cells of both Gram positive and Gram negative bacteria (fig. 1). The inhibition was almost total for *E. coli*, while 3 and 1.5 log cycles reductions were evident against *L. innocua* and *S. aureus* respectively.

Lactoferrin was added directly into the fibers slurry before the papermaking process and, in spite of the relatively harsh process conditions, about 63% of the protein in the slurry could be solubilized under non-denaturing conditions from the finished paper, corresponding to a loading of about 0.28 mg of each protein per cm^2 of the glassine paper produced (tab. 1).

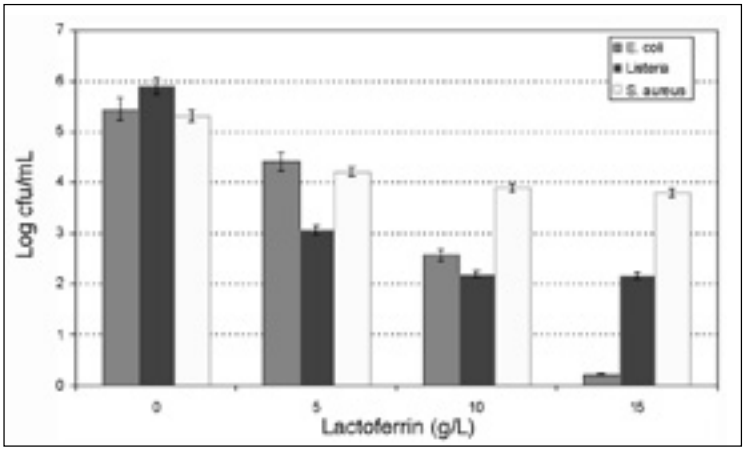


Fig. 1 - Microbial growth (log cfu/mL) of in TSB culture added with lactoferrin.

Protein stability in terms of retention of structural features was tested by measuring tryptophan fluorescence and far-UV circular dichroism, as well as the iron binding capacity of the protein. All of these parameters remained unaltered in the protein released from the paper at high ionic strength. In separate experiments, we found that soluble CMC improved the thermal stability of the high-order structures of lactoferrin.

The efficacy of the active packaging materials was assessed by microbiological

Table 1 - Fiber composition, protein release and recovery of lactoferrin-activated paper.

Short fiber (%)	Long fiber (%)	CMC (%)	Released protein (mg/cm^2)	Recovery (%)
20	80	0	0.080	15.6
80	20	0	0.175	38.9
20	80	10	0.282	62.7
80	20	10	0.241	53.6
50	50	5	0.224	44.9

Table 2 - Final population (log cfu/mL) of cultures grown in presence of lactoferrin-activated paper.

Paper (cm^2)	*L. innocua*	*S. aureus*	*E. coli*
No - control	4.68±0.17	4.56±0.07	4.50±0.03
3	4.02±0.03	4.49±0.04	4.50±0.04
6	3.56±0.09	4.43±0.02	4.47±0.01
9	3.25±0.10	4.29±0.02	4.45±0.10

trials. Lactoferrin-activated paper proved was effective against growing cells, with a maximum of 1.3 log cycles reduction of the final population of *L. innocua* in liquid culture (tab. 2).

This work provides an example of sustainable and active food packaging where an antimicrobial protein could be loaded in a matrix of cellulose fibres and released according to the needs. These results can be considered a promising starting point and will be the focus of further research, aimed at developing innovative cellulosic-based active food packaging.

ACKOWLEDGEMENTS

Work supported in part by funds of the EU project FP7-KBBE-2007-1-NAFISPACK.

REFERENCES

Appendini P., Hotchkiss J. 2002. Review of antimicrobial food packaging. Innov. Food Sci. Emerg. Technol. 3: 113.

Carbonaro M., Iametti S., Mattera M., Bonomi F. 2004. Metal-binding ability of gastrointestinal peptides from *Phaseolus vulgaris*: a spectroscopic study. Proceed. 5th European Conference on Grain Legumes, Dijon (F), pp. 25-26.

Gonzales-Chavez S.A., Arevalo-Gallegos S., Rascon-Cruz Q. 2009. Lactoferrin: structure, functions and applications. Int. J. Antimicrob. Ag. 33: 301.

Mascheroni E., Capretti G., Marengo M., Iametti S., Mora L., Piergiovanni L., Bonomi F. 2010. Modification of cellulose-based packaging materials for enzyme immobilization. Packag. Technol. Sci. 23: 47.

MIGRATION FROM FOOD PACKAGING LAMINATES BASED ON POLYURETHANE

M. AZNAR, E. CANELLAS, C. GASPAR and C. NERÍN

GUIA Group, Department of Analytical Chemistry, I3A,
University of Zaragoza, Spain

ABSTRACT

Polyurethane (PU) adhesives are commonly used in the manufacture of food packaging multilayer materials (laminates). The compounds present in 9 PU adhesives have been identified and a migration test has been carried out to evaluate the migration of the compounds to a food simulant (Tenax). Migration of compounds such as phthalates, triacetin and a residual isocyanate was observed in 3 of the 11 laminates studied. Studies carried out to check the effect of different packaging conditions on migration showed that neither vacuum nor pasteurization had any effect on migration to Tenax.

Key words: polyurethane, adhesives, migration, Tenax.

INTRODUCTION

Multilayer materials based on polyurethane (PU) adhesives are commonly used as food packaging materials. A polyurethane is any polymer consisting of a chain of organic units joined by urethane links, and it is formed through step-growth polymerization by reacting a monomer containing at least two isocyanate functional groups with another monomer containing at least two hydroxyl (alcohol) groups in the presence of a catalyst. PU adhesives can also contain other substances such as surfactants, antioxidants or plastisizers added to improve their properties (Petri *et al.*, 2007). All these compounds could migrate to the food if these PU laminates are used as food contact materials as it has been described for other kind of adhesives (Canellas *et al.*, 2009). The main aim of this work was to identify the compounds present in 9 PU adhesives used in the manufacture of laminates and to study the migration of these compounds to food using Tenax as food simulant. Finally, the

influence of different packaging conditions (vacuum and pasteurization) on the migration to Tenax was determined.

MATERIALS AND METHODS

Samples

Eleven laminates manufactured with 9 different PU adhesives and 6 different substrates were studied. Substrates used were polyamide (PA), polyethylene (PE), polyethylene terephthalate (PET), aluminum (Al), polyethylene-ethylene vinyl alcohol (PE-EVOH) and polyester (Pester). The structure of the laminate was: [substrate 1-PU-substrate 2].

Identification and quantification of the compounds present in the adhesives

For the screening of the samples, 3x0.5 cm cut-outs of laminates were analyzed by SPME-GC-MS and compared with 3x0.5 cm cut-outs of substrates and with the pure adhesive. A PDMS fiber was used for the analysis. The identification of the detected compounds was carried out by comparing the retention time and the mass spectrum of the compounds with those of the pure standards. For the quantification, laminates were extracted with 3 consecutive times with dichloromethane at 40°C (24 hours) and analyzed by GC-MS.

Migration study

Migration study of the compounds identified in the laminates was carried out using Tenax as food simulant. The laminates were thermosealed to manufacture pouches of 5x2 cm. Two replicates of each pouch were filled with 0.4 g of Tenax. Then, these pouches were kept at 40°C for 10 days. Finally, Tenax was extracted with acetone and analysed by GC-MS. Tenax was in contact with the PE side of the laminates.

Influence of the packaging conditions on the migration to Tenax

Migration from laminate 3 [Pester-PU2-PE] was studied under different packaging conditions: standard conditions (SC), vacuum conditions (VC), pasteurization conditions (PC) and vacuum plus pasteurization conditions (VPC). The percentage of relative area related to SC packaging was compared for some of the compounds detected.

RESULTS AND CONCLUSIONS

Identification and quantification of the compounds present in the adhesives

Fig. 1 shows the comparison of the chromatograms obtained for a laminate and the substrates and adhesive that conformed it. This comparison allowed us identifying the compounds coming from the adhesives.

Tab. 1 shows the compounds identified in the 9 PU adhesives. In addition, 7 unknown compounds were also detected. As the results show, some residual isocyanates (2,6-TDI and TODI) still remained in the adhesive after the curing process. A couple of phthalates were also detected, even though it was not possible to elucidate its exact structure. Phthalates are commonly used as plastisizers to provide flexibility and/or elongation to the adhesive. Another

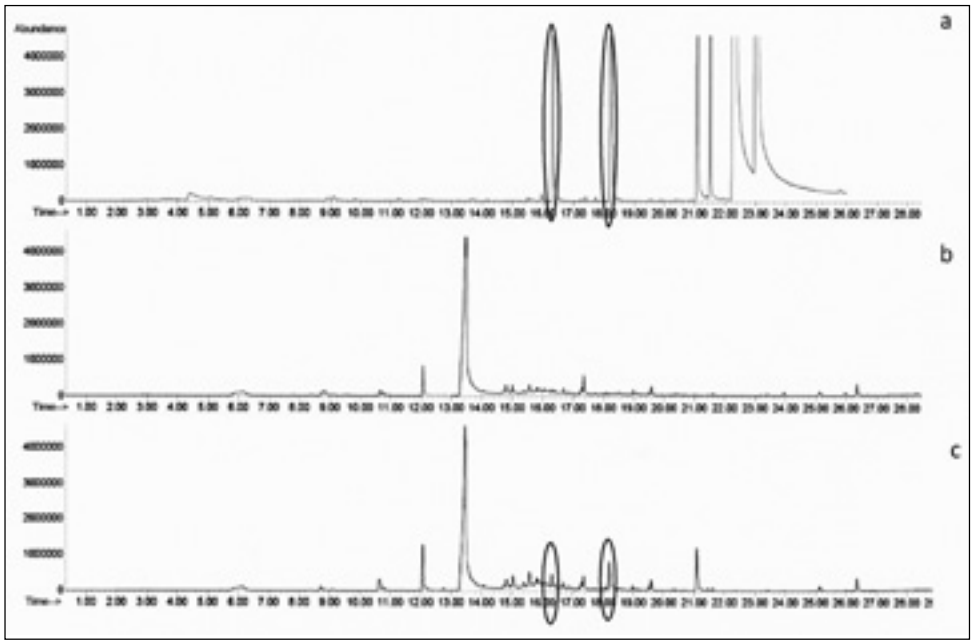


Fig. 1 - Chromatograms of a laminate sample (c), the adhesive (a) and the substrates that conform it (b). Analysis carried out by HS-SPME-GC-MS.

plasticizer, triacetin, was also detected. It was identified BHT, used as antioxidant in all the adhesives. The compound with the highest concentration values was TODI.

Migration study

Tab. 2 shows the migration results. Migration was only found in 3 of the 11 laminates. Nevertheless the existence of migration of compounds such as phthalates, triacetin and 2,6-TDI to Tenax make necessary the control of multilayers materials manufactured with adhesives that are going to be in

Table 1 - Compounds identified in the 9 polyurethane adhesives analyzed.

Compounds (μg/dm² laminate)	PU1	PU2	PU3	PU4	PU5	PU6	PU7	PU8	PU9
Dodecane		0.19							
Triacetin	0.57				3.2				
BHT	0.05	0.11	0.35	0.1	0.26	0.07	0.07	0.13	0.08
Phtalate structure 1		0.46							
Phtalate structure 2		4.4	7.9		23.5	12.4	0.06		
o-Tolidine diisocyanate (TODI)		4.86	34.6		71.4	22.7		28	23.7
2,6-Toluene diisocyanate (2,6-TDI)	<LOQ			9.9	15.3	6.3	14.4	14.1	8.8

LOQ: limit of quantification.

Table 2 - Migration results in Tenax.

Compounds ($\mu g/dm^2$ laminate)	Lam 2 [Polyamide-PU2-PE]	Lam 3 [Polyester-PU2-PE]	Lam 6 [PET- PU5-PE]
Triacetin			2.05
Phtalate sttructure 1	0.15	0.23	
Phtalate sttructure 2	1.54	1.68	4.37
2,6-TDI	2.21	2.49	<LOQ

LOQ: limit of quantification.

contact with food. The experimental migration test carried out at different packaging conditions did not show any difference in migration values among standard conditions and vacuum, pasteurization or vacuum plus pasteurization conditions.

ACKNOWLEDGMENTS

This work has been supported by the European project Migresives. E. Canellas acknowledges her grant to Gobierno de Aragon.

REFERENCES

AOAC 1980. "Official Methods of Analysis" 13th ed. Association of Official Analytical Chemists, Washington, DC.

Bhowmik S.R., Hayakawa K. 1983. Influence of selected thermal processing conditions on steam consumption and on mass average sterilizing values. J. Food Sci. In press.

Canellas E., Aznar M., Nerin C., Mercea P. 2009. Partition and diffusion of volatile compounds from acrylic adhesives used in for food packaging multilayers manufacturing. J. Mater. Chem. DOI:10.1039/C0JM00514B.

Cardello A.V., Maller O. 1982. Acceptability of water, selected beverages and foods as a function of serving temperature. J. Food Sci. 47: 1549.

Petri E.M. 2007. "Handbook of adhesives and sealants" 2nd ed. McGraw-Hill Companies, New York.

Takeguchi C.A. 1982. Regulatory aspects of food irradiation. Paper N. 8, presented at 42nd Annual Meeting of Inst. of Food Technologists, Las Vegas, NV, June 22-25.

Weast R.C. (Ed.). 1981. "Handbook of Chemistry and Physics" 62nd ed. The Chemical Rubber Co. Cleveland, OH.

QUALITY EVALUATION OF SHRIMPS *(PARAPENAEUS LONGIROSTRIS)* AS AFFECTED BY EDIBLE FILMS

C. AVOLA[1], F. LICCIARDELLO[2], A. MAZZAGLIA[1], V. GIANNONE[3], C. ZANTI[1] and G. MURATORE[1]

[1]DOFATA, Università degli Studi di Catania,
Via Santa Sofia 98, 95123 Catania, Italy

[2]DACPA, Università degli Studi di Catania,
Via Santa Sofia 98, 95123 Catania, Italy

[3]P.S.T.S. s.c.pa,
Z.I. Blocco Palma I, Stradale V. Lancia,
95121 Catania, Italy

ABSTRACT

The effect of chitosan blends with different components as coatings for the shelf-life extension of fresh shrimps was evaluated during chilled storage. Blended films were: chitosan solution, chitosan solution added with tapioca starch, cornstarch flour and locust bean gum (LBG). Untreated shrimps were used as control. These coating solutions were characterised by means of pH, viscosity and color measurements. The effects of these edible films on the shelf-life of shrimps were monitored during storage by physical (color and rheological measurements), chemical (pH, total volatile basic nitrogen, TVBN) and microbiological (total bacterial counts) analyses. Sensory analyses were also performed to define the state of freshness of the products. The coating solutions showed similar characteristics, such as pH, viscosity and color, but different effects on the shelf-life of shrimps. Generally the effect of coating solutions on shrimps shelf-life is positive but in necessary to continue the research to improve the results.

Key words: chitosan, coating, edible film, shelf-life, shrimps.

INTRODUCTION

Shrimp is highly perishable and the maintenance of quality depends upon number of factors, including storage time, storage conditions and temperature. Therefore studies on alternative methods to extend the shelf-life, like the use of edible coatings, are needed. Functional edible active coatings may contribute to prolong minimally processed food shelf-life, working as barrier to gases, water vapor, solutes and guaranteeing microbiological safety (Pavlath and Orts, 2009).

The aim of this study was to determine the changes in the quality of shrimps (*Parapenaeus longirostris)* after coating with edible materials.

The effect of chitosan, blends with different components (tapioca starch, cornstarch flour and locust bean gum), as coatings for the shelf-life extension of fresh shrimps was evaluated during chilled storage.

MATERIALS AND METHODS

Chitosan of high molecular weight (Sigma-Aldrich) was dispersed in lactic acid (1% v/v) to prepare solution of proper concentration (0.5% v/v). The other solutions were obtained blending chitosan solution with other coating solutions (Tapioca starch, Cornstarch flour and LBG) in same proportions (50 g of each one) under gently stirring for 20 min (Chillo *et al.*, 2008); final solutions were brought to pH 2.8.

Fresh samples of shrimps were collected from local fishermen in Jonio sea and immediately were transported to the laboratory under refrigeration and dipped into sodium metabisulphite solution (E224) (Omar, 1998). Shelled and headless shrimps were dipped in the test solutions, dried, packaged and stored a 4°C.

The viscosity values of coating solutions (cps) were measured at 25°C with a rotational viscometer (Visco Star R, Selecta, Milan, Italy), using different spindles (R2-R3) and share rates (10-100 rpm).

The color measurements of solutions and samples were performed by colorimeter (NR-3000, Nippon Denshoku Ind. Co. Ltd, Giappone). CIELab parameters (a*, b* and L*) were used to calculate the color ΔE that express the differences of color between the samples, these values were calculated according to the formulae:

$$\Delta E = [(L_f - L_i)^2 + (a_f - a_i)^2 + (b_f - b_i)^2]^{1/2}.$$

For chimical-phisical analyses samples were taken after 3, 6, 9 days of storage, after 1 and 8 days for sensory analyses and 0 and 8 days for microbiological analyses.

The pH value was determinated by a pHmeter, blending 5 g of each sample with 10 ml distilled water.

The total volatile basic nitrogen (TVBN, mg N/100 g fish flesh) was measured using the official method (GUUE n. 338/27, 2005).

The measure of the hardness of shrimps was determinated by penetrometer (TR® 53205 Forlì, Italia) inserting a needle into the sample in a prescribed manner.

For microbiological analyses, shrimps were blended in buffered peptone water (BPW, Oxoid) and homogenized using a stomacher (Bag mixer 500W) for 1 min and serially diluted. Microbial count was effected in duplicate according to the plate

official method (De Medici *et al.,* 1996). Aerobic plate counts were determined by surface spreading homogenate dilutions (0.1 mL) on Plate Count Agar (PCA, Oxoid) and incubated a 30°C for 72 h.

To evaluate the appearance of the flesh and the skin, the odor and the texture of the shrimps, 11 attributes were evaluated, each one receiving a score on a continuous scale of demerit points from 1 to 9, as changes occurred during storage.

RESULTS AND CONCLUSIONS

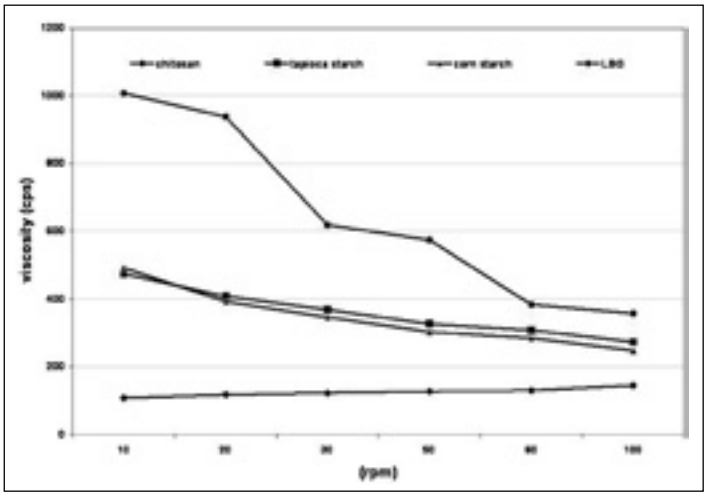


Fig. 1 - Viscosity values (cps) of coating solutions at 25°C versus shear rate.

The tested solutions showed difference in viscosity, in particular the solution of chitosan was characterized by a viscosity lower than the other, while the chitosan-LBG solution viscosity showed greater respect to all solutions (fig. 1).

The values of CIELab parameters, despite the differences in transparency between solutions, once applied on shrimps, had no influence on the samples. ΔE values ranging from a minimum of 6 for the samples treated with chitosan-LBG solution to a maximum of 15 for those treated with chitosan-cornstarch with an upward trend over time with the exception of carob solution.

For all samples pH values showed an upward trend during the period under review but, the pH values remain always below the control.

The development of TVBN concentration (fig. 2) was only contained by the solution of chitosan, despite exceeding the legal limits (30 mg/100 g).

The texture of the shrimp samples treated with the different solutions didn't show significant difference compared to control. The only exception is for samples treated with chitosan-based films that are characterized by higher values of texture at the sixth day of conservation.

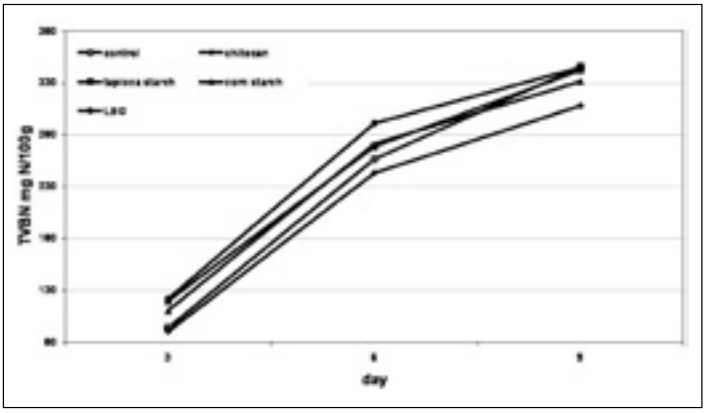


Fig. 2 - Evolution of TVBN during storage.

Sensory evaluation

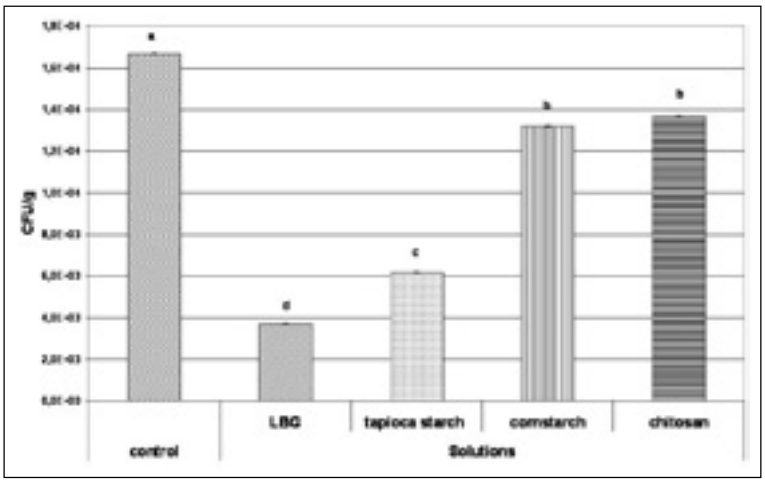


Fig. 3 - Mean values of total aerobic mesophilic bacteria (CFU/g) in samples of shrimp after 8 days of storage ($P<0.01$, Duncan test).

shows that the treated samples show differences compared to untreated samples, particularly on day 1 of storage samples coated with chitosan and chitosan-tapioca presenting a lower intensity of the parameter smell of ammonia, while at day 8 the sample coated with chitosan-cornstarch getting the best overall assessment.

Total mesophylic aerobic count, expressed as CFU g^{-1}, was the average of three repetitions and it was in a range from $3.70x10^3$ CFU/g, samples with chitosan-LBG solution, to $1.66x10^5$ CFU/g, control (fig. 3). The average level of bacterial contamination in the samples was very low. Moreover, the coating solutions used to improve the shelf-life has made no additional bacterial contamination than the untreated product.

This work is a preliminary characterization of the different coating materials to be used for sea food products. Further research is necessary to assess the use of coating solutions formulated with the addition of antioxidants and antimicrobials compounds.

REFERENCES

Chillo S., Flores S., Mastromatteo M., Conte A., Gerschenson L., Del Nobile M.A. 2008. Influence of glycerol and chitosan on tapioca starch-based edible film properties. J. Food Eng. 88: 159.

De Medici D., Fenicia L., Orefice L., Stacchini A. 1996. Metodi di analisi per il controllo microbiologico degli alimenti. Rapporti ISTISAN 96(35): 166.

Gazzetta Ufficiale dell'Unione Europea n. L 338/27 del 22.12.2005. Decisione 2074/2005 della commissione del 5 dicembre 2005 che fissa i valori di ABVT (azoto basico volatile totale) per alcune categorie di prodotti della pesca e i relativi metodi di analisi.

Pavlath A.E., Orts W. 2009. Edible films and coatings: why, what, and how?. Edible films and coatings for food applications, 1-19.

Omar M.I.V. 1998. Utilization of sodium metabisolphite for preservation of frozen-thawed shrimp (*Pandaleus borealis*). Fisheries training programme.

IRON NANOPARTICLES FILM ON *ASPERGILLUS FLAVUS* INHIBITION

DIEGO ALVARENGA BOTREL[1]*, NILDA DE FÁTIMA FERREIRA SOARES[2], REGIANE VICTÓRIA DE BARROS FERNANDES[1] and NATHÁLIA RAMOS DE MELO[3]

[1]Federal University of Viçosa, CRP, Rio Paranaíba-MG, Brazil

[2]Federal University of Viçosa, DTA, Viçosa-MG, Brazil

[3]Fluminese Federal University, VEA, Volta Redonda-RJ, Brazil

*corresponding author: diego.botrel@ufv.br

ABSTRACT

Several treatments are applied to reduce or remove oxygen from packages and in contact with foods, aiming shelf-life extending. Metallic iron has been used as oxygen absorber agent in foods systems. This study aimed evaluates the action of iron nanoparticles cellulosic matrix films on *in vitro* inhibition of *Aspergillus flavus*. Iron nanoparticles show larger contact surface which improve its oxygen absorber action. Plates containing *A. flavus* spores at center were packed inside the following systems: PE/Nylon package without addition of film (C1), PE/Nylon package with addition of pure cellulosic matrix film (C2), PE/Nylon package with addition of iron nanoparticles film (T). The films were put inside the packages. It was measured the diameter variation of the colonies during 13 days. The oxygen concentration reduction inside the packages promoted by iron nanoparticles films showed significant effect ($p<0.05$) on inhibition of colonies growing. To the end of this period, the oxygen concentration inside all the packages reached values under 1% to all treatments, due the consumption by the fungus and the low permeability of the PE/Nylon package used. However, the oxygen level inside the packages submitted to T treatment was reduced more quickly, showed by inhibition on fungus growing. The mean of the colonies diameter values of *A. flavus* was significantly different ($p<0.05$), where C1 and C2 treatments did not differed each other ($p>0.05$), showing the higher values. The diameter means to the end of 13 days were 30, 28, 21 mm to C1, C2 and T, respectively. Use of iron nanoparticles films inside PE/Nylon packages was effective on growth inhibition of *A. flavus* also avoiding the spore formation. This treatment can be used on growth inhibition of *A. flavus* on food packaged and contribute to the food safety.

Key words: active packaging, *Aspergillus flavus*, food safety, oxygen absorber.

INTRODUCTION

Several treatments are applied to reduce or remove oxygen from inside packages containing foods, aiming shelf-life extending. Composites for oxygen absorbing are ferrous compounds, catechol, ascorbic acid and its analogues, oxidatives enzymes as glucose oxidase, unsaturated hydrocarbon and polyamides (Brody *et al.*, 2001). Most of the commercially available oxygen scavengers are based on a sachet containing iron as an oxygen absorber, however, more efficient and safe alternatives on oxygen removal have been researched. Thus, oxygen removing agents can be incorporated into packaging material, such as polymer film (Ahvenainen, 2003). *Aspergillus flavus is a* fungus that contaminates food products and it is responsible by spoilage and mycotoxins production. The production of this toxins occurs in favorable ambient to development of the fungus. Being aerobic organisms, the oxygen removal of packages can ensure food safety and avoid toxins production. New technologies on packaging systems are the most promising advantages that nanotechnology offers to food industry (Garber, 2007). This study aimed evaluate the action of cellulosic matrix films added with iron nanoparticles on *in vitro* growing inhibition *Aspergillus flavus* under temperature of 25°C.

MATERIALS AND METHODS

The methodology applied to zero valent nanoparticles production was proposed by Wang *et al.* (2006). Films were produced by casting system (solvent application), according methodology described by Soares (1998), with some modification.

Aspergillus flavus spores, previously cultivated on 25°C during seven days in potato dextrose agar (PDA), were inoculated in the center of petri dishes containing that same media. Petri dishes were packaged in Polyethylene/Nylon package and submitted to the following treatments: control (without adding film inside the package) (C1); cellulosic matrix film added inside the package (C2); cellulosic matrix films incorporated with iron nanoparticles inside the package (T). The PE/Nylon packages containing the petri dishes submitted to those treatments were incubated on 25°C during 13 days. The colonies diameter was measure over time according methodology described by Northolt *et al.* (1977). Measurements were done in duplicate.

RESULTS

Aspergillus flavus growth was evaluated, *in vitro*, during 13 days of storage on 25°C. Fig. 1 illustrates the colonies diameter increase over the time according to treatments applied.

The oxygen concentration reduction inside the packages promoted by the film added with iron nanoparticles presented significant effect ($p<0.05$) over the colonies growing, slowing the fungus development.

Colonies diameter, after 13 days, reached averages values of 30, 28 and 21.7 mm respectively to control, pure cellulose matrix film and film added with iron nanoparticles. At the end of this period, the oxygen concentration inside the packages

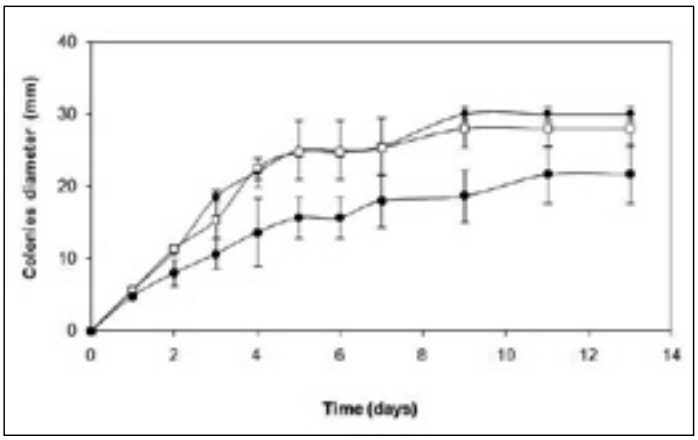


Fig. 1 - Aspergillus flavus colonies diameters values during 13 days of storage on 25°C inside PE/nylon packages, according to the treatments: control (without adding film inside the package) (–◆–); cellulosic matrix film added inside the package (–□–) and cellulosic matrix films incorporated with iron nanoparticles inside the package (–●–).

reached values below 1% to all treatments, probably due fungus consumption and low permeability of the PE/Nylon package used.

It was observed only *Aspergillus flavus* mycelial growth (white colonies) when it was submitted to treatment T (iron nanoparticles films) (fig. 2).

At initial growing stage, *Aspergillus flavus* spores present yellowish green color, as the fungus gets older, the spores become dark green (CIFR, 2005). According to Klich (2007), there is a strong relationship between the spores formation e the aflatoxin production, what can lead to the conclusion that the oxygen absorber films may reduce toxins production, based on slowing the fungus growing, during the 13 days of experiment. Aflatoxin production was not verified in this work.

CONCLUSION

Cellulose matrix films added with iron nanoparticles presented efficient action over *Aspergillus flavus* growing reduction compared to control treatment. It is im-

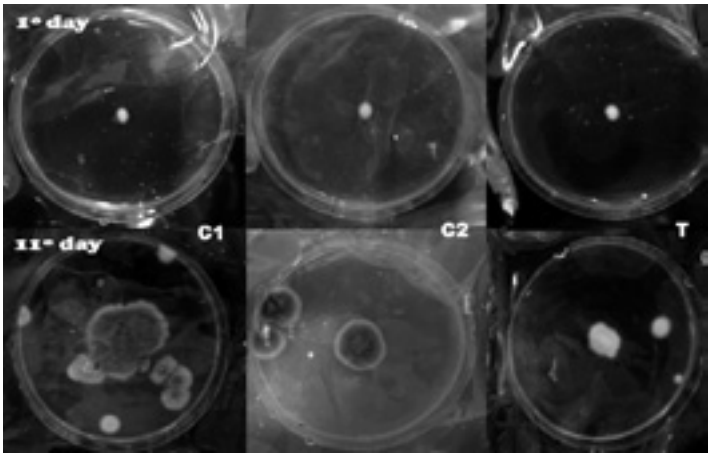


Fig. 2 - *Aspergillus flavus* colonies growth, inoculated in the center of petri dishes, at 1 day and 11 days of storage time on 25°C, when packaged in PE/Nylon bags associated with the following treatments: control (without adding film) (C1), cellulosic matrix film added inside the package (C1) and cellulosic matrix films incorporated with iron nanoparticles inside the package (T).

portant the development of systems that can avoid fungi growth since mycotoxins are great problems related to food. It can be concluded that oxygen absorber films has a potential use as food package to promote food safety.

ACKNOWLEDGEMENTS

CAPES, CNPq, FINEP, FAPEMIG, FAPERJ.

REFERENCES

Ahvenainen R. Novel Food Packaging Techniques. Boca Raton: CRC Press, 2003, 590p.

Brody A.L., Strupinsky E.R., Kline L.R. Active packaging for food applications. Boca Raton: CRC Press LLC. 2001, 224p.

CIFR Center for Integrated Fungal Research. *Aspergillus flavus*, 2005. <http://www.aspergillusflavus.org/aflavus>.

Garber C. Nanotechnology food coming to a fridge near you, Nanowerk LLC, 2007. <http://www.nanowerk.com/spotlight/spotid=1360.php>.

Klich M.A. Environmental and developmental factors influencing aflatoxin production by Aspergillus flavus and *Aspergillus parasiticus*. 2007. Mycoscience. 48: 71.

Northolt M.D., Van Egmond H.P., Paulsch W.E. 1977. Differences between *Aspergillus flavus* strains in growth and aflatoxin B1 production in relation to water activity and temperature. J. Food Protect. 40: 778.

Soares N.F.F. 1998. Bitterness reduction in citrus juice through naringinase immobilized into polymer film. Ph.D. Thesis, Cornell University, New York.

Wang W., Jin Z., Li T., Zhang H., Gao S. 2006. Preparation of spherical iron nanoclusters in ethanol-water solution for nitrate removal. Chemosphere. 65: 1396.

ACTIVE SACHET: DEVELOPMENT AND EVALUATION FOR THE CONSERVATION OF HAWAIIAN PAPAYA QUALITY

PAULA JUDITH PEREZ ESPITIA[1], NILDA DE FÁTIMA FERREIRA SOARES[1]*, LAURA COSTA MOREIRA BOTTI[1], WASHINGTON AZEVEDO DA SILVA[1], NATHÁLIA RAMOS DE MELO[2] and OLINTO LIPARINI PEREIRA[3]

[1]University Federal of Viçosa (UFV), DTA. Viçosa, MG, Brazil
[2]Fluminense Federal University (UFF), VEA. Volta Redonda, RJ, Brazil
[3]University Federal of Viçosa (UFV), DFP. Viçosa, MG, Brazil
*corresponding author: nfsoares@ufv.br

ABSTRACT

The aim of this work was to develop an active sachet incorporated with 20% of cinnamon essential oil (EO), to evaluate the antifungal effect of the developed sachet and to determinate the release percentage of the cinnamon EO during the storage period of the fruit. Active sachets were incorporated with 20% (m/m) of EO in the polymeric resin and put in sacks of nonwoven fabric. Hawaiian papaya fruits in 1/4 ripe maturation stage were sanitized with sodium dichloroisocyanurate solution for 10 min and packaged in paper bags containing in its interior the active sachet. Paper bags containing just papaya fruits in the interior were used as controls treatment. The packaged papayas were put in cardboard box and stored at room temperature for 12 days. The growth of filamentous fungi was analyzed at 0, 3, 6, 9 and 12 days of storage. Parallel to this, paper bags containing a sachet incorporated with active cinnamon EO was put inside of PE/NYLON plastic bags and the active compound released (cinnamaldehyde) in the free space was quantified in GC-MS, at the same period of time. The treatment with 20% of cinnamon EO presented values significantly smaller for the growths of filamentous fungi compared with the control treatment on the 6th and in the 9th day, whereas on the 12th day the growth of the microorganisms was the same in the control and with 20% cinnamon EO treatments. The cinnamaldehyde release was lineal and gradual during the storage. Therefore, the active sachet incorporated with cinnamon EO has potential use in the fruit conservation area, since its gradual release allows the control of filamentous fungi growth in the surface of Hawaiian papaya.

Key words: active packaging, essential oil, fitopathogenic fungi, food conservation.

INTRODUCTION

Research in the food-packaging area has been directed to the development of new packaging materials according to the increase in consumer concern for better food quality, convenience and safety of food products (Chen *et al.*, 2003). New preservation methods such as active packaging are being studied and developed to maintain food quality (Valverde *et al.*, 2005). Active packaging interacts with the food in a desirable way, aiming to improve its features; this area is becoming an emerging food technology that allows preventing microbial growth in the product surface by means of interactions between the food and the packaging materials or interactions with substances released by the packaging into the headspace (Soares *et al.*, 2009). In doing so, active packaging with antimicrobial activity can take several forms, such as polymeric films, that can be edible or not, coatings and sachets. However, the most successful commercial application of antimicrobial packaging has been the sachets that are enclosed loose or attached to the interior of a packaging (Appendini and Hotchkiss, 2002).

Essential oils (EO) are natural extracts of substances that have been used from antiquity as spices for flavoring foods and beverages. Obtained principally from the leafy part of plants, EO have been added to meat, fish and food products for many years and at present are categorized as flavorings and Generally Recognized as Safe (GRAS) (Lopez *et al.*, 2007). Several studies have suggested the use of natural compounds such as EO as possible substitutes for commercial fungicides, indicating their potential application incorporated into packaging material (Zivanovic *et al.*, 2005).

The aim of this work was to develop an active sachet incorporated with 20% of cinnamon EO, to evaluate the antifungal effect of the developed sachet and to determinate the release percentage of the cinnamon EO during the storage period of the fruit.

MATERIALS AND METHODS

System elaboration active packaging: Sachets

The experiment was prepared at the Packaging Laboratory of UFV. Accurel® (a high-absorption polymeric resin) and nonwoven fabric were used for the construction of sachets. Cinnamon (*Cinnamomum zeylanicum*) EO was incorporated in the sachets at concentration of 20% (w/w). The essential oils were supplied by Petit Marie® (SP, Brazil). The sachets of nonwoven fabric had dimensions of 6x7 cm and was hot sealed.

Papaya fruit

The papaya fruit (*Carica papaya* L.) used in the experiments were obtained in the local market with a 25% yellow surface (1/4 ripe). The fruits were treated with a solution of sodium dichloroisocyanurate (Sumaveg®) 200 mg·L-1 (3% active chlorine) for 10 min.

Storage conditions and microbiological analysis

The papaya fruits were packaged in pouches containing an individual sachet incorporating cinnamon EO and in a pouch with no sachet (control treatment). The treatments were stored at 23±1°C, as in typical retail conditions, for 12 days.

The growth of filamentous fungi was analyzed at 0, 3, 6, 9 and 12 days of storage in accordance with Vanderzant and Splittstoeser (1992).

Determination of volatile compound

Pouches containing an individual sachet incorporated with cinnamon EO was put inside of PE/NYLON plastic bags and the active compound liberated (cinnamaldehyde) in the free space of the bag was quantified in GC-MS, at the same period of time as the microbiological analysis (0, 3, 6, 9 and 12 days of storage). The analysis was developed according to Singh et al. (2006). GC-MS (model 17-A, Shimadzu, Japan, 2006), DB-5 column (Agilent Technologies®) with dimensions of 30 m x 250 μm x 0,25 μm and Helium as a carrier gas with a flux of 1 mL·min-1 was used in the experiment. The injector temperature was 250°C and the interface was 200°C, the control of the column temperature was 80°C (1 min), 80°-160°C (4°C·min-1), 160°-250°C (15°C·min-1), splitless (manual injection of 1 μL of the headspace).

Statistical analysis

The processed data were obtained from three different observations taken from three different experiments. Data were subjected to statistical analyses performed with Statistical Analysis System software package v.9.1.

RESULTS AND CONCLUSIONS

The treatment with 20% of cinnamon EO presented values significantly smaller for the growths of filamentous fungi compared with the control treatment on the 6^{th} and in the 9^{th} day, whereas on the 12^{th} day the growth of the microorganisms was the same in the control and with 20% cinnamon EO treatments. The treatments with cinnamon OE presented a reduction of 2.5 log cycles on the 6^{th} day and reductions of 2.2 log cycles, for the 9^{th} day of storage. Also was observed that cinnamaldehyde release was lineal and gradual during the storage, being in the third day of analysis a release of 38.92% in the 6^{th} day and 79.51% in the 9^{th} day of storage.

In conclusion, the active sachet incorporated with cinnamon EO has potential use in the fruit conservation area, due to the gradual liberation of the antifungal substance that allows the control of filamentous fungi growth in Hawaiian papaya and since the EO is safety and natural product.

ACKNOWLEDGMENTS

The authors wish to thank CAPES, CNPq, Fapemig, FINEP, FAPERJ.

REFERENCES

Appendini P., Hotchkiss H. 2002. Review of antimicrobial food packaging. Innov. Food Sci. Emerg. 3: 113.

Chen S.C., Lin C.-A., Fu A.-H., Chuo Y.W. 2003. Inhibition of microbial growth in ready-to-eat food stored at ambient temperature by modified atmosphere packaging. Packag. Technol. Sci. 16(6): 239.

López P. Sánchez C., Batlle R., Nerín C. 2007. Development of flexible antimicrobial films using essential oils as active agents. J. Agr. Food Chem. 55(21): 8814.

Singh G., Maurya S., Lampasona M.P.De., Catalan C. 2006. Chemical constituents, antifungal and antioxidative potential of *Foeniculum vulgare* volatile oil and its acetone extract. Food Control. 17 (9): 745.

Soares N.F.F., Pires A.C.S., Camilloto G.P., Santiago-Silva P., Espitia P.J.P, Silva, W.A. 2009. Recent patents on active packaging for food application. Recent Pat Food, Nutrition & Agriculture. 1(2): 171.

Valverde J.M., Guillén F., Martínez-Romero D., Castillo S., Serrano M., Valero D. 2005. Improvement to table grapes quality and safety by the combination of modified atmosphere packaging (MAP) and eugenol, mentol and thymol. J. Agr. Food Chem. 53(19): 7458.

Vanderzant C., Splittstoeser D.F. 1992. Compendium of methods for the microbiological examination of foods. 3th. Ed. Amercian Public Health Association.

Zivanovic S., Chi S., Draughon A.F. 2005. Antimicrobial activity of chitosan films enriched with essential oils. J. Food Sci. 70(1): 45.

EFFECTS OF DIFFERENT SEALING CONDITIONS ON THE SEAL STRENGTH OF POLYPROPYLENE FILMS COATED WITH A BIO-BASED THIN LAYER

S. FARRIS[1]*, C.A. COZZOLINO[2], L. INTROZZI[1] and L. PIERGIOVANNI[1]

[1]Department of Food Science and Microbiology, Packaging Laboratory, University of Milan, Via Celoria 2, 20133 Milano, Italy

[2]Department of Scienze Ambientali Agrarie e Biotecnologie Agro-Alimentari, University of Sassari, Viale Italia 39/A, 07100 Sassari, Italy

*corresponding author: stefano.farris@unimi.it

ABSTRACT

This paper presents the results of an investigation through the Design of Experiment (DoE) technique on the influence of temperature, dwell time and bar pressure on the heat-seal strength of oriented polypropylene films coated with a gelatin-based thin layer. This approach allowed achieving a thorough understanding of the effect of each independent factor on the two different responses (maximum force and strain energy).

Key words: biomacromolecules, design of experiment (DoE), gelatin, sealing coating.

INTRODUCTION

The obtainment of bio-coatings with similar properties to those of synthetic origin is an important task in order to make them marketable. A bio-layer can provide additional benefits after deposition, for example affording special features such

Table 1 - Factors and their levels for the 2^3 full factorial design.

Variable	Unit	Symbol		Levels		
		Coded	Uncoded	-1	0	+1
Plates temperature	°C	X_1	T	70	90	110
Dwell time	s	X_2	t	0.5	1.0	1.5
Plates pressure	bar	X_3	p	2.5	3.5	4.5

as the adhesive attitude of gelatin, which can be thus exploited to obtain natural sealants for food packaging applications. The aim of this the work was to evaluate the heat seal strength of polypropylene strips coated with a bio-sealant, as a function of the sealing temperature, pressure and dwell time, by means of the DoE technique (Farris *et al.*, 2009).

MATERIALS AND METHODS

Pigskin gelatin powder, acetic acid esters and glycerol were used to prepare the starting water solution. It was then used to coat an oriented polypropylene-OPP as described in literature (Farris *et al.*, 2008).

Seal strength. It was measured through the so-called T-peel test (ASTM F 88-07a) by means of a dynamometer (ASTM F 88-07). The parameters considered were Maximum Force (N) and Strain Energy (Nmm).

Design of Experiment (Tracton, 2006). Three independent variables were considered within a 2^3 full factorial design: temperature (°C), dwell time (s) and bar pressure (bar), (X_1, X_2 e X_3 respectively) were set at two coded levels (-1 and +1) as reported in tab. 1. Two dependent variables were considered as responses: maximum force (Y_1) and strain energy (Y_2). The final worksheet of the developed design is reported in tab. 1.

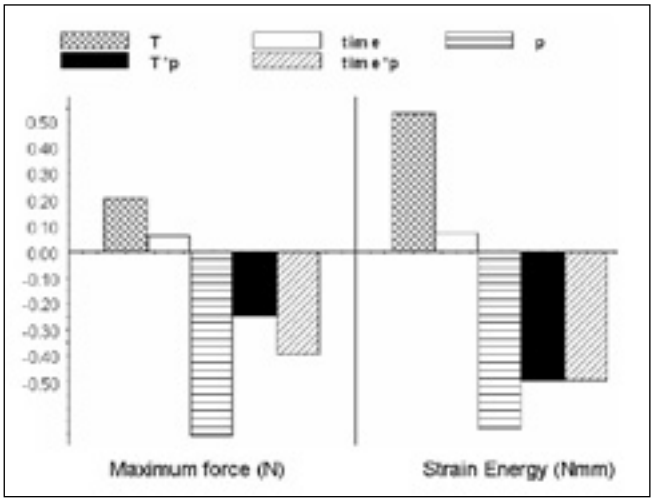


Fig 1 - Coefficient overview plot.

RESULTS AND CONCLUSIONS

The best semi-empirical model was obtained for the strain energy response in term of fitting capability (R^2 = 0.932) and prediction ability (Q^2 = 0.920).

Fig. 1 shows that both responses were inversely influenced especially by

Table 2 - Worksheet of the 2^3 full factorial design.

		Variable levels			Responses	
Exp. N.	Run order	X_1	X_2	X_3	Y_1 [a]	Y_2 [b]
1	13	70	0.5	2.5	0.4075	7.7466
2	8	110	0.5	2.5	0.5534	15.1154
3	14	70	1.5	2.5	0.5423	11.3884
4	29	110	1.5	2.5	0.6513	19.6056
5	12	70	0.5	4.5	0.4448	9.3578
6	23	110	0.5	4.5	0.3639	9.9506
7	26	70	1.5	4.5	0.3325	6.9899
8	21	110	1.5	4.5	0.2637	6.1143
9	27	90	1	3.5	0.3309	9.3118
10	28	90	1	3.5	0.3934	10.2752
11	5	90	1	3.5	0.3938	9.7227
12	1	70	0.5	2.5	0.4653	7.5287
13	10	110	0.5	2.5	0.563	15.8183
14	11	70	1.5	2.5	0.6615	12.8105
15	22	110	1.5	2.5	0.5973	16.2851
16	15	70	0.5	4.5	0.4248	10.1362
17	24	110	0.5	4.5	0.3454	9.7927
18	9	70	1.5	4.5	0.3066	6.4393
19	25	110	1.5	4.5	0.4637	8.4886
20	16	90	1	3.5	0.4005	9.5027
21	3	90	1	3.5	0.5954	10.4872
22	2	90	1	3.5	0.6147	9.7227
23	17	70	0.5	2.5	0.3195	6.6536
24	30	110	0.5	2.5	0.575	13.9764
25	32	70	1.5	2.5	0.489	11.647
26	4	110	1.5	2.5	0.5388	17.5018
27	20	70	0.5	4.5	0.4603	10.0543
28	7	110	0.5	4.5	0.4362	10.1353
29	33	70	1.5	4.5	0.3136	7.3019
30	19	110	1.5	4.5	0.3529	7.2927
31	31	90	1	3.5	0.3717	9.4005
32	18	90	1	3.5	0.4661	11.736
33	6	90	1	3.5	0.5341	11.237

[a] Maximum force (N). [b] Strain energy (Nmm).

the main effect *'bar pressure'* and the 'interaction factor' *time*pressure*. *'Temperature'* and '*temperature*pressure'* were significant only for the strain energy response.

The contour plots of fig. 2 allow an intuitive interpretation of the relationship between factors and responses. The obtained results show that 'bar pressure' is of primary importance in controlling the sealing performance rather than 'temperature' and 'dwell time', oppositely to plastic films. This finding can be explained in terms of "squeezing effect": as the bar pressure is increased, the bio-coating comes

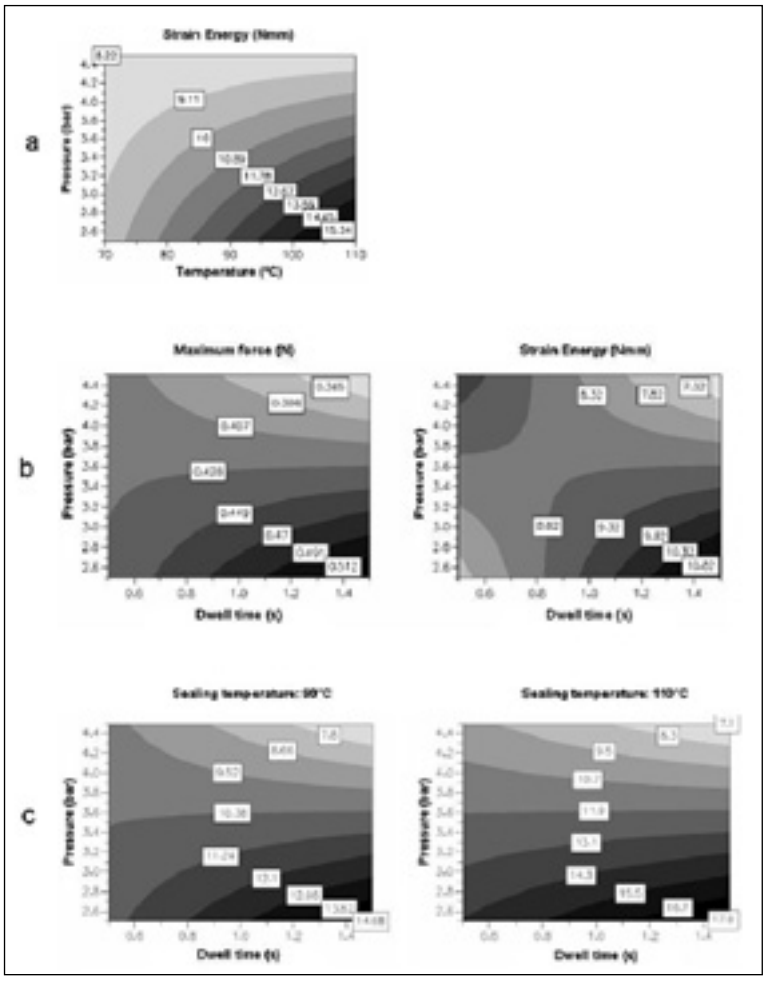


Fig 2 - Representative response contour plots:
(a) *'temperature*pressure';* response: strain energy. Time: 1 s.
(b) '*time*pressure*'; responses: maximum force (left) and strain energy (right). Temperature: 70°C.
(c) *'time*pressure'*; response: strain energy. Temperature: 90°C (left) and 110°C (right).

out from the sealing contact area. This effect is further magnified by an increase in temperature, which plays a detrimental effect on the ultimate sealing properties of the bio-based coating.

On the basis of the results, it can be concluded that a new class of coatings with unique features may be generated from macromolecules of natural origin.

REFERENCES

[1] ASTM. Standard test method for seal strength of flexible barrier materials. Designation F 88-07. American Society for Testing and Materials. DOI: 10.1520/F0088-07A.

[2] Farris S., Cozzolino C.A., Introzzi L., Piergiovanni L. Effects of different sealing conditions on the seal strength of polypropylene films coated with a bio-based thin layer. Packag. Technol. Sci. 22: 359-369. DOI: 10.1002/pts.861.

[3] Farris S., Piergiovanni L., Ronchi G., Rocca R. Edible matrices and relevant applications and preparation method. WO 2008/075396.

[4] Tracton AA. 2006. "Coatings Technology Handbook". CRC Press: Boca Ranton, FL.

IMPROVEMENT OF CO_2 RETENTION OF PET BOTTLES FOR CARBONATED SOFT DRINKS

F. LICCIARDELLO[1], C. CORIOLANI[2], and G. MURATORE[3]

[1]DACPA, Section of Agrochemical Science, Faculty of Agricultural Science, University of Catania, Via S. Sofia 98, 95123 Catania, Italy

[2]Sibeg s.r.l., Z.I. V. Strada 28, 95121 Catania, Italy

[3]DOFATA, Section of Food Technologies, Faculty of Agricultural Science, University of Catania, Via S. Sofia 98, 95123 Catania, Italy

ABSTRACT

One of the major aims pursued by the carbonated soft drinks industry is to extend the shelf-life of PET-packaged products, in order to guarantee the consumers with the original characteristics and quality. Most of the responsibility in the shelf-life extension of such products has to be attributed to the bottle material performances, such as the barrier properties to gases with special regards for the ability to maintain the internal CO_2 through the shelf-life. Tests on carbonated soft drinks bottles are performed at every change (design, volume, etc.) of the package, in order to verify the performances of the new bottle with special regards for the CO_2 retention properties.

The research aims at the evaluation of new bottles addressed to the packaging of a sugar free and a caffeine free carbonated soft drink (Coca-Cola Light e Coca-Cola Caffeine Free), recently launched on the Italian market. The start of commercialisation for these new products is the result of a project named "Silver and Gold", which takes the name from the new colours of the new bottles. Such bottles are produced using the same PET resin as the standard ones, with the only exception of master batch dyes used in the test materials. The CO_2 retention performances of Silver and Gold bottles were investigated in comparison with the standard bottle used as a control. Results showed that CO_2 retention was improved in the Silver and Gold bottles, which retained averagely 88% of the initial CO_2 level after 14 weeks of storage, approximately 10% more than the standard bottles.

Key words: carbonated soft drinks, CO_2 retention, PET additives, PET bottles, shelf-life.

INTRODUCTION

One of the major aims pursued by the carbonated soft drinks industry is to extend the shelf-life of PET-packaged products, in order to guarantee the consumers with the original characteristics and quality. The shelf-life of soft drinks is determined by the CO_2 level, which decreases due to permeation through the packaging material. Therefore, most of the responsibility in the shelf-life extension of such products has to be attributed to the bottle material performances, such as the ability to maintain the internal CO_2 through the shelf-life, that is, in other words, its barrier property to CO_2. Standard shelf-life of "The Coca Cola Company" products is fixed, as a function of the bottle size, to: 6 months: for PET bottles >1 litre; 5 months: for PET bottles <1 litre; 12 months: for glass bottles.

Sibeg s.r.l. follows specific guidelines, recommended by "The Coca-Cola Company" (Packaging Authorization for Non-Refillable Plastic Bottles), containing requirements, tools, specification, and test procedures needed to authorize a new package.

Apart from the controls routinely performed on the preforms (incoming inspection), tests on bottles addressed to the packaging of carbonated soft drinks are performed at every change (design, volume, etc.), in order to verify the correspondence of the bottle performances to standard specifications, with special regards for the CO_2 retention properties. Normally, the verification of gas retention properties is carried out in cases of substitution of the preform/bottle with a lighter one or in cases of change of supplier or resin (Coriolani *et al.*, 2006).

The research aimed at assessing the CO_2 retention performances of new bottles addressed to the packaging of Coca Cola Light and Coca Cola caffeine-free. These products in the new package were launched on the market as a result of marketing project named "Silver and Gold", which takes the name from the new colours of the bottles. The study took into account the role of the colour of the bottle on consumers' perception and, in parallel, the eventual effect of such colorants on bottle performances.

MATERIALS AND METHODS

Preforms used for Silver and Gold bottles are made with the same resin as Clear ones, with the only addition of masterbatch dyes. The test was performed on 1,5 L PET bottles obtained from Clear, Silver and Gold preforms, respectively. Samples were bottled and stored in the same conditions (product volume, temperature, gas volume, screw top torque strength), and the values of the Clear bottles were taken as reference, as this type of bottle is the standard one used for Coca Cola.

The CO_2 retention test protocol implied filling the bottles with 4,32 gas volumes (GV), corresponding to approximately 8,6 g/L (1 GV = 1.98 g/L). Samples were stored at 22°±1°C at 50% relative humidity. Twelve bottles were randomly chosen 24 hours after bottling, and the CO_2 content was evaluated on each bottle by means of a Zahm and Nagel piercing device with pressure gauge, collocated on the cap of the bottles. The cap was pierced by the device; the valve was opened in order to discharge the headspace pressure, and the instrument was calibrated to zero. Bottles were then put in a sonicator until stabilization of the manometer. The test was repeated after 2, 4, 6, 9, 12 and 14 weeks.

RESULTS AND DISCUSSION

A typical CO_2 loss for a 2 l bottle with an initial carbonation of 4 GV, ranges to about 0.3 GV after 3-4 days, due to absorption by the PET and to an increase of the volume of the bottle (around 2.5%), then the CO_2 loss rate slows down to 0.04 GV/week. According to guidelines, such level should not fall below 3.3 GV within established storage periods under the above-mentioned standard testing conditions. Storage periods are referred to as the "standard shelf-life" of the bottles and depend on bottle size. For bottles smaller than 1 liter, storage time is 12 weeks, for 1 liter bottles and larger it is set to 14 weeks. These periods correspond to the usual retailer procurement times.

After 14 weeks the Clear bottles showed a loss of 0.92 GV, with a final value of 3.40. The variation for Silver bottles, on the other hand, amounted to 0.52 GV, reaching 3.80 at the end of the test. Similarly, the variation for the Gold bottles ranged to 0.53, as a consequence the final value registered was 3.79 GV (fig. 1).

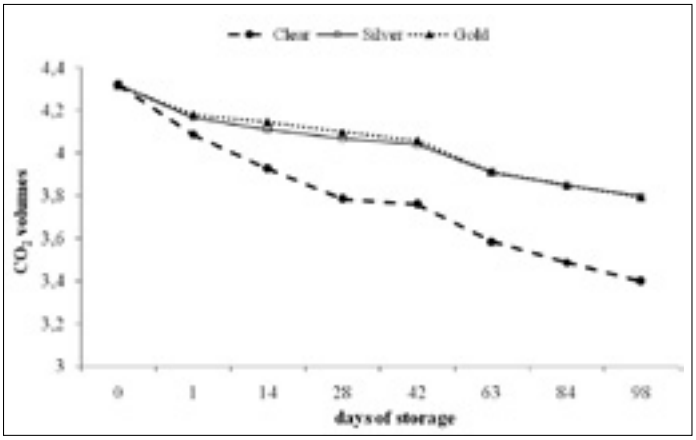


Fig. 1 - CO_2 (gas volumes) decay in Clear, Silver and Gold PET bottles as a function of storage time.

Unexpectedly, Silver and Gold bottles showed improved performances with respect to the reference Clear ones. The improved barrier to CO_2 allowed Silver and Gold samples to retain 88% of the initial gas level, while the standard bottle could provide a retention of 78.7%. It has to be underlined that in any case the Clear bottles perfectly fulfil the requirements; however, a sensible improvement of performances has been achieved.

Bearing in mind that all bottles were produced from the same resin, with the addition of masterbatch dyes in the Silver and Gold samples, the result has to be attributed to the effect of such dyes which, together with an aesthetic function, perform a technological role by interfering with the microstructure of PET and reducing the available spaces for CO_2 to permeate (pores). Such effect resulted in the retention of 10% more gas compared with the PET standard bottles.

To sum up, the use of masterbatch dyes for the production of Silver and Gold bottles not only represents a marketing tool for attracting consumers and contributing to the identity of Coca-Cola light and caffein free, respectively, but also bears the important side effect of prolonging the shelf-life of the beverage by improving the bottle CO_2 retention.

REFERENCES

Coriolani C., Ponzo A., Rizzo V., Licciardello F., Muratore G. 2006. Dependence of the shelf-life of carbonated soft drinks from PET performances as required by "The Coca Cola Company". Special Issue of Italian Journal of Food Science, Proceedings of SLIM 2006, Catania, Italy.

Packaging Authorization for Non-Refillable Plastic Bottles. The Coca Cola Company, 20-Dec-2004.

EFFECT OF UV LIGHT POLYMERIZATION ON THE POLYDIACETYLENE PHOSPHOLIPID NANOVESICLES STRUCTURE

C.P. OLIVEIRA, N.F.F. SOARES*, A.V. TEIXEIRA, T.V. OLIVEIRA and G.W.R. ALMEIDA

Universidade Federal de Viçosa,
Departamento de Tecnologia de Alimentos, Av. P. H. Rolfs s/n,
Campus UFV, CEP: 36570-000 Viçosa, MG, Brasil

*correspong author: nfsoares@ufv.br

ABSTRACT

Conjugated polydiacetylene (PDA) nanovesicles can be used as label-free biosensors. Diacetylene lipids, such as 10-12-pentacosadiynoic acid (PCDA), can undergo polymerization via a 1,4-addition reaction upon UV light to form ene-yne alternating polymer chains, producing liposome – like nanovesicles. The nanovesicles show a bichromic property from blue to red upon external stimuli, such as heat, pH, and mechanical stresses, providing a cheap and convenient sensor. The aim of this work was to investigate the effect of UV light polymerization on the size of PDA/phospholipid nanovesicles. These nanovesicles were prepared by separated dissolution of PCDA and dimyristoylphosphatidylcholine (DMPC) in chloroform at a concentration of 1 mM and mixing in appropriate molar fractions to 10 mL. The chloroform was dryed using N_2 and 10 mL volume of Mille-Q water was added. The suspension was heated at 60°C, sonicated for 1 hour and then filtered through a 0.25 μm PVDF filter. The filtrate was cooled at 4°C overnight. The vesicles were polymerized by exposure to 254 nm UV light for 15 min. The structures were characterized using dynamic light scattering technique where all the intensity correlation functions of dilute solutions were fitted using cumulant analysis. For the samples before the photo-polymerization it was found that the relaxation rate follows a power law regime with the scattering vector with an exponent equals to 2.44. After the polymerization the usual diffusive exponent = 2 was observed. In this last case it was found that the vesicles have a hydrodynamic diameter equals to 260±20 nm. Effects of the exchange of lipid molecules between different vesicles and membrane fluctuations may explain the anomalous behavior observed before polymerization. It is expected that both effects disappear after the polymerization which is coherent to the observed changing in the power law exponent.

Key words: vesicles, biosensor, dynamic light scattering, intelligent packaging.

INTRODUCTION

Self-assembled polydiacetylene (PDA) supramolecules in the form of nanovesicles are attractive owing to their functionalizability and applicability to colorimetric detection systems. In the case of polydiacetylene vesicle formation, the lipids must hydrate and also organize into the correct packing and orientation to undergo the 1,4-addition to the conjugated polymer (Okada *et al.*, 1998). Under external perturbation in the polydiacetylene vesicle, the conjugated PDA backbone can undergo a dramatic reversible or irreversible color change, depending on the chemical structure of PDA (Ahn *et al.*, 2003; Kew and Hall, 2006; Lee *et al.*, 2007; Lee and Kim, 2007). Once PDA vesicles are assembled in solution, it is possible to utilize their colorimetric response to environmental changes to detect important biological recognition events (Reichert *et al.*, 1995; Pan and Charych, 1997; Ma *et al.*, 1998). The colorimetric assay design is straightforward and adaptable for many diverse situations providing a cheap and convenient sensor (Ahn and Kim, 2007).

Independent verification of vesicle formation in a nondestructive manner can be achieved using dynamic light scattering (DLS) techniques. The simplest method for vesicles is to use a particle sizing apparatus, and assume that a uniform Gaussian distribution of diameters exists (Okada *et al.*, 1998). The objective of this work was to investigate the effect of UV light polymerization on the size of PDA/phospholipid nanovesicles using DLS.

MATERIALS AND METHODS

These nanovesicles were prepared by separated dissolution of PCDA and DMPC in chloroform at a concentration of 1 mM and mixing in appropriate molar fractions to 10 mL. The chloroform was dryed using N_2 and a 10 mL volume of Mille-Q water was added. The suspension was heated to 60°C, sonicated for 1 hour and filtered through a 0.25 μm PVDF filter. The filtrate was cooled at 4°C overnight. The vesicles were polymerized by exposure to 254 nm UV light for 15 min. The structures (before and after polymerization) were characterized using dynamic light scattering technique. The intensity correlation functions were obtained using a TurboCorr correlator (Brookhaven Co.) changing the scattering angle from 30° to 120°. All the intensity correlation functions were fitted using cumulant analysis. To avoid concentration effects the solutions were diluted until no important variations in the hydrodynamic diameter were observed.

RESULTS AND CONCLUSIONS

For the samples before the photo-polymerization it was found that the relaxation rate follows a power law regime (Γ) with the scattering vector (q) with an exponent equals to 2.44. After the polymerization the usual diffusive regime $\Gamma \sim q^2$ was observed (fig. 1). In this last case it was found that the vesicles have a hydrodynamic diameter equals to 260±20 nm. Effects of the exchange of lipid molecules between different vesicles and membrane fluctuations may explain the anomalous behavior observed before polymerization. It is expected that both effects disappear after the polymerization which is coherent to the observed changing in the power law exponent.

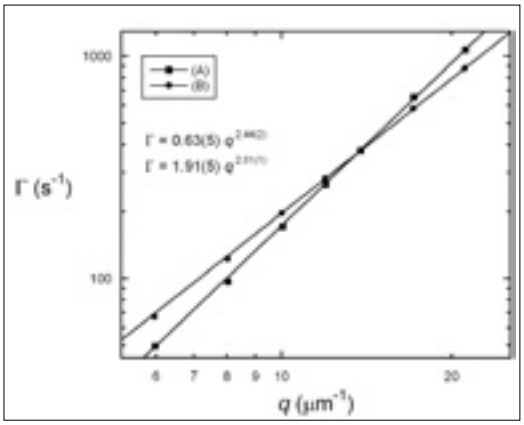


Fig. 1 - Relaxation rate (Γ) versus scattering vector (*q*) before polymerization (A) and after polymerization (B).

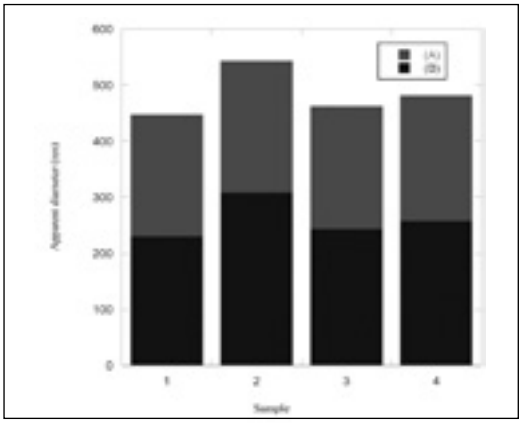


Fig. 2 - Vesicles size before polymerization (A) and after polymerization (B) for four different samples prepared at the same conditions.

Analyzing the apparent hydrodynamic diameter for the samples before polymerization at the smallest scattering vector it was observed that polydiacetylene vesicles decreased their size after polymerization (fig. 2).

The increased stability of the polymerized vesicles is advantageous for biosensor applications of the conjugated PDA nanosized vesicles as label-free biosensors in intelligent food packaging science.

ACKNOWLEDGEMENTS

The authors thank CAPES, CNPq and FAPEMIG for the financial support.

REFERENCES

Ahn D.J., Chae E.-H., Lee G.S., Shim H.-Y., Chang T-E., Ahn K.-D., Kim J.-M., 2003. Colorimetric Reversibility of Polydiacetylene Supramolecules Having Enhanced Hydrogen-Bonding under Thermal and pH Stimuli. J. Am. Chem. Soc. 30: 8976.

Ahn D.J., Kim J.-M., 2008. Fluorogenic Polydiacetylene Supramolecules: Immobilization, Micropatterning, and Application to Label-Free Chemosensors. Accounts of Chemical Research. 41: 805.

Kew S.J., Hall E.A.H., 2006. pH Response of Carboxy-Terminated Colorimetric Polydiacetylene Vesicles. Anal. Chem. 78: 2231.

Lee S.S., Chae E.H., Ahn D.J., Ahn K.H., Yeo J.-K., 2007. Shear-induced color transition of PDA (polydiacetylene) liposome in polymeric solutions. Korea-Australia Rheology Journal. 19: 43.

Lee S., Kim J.-M., 2007. r-Cyclodextrin: A Molecule for Testing Colorimetric Reversibility of Polydiacetylene Supramolecules Macromolecules. 40: 9201.

Ma Z., Li J., Liu M., Cao J., Zou Z., Tu J., Jiang L., 1998. Colorimetric detection of *Escherichia coli* by polydiacetylene vesicles functionalized with glycolipid. J. Am. Chem. Soc. 120: 12678.

Okada S., Peng S., Spevak W., Charych D., 1998. Color and Chromism of Polydiacetylene Vesicles. Acc. Chem. Res. 31: 229.

Pan J.J., Charych D. 1997. Molecular recognition and colorimetric detection of cholera toxin by poly(diacetylene) liposomes incorporating Gm1 Ganglioside. Langmuir 1997, 13, 1365-1367.

Reichert A., Nagy J.O., Spevak W., Charych D., 1995. Polydiacetylene liposomes functionalized with sialic acid bind and colorimetrically detect influenza virus. J. Am. Chem. Soc. 117: 829.

ANTIBACTERIAL COATED PAPER REINFORCED WITH NANOCLAY

NILDA DE FÁTIMA FERREIRA SOARES[1]*, FRANCYS KLEY VIEIRA MOREIRA[2], TATIANA LOPES FIALHO[1] and NATHÁLIA RAMOS DE MELO[3]

[1]University Federal of Viçosa, LABEM, Avenida PH Rolfs, Viçosa, MG, Brazil

[2]University Federal of Sergipe, NEA, São Cristóvão, SE, Brazil

[3]Fluminense Federal University, DEA, Volta Redonda, RJ, Brazil
*corresponding author: nfsoares@ufv.br

ABSTRACT

In this study antibacterial coated paper packaging with improved properties were prepared from incorporation of nanoclay montmorillonite and antimicrobial triclosan into coatings. The materials were characterized by mechanical and antimicrobial tests, water barrier and thermal analysis. It was demonstrated that use of nanoclay increases significantly the tensile strength of coated papers, besides increase the thermal stability of them. All triclosan-containing papers presented inhibitory effect against *Staphylococcus aureus* and *Escherichia coli*. In addition, all coated papers presented superior water barrier due to coating process. The new nanocomposite developed shows great potential for antimicrobial packaging applications.

Key words: antimicrobial packaging, coating, nanoclay, paper, triclosan.

INTRODUCTION

Active packaging are systems that interact with the food and/or environment to extend the self life or to improve sensory and nutritional properties of product, keeping so its quality (Soares, 2008). These new packaging are based in active compounds such as antimicrobial triclosan (TC), which can be incorporated into conventional packaging in order to maximize the preservation of foods. The paper

is a traditional material used as package to several foods, which is usually coated with hydrophobic substances to improve the water-resistance and to increase the shelf-life of the packaged products. The coatings have been explored as inclusion matrices to develop active packaging by addition of antimicrobials to the formulation before coating, thus creating an active layer onto paper surface (Rodríguez *et al.*, 2007). However, it is possible also to incorporate other substances into coatings such as nanoclay organo-montmorillonite (OMMT) to improve the mechanical of the coating-based antimicrobial paper packages.

The aim of this study was to develop antibacterial coated paper packaging with improved properties from incorporation of TC and OMMT into coating formulations.

MATERIALS AND METHODS

Preparation of Coated Papers

Coatings formulations were prepared from a hydrophobic emulsion according to methodology of Soares (2003). Both TC-based compound (Microban®, Microban Products Company, Huntersville, NC, USA) and nanoclay OMMT (Cloisite® 10A, Southern Clay Products, Inc.) were added to the emulsion at concentration of 1wt% (w/w of resin) and the mixtures were homogenized at 10,000 RPM for 5 min. Coating formulations were spread twice over both sides of Kraft paper sheets (grammage of 75 $g.m^{-2}$). For evaporation of solvent, the samples were dried at 45°C for 2h. The treatments evaluated were: paper without coating (PA), coated paper (CP), coated paper containing TC (CP-TC) and coated paper containing TC and OMMT (CP-TCM1).

Characterizations

The antimicrobial efficiency of the paper samples was evaluated against *Escherichia coli* (ATCC11229) and *Staphylococcus aureus* (ATCC6538) according to so-called agar diffusion test. In addition, the samples were characterized by tensile tests (specimens with dimensions of 4x12 cm, speed of 50 $mm.min^{-1}$) using a universal testing machine Instron 3367 with a 1kN load cell and water vapour transmission rate (WVTR) determinations following the standard ASTM E96-95. Surface morphologies were investigated by Scanning Electron Microscopy (SEM) in a Philips XL 30 TMP microscope, operating with a beam of 8kV. Temperature of initial degradation (T_i) and first peak temperature (T_{max1}) of the papers were determined by thermogravimetric analysis in a SDT Q600 equipment TA instruments, using alumina panel, heating rate of $10°C.min^{-1}$ and atmosphere of synthetic air with flow rate of 100 $mL.min^{-1}$. The data were submitted to the analysis of variance and differences among average values were considered to be significant at $p<0.05$ using Tukey's test.

RESULTS AND CONCLUSION

In the results of antimicrobial tests *in vitro*, fig. 1, was observed around of TC-containing papers the formation of an inhibitory zone for the test with *S. aureus* and a zone of reduction in colony density for *E. coli*. These results confirm that TC incorporated into coating can migrate efficiently from microstructure of coated paper out to the culture medium and inhibits the growth of bacteria.

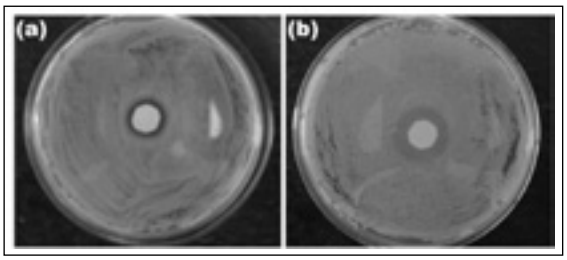


Fig. 1 - Illustration of the antimicrobial efficiency for paper sample CP-TC (TC at concentration 1wt%) against (a) *Staphylococcus aureus* and (b) *Escherichia coli*.

The results of the characterizations are summarized in tab. 1. A comparison between the values for PA and CP reveals that tensile strength (TS) and T_{max1} were increased after coating process. These properties were keeping after incorporation of antimicrobial TC into coating, as evidenced in the values of CP-TC, but were affected by incorporation of nanoclay OMMT. TS property of CP-TCM1 was higher than CP-TC, 59 and 67 MPa, respectively, which represents a significant increase of 30% in this property in comparison to PA ($p<0.05$). The thermal stability of CP-TCM1 also was slightly higher than CP-TC as evidenced in the shift of T_{max1} from 325° to 330°C.

The WVTR values of all coated papers (CP, CP-TC and CP-TCM1) were significantly lowers, around of 40%, when compared to non-coated paper PA ($p<0.05$). In agreement with this result, the SEM micrographs, fig. 2, showed a layer of hydrophobic emulsion onto coated papers that efficiently filled the superficial voids of the materials. The diffusion of water vapour through paper is impaired in this filled structure, which explains the decreases in WVTR values (Laratonda *et al.*, 2005).

The results of this study demonstrate that mechanical and thermal properties of coated paper packaging can be improved by incorporation of nanoclay OMMT at low concentrations into coating. This new nanocomposite developed shows great potential for antimicrobial packaging applications.

Table 1 - Properties of paper-based materials.

Sample	TS (MPa)*	WVTR ($gH_2O.m^{-2}.d^{-1}$)**	T_i(ºC)***	T_{max1}(ºC)***
PA (Control)	$51,2^{c}$	364^{b}	238	315
CP	$57,8^{b,c}$	230^{a}	238	325
CP-TC	$59,1^{b,c}$	189^{a}	238	325
CP-TCM1	$66,7^{a,b}$	192^{a}	238	330

* Values are means of five measurements, ** Values are means of three measurements, *** Typically, one sample was tested. Means in the same column bearing the same letter are not significantly different ($p>0,05$) according to Tukey's test.

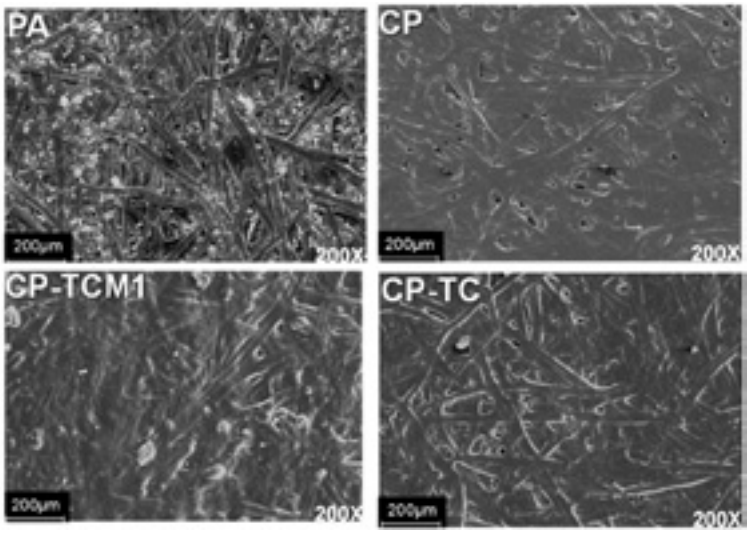


Fig. 2 - Scanning Electrons Microscopy (SEM) micrographs for paper samples PA, CP, CP-TC and CP-TCM1.

ACKNOWLEDGMENTS

The authors thank to CAPES, CNPq, FAPEMIG and FAPERJ for financial supports.

REFERENCES

Larotonda F.D.S., Matsui K.N., Sobral P.J.A., Laurindo J.B., 2005, Hygroscopicity and water vapor permeability of Kraft paper impregnated with starch acetate. J. Food Eng., 71, 402.

Rodríguez A., Batlle R., Nerín C., 2007. The use of natural essential oils as antimicrobial solutions in paper packaging. Part II, Prog. Org. Coat., 60, 33-38.

Soares N.F.F., 1998. Bitterness reduction in citrus juice through naringinase immobilized into polymer film, PhD Thesis, Cornell University, New York.

Soares N.F.F., 2003. Filmes antimicrobianos para produtos alimentícios, PI0305657-0.

EXTENSION OF STRAWBERRY SHELF-LIFE BY IRRADIATED EDIBLE COATING

M.P. JUNQUEIRA-GONÇALVES[1], A. TAPIA[1], C. RODRIGUEZ[1], F.I. ROSCHZTTARDTZ[2], X. VALENZUELA[1] and P. AGUIRRE[3]

[1]Universidad de Santiago de Chile (USACH), Facultad Tecnológica, Dpto. de Ciencia y Tecnología de los Alimentos, Santiago, Chile

[2]Universidad de Santiago de Chile (USACH), Facultad de Ciencias, Depto. de Física, CEDENNA, Santiago, Chile/University of Leeds, UK. Polymers and Complex Fluids, School of Physics and Astronomy

[3]Comisión Chilena de Energía Nuclear (CCHEN), Santiago, Chile

ABSTRACT

The aim of this work was to verify the influence of gamma irradiation on properties modification of a protein edible coating (calcium caseinate and whey protein isolated, added of potassium sorbate (PS) as an antimicrobial agent – film A) to enhance the shelf-life of fresh strawberries. The fruits were divided in four groups: (1) coated with film A without antimicrobial agent, (2) coated with film A, without irradiation (3) coated with film A, treated at 35.0 kGy and (4) uncoated fruits (control). The effects of γ-irradiation on the structure of the edible coating were studied by SEM and rheology properties. In order to evaluate the fruits' shelf-life the following analysis were carried out: total counting of moulds and yeasts, color, weight loss, firmness and sensorial analyses. The results showed that PS was effective against *Botrytis cinerea*, increasing the shelf-life in 200% when compared with the control. The irradiation did not affect the antimicrobial agent. The irradiated coating improved significantly ($p<0.05$) some quality parameters (color, firmness, weight loss and sensorial attributes) in the last day of storage when compared with the non-irradiated. The induced modification on the protein coating solution by γ-irradiation can be an efficient alternative to improve the shelf-life of fresh strawberry.

Key words: edible coating, γ-irradiation, potassium sorbate, milk proteins.

INTRODUCTION

Basically there are two problems to be faced when the objective is to maintain the freshness of strawberries: first, they are alive and many chemical and biochemical reactions are happening. Some reactions, if not controlled can take quickly to the senescence of the fruit and loss of the freshness. Second, it should late to the maximum the risk of microbiological contamination. In general, the shelf-life of the berries is limited by fungal diseases, especially *Botrytis cinerea* (Karabulut *et al.*, 2004). These problems can be controlled by the edible coating that seeks to delay the breathing, the ripening, the humidity loss, the enzymatic browning and the quality alterations came from these processes (Lin and Zao, 2007). When the edible coatings are combined with antimicrobial agents they can reduce, inhibit or delay the growth of microorganisms in the surface of the foods (Cha and Chinnan, 2004). The development of edible films and coatings has been focused upon barriers containing proteins, polysaccharides, and lipids (Perez-Gago *et al.*, 2006), that can be recycled and completely biodegraded in a short time, contributing excessively to the decrease of the environmental pollution (Guilbert *et al.*, 1996). Proteins and polysaccharides generally produce films with good mechanical properties but they present poor moisture barriers, because of their hydrophilic nature (Fabra, 2008). The induced modification using γ-irradiation was found to be an effective method for the improvement of both barrier and mechanical properties of the edible films and coatings based on calcium and sodium caseinates alone or combined with some globular proteins (Cieslà *et al.*, 2004).

MATERIALS AND METHODS

Determination of the minimal inhibitory concentration from the antimicrobial agent

Potassium sorbate (PS) was tested against *B. cinerea*. Different concentrations (0.0-2.0%) were jointed to the coating components (water, milk proteins and glycerol), added to Potato Dextrose Agar and poured into Petri dishes. On the surface of the agar it was added a piece of agar (0.4 cm of radio) containing *B. cinerea* with a growth time of 3 days. The plates were stored at 25°C for 5 days, after which the growth halos of the microorganism were measured.

Coating solution and Gamma Irradiation

The coating solution (Film A) was prepared with calcium caseinate, whey protein isolated, glycerol (1:1.1) and PS in water. The fruits were acquired at a local market, washed, sanitized and divided in four groups: (1) coated with film A without antimicrobial agent, (2) coated with film A, without irradiation, (3) coated with film A, treated at 35.0 kGy (dose rate of 28.7 Gy/min) and (4) uncoated fruits (control). The fruits were packed in a polystyrene tray, covered with PVC film and stored at 4±1°C for 20 days. The coating was irradiated at CCHEN (Comisión Chilena de Energía Nuclear, Santiago, Chile).

Evaluation of the shelf-life of strawberries

Microbiological analysis

The microbiological evaluation of the strawberries was based on counts of fila-

mentous moulds and yeasts. The counting was carried out on YPD Agar (Difco), incubated at 25°C/5 days. Results corresponded to the average counts and were expressed in log cfu g^{-1}.

Color

Superficial color alterations were monitored with a colorimeter (Konica Minolta, CR410). CIE-L*a*b* parameters were used to calculate the color difference (ΔE): $\Delta E=((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$ where, ΔL*, Δa* and Δb* represents the differences between the color parameters of the samples and the color parameters of the standard (uncoated fruits at the first day).

Firmness

Firmness was determined using a Texture Analyzer (Zwick Roell, M.BDO-FBO.5HT). Each experiment was conducted with a compression speed of 1 mm/s, with a compression load cell of 3 g. The firmness were reported as peak force and expressed in N/g of sample.

Weight Loss

Mass variation was determined by weighing the fruits in a semi-analytical balance with 0.001 g readability. The analyses were performed daily.

Sensorial analysis

The sensorial analysis were performed by 15 semi-trained panelists using a nine-point hedonic scale ranging from 1 (most disliked) to 9 (most liked) for the attributes of taste, color, aroma and global appearance. The purchase intention was also determined.

Experimental design

The factor to consider was the edible coating, which were studied in the following levels: Film A, without antimicrobial; Film A and irradiated Film A. The results were submitted to analysis of variance (ANOVA). Statistical analysis was carried out through the program Statgraphics 5.1.

Effect of gamma irradiation on the proteins solution coating

Rheology of protein solutions

The viscosities of both protein solutions were measured at 25°C in a low torque high resolution Rheometer 301 Anton-Paar (50 mm cone plate geometry - 1 degree angle) disposed with a solvent tramp to avoid evaporation. The flow curve of each protein solution is the resulting of singles steady measurements at different rates obtained from fresh samples. This protocol allowed reliability in controlling

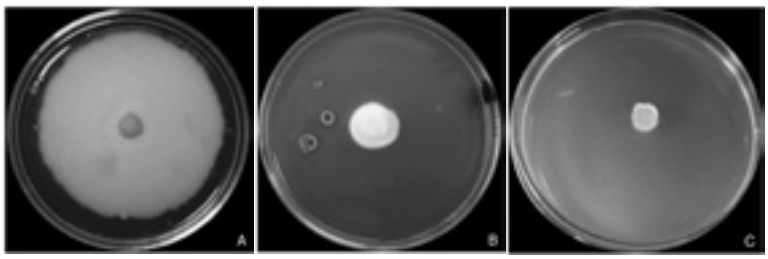


Fig. 1 - Potato dextrose agar with the components of the edible coating. A: without PS; B: added with 0.4% of PS; C: added with 0.5% of PS.

both concentration fluctuations and evaporation phenomenon during sampling measurements.

Scanning Electronic Microscopy (SEM)

A SEM (Jeol – 5410) was used on dried film protein coatings to evaluate the effects of irradiation. Samples were previously lyophilized and covered with AuPd.

RESULTS AND DISCUSSION

Determination of the minimal inhibitory concentration

Fig. 1 depicts potassium sorbate effectiveness against *B. cinerea* at 0.5%.

Evaluation of the shelf-life of strawberries

Fig. 2 shows the total count of molds for the different treatments during the storage period. The results showed that PS was effective against *B. cinerea*, increasing the shelf-life in 200% when compared with the control. The irradiation did not affect the antimicrobial agent. The irradiated coating improved significantly ($p<0.05$) some quality parameters (color, firmness, weight loss and sensorial attributes) in the last day of storage when compared with the non-irradiated. The fig. 3 shows color difference (ΔE) at different treatments in the end of the storage period and fig. 4 depicts the maximum force of penetration in strawberries at different treatments during the storage period. The induced modification of protein properties by γ-irradiation can be an efficient alternative to improve the shelf-life of coated strawberry. These results was consistent with Ouattara *et al.* (2002) and Vachon *et al.* (2003) that the irradiation of the protein coating solution at 32.0 kGy, prior to the coating process of fresh strawberries, reduced the level of fruits contamination during the storage period compared to non-irradiated coating.

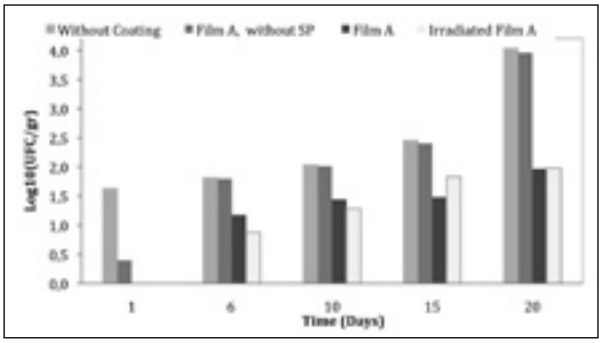


Fig. 2 - Total count of molds in strawberries at different treatments during the storage period.

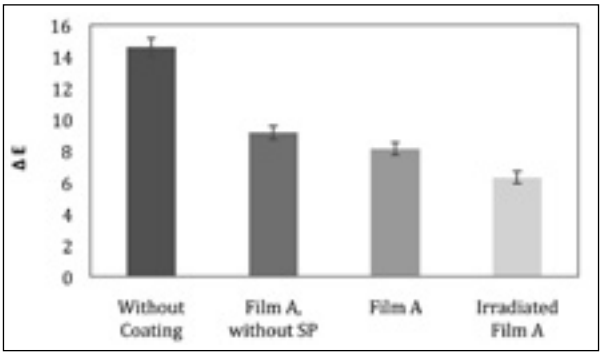


Fig. 3 - Color difference in strawberries at different treatments in the end of the storage period.

Effect of gamma irradiation on the protein solution coatings

Fig. 5 depicts the rheology of the irradiated and non irradiated protein solutions. The viscosity profile of both samples suggests a shear thinning behavior characterized by a well defined plateau regime at high rates

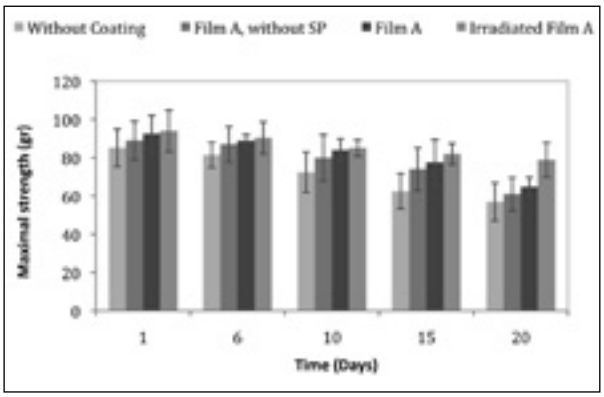


Fig. 4 - Maximum force of penetration in strawberries at different treatments during the storage period.

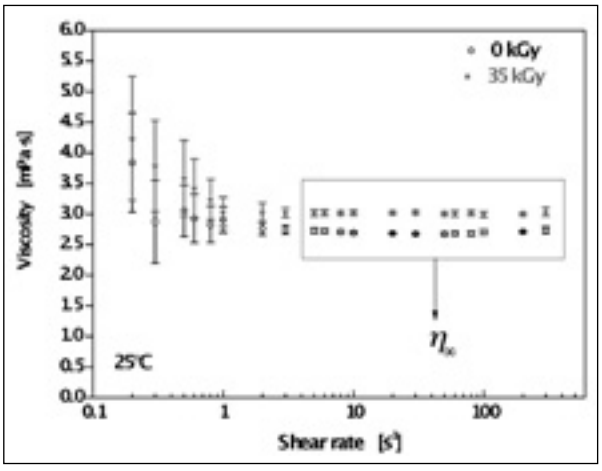


Fig. 5 - Flow curves of unirradiated (open circle) and irradiated (star) proteins solutions measured at 25°C. Inner caption represents the infinite viscosity plateau.

(identified as η in the fig. 5) and shows significant effect of the irradiation doses on the resulting viscosity. The viscosity measured of the irradiated solutions in this region is 3.02±0.03 mPa.s, which, compared to the non irradiated (2.68±0.03 mPa.s) represents an increase of 12%. These values differ from those obtained by Ciésla *et al.* (2004), but confirm the increase in viscosity observed here. The effects on viscosity measured may suggests modifications on the protein structure as cross-link, aggregations or structural conformations changes, but will require further studies to identify the mechanism.

Fig. 6 shows SEM images that indicate a modification on the irradiated coating (6A) when compared which the non-irradiated (6B). The irradiation conduced to a better fruit-coating adhesion and a more uniform structure. A granular structure was observed to the non-irradiated coating.

CONCLUSION

Gamma irradiation (35.0 kGy) induced modifications on protein properties of Film A, leading on an

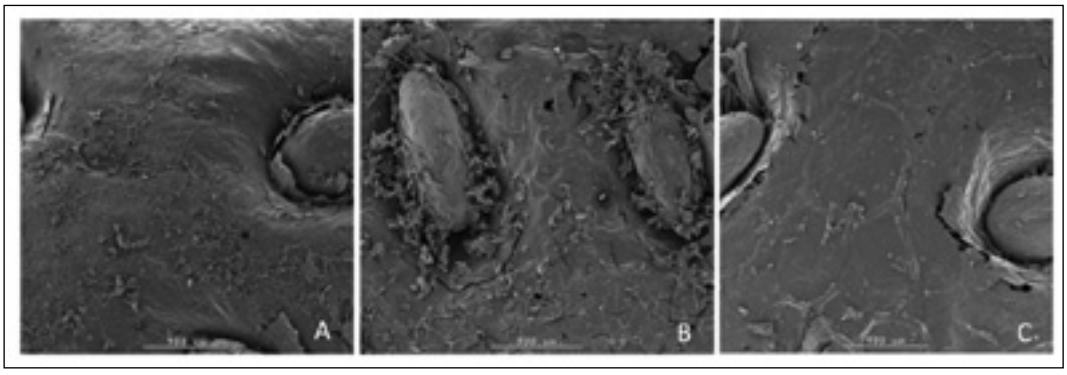


Fig. 6 - SEM images. A: Strawberry coated with irradiated film A; B: Strawberry coated with film A; C: Strawberry uncoated. All images have 35x magnification.

increasing on the fruits shelf-life and a significant improved on the strawberries quality parameters as color, weight loss and firmness in the end of storing.

ACKNOWLEDGMENT

This research was supported by Departamento de Investigaciones Científicas y Tecnológicas (DICYT) de la Universidad de Santiago de Chile (USACH).

REFERENCES

Cha D., Chinnan M. 2004. Biopolymer-based Antimicrobial Packaging: A Review. Critical Reviews in Food Science and Nutrition, 44, 223-237.

Ciésla K., Salmieri S., Lacroix M., Le Tienb C. 2004. Gamma irradiation influence on physical properties of milk proteins. Radiation Physics and Chemistry, 71, 93-97.

Fabra M.J., Talens P., Chiralt A. 2008. Effect of alginate and k-carrageenan on tensile properties and water vapour permeability of sodium caseinate-lipid based films. Carbohydrate Polymers, 74 (3), 419-426.

Guilbert S., Gontard N., Garris G.M. 1996. Prolongation of the shelf-life of perishable food products using biodegradable films and coatings. Lebensmittel-Wissenschaft und-Technologie, 29 (1-2), 10-17.

Karabulut O., Romanazzi G., Smilanick J., Lichter A. 2004. Postharvest ethanol and potassium sorbate treatments of table grapes to control gray mold. Postharvest Biology and Technology. 37, 129-134.

Lin D., Zao Y. 2007. Innovations in the development and application of edible coating for fresh and minimally processed fruits and vegetable. Comprehensive Reviews in Food Science and Food Safety, 6 (3), 60-75.

Ouattara B., Sabato S.F., Lacroix M. 2002. Use of gamma-irradaition technology in combination with edible coating to produce shelf-stable foods. Radiation Physics and Chemistry, 63, 305-310.

Perez-Gago M.B., Serra M., Del Rio M.A. 2006. Color change of fresh-cut apple coated with whey protein concentrate-based edible coating. Postharvest Biology and Technology, 39, 84-92.

Vachon C., D'Aprano G., Lacroix M., Letendre M. 2003. Effect of Edible Coating Process and Irradiation Treatment of Strawberry *Fragaria* spp. on Storage-keeping Quality. Food Microbiology and Safety. 68 (2), 608-612.

ANTIMICROBIAL ACTIVITY OF CHITOSAN IN PORK LOINS

A. PAPARELLA, A. SERIO, F. DI PASQUALE, L. DE NICOLA and C. CHAVES-LÓPEZ

Department of Food Science, University of Teramo, Via C.R. Lerici 1, 64023 Mosciano Stazione, TE, Italy

ABSTRACT

The antimicrobial activity of chitosan at 1 and 2% was evaluated. Starting from the *in vitro* evaluation, the activity was tested against several strains of *Listeria monocytogenes* of different serotypes, genotypes and sources. Some strains of *Salmonella* spp. were also tested to compare the activity of chitosan between Gram positive and Gram negative bacteria. The effectiveness of a chitosan film was also investigated on pork loins, inoculating 3 *L. monocytogenes* isolates individually and as a cocktail, and storing the samples at 4°C in ordinary atmosphere and under vacuum packaging. The impact of the chitosan film on the pH and a_w of the food samples was monitored during the storage time. *In vitro* results showed a good antimicrobial activity against *Listeria monocytogenes* but a lower activity against *Salmonella* spp. In the challenge test, good results were obtained in ordinary atmosphere since *L. monocytogenes* cocktail load was reduced of about 3 Log cfu/g during 7 days shelf-life. The anti-*Listeria* activity was even greater in vacuum packaged samples, where a sharp decrease was observed also in aerobic plate count and lactic acid bacteria.

Key words: chitosan, *L. monocytogenes*, pork loin, antimicrobial activity.

INTRODUCTION

Chitosan is a polysaccharide deriving from deacetylation of chitin. Due to its biodegradability, biocompatibility, atoxicity and antimicrobial activity, it shows wide possibilities of application in food biopreservation (Whang, 1992; Outtara *et al.*, 2000). In acid solution, the positively charged amino groups of chitosan interfere with the negative charges of the macromolecules at the cell surface. The antimicrobial mechanism might be correlated to this interaction that can disturb

the physiological activities of bacteria and kill them. This is the reason why the antimicrobial activity of chitosan may be different between Gram positive and Gram negative bacteria (Zheng and Zhu, 2003; Pranoto *et al.*, 2004). Moreover, the activity is influenced by the concentration and the molecular weight of chitosan and, in a food model, by interaction with nutrition elements and physical-chemical characteristics (Devlieghere *et al.*, 2004).

The aim of this study was to evaluate the antimicrobial activity of chitosan against *Listeria monocytogenes* and *Salmonella* spp. *in vitro.* In addition, the effect of chitosan against *Listeria monocytogenes*, together with the impact on pH and a_w, was evaluated in pork loins.

MATERIALS AND METHODS

The *in vitro* antimicrobial test was conducted on 2 *L. monocytogenes* type strains, 8 *Listeria monocytogenes* and 6 *Salmonella* spp. previously isolated from meat products, using a chitosan of medium molecular weight (Sigma-Aldrich, St. Louis, MO, USA) at 1 and 2% (w/v) in acetic acid 1% (v/v) and water solution. The inhibition rate was estimated as described by Zheng and Zhu (2003).

Pork loins (3 g for each sample) inoculated with 3 *L. monocytogenes* isolates (ATCC 19144, 95986, 58712) (5-6 Log cfu/g), both individually and as a cocktail, were dipped in the chitosan film (1 and 2%) and dried for ten minutes. The samples were stored at 4°C in ordinary atmosphere and under vacuum packaging for 7 days. The antimicrobial activity of chitosan films was evaluated against *L. monocytogenes* (Lm) (on ALOA, Biolife Italiana, Milan, Italy), as well as on total aerobic population (Plate Count Agar, Oxoid, Basingstoke, UK) and lactic acid bacteria (MRS Agar, Oxoid). pH and a_w of the samples coated with the chitosan film were monitored by using Mettler Toledo MP 220 pH-meter and AcquaLab (DECAGON).

RESULTS AND CONCLUSIONS

In vitro results (fig. 1) highlighted a good antimicrobial activity of chitosan against *L. monocytogenes* strains, that increased with growing chitosan concentrations. The strains showed variable sensitivity, and type strain ATCC 7644 was the most

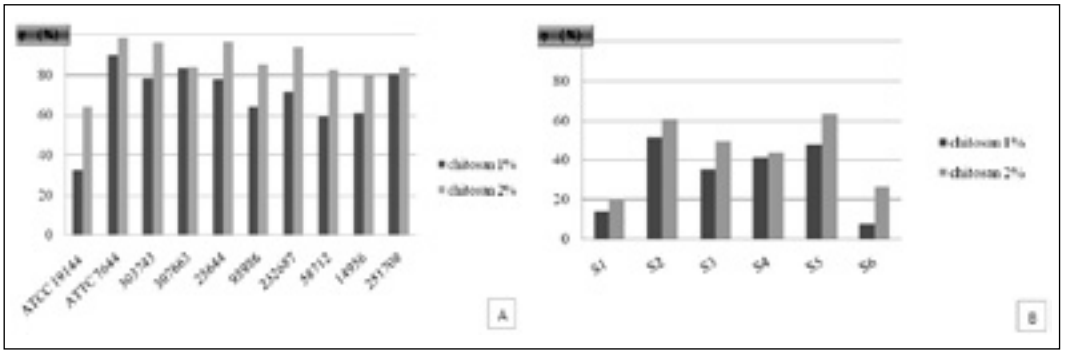


Fig. 1 - Antimicrobial activity of chitosan against *L. monocytogenes* (A) and *Salmonella* spp. (B).

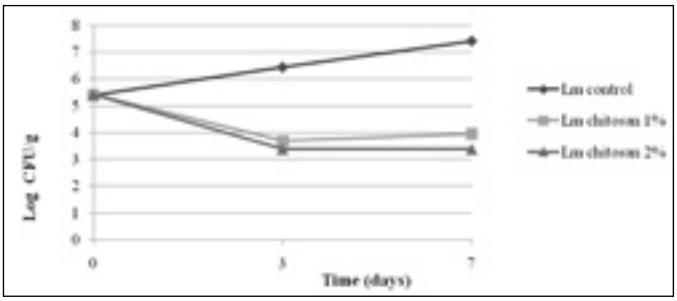


Fig. 2 - Chitosan activity against *L. monocytogenes* cocktail (ALOA count) in pork loin in ordinary atmosphere.

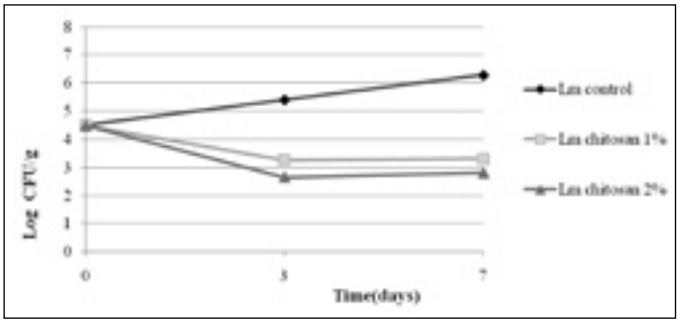


Fig. 3 - Chitosan activity against *L. monocytogenes 58712* (ALOA count) in pork loin in ordinary atmosphere.

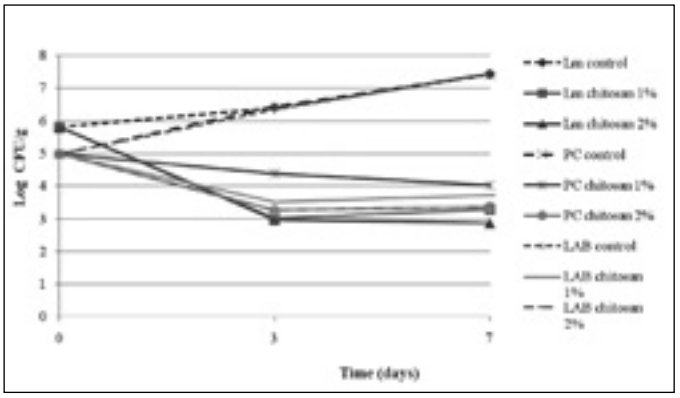


Fig. 4 - Chitosan activity against *Listeria monocytogenes* cocktail (Lm), aerobic plate count (PC) and lactic acid bacteria (LAB), in vacuum packaged pork loin.

sensitive with an inhibition rate of 98.90 at chitosan 2%. *Salmonella* spp. strains were clearly more resistant with respect to *L. monocytogenes*, probably because of the different cell wall composition.

In the challenge study on pork loins, good results were obtained in ordinary atmosphere, since *L. monocytogenes* cocktail load was reduced of about 3 Log cfu/g during 7 days shelf-life (fig. 2). As observed in fig. 3, the experiments carried out with single strains in ordinary atmosphere confirmed that the antimicrobial effect of chitosan was already attained after 3 days.

The effect of chitosan was even more evident in vacuum packaged loins. Figure 4 shows that *L. monocytogenes*, as well as aerobic plate count (PC) and total lactic acid bacteria (LAB), sharply decreased over time (3 Log cfu/g reduction). Physical-chemical analyses demonstrated that a_w values of all the samples did not change substantially during storage; on the other hand, pH of the samples coated with chitosan remained below 6.0 during the storage time, while pH of untreated control reached 7.0 due to the spoiling process. The results obtained in the challenge test were particularly interesting, confirming the bacteriostatic activity of chitosan against *L. monocytogenes* both in ordinary atmosphere and under vacuum conditions. Microbiological data, together with the valuable effect on pH stability, suggest that chitosan may be particularly useful for shelf-life extension of fresh meats.

REFERENCES

Devlieghere F., Vermeulen A., Debevere J. 2004. Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. Food Microbiol. 21: 703.

Outtara B., Simard R.E., Piette G., Begin A., Holley R. 2000. Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial prepared with chitosan. Int. J. Food Microbiol. 62: 139.

Pranoto Y., Rakshit S.K., Salokhe V.M. 2005. Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. LWT Food Sci. Technol. 38: 859.

Wang G.H. 1992. Inhibition and inactivation of five species of foodborne pathogens by chitosan. J. Food Prot. 55(11): 916.

Zheng L.Y., Zhu J.F. 2003. Study on antimicrobial activity of chitosan with different molecular weight. Carboh. Polym. 54: 527.

CHARACTERIZATION OF CHITOSAN MEANT FOR ANTIMICROBIAL FOOD PACKAGING

R. SENDÓN[1], A. RODRÍGUEZ-BERNALDO DE QUIRÓS[1], C. BUENO-SOLANO[2], D. PEREIRA[1], A. SANCHES-SILVA[3], H.S. COSTA[3], D.I. SÁNCHEZ-MACHADO[2], H. SOTO VALDEZ[4], I. ANGULO[5], G.P. AURREKOETXEA[5], J. LÓPEZ-CERVANTES[2] and P. PASEIRO[1]

[1]Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Pharmacy, University of Santiago de Compostela, 15782, Spain

[2]Department of Biotechnology and Food Sciences, Technological Institute of Sonora, 5 de Febrero 818, Cd. Obregón, 85000 Sonora, México

[3]Departamento de Alimentação e Nutrição, Instituto Nacional de Saúde Doutor Ricardo Jorge, I.P., Av. Padre Cruz, 1649-016 Lisboa, Portugal

[4]Centro de Investigación en Alimentación y Desarrollo, A.C., CTAOV, Apdo. Postal 1735 Hermosillo, 83000 Sonora, México

[5]Gaiker, Technological Center. Parque Tecnológico Edificio 202, 48170 Zamudio, Spain

ABSTRACT

Chitosan (CAS n. 9012-76-4) is a natural polysaccharide obtained by the partial deacetylation of chitin. It is a linear polymer of β (1-4) 2-acetamido-2-deoxy-D-glucose and 2-amino-2-deoxy-D-glucose in different proportions. Chitin is the most abundant polysaccharide after cellulose and the main source is the shells of crustaceans.

It has been demonstrated that some important properties are directly related to the antimicrobial activity of chitosan. Some of these properties are the molecular weight (Mw), the degree of polymerisation (DP) and the degree of deacetylation (DD).

In this work several analytical techniques (FTIR (Fourier-transform Infrared Spectroscopy), NMR (Nuclear Magnetic Resonance Spectroscopy) and SEM (Scanning Electron Microscopy)) were attempted to characterize two different samples obtained from shrimp waste. It can be concluded that sample 1 should be more suitable to be added as an active agent to a film.

Key words: chitosan.

INTRODUCTION

Antimicrobial food packaging is a form of active packaging. This could play an important role in extending shelf life of products and reduce the risk from pathogens. Chitosan has been found to be nontoxic, biodegradable, biofunctional, biocompatible and moreover to have antimicrobial characteristics (Dutta *et al.*, 2009). Several works have described the use of chitosan as an antimicrobial agent incorporated directly to the film and as a coating to protect fresh vegetables and fruits from fungal degradation (Appendini *et al.*, 2002).

Chitosan (CAS n. 9012-76-4) is a natural polysaccharide obtained by the partial deacetylation of chitin. It is a linear polymer of β (1-4) 2-acetamido-2-deoxy-D-glucose and 2-amino-2-deoxy-D-glucose in different proportions. Chitin is the most abundant polysaccharide after cellulose and the main source is the shells of crustaceans.

It has been demonstrated that some important properties are directly related to the antimicrobial activity of chitosan. Some of these properties are the molecular weight (Mw), the degree of polymerisation (DP) and the degree of deacetylation (DD). DD values obtained may change, depending on the nature and level of impurities, source and polymer morphologies (Kasaai, 2009).

In this work several analytical techniques were used to characterize two different samples obtained from shrimp waste.

MATERIALS AND METHODS

Standards

Four commercially available chitosan were used. All were from Aldrich (Schnelldorf, Germany): Medium molecular weight chitosan (QM), viscosity: 200,000 cps, molecular weight (Viscosity based): 190,000-310,000 Da and deacetylation degree: 75-85%. Low molecular weight chitosan (QL), viscosity: 20,000 cps, molecular weight (Viscosity based): 50,000-190,000 Da and deacetylation degree: 75-85%. Chitosan shrimp (QS), deacetylation degree: ≥75-

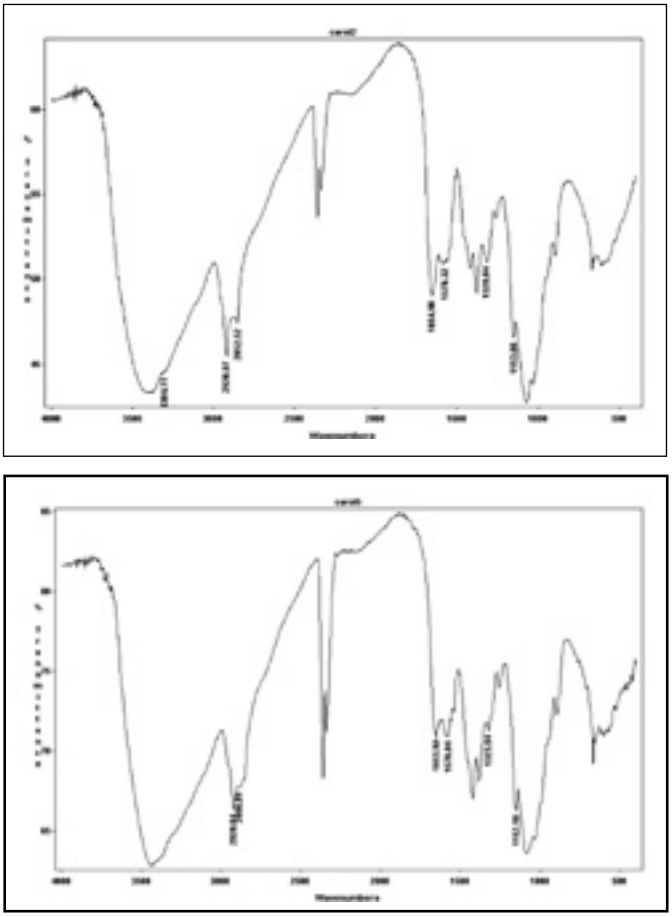


Fig. 1 - FTIR spectra of A: QM standard and B: 1Q.

85%. Chitosan crab (Q), viscosity: >200,000 cps, molecular weight (Viscosity based): 190,000-375,000 Da.

KBr used in FTIR experiments and CD_3COOD and D_2O used in NMR experiments were all from Aldrich (Schnelldorf, Germany).

Samples

Shrimp waste (heads and cephalothorax) samples were collected from local shrimp processing factories in South Sonora, Mexico. The waste was minced, fermented and centrifuged. Three fractions were obtained: chitin-rich fraction, protein hydrolysate and lipid fraction. Then, the chitin was converted to chitosan using a thermochemical method with NaOH (45%) at 110°C.

FTIR (Fourier-transform Infrared) spectroscopy

IR spectral studies were performed in a Mattson Genesis II (USA), controlled by Winfirst© software.

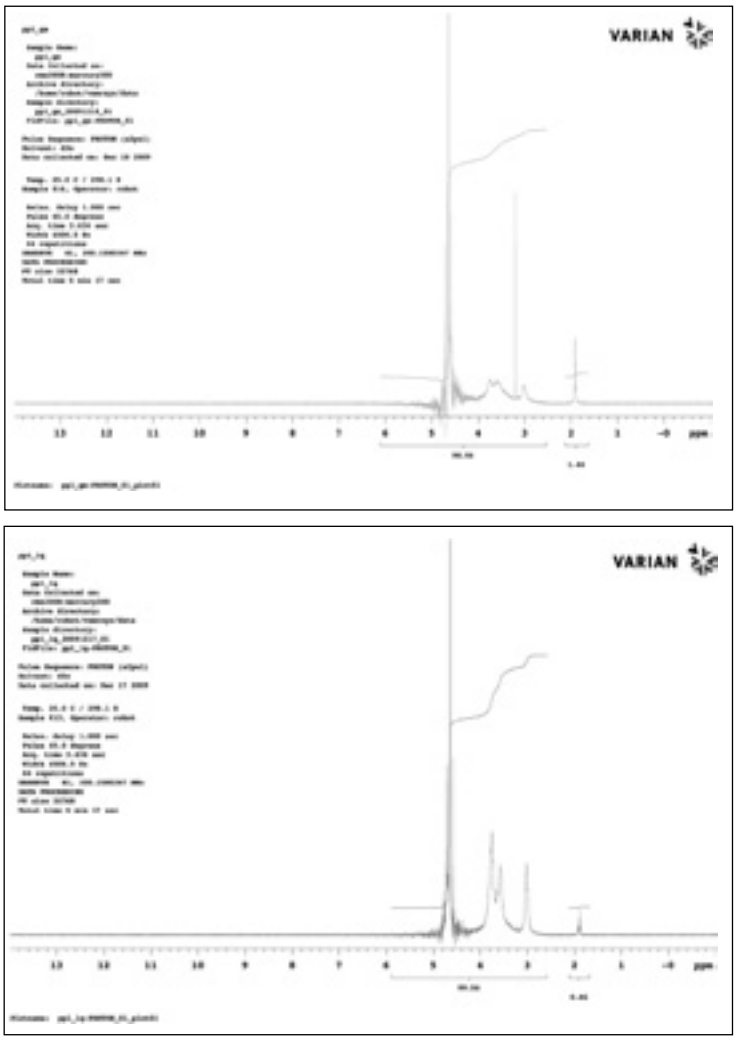


Fig. 2 - ^1H NMR spectra of A: QM standard and B: 1Q.

The sample was prepared as a thin pellet made from a mixture of KBr and the chitosan powder (3 mg). All spectra were recorded in the range of 400-4,000 cm^{-1}.

NMR (Nuclear Magnetic Resonance) spectroscopy

All spectra were recorded using a Varian Inova 750 spectrometer at 300 mHz.

Two experiments were tentatively carried out: ^1H NMR and ^{13}C NMR. In both cases approximately 5 mg of each sample were disolved in 1% (v/v) CD_3COOD in D_2O.

SEM (Scanning Electron Microscopy)

Samples were spread on a carbon conducting adhesive tape pasted on a metallic stub, subjected to gold covering and observed employing a LEO-435VP (Cambridge, UK) scanning electron microscope (EDX, Oxford 300).

RESULTS AND DISCUSSION

All data were compared with four commercially available standards, which were submitted to the same analysis than samples were.

From the relationship of A_{1655}/A_{3450} (Amide I/hydroxyl group) observed in the IR spectra (fig. 1) it can be concluded that the DD of samples is higher than standards.

In the ^{1}H RMN experiments, the intensity of ≈ 2 ppm signal corresponds to methyl protons of the N-acetyl groups. So as can be observed from fig. 2, less intensity of this signal appears for sample 1 (1Q), showing that the DD this sample is higher.

The ^{13}C RMN spectra obtained were not sensitive at the concentration tested; but despite that it is not conclusive, standards showed weak signals at ≈ 25 and ≈ 176 ppm (corresponding to $-CH_3$ and –C=O groups respectively) whereas these peaks were not observed in neither of samples.

The SEM images show that the particle size for both samples should be reduced.

So it can be concluded that sample 1 should be more suitable than sample 2, to be added as active agent to a film.

ACKNOWLEDGEMENTS

This work was funded under the Project no. 95935 from FONCICYT C002-2008-1/ALA – 127 249. R. Sendon is grateful to the "Parga Pondal" Program financed by "Consellería de Innovación e Industria, Xunta de Galicia" for her postdoctoral contract. Authors are also grateful to Ms. Patricia Blanco Carro, Ms. Cristina Casal Romero and Mr. Gonzalo Hermelo Vidal for their excellent technical assistance.

REFERENCES

Appendini P., Hotchkiss J.H. Review of antimicrobial food packaging. Innovative Food Science & Emerging Technologies. 2002. (3), 113-126.

Dutta P.K., Tripathi S., Mehrotra G.K., Dutta J. Perspectives for chitosan based antimicrobial films in food applications. Food Chemistry. 2009. (114), 1173-1182.

Kasaai M.R. Various Methods for Determination of the Degree of N-Acetylation of Chitin and Chitosan: A Review. Journal of Agricultural and Food Chemistry. 2009. (57), 1667-1676.

DETERMINATION OF α-TOCOPHEROL IN SHRIMP WASTE TO EVALUATE ITS POTENTIAL TO PRODUCE ACTIVE PACKAGING

A. SANCHES-SILVA[1], H.S. COSTA[1], C. BUENO-SOLANO[2], R. SENDÓN[3], D.I. SÁNCHEZ-MACHADO[2], H. SOTO VALDEZ[4], C. COLÍN-CHÁVEZ[4], I. ANGULO[5], G.P. AURREKOETXEA[5], P. PASEIRO[3] and J. LÓPEZ-CERVANTES[2]

[1]Departamento de Alimentação e Nutrição, Instituto Nacional de Saúde Doutor Ricardo Jorge, I.P., Av. Padre Cruz, 1649-016 Lisboa, Portugal

[2]Department of Biotechnology and Food Sciences, Technological Institute of Sonora, 5 de Febrero 818, Cd. Obregón, 85000 Sonora, México

[3]Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Pharmacy, University of Santiago de Compostela, 15782 Spain

[4]Centro de Investigación en Alimentación y Desarrollo, A.C., CTAOV, Apdo. Postal 1735 Hermosillo, 83000 Sonora, México

[5]Gaiker, Technological Center. Parque tecnológico Edificio 202, 48170 Zamudio, Spain

ABSTRACT

The possibility of using the lipid fraction from fermented shrimp waste as a source of natural α-tocopherol to produce packaging with antioxidant properties was evaluated. A fast reverse-phase ultra-performance liquid chromatographic (RP-UPLC) method coupled with a diode array detector was developed to determine α-tocopherol in the lipid fraction of shrimp waste. The α-tocopherol level found using acetonitrile as extraction solvent was 50.5 mg/100 g sample, indicating.

Key words: α-tocopherol; antioxidant; shrimp waste; UPLC-DAD.

INTRODUCTION

The commercial food processing from sea foods is generating great amounts of by-products such as shrimp heads and cephalothoraxes. In order to eliminate the effects of its accumulation in the environment, it is important to carry out a complete characterization to know their re-use potential. The production of shrimp in Sonora (Mexico), in 2007 was about 65,000 t, of which 35% is considered as waste (22,750 t), therefore it has a lipid phase potential of 682.5 t.

Additionally, there is a growing interest on active packaging with natural compounds because it increases the shelf-life of foods. α-tocopherol is a molecule of natural origin and it has great scientific and commercial interest due to its antioxidant activity. The possibility of using it in active packaging has already been the aim of previous studies and now the possibility of using the lipid fraction from fermented shrimp waste as a source of natural α-tocopherol is being evaluated, in order to produce packaging with antioxidant properties.

A reverse-phase ultra-performance liquid chromatographic (RP-UPLC) method coupled with a diode array detector was developed to determine α-tocopherol in the lipid fraction.

MATERIAL AND METHODS

Shrimp waste (heads and cephalothoraxes) samples were collected from local shrimp processing factories in South Sonora, Mexico. The waste was minced, fermented and centrifuged. Three fractions were obtained (chitin-rich fraction; protein rich liquor and lipid fraction) and an upper solid lipid fraction was separated, and used to determine α-tocopherol content.

The sample (0.12 g) was extracted with 5 mL of extraction solvent and vortexed for 20 s. Two solvents, methanol and acetonitrile (ACN) were tested. Afterwards, samples were mixed in an ultrasonic bath and centrifuged for 10 min at 3,000 rpm. The upper phase was filtered by a GHP filter (0.2 μm) and 10 μL were injected in the UPLC© column.

Chromatographic separation was performed with a UPLC® C18 BEH (50x2.1 mm I.D., 1.7 μm particle size) at 30°C and using a gradient elution method, which employs ACN and water as mobile phase. Within the first 1.5 min the mobile phase changes from 10% water/90% ACN for 100% and then keep isocratic at 100% ACN.

The flow-rate was 0.3 mL/min and detection was performed at 295 nm. The wavelength used for determination was selected on basis of its maximum absorbance peak in UV scanning.

RESULTS AND CONCLUSIONS

The chromatographic conditions allowed to obtain a good resolution of the α-tocopherol peak at 3.9 min (fig. 1). Calibration curve was linear over the concentration range of 1.022 and 102.2 μg/mL and showed excellent correlation coefficients ($r^2 = 0.999$). This indicates suitability for α-tocopherol quantification.

For extraction of the lipid fraction from fermented shrimp waste two solvents were tested. In the extraction procedure with methanol the α-tocopherol level found was 49.8 mg/100 g sample, while in the extraction with acetonitrile it was 55.7

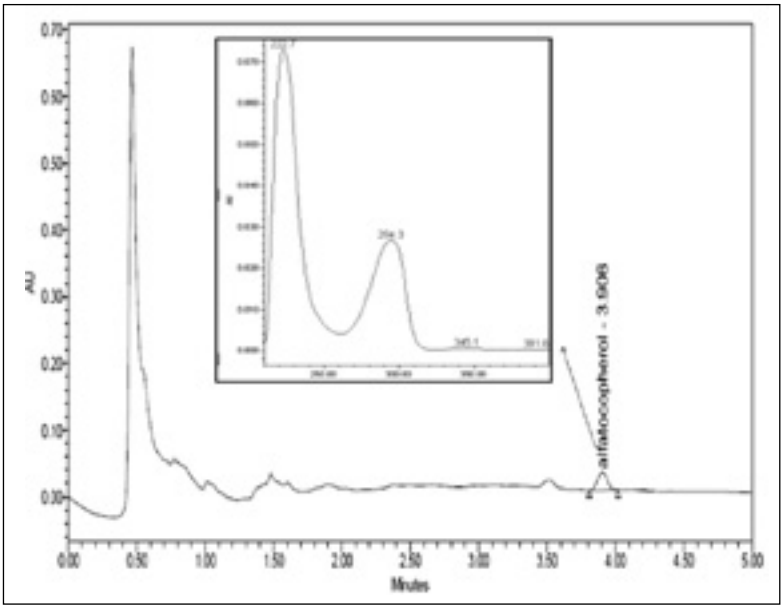


Fig. 1 - UPLC© chromatogram of a sample (lipid fraction from fermented shrimp waste) showing the α-tocopherol peak at 3.9 min and the respective UV spectrum.

mg α-tocopherol/100 g sample. Another test performed an extraction for 18 h with methanol. The amount found was 50.5 mg α-tocopherol/100 g sample. The levels found in this study with both extraction solvents are higher but in agreement with those found in the literature (López-Cervantes *et al.*, 2006).

ACKNOWLEDGEMENTS

This work was funded under the Project n. 95935 from FONCICYT C002-2008-1/ALA – 127 249. Authors are grateful to the "Fundação para a Ciência e Tecnologia", Portugal, for the Postdoctoral contract of Ana Sanches Silva in the frame of the Program "Science 2007".

REFERENCES

López-Cervantes J., Sánchez-Machado D.I., Ríos-Vázquez N.J., 2006. "High Performance liquid chromatography method for the simultaneous quantification of retinol, α-tocopherol, and cholesterol in shrimp waste hydrolysate". Chromatogr. A. 1105 (1-2): 135.

VALORISATION OF INDUSTRIAL LIGNIN AS ADDITIVE WITH ANTIOXIDANT PROPERTIES IN PLA

ABDERRAHIM LOUAÏFI[1], ALAIN GUINAULT[2], SANDRA DOMENEK[1]*, VIOLETTE DUCRUET[3] and STÉPHANIE BAUMBERGER[4]

[1]AgroParisTech, UMR 1145 (AgroParisTech/INRA), 1 Avenue des Olympiades, 91300 Massy, France

[2]CNAM, Laboratoire des Matériaux Industriels Polymères, 292 rue Saint Martin, 75000 Paris, France

[3]INRA, UMR 1145 (AgroParisTech/INRA), 1 Avenue des Olympiades, 91300 Massy, France

[4]Institut Jean-Pierre Bourgin, UMR 1318 AgroParisTech/INRA, Pôle Systèmes Cellulaires, Morphogénèse et Signalisation, 78026 Versailles, Cedex, France

*corresponding author: sandra.domenek@agroparistech.fr, Tel.: +33(0)1 69 93 50 68

ABSTRACT

Lignins are complex phenolic polymers. They are one of the main byproducts of mainly industries. The valorisation of lignin is therefore a key issue in the economic performance of these industries. One of the functional properties of lignin is an antioxidant action, the efficiency of which is closely linked to the lignin structure. Small phenolic compounds seem to play an important role in the antioxidant potential. In this work we studied the effect of the dispersion of an alkali lignin sample (Protobind 1000, Granit SA) in poly(lactic acid) on the properties of thermoformed films. The lignin was incorporated in the polymer with the help of a twin screw extruder and film samples were obtained by thermocompression. The effect of the fabrication process on lignin structure was investigated by size exclusion chromatography (SEC) and GC-MS. The barrier properties of the different samples were investigated through permeability measurements. The antiradical activity was assessed by DPPH radical scavenging tests. The analysis of lignin structure in the film samples showed that the process increase the proportion of small phenolic compounds, such as ferulic and ρ-coumaric acids.

Key words: Lignin, poly(lactic acid), twin screw extrusion, antioxidant effect.

INTRODUCTION

Lignins are phenolic biopolymers that considered as the second most important substance produced in nature. Being byproducts of the paper industry and the agro-fuels, the valorization of these compounds represents a challenge for these industries. Packaging is possibly a market for these lignins, when used as additives in polymers for the formulation of packages with advanced, active properties. A promising way is taking profit of the antioxidant properties of the constituents of lignins (Pouteau *et al.*, 2003). The aim of this work was to investigate the feasibility of PLA-blends by extrusion processes containing lignins, the effects of the process on the lignin's structure and maintaining antioxidant properties in the final material.

Experimental

The PLA 2002D pellets were provided from NatureWorks (USA). The lignin Protobind 1000, was provided from GRANIT SA (Switzerland).

The blend of dried PLA-Lignin was made by twin screw extrusion (Thermo-Hake Rheomex) at 180°C. The premix of PLA-Lignin (9/1:m/m) was made separately. The formation of film of thickness about 200-300 μm is made by 3 g of PLA-Lignin pellets, thermocompressed (Télémécanique, N1) at 185°C. The oxygen transmission rate was measured at 23°C and 0%RH using Systech 8001 (France) apparatus. The measurements were done in duplicate.

Size Exclusion Chromatography was performed on samples (500 mg PLA-Lignin) that have been acetylated and dissolved in THF (10 mL) (Carlo Erba SDS). The SEC analysis was made on a column of poly(styrene divinylbenzene) (600×7.5 mm) with THF as an eluent at 1 mL/min flow rate. The detection was performed by a Shodex RI 101 refractometer and a UV Dionex Ultimate 3000 diode array detector.

The Gas chromatography-Masse spectroscopy was performed on samples (500 mg of PLA-Lignin) that have been dissolved in 35 mL of containing BHT (50 mg/L). The PLA was precipitated in 200 mL of methanol (CarloErba). The precipitate was separated from methanol by centrifugation (1h, 15,000 rpm). The methanol was eliminated from the supernatant by evaporation under vacuum. The residue was solubilised in 2 mL dichloromethane/ethyl-acetate (1/1:v/v) before silylation and injection (1 μL) on a Saturne 2100 GC-MS instrument (Varian) equipped with a polydimethylsiloxane column (Supelco, 30 mx0.25 mmx0.25 μm) (Lepifre *et al.*, 2004). The quantification of phenolics compounds was done by determining the response coefficient (K).

DPPH radical scavenging tests were performed as shown by Brand-Williams *et al.*, 1995. The solutions of antiradical molecules were obtained by diffusion of phenol molecules from 1 cm^2 of film of PLA, PLA-Lignin or rod or 6.15 mg of pure lignin in 3 mL of ethanol/water (95/5: v/v) during 24h. Thereafter, the solution of ethanol/water was evaporated under vacuum and the remaining phenols were recovered in 77 μL of methanol.

RESULTS

The film formation process was optimized in order to incorporate as much lignin as possible while preserving a rather homogeneous morphology. A 6.6% maximal lignin content was obtained with lignin finely dispersed as ~1 μm aggregates in

the PLA matrix (fig. 1). Drying PLA and lignin before blending allowed preventing the polymer hydrolysis (tab. 1).

The incorporation of lignin didn't affect the film oxygen permeability up to a lignin content of 4.5% while a slight decrease is observed at 6.6% (fig. 2). Size exclusion chromatograms of the native isolated lignin compared to lignin after incorporation in PLA (fig. 3) indicates that low molar mass compounds are formed during processing. Indeed, the relative intensity of the peak eluted at 21 min dramatically increases after processing. Moreover, the distribution of the polymer population is shifted to lower molar masses after processing (M_w reduced from 2,600 to 1,300 g mol^{-1}). This suggests that partial lignin depolymerisation occurs during processing.

The analysis of the low molar mass phenolic fraction showed that this fraction mainly contains ferulic acid and ρ-coumaric acid. The proportions are shown in tab. 2.

The results show that the thermomecanical process can generate low-mass phenolic compounds from lignin polymers. These compounds, which are naturally linked to grass lignin in plants, are known for there antioxidant activity. In order to assess the possible antioxidant effect of the film in contact with a liquid medium, radical scavenging tests were performed with aqueous ethanolic extracts of the materials. The radical scavenging effects of the ethanol/water extracts recovered from native PLA, native lignin, PLA-lignin rod and PLA-lignin film are compared in fig. 4. Whereas no effect is

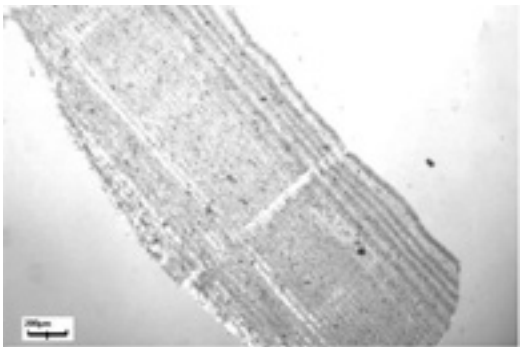


Fig. 1 - Observation under an optical microscope (magnification 2.6) of a section through PLA-Lignin (6.6%) extruded rod.

Table 1 - Molecular weights (M_w) and polydispersity index of native PLA and PLA extruded with lignin.

	M_w	Ip
Native PLA	139,000	1.91
PLA- Protobind (6.6%)	132,000	1.87

Table 2 - Levels of ferulic acid and ρ-coumaric acid in ppm of total lignin fraction calculated from chromatograms in GC-MS.

	Lignins (ppm)	Rod (ppm)	Films (ppm)
Ac. PC	380	330	610
Ac. FE	250	290	510

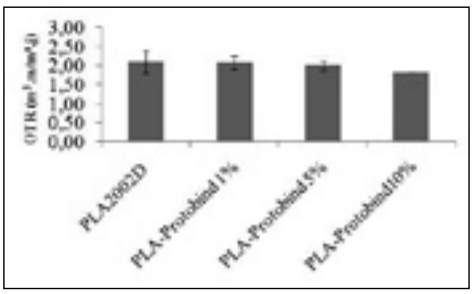


Fig. 2 - Oxygen permeability of PLA-Lignin films.

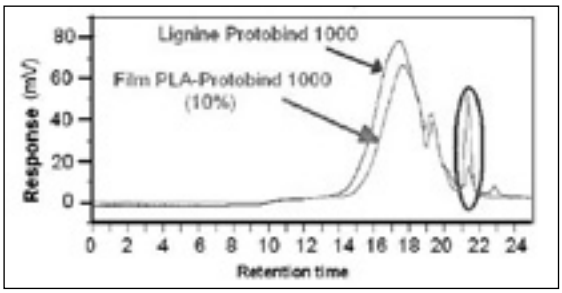


Fig. 3 - HP-SEC chromatogram (UV detection) of the mixture PLA-Lignin.

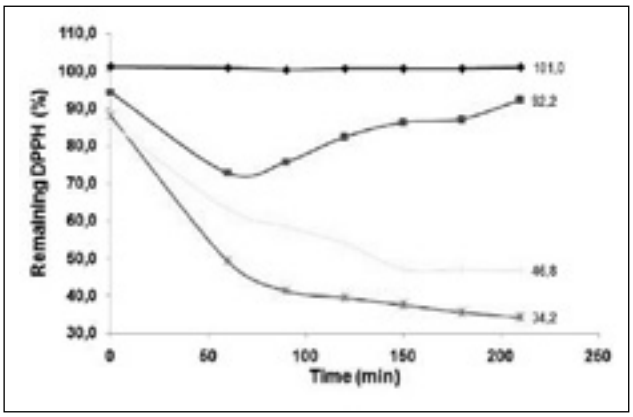


Fig. 4 - Kinetic of scavenging of free radical DPPH° in the presence of extracts of PLA (◆), Lignin (■), rod of PLA-Lignin (+) and film of PLA-Lignin (∗).

observed with PLA alone, the extracts recovered in the presence of lignin exhibits a radical scavenging activity that increases when lignin is submitted to a thermomechanical treatment and reaches a maximum with the PLA-lignin film.

CONCLUSION

This studies show that it was possible to get compatible PLA-lignin composites films using a twin-screw extrusion blending and thermocompression. Acting as a filler finely dispersed in the PLA matrix, lignin does not dramatically change oxygen barrier properties of the films. The PLA-lignin film exhibited a pronounced radical scavenging effect due the phenolic extractable compounds brought by lignin. Naturally covalently linked to lignin, these compounds seem to be released during the film processing, increasing its antiradical activity. Thus, PLA-lignin films are potentially good candidates regarding biodegradable active packaging.

REFERENCES

Brand-Williams W., Cuvelier M.E., Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT, 28, 25-30.

Lepifre S., Baumberger S., Pollet B., Cazaux F., Coqueret X., Lapierre C. 2004. Reactivity of sulphur free alkali lignins within starch films. Ind. Crops Prod., 20, 219-230.

Pouteau C., Dole P., Cathala B., Averous L., Boquillon N. 2003. Antioxidant properties of lignin in polypropylene. Polym. Degrad. stability, 81(1), 9-18.

RADIO-FREQUENCY TECHNOLOGY FOR FRESH STUFFED PASTA PASTEURIZATION/PRE-DRYING PROCESS: PRELIMINARY RESULTS

F. ALBERTI[1], N.C. QUAGLIA[1], L. SPREMULLI[2], A. DAMBROSIO[1], E. TODARO[2], C. TAMBORRINO[1], V. LORUSSO[1] and G.V. CELANO[1]

[1]Department of Health and Animal Welfare,
Faculty of Veterinary Medicine, University of Bari "Aldo Moro"
Strada prov. per Casamassima Km 3, 70010 Valenzano, BA, Italy

[2]STC Science Technology & Consulting,
Via A. Murri 22 Zona Ind., 72023 Mesagne, BR, Italy

ABSTRACT

Radio frequency (RF) applications to food process are well known due to the possibility of quickly and uniformly heating food matrix (1).

Fresh stuffed pasta conventional production technology consists of a first steam pasteurization for a period varying from 2 to 10 min depending on pasta size and weight, initial microbial density and wet steam or superheated steam, followed by a drying phase with forced hot air at a temperature not exceeding 65°-70°C to increase pasta consistency and stabilize the shape.

Fresh semolina pasta RF pasteurization/pre-drying process is already used by some companies a reduction of microbiological parameters, reduced time pasteurization, a better cooking bheavior, keeping g a great intensity taste and aroma the typical of fresh semolina pasta.

Fresh stuffed pasta production has several healthy and technology problems with respect to fresh semolina pasta, thus RF pasteurization/pre-drying process requires a proper implementation and evaluation of effects on different types of products.

In this study we have examined three different types of fresh stuffed pasta, cappelletti stuffed with ham, fagottini stuffed with cheese and tortellini stuffed with meat. Three different RF treatments were carried out: 3 kV were applied to fresh pasta stuffed with ham and meat and 2 kV to fresh pasta stuffed with ricotta cheese for a total time of 10 and 8 min, respectively.

RF plant consists of a single steel tunnel, in which electrodes are placed and kept at 27.12 MHz, were simultaneously pre-drying and pasteurization of fresh pasta occurs (2).

Microbiological analysis of fresh stuffed pasta different samples, pre-and post-pasteurization, were made by the Tempo® System (bioMériux, France).

Preliminary results in different types of fresh stuffed pasta show that RF pasteurization/pre-drying process cause reduction of some microbiological parameters.

Key words: fresh stuffed pasta, pasteurization, pre-drying, radio frequency.

INTRODUCTION

The goals of pasteurization and sterilization are to eliminate pathogenic microorganisms and reduce food spoilage to extend food shelf-life. The availability of water in pasta encourages microbial activity and hence the occurrence of biochemical phenomena that cause alteration of the product until the complete loss of its edibility.

Production and storage techniques of fresh pasta therefore, aim to either reduction or inactivation of microorganisms involved in the biochemical process able to alterate the product.

Radio frequency technique is a viable alternative to existing pasteurization of fresh stuffed pasta as it is able to heat the product quickly allowing a simultaneous drying (3;4).

MATERIALS AND METHODS

Three different types of fresh stuffed pasta using ham, ricotta cheese and meat, were prepared and treatment in the RF plant for pasteurizing and pre-drying.

RF plan is formed by a steel tunnel including some electrodes for the RF voltage application. The tunnel containt two continous module where pre-drying and pasteurization occur simultaneously. In the first modul the steam is also injected in order to facilitate starch gelatinization and reduce the effect of drying excess on pasta.

Table 1 - Operating conditions of the RF oven during the experiment.

Fresh pasta samples	V1 (KV)	V2 (KV)	v (m/min)	T1 (°C)	T2 (°C)	P1 (kW)	P2 (kW)
Ham (cappelletti)	3	3	1,5	70	81	4	5
Meat (tortellini)	3	3	1,5	68	86	4	6
Ricotta cheese (fagottini)	2	2	1,6	80	90	4	3

A voltage of 3 kV for 10 min was applied during the process on the fresh stuffed pasta with ham and meat, and 2 kV for 8 min was used for the ricotta cheese stuffing. In both cases the frequency was 27.12 MHz (5). Temperature for both modules was 90°C and measured humidity was 52% in the first module and 22% in the second.

After the pasteurization/pre-drying process the pasta was cooled in a section closely linked to the RF oven, and prepared in appropriate polymeric food packaging where a modified and protective atmosphere, N 65% + CO_2 35%, was created.

In tab. 1 are reported the operating conditions of the RF oven. V1 and V2 are the voltages applied to the first and second module, respectively, v is the velocity of the conveyor belt on which the pasta is placed, T1 e T2 are the temperatures of the pasta in the first and second module, and P1 and P2 relate the energy absorbed from fresh pasta in the two radiofrequency modules.

Pasteurized and virgin pasta samples were analyzed by using Tempo® System (bioMériux, France) to evaluate total aerobic mesophilic plate count (Tempo TVC, bioMériux, France), total coliform (Tempo TC, bioMériux, France), coagulase-positive staphylococci (Tempo STA, bioMériux, France), yeast and moulds (Tempo YM, bioMériux, France). On the some samples water activity and % moisture measured.

RESULTS AND CONCLUSIONS

In tab. 2 the average values of microbiological parameters mentioned in the previous section and the values of water activity (a_w) and % moisture (RH), are reported

Table 2 - Average microbiological, water activity (a_w) and % moisture (RH) values of fresh unpasteurized stuffed pasta.

Fresh samples pasta	a_w	RH (%)	TVC cfu/g	TC cfu/g	STA cfu/g	YM cfu/g
Ricotta cheese (fagottini)	0.97	32.3	$1.3x10^7$	$6.2x10^4$	$9.1x10^4$	$5.7x10^3$
Meat (tortellini)	0.98	33	$3.8x10^6$	$6x10^4$	$8.7x10^4$	$1.8x10^4$
Ham (cappelletti)	0.97	32	$4.7x10^6$	$1.3x10^4$	$1.7x10^5$	$1.2x10^4$

Table 3 - Average microbiological, water activity (a_w) and % moisture (RH) values of fresh pasteurized stuffed pasta.

Fresh samples pasta	a_w	RH (%)	TVC cfu/g	TC cfu/g	STA cfu/g	YM cfu/g
Ricotta cheese (fagottini)	0.94	27.7	$5.5x10^4$	<10	<10	<100
Meat (tortellini)	0.93	27.15	$6.7x10^3$	<10	<10	<100
Ham (cappelletti)	0.93	27.4	$8.6x10^3$	<10	<10	<100

for unpasteurized fresh stuffed pasta. By comparing the experimental results in tab. 2 and 3, it can be observed that radio frequency process induces a significant reduction of the microbiological parameters.

Additional experimental work is required to standardize the RF technique for predicting an accurate evaluation of shelf-life.

This optimization technique should may allow the preparation of healthy fresh stuffed pasta preserving the original taste together with a strong reduction of production costs.

In conclusion we have experimentally demonstrated the advantage of RF technique in the preparation of fresh stuffed pasta with respect to the conventional technique, in terms of reduction of microbiological parameters. Preliminary results strongly encourage to spend additional efforts to achieve the standardization of RF processing for fresh stuffed pasta.

ACKNOWLEDGEMENTS

Study carried out in collaboration with pasta factory "Soave s.r.l." Via dell'Industria Zona Industriale 72021 Francavilla Fontana (BR) Italy, project POR Puglia 2007-2013: "Application of radiofrequency technology for drying and pasteurization of fresh stuffed pasta".

REFERENCES

(1) Guo Q., Piyasena P., Mittal G.S., Si W., Gong J. 2006. Efficacy of radio frequency cooking in the reduction of Escherichia coli and shelf stability of ground beef. Food Microbiology, 23 (2): 112-118.

(2) STC Science Technology & Consulting patent BR2006A000008.

(3) Wang Y., Wig T.D., Tang J., Hallberg L.M. 2003. Sterilization of Foodstuffs Using Radio Frequency Heating. Journal of Food Science, 68 (2): 539-544.

(4) Piyasena P., Dussault C. 2003. Continuous radio-frequency heating of a model viscous solution: Influence of active current, flow rate, and salt content on temperature rise. Canadian Biosystems Engineering, 45: 327-334.

(5) Wang Y., Wig T.D., Tang J., Hallberg L.M. 2003 Dielectric properties of foods relevant to RF and microwave pasteurization and sterilization Journal of Food Engineering, 57: 257-268.

(6) Orsat V., Vijaya Raghavan G.S. 2005. Radio frequency processing In: Emerging Technologies for food processing, Eds DA-WEN SUN, 445-463.

SESSION III

"Shelf Life Modelling and Prediction"

Chairmen:

D.S. Lee (Kyungnam University, KR)
L. Piergiovanni (University of Milano, I)

ANTIFUNGAL ACTIVITY OF THE ESSENTIAL OIL OF CINNAMON *(CINNAMOMUM ZEYLANICUM)*, OREGANO *(ORIGANUM VULGARE)* AND LAURAMIDE ARGINE ETHYL ESTER (LAE) AGAINST THE MOLD *ASPERGILLUS FLAVUS* CECT 2949

S. MANSO, C. NERÍN and R. GÓMEZ-LUS

I3A, (CPS), Dpto. Química Analítica,
Univ. de Zaragoza, María de Luna 3,
50018 Zaragoza, Spain

ABSTRACT

Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) for oregano EO, cinnamon EO and LAE were determined using a direct-contact antifungical assay by macrodilution method. Cinnamon gave the strongest inhibition followed by oregano and LAE with similar results. LAE showed no antifungal activity in vapor phase, so growth and kill-kinetics were studied by direct contact.

Colony diameter of *Aspergillus flavus* was measured by agar dilution method at different concentrations of cinnamon and oregano EOs, resulting cinnamon EO much more active than oregano EO. In the vapor phase test, the influence of the time of exposure to the cinnamon EO and oregano EO was evaluated, showing relevant differences in their behavior. Kill kinetics showed a different behavior among the three agents.

Key words: *Aspergillus flavus* CECT 2949, cinnamon essential oil, colony radial growth, lauramide argine ethyl ester, oregano essential oil, volatile effect.

INTRODUCTION

During the last decades, prevention of foodborne spoilage has increased the research of new alternatives leaving behind the traditional ones (Quiroga *et al.*, 2009). Many studies in the scientific literature demonstrated the powerful of essential oils as antimicrobial agents (Burt, 2004); (Kalemba and Kunicka, 2003). Their advantages have been tested as antimicrobial packaging where the agents can either interact directly with the spoiled organisms or interact with the environment inside the package (Lopez and Sanchez *et al.*, 2007); (Rodriguez and Nerin *et al.*, 2008); (Nielsen and Rios, 2000).

Antifungal activity of the essential oils of *Cinnamomum zeylanicum* and *Origanum vulgare* and the non volatile agent Lauramide argine ethyl ester (LAE) was evaluated against the mold *Aspergillus flavus* CECT 2949. Many previous works concerning the antimicrobial activity of essential oils have been reported, however, LAE is a recent substance still in investigation (Luchansky and Call *et al.*, 2005) (Porto-Fett and Campano *et al.*, 2010). Due to the scarce knowledge reported in fungi compared to bacteria, different methodologies have been applied to increase information about these shelf-life extenders.

MATERIALS AND METHODS

Microbial culture and growth conditions

Strain of *Aspergillus flavus* CECT 2949 from the Spanish Collection CECT of the University of Valencia, Spain, was used. The fungus was stored at -18°C in sterilized skimmed milk. Fungal conidia were harvested after inoculation on Malt Extract Agar (MEA) for 10 days at 25°C and transferred to a test tube with physiological saline solution (NaCl 0,9%). A final concentration of 10^6 cfu/mL was reached and confirmed by plate counting in culture media.

Active agents

The essential oils of Cinnamon (*Cinnamomum zeylanicum*, CAS 805-91-6) and oregano (*Origanum vulgare*, CAS 8007-11-2) were supplied by Argolide (Spain) and the agent Lauramide arginine ethyl ester (LAE, CAS 60372-77-2) by Lamirsa (Spain).

Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC)

MIC was determined by macrodilution method in yeast extract broth. Serial dilutions of each active agent were prepared (Pinto and Salgueiro *et al.*, 2007). Both essential oils were diluted into etanol (v/v) and LAE into sterilized water (w/v) and mixed into yeast extract broth to reach a range of 1,600-100 ppm final concentration. A 10^6 cfu/mL of *Aspergillus flavus* suspension was added to each sample. Test tubes with a 2 mL of total volume were incubated for 48h at 25°C in continuous shaking. The minimal inhibitory concentration was determined by visual method, as the lowest concentration where no growth was observed. Then, 100 µL of these non-growth concentrations were seeded with a Drigalsky sterile loop into different Petri dishes containing 15 mL of Malt Extract Agar (MEA). After 3-5 days of incubation period at 25°C, minimal fungicidal concentration was determined as the lowest concentration where no colony was developed. This test was carried out three times by duplicate.

Volatile effect of cinnamon and oregano versus time

Lauramide arginine ethyl ester (LAE) did not show any activity in vapor phase, so, cinnamon and oregano were employed as active agents. 100 μL of 10^6 cfu/mL of *Aspergillus flavus* suspension was inoculated into the petri dishes. Then, 5 μL of each essential oil was applied into the cover lid, and the Petri dishes were incubated with the lid upside down (Lopez and Sanchez *et al.*, 2005). Four exposure times were evaluated (24h, 48h, 72h, 96h) and 3 replicates were prepared for each time and each essential oil. So, a total of 24 Petri dishes were carried out in the same way. Two controls without active agent were performed to assure the correct growth of the fungus. Every 24h the covers of 3 replicates of each sample were removed. After 12 days of incubation period at 25°C, inhibition area (cm) was measured.

Colony radial growth

The experiment was carried out in direct contact with the culture media (MEA). The three active substances were used in a range of 1-4% of concentration. Dilutions of essential oils (v/v) were made into ethanol and LAE (w/v) into sterilized water. Three replicates of a total volume of 100 μL were prepared into eppendorf tubes and poured into 15 mL of malt extract agar (MEA) previously liquefied and maintained at 45°C of temperature. The mixture was homogeneously shaken in all directions. Once the medium containing the active agent was solidified, a 10 μL drop of 10^6 cfu/mL fungal suspension was inoculated into the middle of each Petri dish. Controls were performed with addition of ethanol in the culture media instead of active agent. The incubation period at 25°C stopped at 9 days, point where the control reached the border of the dish. Antifungal activity was measured in relation to the colony diameter (cm) developed (Boyraz and Ozcan, 2006).

Kill curves

An initial concentration of 10^5 cfu/mL of fungal suspension was mixed with the minimal fungicidal concentration (MFC) for oregano and LAE. Cinnamon needed a higher concentration, so MFC and two times MFC were tested. The mixture was diluted into yeast extract, placed into 2 mL test tube of total volume and incubated at 25°C in continuous shaking. Different time points were established depending on the active agent evaluated, then, 100 μL of each mixture were inoculated into 15 mL MEA Petri dishes. After 3-5 days of incubation period, fungal colony was counted (cfu/mL).

RESULTS

Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC)

As shown in tab. 1, the lowest concentrations were obtained for cinnamon essential oil, while LAE and Oregano gave the same results.

Table 1 - MIC and MFC of LAE, *cinnamon* and *oregano* EO.

Cinnamon		LAE		Oregano	
MIC (ppm)	MFC (ppm)	MIC (ppm)	MFC (ppm)	MIC (ppm)	MFC (ppm)
100	200	400	800	400	800

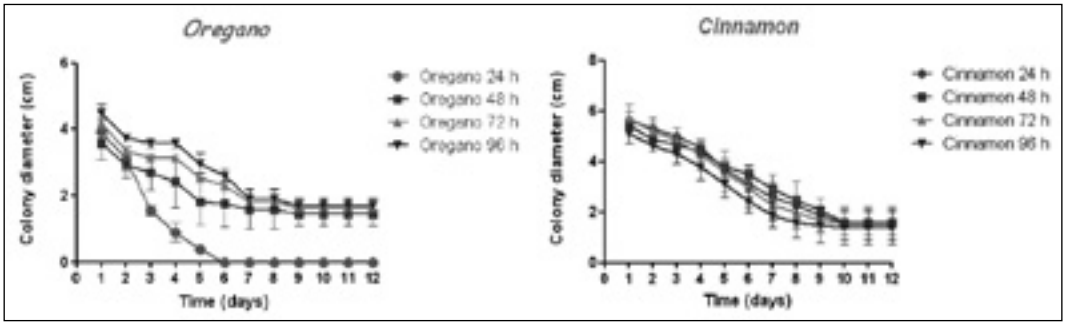


Fig. 1 - Antifungal activity of oregano (1a) and cinnamon (1b) EO versus time.

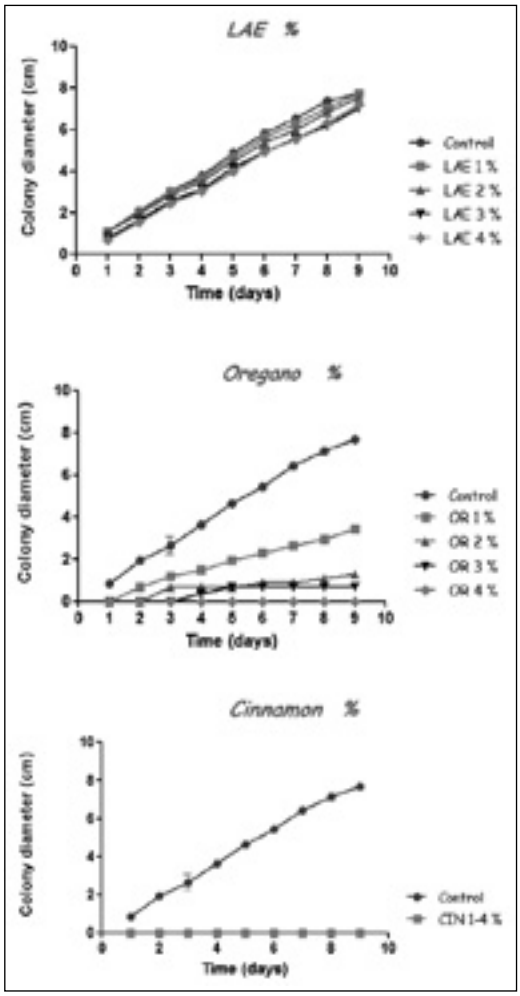


Fig. 2 - Colony diameter (cm) against different concentrations of LAE (fig. 2a), oregano (fig. 2b) and cinnamon (fig. 2c) EO.

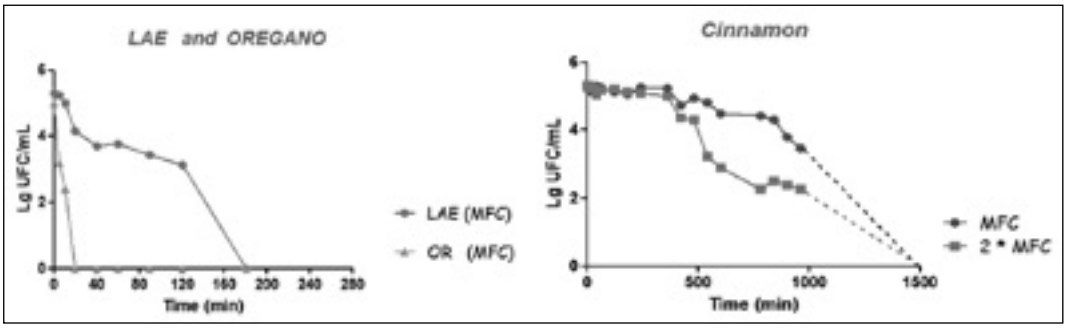
Volatile effect of cinnamon and oregano versus time

On one hand (fig. 1a), oregano's activity manifested a dependence of time of exposure. As we can see, replicates exposed for only 24h showed a faster decrease of the inhibition area, reaching the complete inhibition at sixth day. Not significance differences were obtained between replicates exposed for 48h, 72h and 96h, that is, oregano's antifungal activity against *Aspergillus flavus* in vapor phase needs at least 24h of exposure.

On the other hand (fig. 1b), cinnamon did not show differences between replicates exposed for different times. This behavior could be explained by a faster release of volatile compounds in the first 24h.

Colony radial growth

Colony diameter were used as a parameter of antifungal activity in direct contact with culture media. LAE did not show any activity (fig. 2a), non differences were obtained between control and any concentration. Oregano showed low activity at 1% concentration, however, colony diameter were strongly reduced with higher concentrations, obtaining total inhibition at 4% (fig. 2b). As we can observed, cinnamon was the most active agent in this test since non growth was found at any concentration (fig. 2c).



Kill curves

An initial kinetic study was performed in direct contact with yeast extract broth. Oregano (fig. 3a) evidenced the fastest activity where non colony was counted after 20 min of assay and LAE showed the same result after 3h (fig. 3a). However, cinnamon (fig. 3b) needed longer contact, in about 16h MFC produced a growth reduction of 2 logaritmic units while two times the MFC gave a reduction of 3 units.

CONCLUSIONS

Although both essential oils gave antifungal activity in all experiments, differences between them were found. In particular, the results in vapor phase test showed signs of a faster release of volatile compounds in the case of cinnamon. Stronger activity was also found for cinnamon in MIC and MFC determinations and in the test performed in direct contact with culture media. These results are in correlation with other authors (Lopez and Sanchez *et al.*, 2005; 2007) and are essential to perform the antimicrobial packaging, considering cinnamon essential oil more active than oregano essential oil in the case of fungi.

The advantage of LAE in relation to the essential oils is the fact that organoleptic modifications of products are minimized. However, LAE has not any activity in vapor phase, needing the presence of water to be dissociated and to be able to act, medium where it has a strong activity (Kawamura, 2008). This fact means that the substance requires direct contact with the product and the presence of water. In conclusion, choice of antifungal agent will depend on the kind of food product and package performed.

ACKNOWLEDGMENTS

This work has been financed by the project AGL-2008-04363 from the Spanish Ministry of Science and Innovation and by the European Project Nafispack.

REFERENCES

Boyraz N., Ozcan M. (2006). "Inhibition of phytopathogenic fungi by essential oil, hydrosol, ground material and extract of summer savory (*Satureja hortensis* L.) growing wild in Turkey". International Journal of Food Microbiology 107(3): 238-242.

Burt S. (2004). "Essential oils: their antibacterial properties and potential applications in foods - a review". International Journal of Food Microbiology 94(3): 223-253.

Kalemba D., Kunicka A. (2003). "Antibacterial and antifungal properties of essential oils". Current Medicinal Chemistry 10(10): 813-829.

Kawamura Y., Whitehouse B. (2008). Chemical and Technical Assessment of ethyl lauroyl arginate for the 69th JECFA.

Lopez P., Sanchez C., *et al.* (2005). "Solid- and vapor-phase antimicrobial activities of six essential oils: Susceptibility of selected foodborne bacterial and fungal strains". Journal of Agricultural and Food Chemistry 53(17): 6939-6946.

Lopez P., Sanchez C., *et al.* (2007). "Development of flexible antimicrobial films using essential oils as active agents". Journal of Agricultural and Food Chemistry 55: 8814-8824.

Luchansky J.B., Call J.E., *et al.* (2005). "Viability of *Listeria monocytogenes* on commercially-prepared hams surface treated with acidic calcium sulfate and lauric arginate and stored at 4°C.". Meat Science 71(1): 92-99.

Nielsen P.V., Rios R. (2000). "Inhibition of fungal growth on bread by volatile components from spices and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil". International Journal of Food Microbiology 60(2-3): 219-229.

Pinto E., Salgueiro L.R., *et al.* (2007). *"In vitro* susceptibility of some species of yeasts and filamentous fungi to essential oils of *Salvia officinalis*". Industrial Crops and Products 26(2): 135-141.

Porto-Fett A.C.S., Campano S.G., *et al.* (2010). "Control of *Listeria monocytogenes* on commercially-produced frankfurters prepared with and without potassium lactate and sodium diacetate and surface treated with lauric arginate using the Sprayed Lethality in Container (SLIC (R)) delivery method". Meat Science 85(2): 312-318.

Rodriguez A., Nerin C., *et al.* (2008). "New cinnamon-based active paper packaging against Rhizopusstolonifer food spoilage". Journal of Agricultural and Food Chemistry 56(15): 6364-6369.

DEVELOPMENT AND USE OF MICROBIOLOGICAL SPOILAGE MODELS IN THE FOOD INDUSTRY

L.K. EVERIS* and G.D. BETTS
Campden BRI, Chipping Campden,
Gloucestershire GL55 6LD, UK

*corresponding author: l.everis@campden.co.uk

ABSTRACT

The use of predictive models in the food industry as a quick, easy and inexpensive way of establishing the stability of product formulations and assessment of likely shelf-life is increasing. The development of reliable, fully validated models is therefore of great benefit to the food industry. Campden BRI has produced many models that cover a wide range of spoilage organisms and product commodities.

Key words: food industry, Forecast, kinetic, models, spoilage, time to growth.

INTRODUCTION

Over the past few years the food industry has started to use predictive microbiological models to help in many areas of food manufacture, such as new product development, evaluation of recipe changes and determination of appropriate shelf-life and storage conditions. The type of model required will depend on the food category under consideration.

For perishable, short-shelf-life food products, kinetic growth models that are able to give reliable estimations of lag time and growth rate are most appropriate. In such products, a certain amount of growth of spoilage organisms can be tolerated provided the levels do not exceed any microbiological criteria that have been set. Use of predictive models can ensure that the product formulation or storage conditions chosen will be appropriate to control the growth, so that these criteria are achieved.

For ambient stable products such as acid preserved foods or drinks and fruit based products, it is not the rate of growth which is important but more the ability for growth to be initiated. In these long shelf-life products, the formulation conditions must be designed to prevent any growth throughout life, as once growth begins it is inevitable that the product will spoil. A different modelling approach based on growth/no growth or likelihood of growth occurring is needed for these foods. These models can be used to predict whether spoilage is likely to occur rapidly (within 0-4 weeks), slowly (within 1-6 months) or not at all.

Development of dynamic kinetic growth models for a range of spoilage groups (*Pseudomonas*, Enterobacteriaceae, lactic acid bacteria, *Bacillus*) and for specific products such as fish and meat where a mixed spoilage consortium comprising several genera of organisms are present would be of great benefit for the food industry. Making these models to be dynamic allows fluctuating temperature conditions to be considered. This allows more realistic predictions to be obtained, as it ensures that the temperature profile represents the conditions that products will encounter during distribution and sale. Development of models that allow the likelihood of spoilage of long life ambient products to be predicted can save the food industry valuable time in product development and can save the industry money by reducing both microbial testing costs and product spoilage and hence wastage.

Two of the models produced by Campden BRI, the Enterobacteriaceae model and acid preserved foods spoilage model, will be discussed in further detail.

MATERIALS AND METHODS

Suitable microbiological broth media were chosen depending upon the model to be produced and the microorganisms to be used, for example Tryptone Soya Broth (TSB, Oxoid CM 0129) for Enterobacteriaceae modelling or de Mann Rogosa Sharpe Broth (MRSB, Lab M, lab 94) for modelling cocktails of spoilage yeasts, moulds and lactics. The relevant amount of salt or sugar was then added to the base medium to give the required concentration. The pH of the broth was adjusted using hydrochloric acid or sodium hydroxide and preservatives were added where relevant.

Once prepared, these broths were inoculated with a cocktail of organisms grown to late exponential phase. The microorganisms used were appropriate to the spoilage group or product type under consideration. For the Enterobacteriaceae model the following organisms were used: *Proteus mirabilis* (CRA 615), *Klebsiella pneumoniae* (CRA 1483), *Citrobacter freundii* (CRA 3777), *Enterobacter cloacae* (CRA 4933) and *Hafnia alvei* (CRA 4936).The organisms were inoculated at a level of 10^2-10^3 cfu/mL and these were enumerated over time using standard microbiological procedures. This data was modelled using the Baranyi parameterisation of the four-parameter Gompertz model and a quadratic response surface model in a single global fitting approach. The single fitting approach does not require complete curves to be generated, which means that it is more data efficient.

For spoilage organisms of relevance to cold filled acidified foods the following organisms were used: yeasts: *Pichia membranefaciens* (VYAPi 01-02) and *Zygosaccharomyces bailli* (VYASa 07-01), moulds: *Monascus ruber* (VMEuMo 01-02), *Penicillium roqueforti* (VMMope 16-07) and *Penicillium verrucosum* (VMMope 20-07) and lactic acid bacteria: *Lactobacillus buchneri* (VBLLa 18-01). In

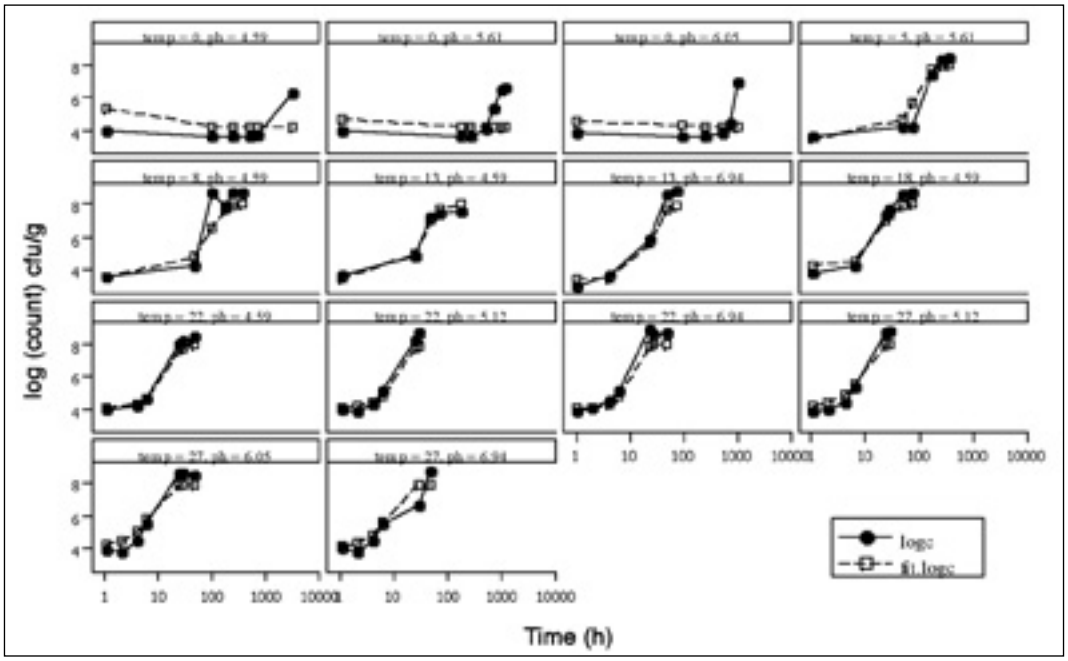


Fig. 1 - Growth curves at salt = 1.0%.

this case, the organisms were inoculated at a level of approximately 10^5 cfu/mL, and the time for the broths to show turbidity as a sign of growth was assessed. A different type of modelling technique was used to develop the models for the acidified foods as no growth curves were generated (Everis and Betts, 1999). Due to the increased number of parameters involved, i.e. pH, a_w, salt, sorbate and benzoate, the possible number of combinations required was large. A matrix of 1306 conditions was used to produce the cold fill spoilage model and the time to growth data was fitted using a Classification and Regression Trees approach (CART). This CART model is purely descriptive, working with classes or categories rather than with actual times to growth. So, for the purpose of fitting a CART model, times to growth were categorised as: under 15 days, under 1 month, under 3 months, under 5 months and over 5 months, i.e. no growth. The application of this technique is carried out by a computer program and the output is a logical classification tree. Each branch of the tree corresponds to a test and a decision as to whether a certain factor is above or below a critical value, calculated by the program. The tree provides a complete description of the classification scheme. The program places restrictions on the number of branches and end-point nodes so that the tree becomes manageable. It is then a question of applying the set of rules defining the tree to a new set of data, to see whether the rules still hold.

All Campden BRI spoilage models are fully food validated. Relevant foodstuffs were inoculated with the organisms used in the production of the model and were stored at the appropriate temperatures. Either the level of organisms present or the turbidity and changes in physical appearance were then noted. The data generated from these studies were then compared to the broth data.

Table 1 - Results for cold fill spoilage organisms in terms of predictions of growth/no growth in 270 days.

Trial result	No. of trials	Predicted	Not predicted
Growth	347	339 (97.7%)	8 (2.3%)
No growth	998	790 (79%)	208 (21%)

Days	1 to 14	15 to 30	31 to 60	61 to 270	Nogrowth
N. of broths exhibiting growth	190	60	54	43	998
N. predicted correctly	158 (83%)	40 (67%)	29 (54%)	38 (88%)	790(79%)
N. incorrectly predicted	32 (17%)	20 (33%)	25 (46%)	5 (12%)	208(21%)

Table 2 - Spoilage models available in Forecast.

Model	Temperature (°C)	NaCl (% aq)	Equivalent a_w	pH	Other Conditions
	Kinetic models:				
Pseudomonas	0-15	0.0-4.0	1.00-0.977	5.5-7.0	Fluctuating temperature, pH, salt
Bacillus spp.	5-25	0.5-10	0.997-0.935	4.0-7.0	Fluctuating temperature, pH, salt
Enterobacteriaceae	0-27	0.5-10	0.997-0.935	4.0-7.0	Fluctuating temperature, pH, salt
Yeasts (chilled foods)	0-22	0.5-10	0.997-0.935	2.6-6.0	Fluctuating temperature, pH, salt
Lactic acid bacteria	2-30	0.5-10	0.997-0.935	3.0-6.0	Fluctuating temperature
Meat spoilage	2-22	0-6	1.00-0.964	4.6-7.0	0-240 KNO_2 (ppm) Fluctuating temperature, pH, salt
Fish spoilage	2-22	0-6	1.00-0.964	4.5-8.0	Fluctuating temperature, pH, salt
Fresh produce TVC	2-25	-	-	-	
Fresh produce Enterobacteriaceae	2-25	-	-	-	
Fresh produce lactic acid bacteria	2-25	-	-	-	
Fresh produce *Pseudomonas*	2-25	-	-	-	
	Time to growth models				
Yeasts (fruit/drinks)	0-22	-		2.0-7.0	0-60% Sucrose (w/v) 0-20% Ethanol (v/v) Potassium sorbate 0-1,000 (ppm)
Bacillus	8-45	0.5-10	0.997-0.935	4.0-7.0	
Cold fill spoilage (yeasts, moulds, lactics)	25	0.5-18	0.85-1.00	2.8-5.0	Benzoate Sorbate 0-2000 (in total)
Cold fill pathogens *(E. coli, S. aureus, Salmonella)*	25	0.5-16	0.87-1.00	3.9-5.0	Benzoate Sorbate 0-2,000 (in total)
Hot fill spoilage *B. polymyxa* *B. coagulans* *B. cereus* *C. tyrobutyricum* *C. pasteurianum* *C. butyricum*	25	0.5-18	0.86-1.00	3.7-5.2	Benzoate Sorbate 0-2,000 (in total)
	Thermal death model				
Enterobacteriaceae	52 to 64	0-8	1.00-0.95	4.0-7.0	Predicts D value

RESULTS AND DISCUSSION

For the Enterobacteriaceae kinetic growth model there was a good fit to the data, with a residual square (R^2) value of 80% and a root mean square value (rms) residual error of 0.86 $\log_{10}$ (count). There was good agreement between fitted and observed $\log_{10}$ (counts) and this is illustrated graphically in fig. 1 for the 1.0% salt conditions (Everis and Betts, 2008).

This CART model fitted the cold fill spoilage data well, with 84% of the data predicted correctly overall. It was better at predicting where growth occurred, predicting 98% of them correctly whereas only 78% of the no growth situations were correctly modelled. (tab. 1). The full list of spoilage models available at Campden BRI for industrial applications is given in tab. 2.

CONCLUSIONS

The spoilage models described here cover a wide range of spoilage organisms, groups of organisms and product types. They are suitable for use with a wide range of product types including chilled foods, meats, fish, drinks, ambient stable acidified foods and fresh produce. These models are widely used to aid the food industry with developing stable product formulations, assessing potential shelf-life and in trouble shooting when there are deviations with parameters such as pH, salt or temperature of storage.

REFERENCES

Everis L. and Betts G. (1999). Unpublished. The final report of the acid preservation club. Campden BRI, Chipping Campden, Glos. UK.

Everis L. and Betts G. (2008). Modelling the increase and growth of spoilage microorganisms in food: Production of an Enterobacteriaceae model for predictions under fluctuating conditions. Campden BRI, Chipping Campden, Glos. UK R&D Report N. 269.

SURVIVAL ANALYSIS APPLIED TO SENSORY SHELF-LIFE PREDICTION OF READY TO EAT VEGETABLE PRODUCTS

B. ALFARO[1]*, M. NUIN[1] and B. IBAÑEZ[2]

[1]AZTI-Tecnalia, Food Research Divison,
Parque Tecnológico de Bizkaia, Asontodo Bidea,
Edf. 609, 48160 Derio, Bizkaia, Spain

[2]Fundación Vasca de Innovación
e Investigación Sanitarias (BIOEF), Sondika Bizkaia, Spain
*corresponding author: balfaro@azti.es

ABSTRACT

The purpose of this study was to estimate and compare the sensory shelf-life of eight types of ready-to-eat (RTE) heat stable vegetable products using different methodologies in sensory analysis. Sample data compromise 81 consumers who tested eight types of vegetable meals packed in returnable plastic containers stored at 23° and 4°C with six different storage times for each temperature. The methodologies used were acceptability scoring and the evaluation of consumer rejection by survival analysis (Turnbull nonparametric estimation and Weibull Accelerated Failure Time, AFT). Results showed that the shelf-life estimated for the overall products at 23°C by Weibull distribution for probability of product failure of 50% was 32.6 months ($CI_{95\%}$ 30.9-34.5), which is about two years and a half. In addition, differences in shelf-life among the eight products were also found. In conclusion, sensory shelf-life estimation using different methodologies may provide valuable information regarding the quality of a food product.

Key words: ready to eat food, sensory evaluation, shelf-life prediction, survival analysis.

INTRODUCTION

Nowadays convenience food plays an increasing important role in the food market. Food products not affected by microbiological spoilage, such as RTE heat stable foods, have a shelf-life limited by changes in sensory properties eventually, making

the product unacceptable for consumers. Therefore, sensory evaluation techniques are necessary for the optimal shelf-life prediction of microbiologically stable products (Hough *et al.*, 2003). Shelf-life refers to the end of consumer quality, and it is the time at which a percentage of consumers are displeased with the product. Several statistical approaches have been proposed in the literature focused on shelf-life estimation by sensory analyses. They have been based on sensory scores using trained panellists (Fritsch *et al.,* 1997) or untrained consumers (Meilgaard *et al.*, 1991), while others work with consumer acceptance/rejection responses (Cardelli *et al.*, 2001). Survival analysis, such as that based on the so-called Weibull model, was introduced to the sensory shelf-life studies by Gacula and Sing (1984). This methodological approach models the time until the refusal of the product by a consumer is observed, and both shelf-life estimates as well as comparisons among shelf-life of different products can be derived.

The first aim of this study was, using survival analysis, to estimate the sensory shelf-life of eight types of new ready meals vegetable products, stored at room temperature or refrigerated. The second objective was to compare different methodologies in sensory analysis: acceptability scoring versus consumer rejection by survival analysis.

MATERIALS AND METHODS

Eight RTE vegetable meals were studied: Artichokes with cured ham, Beans, Chard with potatoes, Thistle with almonds, Carrot cream soup, Peas with cured ham, Green beans, and Vegetable stew packed in returnable plastic containers and heat treated.

Samples of the products were stored at room temperature (23°C) and refrigeration (4°C) during the period of the experiment; using trays stored in refrigeration as reference (control). Sample data compromise 81 consumers who tested eight types of vegetable meals stored at 23° and 4°C with six different storage times for each temperature. About fifty consumers were recruited for each sampling times except for the first two sampling, when thirty and forty were recruited, respectively. For each sample, consumers had to taste it and answer the question: "Would you normally consume this product? Yes or No?" Additionally, the consumer panel evaluated the overall acceptance of the eight RTE food products using a 9-point hedonic scale, with 1=extremely dislike, 5=neither like nor dislike, and 9=extremely like. Every consumer assessed one sample of each of the eight products at each sampling time. Blind control samples (4°C) were also presented at each sensory analysis to some of the panellists.

For the analysis of the overall quality score the repeated measures modelling approach within the mixed modelling framework was employed. Also the storage time and the effect of the room temperature products with respect to control samples (4°C) where therefore assessed. The same methodology was used to analyze the evolution by product at each temperature, and to study possible product preferences among consumers.

To estimate and compare the shelf-life of the products, two different methods based on survival analysis were used (Turnbull nonparametric estimation and Weibull Accelerated Failure Time, AFT). Shelf-life was estimated as the time at which 50% of consumers found the product unacceptable. All analyses were performed using the freely available statistical software R 2.5.1 and a significance level of α=0.05.

RESULTS AND CONCLUSIONS

Results showed that the storage time in samples stored at room temperature (23°C) caused a significant decrease on the consumer's opinion (p<0.001) with mean scores ranging from 6.30 in the first sampling time to 5.30 in the last sensory test (fig. 1). In contrast, the decrease observed for the control group (4°C) was not found significant (p=0.898).

The shelf-life estimated for overall products at 23°C by Weibull distribution and Turnbull was similar being 32.6 months ($CI_{95\%}$ 30.9-34.5 according to the Weibull model and 32 months for the Turnbull method (tab. 1). The shelf-life estimated for Product 1 at 23°C by Weibull distribution for probability of product failure of 50% was 37.3 months ($CI_{95\%}$ 31.8-43.8),

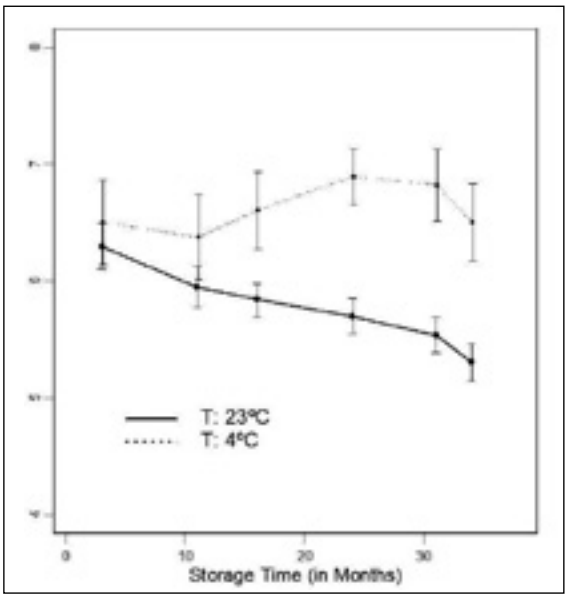


Fig. 1 - Acceptability scores of vegetable products stored at 23° and 4°C for increasing time (in months). Points represent mean values of determination.

Table 1 - Shelf-life (in months) for ready to eat vegetables products stored at 23° and 4°C estimated using non-parametric estimation (Turnbull) and the parametric model (Weibull distribution) for a 25 and 50% consumer rejection.

	Shelf-life (months) Consumer's rejection ($CI_{95\%}$)[a]			
	Non-parametric (Turbull)		Parametric model (Weibull)	
	for 25%	for 50%	for 25%	for 50%
Model I				
All products, 23°C	22	32	21.4(19.5,23.4)	32.6(30.9,34.5)
All controls, 4°C	>33	>33	55.3(37.7,81.3)	84.5(56.5,126.5)
Model II				
By product 23°C				
Product 1	31	>33	25.5(21.5,30.2)	37.3(31.8,43.8)
Product 2	15	29	16.1(13.7,18.9)	23.6(20.8,26.7)
Product 3	33	>33	21.7(18.5,25.4)	31.7(27.5,36.6)
Product 4	22	29	18.2(15.5,21.3)	26.6(23.3,30.3)
Product 5	30	>33	26.2(22.1,31.1)	38.4(32.4,45.4)
Product 6	22	29	16.4(14.0,19.2)	24.0(21.1,27.3)
Product 7	34	>34	31.7(26.2,38.3)	46.4(38.2,56.3)
Product 8	29	>33	28.5(23.5,34.4)	41.6(34.4,50.5)

[a] Confidence interval expressed as a percentage of a 95%.

which is about three years. When the probability of Product 1 failure of 25% was considered, shelf-life estimation was 25.5 months ($CI_{95\%}$ 21.5, 30.2), which is about two years. Significant differences between products were observed due to their different formulation.

The shelf-life estimate can be highly affected by the cut-off point chosen by the researcher or the food company (25%, 50%).In conclusion, shelf-life estimations using survival methods, together with the analysis of the degree of consumers' acceptance via repeated measures methods, may provide valuable information regarding the quality and shelf-life of a food product.

ACKNOWLEDGMENTS

Authors would like to thank the Spanish food company Conservas Hijos de M. Sánchez Basarte, S.A. (Gutarra) for the partially financial support of this research.

REFERENCES

Cardelli C., Labuza T.P. 2001. Application of Weibull hazard analysis to the determination of the shelf-life of roasted and ground coffee. Lebensm.-Wiss.u.-Technol., 34: 273.

Fritsch C.W., Hofland C.N., Vickers Z.M. 1997. Shelf-life of sunflower kernels. J. Food Sci., 62: 425.

Gacula M.C., Singh J.J. 1984 Shelf-life testing experiments. In Statistical methods in food and consumer research. Nueva York: Academic Press.

Hough G., Langohr K., Gómez G., Curia A. 2003. Survival analysis applied to sensory shelf-life of foods. J. Food Sci., 68: 359.

Meilgaard M., Civille G.V., Carr B.T. 1991. Sensory evaluation techniques. CRC Press, London.

SHELF-LIFE OF BLUE MOLDED CHEESES PACKAGED IN POLYLACTIC ACID TRAYS

P. FAVA* and A. MUSETTI

Department of Agricultural and Food Science,
University of Modena and Reggio Emilia,
Via Amendola 2, pad. Besta, 42122 Reggio Emilia, Italy

*corresponding author: patrizia.fava@unimore.it

ABSTRACT

The shelf-life of hard and semi-hard cheeses is usually prolonged by means modified atmosphere packaging in very high barrier materials, in order to prevent as long as possible the interactions between cheese and oxygen, responsible for microbiological and oxidative decay. However, the complete exclusion of oxygen into the headspace of packages containing "respiring cheeses" may be detrimental for their right and prolonged preservation. In this work the possible use of poly(lactic acid) (PLA) for the packaging of Gorgonzola cheese will be discussed. A predictive simple model was used to foresee the atmosphere evolution inside the PLA trays, as a function of the cheese respiration rates and the package OTR. According to the result obtained, slices of Gorgonzola were packaged in the selected PLA trays and stored at 4°C for a month. The analytical controls performed at fixed interval of time (headspace composition, pH, color, weight loss) demonstrated that the use of PLA allows a good refrigerated shelf-life of Gorgonzola up to 4 weeks.

Key word: shelf-life, mold ripened cheeses, PLA, passive modified atmosphere.

INTRODUCTION

Poly(lactic acid) (PLA) is a biodegradable thermoplastic polyester with excellent functional properties (mechanical, optical, flexibility and long term stability) that enable its industrial application. However, PLA doesn't possess high barrier against oxygen and water vapor permeation (Lehermeier *et al.*, 2001) Those are recognized

to be the most important limiting factors affecting the shelf-life of many packaging-dependent food products sensitive to oxidative processes that usually require modified atmosphere packaging (MAP) to provide proper protection. Some efforts to overcome these drawbacks have been studied (Iotti *et al.*, 2009), but at present this packaging material has proved to be suitable only for packaging of foods with relatively short shelf lives (Haugaard *et al.*, 2002; 2003). However, it is well known (Piergiovanni *et al.*, 1993; 1999) that for a lot food products the complete exclusion of oxygen into the packages headspace may be detrimental for their right and prolonged preservation. Cheeses like Italian Gorgonzola need to respire in order to maintain the right metabolism of the molds present into the paste, but the excess of oxygen may contribute to the reduction of the shelf-life length In this work the possible use of poly(lactic acid) (PLA) for the extension of Gorgonzola cheeses shelf-life will be discussed.

MATERIALS AND METHODS

Packaging and storage of cheese

Ripened Gorgonzola was supplied by a single producer (Emilio Mauri SpA, Pasturo, Italy). The cheeses were portioned and packaged in thermoformed, transparent PLA trays, closed with a lid made of 30 μm PLA film (Biophan, Treofan Germany GmbH & Co, Raunheim, Germany). The gases transmission (TR) rates of the PLA lidded trays were: O_2TR 17 cm^3 day^{-1} (air, 4°C), CO_2TR 45 cm^3 day^{-1} (air, 4°C). For comparison purposes, a medium high barrier package used by Mauri (technical data not allowable) was used. The cheeses in PLA were packaged in a MA consisting of 100% nitrogen. The packaged were stored in a cabinet, in dark conditions, at a temperature of 4±1°C.

Headspace gas composition/Packages gas transmission rates

Headspace gas composition, expressed as % oxygen and % carbon dioxide was determined by gaschromatographic analysis, sampling 50 μL of inner atmosphere through a septum placed on the packages. A GC-HWD (GC 320 – GL Sciences) equipped with a CTR I column (Alltech SpA, Milan, Italy) was used. Chromatographic condition: column and injector temperature 55°C, detector temperature 120°C, carrier gas Helium (100 kPa). The packages gas transmission rates (O_2TR and CO_2TR) were determined by a permeability fractometer GPM500 (Lyssy, Zollikon, Switzerland) equipped for the container permeability measurements and connected with the same GC system described above.

Color

The cheese color was evaluated by means a Minolta Chromameter serie CR-400 (Konica Minolta Sensing Inc.), determining L*,a*,b* of the CIE Lab color space.

Weight loss

Weight loss of cheeses was evaluated by gravimetric measurements of the entire packages and expressing the results as percentage decrease against storage time.

Chemical analysis

Chemical analysis and characterization of the cheese were performed according to Italian Official Methods for cheese analysis.

RESULTS

Experimental trials have been carried out to study the respiration needing of Gorgonzola and to establish if the O_2 and CO_2 transmission rate of PLA trays were apt to guarantee the right product oxygenation and also the creation of an equilibrium atmosphere inside the package with a CO_2 percentage ranged between 10 and 15%, condition which demonstrated to avoid an excessive microbial proliferation into the product (data not shown). A predictive simple model (Piergiovanni *et al.*, 1993) was used to foresee the atmosphere evolution inside the PLA trays, as a function of the cheese respiration rates and the package OTR. The results obtained are compared with those experimentally determined by the headspace analyses carried on the same trays but containing the cheese, as shown in fig. 1. This comparison demonstrates that it is possible, knowing the particular food products metabolism and the diffusional properties of a package, to foresee the gases evolution inside the package itself and also select of the best packaging material for specific food packaging application. The gases evolution inside the PLA trays containing Gorgonzola is shown in fig. 2. These data demonstrated that Gorgonzola actively interacts with the gaseous atmosphere, consuming the oxygen which permeates daily inside the packages and which percentage was very low and constant) and contributing to create an adequate CO_2 partial pressure, which slowed the molds proliferation inside the cheese, maintaining however their typical blue-green color. In fact, ones of the most important quality attributes of a food products are the color and the appearance and for this reason the results obtained from the instrumental color determination are shown. In fig. 3 the evolution

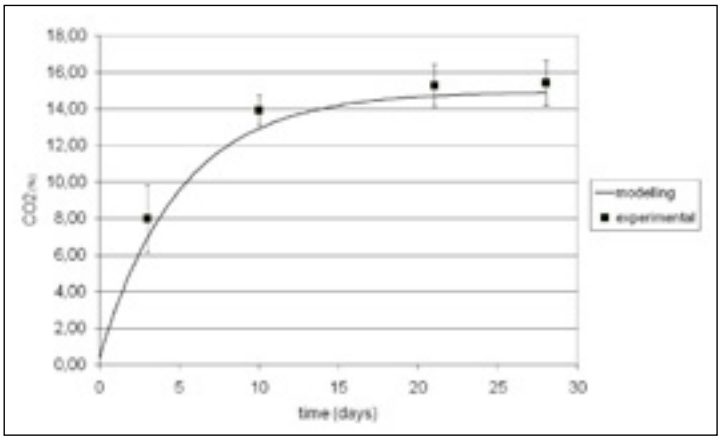


Fig. 1 - Comparison between experimental CO_2 evolution inside PLA trays containing Gorgonzola cheese (■) and theoretical CO_2 evolution as resulted by the application of a predictive model (continuous line).

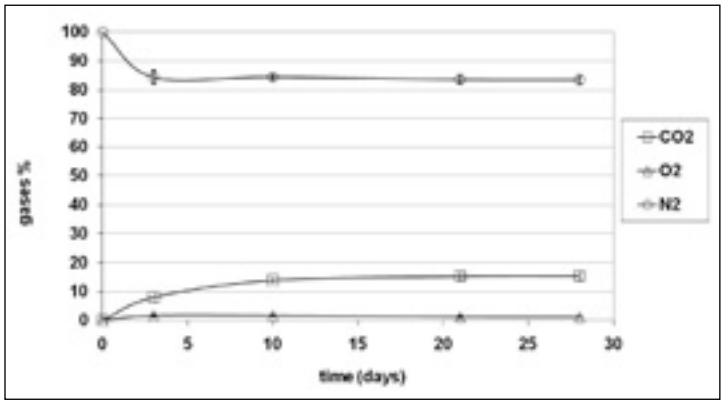


Fig. 2 - Atmosphere evolution inside PLA trays containing Gorgonzola, stored at 4°C.

of L*,a*,b* parameters measured on the Gorgonzola surface are reported. It is well clear that no significant difference was determined along the storage, due to lipid oxidation or to a dryness of the surface, considering the poor water vapor barrier of PLA. Nevertheless this negative performance of the biomaterial used in the present experimental work, the weight loss of Gorgonzola packages at the end of storage was around 0.8-1.0%, a value considered acceptable from the commercial point of view.

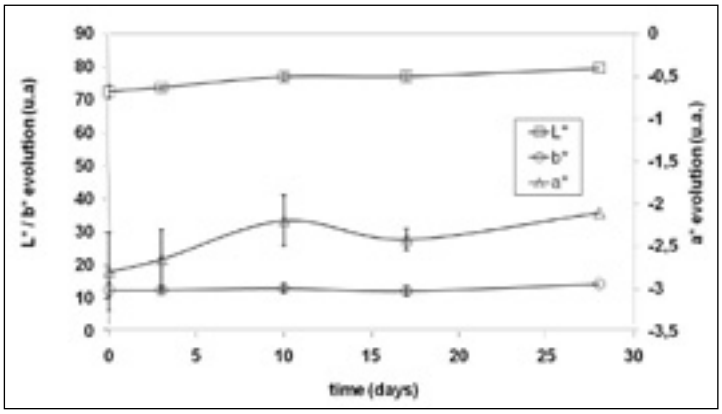


Fig. 3 - Color evolution of Gorgonzola during refrigerated storage in PLA trays.

The evolution of chemical indexes determined on Gorgonzola, in particular those related to molds sustained proteolysis, was negligible and for this reason these data are not commented.

CONCLUSION

The results of storage experiment indicate that PLA packages can be used for storage of Gorgonzola cheese for a period of at least 4 weeks, with limited and not significant modification of its chemical, microbiological and sensorial profile. It can be concluded that the right selection of the PLA packages dimensions and shape, combined with an initial MA containing only 100% of nitrogen, could be a valid alternative to traditional paper wrapping of the mold ripened cheeses, but also to packages made with plastic polymers, contributing to reduce the impact of food packages on the environment.

REFERENCES

Haugaard V.K., Weber, C.L., Danielsen B., Bertelsen G. 2002. Quality changes in orange jiuce packaged in materials based on polylactate (PLA). Eur. Food Res. Technol., 214:423.

Haugaard V.K., Danielsen B., Bertelsen G. 2003. Impact of polylactate and poly(hydroxybutyrate) on food quality. Eur. Food Res. Technol., 216:233.

Iotti M., Fabbri P., Messori M., Pilati F., Fava P. 2009. Organic-Inorganic Hybrid Coatings for the Modification of Barrier Properties of Poly(lactic acid) Films for Food Packaging Applications. J. Polym. Environ., 17:10.

Lehermeier H.J., Dorgan J.R., Way J.D. 2001. Gas permeation properties of poly(lactic acid). J. Memb. Sci., 190:243.

Piergiovanni L., Fava P., Moro M. 1993. Shelf-life extension of Taleggio cheese by modified atmosphere packaging. Ital. J. Food Sci., 5(2):115.

Piergiovanni L., Fava P., Ceriani S. 1999. A simplified procedure to determine the respiration rate of minimally processed vegetables in flexible permeable packaging. 11(2):99.

ESTIMATION OF SHELF-LIFE OF HOT DOG SAUSAGE

PATRÍCIA MOISES DAVILA BATTISTELLA[1], PAULO ROGÉRIO FRANCHIN[2], PAULO JOSÉ OGLIARI[1], CLEIDE ROSANA VIEIRA BATISTA[1]

[1]Federal University of Santa Catarina
[2]BRF Brasil Foods SA

ABSTRACT

This study aimed to determine shelf-life of vacuum packed hot dog sausages stored at 2°, 6.5°, 15° and 21°C. Besides, it also aimed to identify the main causes of spoilage and to apply survival analysis for evaluating the results. End of shelf-life was determined when lactic bacteria counts reached 10^7 cfu/g with viscosity formation, harming the appearance of sausages. Contamination by such bacteria occurred due to manipulation after thermal treatment during packing process and, also, due to thermo tolerance of these microorganisms. Samples were, then, exposed to different temperatures and monitored at different periods. Packs considered unsuitable for consumption according to microbiological evaluation, combined with sensorial evaluation were considered as "fail" and suitable packs were considered "censored". With survival analysis it was possible to establish a shelf-life of 43 days for sausages kept at 2°C, 21 days for sausages kept at 6.5°C, 5 days for sausage kept at 15°C and 2.6 days for sausage kept at 21°C. So, it can be concluded that lactic bacteria are the main cause of sausage deterioration and that survival analysis can be used for shelf-life determination of food products.

Key words: lactic bacteria, shelf-life, survival analysis.

INTRODUCTION

Industrialized products are more and more practical, ready to eat, being the only work to open the pack. Besides practicality, industrialized food products also present a larger shelf-life than raw and not processed food products, making storage easier.

Shelf-life is an important attribute of food products. It can be defined as the

time since production and packing until it is no more viable for human consuming. So, shelf-life is related to total quality of food products and directly linked to production, projection, ingredients characteristics, handling process and storage. Besides, shelf-life depends on type of food, being essential that manufacturers identify intrinsic and extrinsic parameters that influence this period.

Prediction of shelf-life is not an easy task, being the result not always accurate. However, it is very important to obtain maximum information about food product under analysis once it will allow a more precise shelf-life estimation and guiding regarding to the more adequate conditions for conserving such food product.

Microbiological analysis of total plate count and lactic bacteria count, physicochemical analysis of pH and sensorial analysis were performed in order to evaluate the spoilage process that occurs during storage. The present work aimed to estimate shelf-life of vacuum packed hot dog sausages stored at 2°, 6.5°, 15° and 21°C using survival analysis.

MATERIALS AND METHODS

Sampling

The present study was performed with 135 samples of sausage collected immediately after packing. Each sample represents 1 (one) vacuum-packed pack containing 1 kg of product. The packs were used for shelf-life, being stored in refrigerator with controlled temperature at 2°, 6.5°, 15°, and 21°C. Visual inspections were periodically performed in order to detect viscosity inside the packs. Microbiological, physicochemical and sensorial analyses were carried out according to the pre-determined time intervals.

Samples presenting excessive viscosity or microbiological countings higher than 10^7 cfu/g were considered unsuitable for consumption. These samples were labeled as "*fail*". Samples considered suitable for consumption were labeled as "*censor*" for statistical analysis.

Laboratorial Analyses

Following microbiological analysis were performed for each sample: total plate count (PCA – Plate Count Agar, Merk, Germany) and lactic bacteria count (MRS – Lactobacilli MRS Agar, Acumedia, Michigan). pH and presence of viscosity were also determined in each sample.

Statistical Analyses

Statistical analyzes were performed using Statistica 7.1 software. Survival analysis and Kaplan Meier estimator were used for evaluating of shelf-life.

RESULTS AND CONCLUSIONS

Samples were analyzed in determined time periods to detect the main microbiological, physicochemical and sensorial parameters involved in spoilage process during storage.

As it can be noticed by results, values tend to present the same behavior for all temperatures. Total plate counts, lactic bacteria counts and pH showed significant variation concerning storage time.

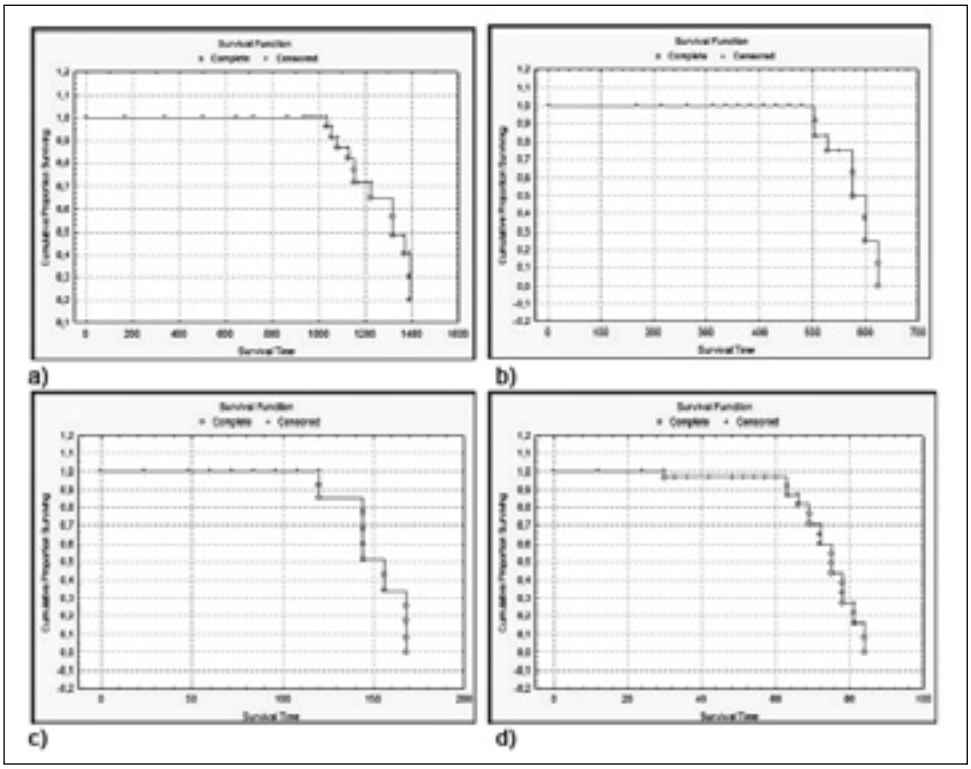


Fig. 1 - Survival plots of sausages stored at 2°C for 1,392 hours (a): 6.5°C for 642 hours (b); 15°C for 168 hours (c) and 21°C for 84 hours (d).

These results suggest that total plate counts are due to multiplication of lactic bacteria once values tended to present the same behavior. Lactic bacteria naturally inhabit the environment of cooked and refrigerated meat products packed under anaerobic conditions, as reported by Noskowa (1978), Giannuzzi *et al.* (1998), Sakala *et al.* (2002), Blickstad *et al.* (1983), Von Holy *et al.* (1991) and Ferreira (2004).

According to Noskowa (1978), multiplication of lactic bacteria is due to the high carbohydrate content present in sausages. Such microorganisms present an irregular behavior regarding to its heat stability. By this way, if Noskowa's approach would consider, it is possible that lactic acid bacteria survived to the process.

Cayré *et al.* (2005), also report such behavior, reinforcing the possibility of survival of these microorganisms to the cooking process due to the heat tolerance, besides being tolerant to NaCl, nitrite and cure process. Results obtained in the present work are in agreement with the findings presented by the cited authors and explain the post-processing multiplication during storage of sausages.

Also, it was observed a decreasing in pH values for both tests. According to Borch *et al.* (1996) and Metaxopoulus *et al.* (2002) lactic or acetic acid production by lactic acid bacteria explains such decreasing, observed in samples during storage.

The observed viscosity occurs due to the acid production by bacteria. Such viscosity that occurs out of products is positive in wet surfaces, typical environment of sausage packs. This occurrence makes evident an unpleasant aspect of

the product to the consumer becoming a rejection parameter. These results are in agreement with Borch *et al.* (1996).

The present work allow to clarify that it is possible to apply the survival analysis for studying shelf-life of food products, being possible to verify the probability of consumer to reject a product after a period of storage.

The fig. 1 shows product stored at 2°C, shelf-life is approximately 1,032 hours (43 days), product stored at 6.5°C, shelf-life is approximately 504 hours (21 days), product stored at 15°C, shelf-life is approximately 120 hours (5 days), product stored at 21°C, shelf-life is approximately 30 hours (1.25 days). However, it can be concluded that the statistical method used in the present work can provide storage options and probabilities data for survival of products presented to the consumers. It also allows to evaluate and to determine the real shelf-life of products that for some reason were stored at adverse conditions.

REFERENCES

Blickstad E., Molin G. 1983. The microbial flora of smoked pork loin and frankfurter sausage stored in different atmospheres at 4ºC. J. Appl. Bacteriol. 54: 45-56.

Borch E., Kant-Muermans M.L., Blixt Y. 1996. Bacterial spoilage of meat products. Int. J. Food Microbiol. 33: 103-120.

Cayré M.E., Garro O., Vignolo G. 2005. Effect of storage temperature and gas permeability of packing film on the growth of Lactic Acid Bacteria and *Brochothrix thermosphacta* in cooked meat emulsions. Food Microbiology. 22: 505-512.

Ferreira L.D. 2004. Utilização da microbiologia preditiva na avaliação do crescimento de bactérias ácido láticas em presunto fatiado Sc. D. Thesis. Federal University of Santa Catarina. Florianópolis.

Giannuzzi L., Pinotti A., Zaritzky N. 1998. Mathematical modelling of microbial growth in packaged refrigerated beef storage at different temperature. J. Food Microbiol. 39: 101-110.

Metaxopoulos J., Mataragas M., Drosinos E.H. 2002. Microbial interaction in cooked cured meat products under vacuum or modified atmosphere at 4ºC. J. Appl. Microbiol. 93: 363-373.

Noskowa G.L. 1978. Microbiologia de las Carnes Conservadas por el Frio. Editorial Acribia S.A., Zaragoza-España.

Sakala R.M., Hayashidani H., Kato Y., Hirata T., Makino Y., Fukushima A., Yamada T., Kaneuchi C., Ogawa M. 2002. Change in the composition of the microflora on vaccum-packaged beef during chiller storage. Int. J. Food Microbiol. 74: 87-99.

Von Holy A., Cloete T.E., Dykes D.A. 1991. Quantification and characterization of microbial populations associated with spoiled, vacuum-packed vienna sausages. J. Food Microbiol. 8: 95-104.

ANTIMICROBIAL ACTIVITY OF DECAFFEINATED GREEN TEA EXTRACT, GREEN TEA ANTIOXIDANT EXTRACT AND LAURAMIDE ARGINE ETHYL ESTER

R. BECERRIL[1], R. GÓMEZ-LUS[2] and C. NERÍN[1]*

[1]Universidad de Zaragoza, Departamento de Química Analítica,
Instituto de Investigación
en Ingeniería de Aragón (I3A), Mª de Luna 3,
Edificio Torres Quevedo, Campus Rio Ebro,
50018 Zaragoza, Spain

[2]IUCA, Dept. Microbiología, Facultad de Medicina,
Universidad de Zaragoza, Spain

*corresponding author: cnerin@unizar.es

ABSTRACT

The selection of the active substance is critical in the development of antimicrobial packaging, as it has to demonstrate high antimicrobial properties against significant bacterial in food contamination. The aim of this work is to evaluate the antibacterial activity of lauramide argine ethyl ester (LAE), decaffeinated green tea extract (GTD) and green tea antioxidant extract (GTA) against the foodborne *Listeria innocua*. For this purpose, the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), growth kinetics and kill-kinetics were determined. The results obtained showed that LAE was much more active than the two Green Tea extracts since MIC and MBC obtained were about 100 times lower. In addition, at inhibitory concentration LAE showed a fast bactericidal activity and at subinhibitory concentration produced an increasing in the lag phase of growth.

Key words: antimicrobial, green tea, *Listeria innocua*, Lauramide argine ethyl ester (LAE).

INTRODUCTION

Active packaging is considered a potential method to extend the food shelf-life or to improve its safety while maintaining its quality (Suppakul, 2003; 2004). Food- packaging films containing active substances can create an antimicrobial environment in which foodstuff is protected against microbiological proliferation. Previous research papers showed that antimicrobial packaging materials can be very efficient with bacterial growth inhibition (López *et al.*, 2007; Gutiérrez *et al.*, 2009). However, in the development of active packaging the selection of the active substance is critical, as it has to demonstrate high antimicrobial properties against a wide range of microbial. The aim of this study is to evaluate in vitro antimicrobial activity of Lauramide Arginine Ethyl Ester (LAE) and Decaffeinated Green Tea (GTD) and Antioxidant (GTA) extract.

MATERIALS AND METHODS

Antimicrobial substances

Two extract of Green Tea (Chemical Abstracts Service (CAS) Registry number 84650-60-2), Decaffeinated (GTD) and Antioxidant (GTA) supplied by University of MILAN and Lauramide Argine Ethyl Ester (LAE: Chemical Abstracts Service (CAS) Registry number 60372-77-2) supplied by LAMIRSA (Barcelona, Spain) were evaluated.

Bacterial strains

A common gram positive bacteria, *Listeria innocua* DSM 20649 (Deutsche Sammlung von Mikroorganismen) were used as representative bacteria.

Antimicrobial susceptibility test

To study the inhibitory concentrations, a broth dilution method described by Becerril *et al.* was used (Becerril *et al.*, 2007). Briefly, an aqueous solution of antimicrobial substances was prepared and mixed in Soya Triptone broth (TSB) medium containing 10^5 colony forming unit (CFU)/mL of *Listeria innocua.* After the incubation period, the bacterial growth was determined by measuring the optical density at 625 nm. Surviving bacterial were determined by conventional plating. The minimal inhibitory concentration (MIC) was defined as the lowest concentration in which bacterial growth was not detected.

The minimal bactericidal concentration (MBC) was defined as the lowest concentration of the active substance that produces total death of bacteria growth. Both were expressed as milligrams of EO per litre (mg/L).

Time-kill and growth kinetic study

TSB containing 10^5 CFU/mL was mixed with the appropriate dilution of LAE. Immediately after time 0 a 10 μL aliquot was removed, diluted and spread on TSA agar plates. After the incubation period CFU were determined by conventional plating.

This process was repeated at 2.5, 5, 7.5 and 10 min to determine the time-kill kinetics, and every hour for 15 h to determine the growth kinetic.

The concentration of LAE used was equal to MBC in the kill kinetic study and 6 mg/L in the growth kinetic study.

Controls without antimicrobial substances were carried out in both experiments.

Kill and growth curves were determined by recording the number of CFU/mL versus incubation time in the presence or absence of LAE.

A program implemented in Microsoft Excel (D-model; Institute of Food Research, Norwich, UK) was used to fit the equation of Baranyi (Baranyi and Roberts, 1994) to the growth data.

RESULTS

Antimicrobial susceptibility test

MIC and MBC values of antimicrobial agents are presented in tab. 1. LAE possessed much stronger anti-*L. innocua* activity than GTD or GTA, since MBC of GTA and GTD was 100 times higher than the MBC of LAE.

LAE is a cationic surfactant that can degrade or solubilize cell membranes at low concentrations, therefore a low MIC and MBC is expected (Rodríguez *et al.*, 2004).

Table 1 - MIC and MBC of lauramide argine ethyl ester (LAE), green tea decaffeinated (GTD) and green tea antioxidant (GTA) obtained against *Listeria innocua.*

Antimicrobial agent	MIC (mg/L)	MBC (mg/L)
LAE	25	25
GTD*		2,000
GTA*		2,000

* MIC of GTA and GTD were not determinate because absorbance at 625 nm was impossible to measure.

Green tea is a natural substance with demonstrated antimicrobial properties (Hamilton-Miller, 1995), however, the results showed that the extract used exhibit poor antimicrobial activity. Due to their natural origin, the composition of green tea is very complex and variable, so different extracts can show different antimicrobial characteristics (Chou *et al.*, 1999).

Following the results obtained in this research GTA and GTD were discarded to be used as antimicrobial substances in active packaging. For this reason only growth and kill kinetics of LAE were determined.

Time-kill kinetic and growth kinetic study

According to fig. 1, the time-kill curve results showed that LAE at 25 mg/L (MBC) demonstrated fast bactericidal activity against *Listeria innocua*. At 5 min, LAE produces a reduction of 99.9% CFU/mL. This fast activity is due to their mechanism of action that implies the disruption of cell membranes and consequently the cell death (Rodríguez *et al.*, 2004).

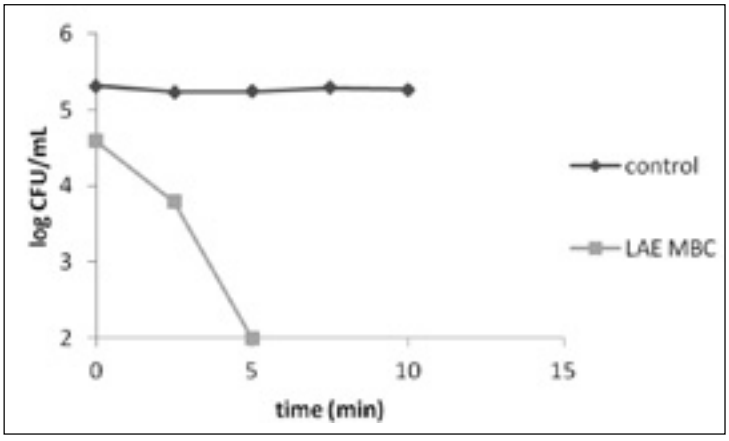


Fig. 1 - *Listeria innocua* time-kill curve.

Tab. 2 shows the kinetics parameters obtained in the growth curve after fit to the equation of Baranyi. The lag phase of *L. innocua* without treatment was not detected due to

Table 2 - Kinetic parameters obtained to *L. innocua* including exponential growth rate, lag time, maximum population density (Yend), and the coefficient of determination (R^2).

	Growth Rate (h^{-1})	Lag (h)	Yend (log CFU/mL)	R^2
Control	0.45		9.78	0.966
LAE (6 mg/L)	0.45	2.59	9.67	0.987

higher initial inoculum concentration used, however, *L. innocua* treated with LAE showed a lag phase of 2.59 h. The growth rate was not reduced by the treatment.

LAE showed a high and fast antimicrobial activity, suggesting being an excellent candidate to be used as antimicrobial substance in active packaging.

ACKNOWLEDGMENTS

This research has been financed by the European project NAFISPACK.

REFERENCES

Baranyi J., Roberts T. 1994. A dynamic approach to predicting bacterial growth in food. Int. J. Food Microbiol., 23(3-4):277-94.

Becerril R., Gómez-Lus R., Goñi P., López P., Nerín C. 2007. Combination of analytical and microbiological techniques to study the antimicrobial activity of a new active food packaging containing cinnamon or oregano against *E. coli* and *S. aureus*. Analytical and Bioanalytical Chemistry, 388(5):1003-11.

Chou C.-C., Lin L.-L., Chung K.-T. 1999. Antimicrobial activity of tea as affected by the degree of fermentation and manufacturing season. International Journal of Food Microbiology, 48(2):125-30.

EC Regulation N. 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. Off. J. Eur. Union. L338(4-17).

Gutiérrez L., Sánchez C., Batlle R., Nerín C. 2009. New antimicrobial active package for bakery products. Trends in Food Science & Technology, 20(2):92-9.

Hamilton-Miller J.M. 1995. Antimicrobial properties of tea (*Camellia sinensis* L.). Antimicrobial Agents Chemotherapy, p. 2375-7.

López P., Sánchez C., Batlle R., Nerín C. 2007. Development of flexible antimicrobial films using essential oils as active agents. Journal of Agricultural and Food Chemistry, 55(21):8814-24.

Rodríguez E., Seguer J., Rocabayera X., Manresa A. 2004. Cellular effects of monohydrochloride of l-arginine, N-lauroyl ethylester (LAE) on exposure to *Salmonella typhimurium* and *Staphylococcus aureus*. Journal of Applied Microbiology, p. 903-12.

Suppakul P., Miltz J., Sonneveld K., Bigger S.W. 2003. Active packaging technologies with emphasis on antimicrobial packaging and its applications. Journal Food of Science, 68:408-20.

SHELF-LIFE ASPECTS OF TOASTED BREAKFAST CEREAL WITH AND WITHOUT FRUIT

I.S.M. MACEDO, M.J. SOUSA GALLAGHER, J.C. OLIVEIRA and E.P. BYRNE

Department of Process and Chemical Engineering, University College Cork, Ireland

ABSTRACT

Toasted-breakfast-cereal is a high quality, high nutritional dry food made of granulated particles. Quality characteristics are important for consumer acceptance and these can be influenced by environmental conditions throughout storage, which can be detrimental for product quality and shelf-life. The aim of this study was to identify the critical quality parameters, determine the influence of environmental conditions on quality parameters and study if the presence of fruit affected the cereals. Toasted-breakfast-cereal was stored according to a mixed full factorial experimental design with two and three levels at three factors (temperature T (°C), relative humidity RH (%) and presence of fruit) to measure the effect of these factors on the quality of cereal. Cereal samples with and without fruit were placed in air-tight containers, above saturated salt solutions individually stored at constant temperatures. Moisture content; water activity; firmness and colour (L-, a- and b-values; browning index and Delta E) were evaluated (at least in triplicate), under the different conditions throughout storage. Statistical analysis of the quality parameters was performed using ANOVA, the Taguchi method and correlations. Moisture content and water activity were found to be the critical quality parameters during storage. Relative humidity is the environmental factor studied that had the greatest effect on the critical quality parameters. The presence of fruit exhibited a significant effect on the critical quality parameters, resulting in a product with lower moisture content. Knowledge of how the environmental conditions affect the critical quality parameters during storage can help the selection of appropriate packaging materials, and facilitate the design of an optimum package to guarantee high quality product during the desired shelf-life. Therefore, a package with good barrier properties to water would be recommended for storage of toasted-breakfast-cereal with and without fruit.

Key words: critical quality parameters, environmental conditions, fruit, storage and toasted-breakfast-cereal.

INTRODUCTION

The quality of dried breakfast cereals depends not only on its original state but also on the extent of changes during processing and storage. During storage and distribution, foods are exposed to a wide range of environmental conditions. Environmental factors such as temperature, humidity, oxygen and light can trigger several reaction mechanisms that may lead to food degradation. As a consequence of these mechanisms, foods may be altered to such an extent that they are either rejected by the consumer, or they become harmful to the person consuming them (Man and Jones, 1994). Some characteristics (composition, physical and chemical) are considered important to assess the quality of food product (Yan *et al.*, 2008). Among all the characteristics water activity, moisture content, texture and colour can be selected to analyse the quality of cereals. Moisture content influences the physical characteristics, chemical stability and microbial growth rates of a product. The texture of cereals is also important, consumers do not want moist aggregates, and a crunchy product is required. Colour is one of the most important appearance attributes of foods as it influences consumer's acceptability.

The main objectives of this study were to i) identify the critical quality parameters during storage; ii) determine the influence of environmental conditions on quality parameters and iii) study if the presence of fruit affected the cereals.

MATERIALS AND METHODS

Cereal samples

Toasted breakfast cereals with and without fruit is an industrial manufactured product, which was provided by Stable Diet (Co. Wexford, Ireland).

Experimental Design

Toasted-breakfast-cereal was stored according to a mixed full factorial experimental design with two and three levels (20°, 30° and 40°C; 33, 55 and 75; without fruit and with fruit) at three factors (temperature T (°C), relative humidity RH (%) and presence of fruit) to measure the effect of these factors on the quality of cereal. Cereal samples with and without fruit were placed in air-tight containers, above saturated salt solutions individually stored at constant temperatures.

Moisture content; water activity; firmness and colour were evaluated, under the different conditions throughout storage. Statistical analysis of the quality parameters was performed using ANOVA, the Taguchi method and correlations.

Measurement of Quality Parameters

The quality parameters were measured throughout 15 days of storage. A total of 127 samples were measured and analysed at least in triplicate for each of the quality parameters, at 18 of the different conditions throughout the 7 sampling times during storage.

Moisture Content: The moisture content of samples was measured by weight loss through heating at 104°C for 8 hours at least. The values were expressed in % (wb) ($g_{water}/g_{dry\ product}$) and they correspond to an average of three replicates.

Water Activity: It is defined as the ratio of the vapour pressure of water in the food to the vapour pressure of pure water at the same temperature.

Texture: The firmness of cereal was determined using a TA-XT2i texture analyzer

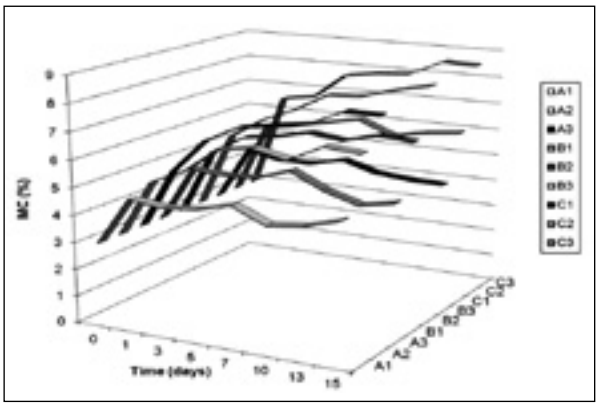


Fig. 1 - Changes of MC of cereals without fruit throughout storage under different conditions of temperature and relative humidity. A1: 20°C, 33%; A2: 20°C, 55%; A3: 20°C, 75%; B1: 30°C, 33%; B2: 30°C, 55%; B3: 30°C, 75%; C1: 40°C, 33%; C2: 40°C, 55%; C3: 40°C, 75%.

(Stable Micro Systems, Godalming, United Kingdom) equipped with a 50 N load cell, using a 75 mm compression plate probe. Firmness was expressed as maximum compression force (N) and an average of ten replicates was calculated.

Colour: Colour changes of the samples were monitored by the colorimeter Chroma Meter CR-300 (Konica Minolta Sensing, Inc., Osaka, Japan), using CIELAB L, a, b parameters. Hunter L, a and b were measured throughout time and Browning Index and Delta E calculated. Colour was measured in 10 different positions for each sample and the averages values were reported.

Statistical Analysis: Data analysis of quality parameters during storage under different conditions were performed using Statistic software (release 7, edition 4, StatSoft, Tulsa, OK, USA) to carry out ANOVA, correlations and the Taguchi method.

RESULTS AND CONCLUSION

Moisture content (MC) and water activity (a_w) were found to be the critical quality parameters, according to F-test. The effect of storage conditions on MC is shown in fig. 1; MC increased with time from an average 2.9% (w.b.) to 4.2 or 8.5% (w.b.), with the biggest increase taking place at high RH (75%).

MC and a_w were shown to have a high positive correlation factor (0.86), meaning that further work would only require one of these critical parameters to be assessed.

The effect of relative humidity and temperature on MC was visualized by the response surface plots, in fig. 2. Relative humidity had a strong positive linear effect on MC, whereas temperature did not influence the response of MC.

The importance of all factors on MC, following ANOVA and the Taguchi method are shown in fig. 3.

The largest contribution to the total sum of squares is caused by relative humidity (RH) (1st bar), therefore RH was found to be the most influential environmental fac-

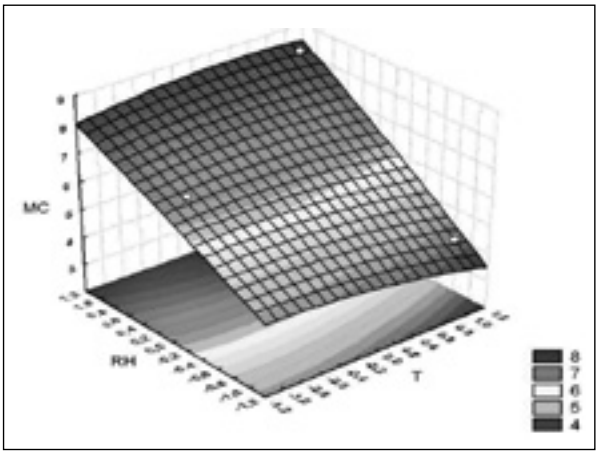


Fig. 2 - 3-D response surface plots showing the effect of temperature and relative humidity on MC.

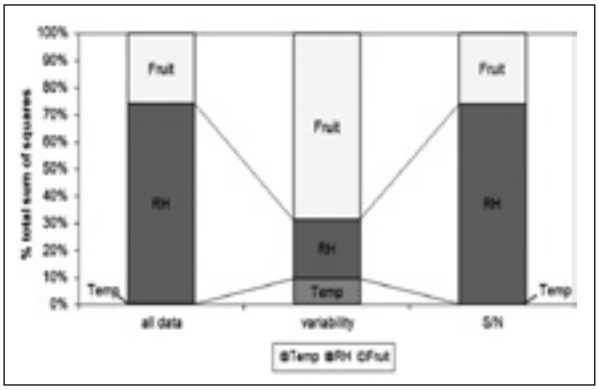


Fig. 3 - Importance of factors on MC (1st bar), variability of the system (2nd bar) and the S/N ratio (3rd bar).

tor studied. The presence of fruit also exhibited a significant effect on MC. Regarding variability of all factors on MC (2nd bar), the presence of fruit was the only factor that introduced significant noise to the system, nevertheless, the S/N ratio (3rd bar) showed that the system was robust.

The optimum conditions to target minimum moisture content (maximum value of S/N ratio) were found using the Taguchi method. The variation of the S/N ratio for each factor at different levels is shown in fig. 4.

The presence of fruit in the toasted breakfast cereal and the exposure to relative humidity showed a maximum and a minimum of S/N ratio for the levels used, whereas temperature did not show much variation.

Overall, moisture content and water activity were found to be the critical quality parameters and the relative humidity was found to be the most influential factor studied. The optimum conditions were found to be for the toasted breakfast cereal with fruit at intermediate/high temperature and low relative humidity. Therefore, a package with good barrier properties to water would be recommended for storage of cereals with or without fruit.

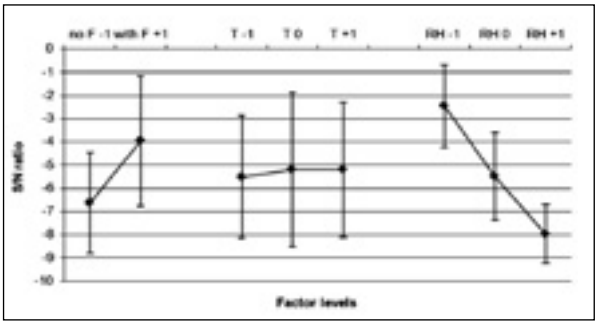


Fig. 4 - Average values of S/N ratio for each factor at different levels.

ACKNOWLEDGMENTS

The authors would like to acknowledge financial support provided under the National Development Plan, through the Food Institutional Research Measure (FIRM 06/RDC/428), administered by the Dept. of Agriculture, Fisheries & Food, Ireland. A special thanks to Stable Diet (Co. Wexford, Ireland) for providing the products used in this study.

REFERENCES

Man C.M.D., Jones A.A. (1994). Shelf-life Evaluation of Foods. Blackie Academic & Professional, first edition, Glasgow.

Yan Z., Sousa-Gallagher M.J., Oliveira F.A. (2008). Identification of critical quality parameters and optimal environment conditions of dried banana during storage. Journal of Food Engineering, 85, 168-172.

INNOVATIVE APPROACHES AND INSTRUMENTS IN MODELLING AND MONITORING THE MOISTURE DIFFUSION IN PACKAGES AND MOISTURE ADSORPTION BY DRIED PRODUCTS

P. LAMIANI, S. LIMBO and L. PIERGIOVANNI

Università degli Studi di Milano,
Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche,
Via Celoria 2, 20133 Milano, Italia

ABSTRACT

Shelf-life forecast models for moisture depending products assume that the relative humidity of the package headspace (which changes with the entrance of water vapor from the outside) is immediately in equilibrium with the product.

In the present research *fetta biscottata* (a typical Italian bakery product) has been used as a model system to evaluate how different quantities of product, different headspace volumes, and different packaging solutions influence the equilibrium between the relative humidity of the headspace and the relative humidity of the product.

From the data obtained it can be concluded that the relative humidity of the headspace of the packaging is not immediately in equilibrium with the product. The theoretical assumption generally used in the shelf-life modeling has not been verified for this type of product.

Key words: humidity sensors, bakery products, shelf-life modelling.

INTRODUCTION

Shelf-life models are mathematical equations which describe the relationship between food, package, and environment. These models are based on different degradation factors and are useful to predict the shelf-life of food, to design food packages, and to provide useful insights.

Many chemical, biological, and physical changes depend on moisture content or water activity in the foods, whose changes may be dictated by the packaging protection from the environment in the distribution channel. Water activity is ruled by the

moisture content in the food, which may change with time through the permeation process of water vapor across the packaging film. The equilibrium relationship between moisture content and water activity is explained by the adsorption/desorption isotherm and several mathematical equations have been developed to express this equilibrium relationship (Lee *et al.*, 2008). These equations are used in forecast models to determinate the shelf-life of moisture depending products (Labuza and Schmidl, 1985). All these models assume that the relative humidity of the package headspace (which change with the entrance of water vapor from the outside) is immediately in equilibrium with the product (Fava *et al.*, 2000). This assumption has not yet been verified experimentally and cannot be valid for all the products and the packages.

The aim of the present work was to study if different quantities of product, different headspace volumes, and different packaging solutions may influence the equilibrium between the relative humidity of the headspace and of the product.

MATERIALS AND METHODS

An acquisition system composed by humidity sensors and a specific acquisition software to record simultaneously different moisture and temperatures values has been built in collaboration with RDE Company srl. Rigid trays (Sirap Gema) with capacity of 1,400 and 1,030 cm^3 sealed with a OPP 20 mm film (Moplefan) have been used as a model package system. The water vapor transmission rate (WVTR) of the complete package has been determined, by means of a gravimetric method, as equal to 1.52±0.02 g $pack^{-1}$ $24h^{-1}$ (38°C and 98% ΔRH).

Fetta biscottata (a typical Italian bakery product, with a water activity of 0.22 and a moisture content of 3.71±0.27 g/100 g) has been packed, in air, inside the trays in different quantities. Humidity sensors have been placed inside the product and in the headspace, in order to record the relative humidity in different points of the package. The packages were submitted to an accelerated storage test in a thermostatic cell at 38±0.5°C and 98% RH. The experiments were repeated at least two times for each sample.

Different quantities of the dry product (*fetta biscottata*) have been packaged in the two type of trays, obtaining the combinations summarized in tab. 1.

RESULTS AND CONCLUSIONS

During the accelerated storage the moisture adsorption by *fetta biscottata* and the moisture increase in the headspace of the packages have been measured and

Table 1 - Packaging combinations and RH differences among packaging solutions.

Sample number	Number of fetta biscottata	Product weight [g]	Product volume [cm^3]	Tray volume [cm^3]	Headspace volume [cm^3]	Headspace volume/ Product weight [cm^3 g^{-1}]	Δ% RH
1	4	32.48	182.8	1,030	847.2	26.1	4.5±0.7
2	4	32.48	182.8	1,400	1,217.2	37.5	5.6±0.8
3	6	48.72	274.2	1,030	755.8	15.5	2.7±0.7
4	6	48.72	274.2	1,400	1,125.8	23.1	4.2±0.9
5	8	64.96	365.6	1,030	664.4	10.2	1.4±0.4
6	8	64.96	365.6	1,400	1,034.4	15.9	2.7±0.4
7	12	97.44	548.4	1,400	851.6	8.7	1.2±0.2

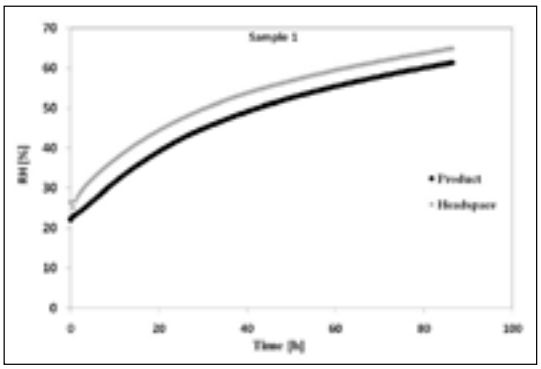


Fig. 1 - Moisture change in sample 1.

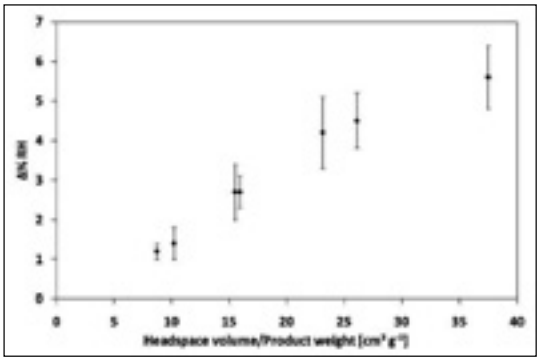


Fig. 2 - Relationship between headspace volume and Δ% RH.

recorded every 30 min for each combination. In fig. 1 the relative humidity change for sample 1 is shown. The "Headspace" curve represents the moisture diffusion in the headspace, while the "Product" curve represents the moisture adsorption by *fetta biscottata.* For all the samples the difference between the curves can be considered constant in the RH range from 0 to 65% and shows the delay of the product in absorbing the moisture. Experiments conducted until 95% RH demonstrated that the two curves joined after a quite long time (data not shown).

To analyze the effect of the different packaging combinations, the ratio between the headspace volume and the product weight was related to the difference (in average) between the headspace and the product RH values. Data in tab. 1 show that the higher the ratios, the bigger the differences.

In fig. 2 the differences in RH values are plotted versus the volume/weight ratio, showing a clear trend and an increasing uncertainty in the measures.

From our data, obtained combining different weights of *fetta biscottata* and different headspace volumes, it can be concluded that the relative humidity of the headspace of the packaging (which changes for the water vapor permeability) is not immediately in equilibrium with the product. The theoretical assumption generally used in the shelf-life modeling has not been verified for this product and, in future works, it will be studied how these differences influence the shelf-life prediction for fetta biscottata and for other moisture depending products and different packages.

ACKNOWLEDGEMENTS

To RDE Company srl (Cormano, Milano, Italy) for the support and the hardware and software knowledge.

REFERENCES

Lee D.S., Yam K.L., Piergiovanni L. (2008). Shelf-life of packaged food products, Chp. 16 in "Food Packaging, Science and Technology". CRC Press. Boca Raton, Florida, USA.

Labuza T.P., Schmidl M.K. (1985). Accelerated shelf-life testing of foods. Food Technology, 39(9):57.

Fava P., Limbo S., Piergiovanni L. (2000). La previsione della shelf-life di prodotti alimentari sensibili agli scambi di umidità. Industrie Alimentari, 39(2):121.

PACKAGING SAFETY OF PACKAGED FOOD PRODUCTS IN OPINION OF POLISH CONSUMERS

M. LISIŃSKA-KUŚNIERZ

Cracow University of Economics, Faculty of Commodity Science, Packaging Department, Rakowicka 27 St., 31-510 Cracow, Poland

correspondig author: liskusm@uek.krakow.pl

ABSTRACT

Presented research concerns results of studies conducted among polish consumers determining importance of packaging safety in comparison to other food products packaging features. Additionally the research concerns pointing food products packaging dangerous features.

Conducted research demonstrate that for consumers the most important feature of food products packaging is safety of packaged food in a scope of lack of negative interactions. Studies also showed urgent need of consumer education in a range of their right to safety assurance and health protection.

Key words: consumer opinion, food product, packaging safety.

INTRODUCTION

The basis of every country consumers' politics in a range of safety and health protection is assurance of offering consumers packaged products which will not caused danger to their health and life. Joining worlds' politics current of consumers health protection World Packaging Organization (WPO) fixed guidelines for packaging and safety of packaged food. Guidelines require to specify packaging safety characterizing properties, creating mechanisms preventing from hazardous materials present on the market and implementing procedure of quick alarming and withdrawing dangerous materials from the market (Position Paper. Packaging and Food Safety, 2009; LEE e*t al.,* 2008).

All transactors constituting supply chain are obliged to comply legislation require-

ments concerning packaging and packaged products safety. Among regulations directly in force in European Union members concerning problem of packaging safety are: Regulations of the European Parliament and of the Council 178/2002 and 1935/2004 and Commission Regulation 2023/2006, 282/2008 and 450/2009. Moreover on the rule of facultative requirements of management systems considering safety aspects are implemented (ISO 22000, 2005; BRC/IoP Global standard for packaging and packaging materials, 2008). With the support of results of studies conducted in 2008 by World Packaging Organization (WPO) it might be stated that increase of consumer awareness on a field of health safety and the rank of packaging utility will be in the greatest degree influence on directions of packaging science development. More that 90% of WPO members taking part in the study indicated that increase of consumer awareness on a field of safety is a factor of importance or great importance in branch development shaping (Market statistics and future trends in global packaging, 2008).

Subjects and methodology of the research

In objective to recognition requirements which are posed to food products packaging by polish consumers the survey was conducted on a group of 900 persons in age 19-30, living in million habitants three agglomerations, who increases professional qualifications in different forms of education. The group of young people was intentionally selected assuming that they are a group of aware consumers, respondents just has started their professional carrier and are active in a field of education. In studied consumers population women constitute 57%, men 43%.

The study mainly includes: significance evaluation of food products packaging features, not taking under consideration the type of packaging and also taking into account packaging material (plastics, glass, metal, paper and cardboard). Signification point evaluation relates to such features of packaging as: shape, aesthetics, quality of conformance, weight, opening easiness, ergonomics, economy, ecology, safety in respect of product – lack of negative interactions, effectiveness of product protection during exploitation and effectiveness of product protection during logistic processes.

In the survey importance of packaging features was valued by respondents on the scale from 0 to 5, assuming that: 0-not important, 1-very hardly important, 2-less important, 3-average important, 4-important, 5-very important. Moreover in the survey consumers were asked to indicate dangerous features of food products packaging.

RESULTS AND DISCUSSION

Obtained results from consumers' valuation of particular food products packaging properties and packaging made from different packaging materials is presented in tab. 1 and 2.

Conducted research demonstrated that for consumers the most important feature of food products packaging is safety of packaged food in a scope of lack of negative interactions. In a group of packaging features with important rank for consumers are such as: quality of conformance, opening easiness, effectiveness of product protection during exploitation. However, in a group of features with average importance are: effectiveness of product protection during logistic processes, economy, ecology and aesthetics. Least important according to consumers were such features

Table 1 - Importance of particular food products packaging features and packaging made from different packaging materials in consumer evaluation (points).

Packaging features	Food products packaging	Plastic packaging	Glass packaging	Metal packaging	Paper and cardboard packaging
Shape	2,85	2,94	3,43	3,10	3,05
Aesthetics	3,49	3,70	3,71	3,42	3,49
Quality of conformance (lack of defects)	4,01	3,92	4,03	3,89	3,79
Weight (lightness)	3,23	3,18	3,27	3,47	3,07
Opening easiness	4,09	3,99	3,92	4,14	3,72
Ergonomics	3,40	3,28	3,29	3,23	3,21
Economy (share of packaging price in product price)	3,65	3,62	3,61	3,56	3,39
Ecology (environmental friendliness)	3,51	3,46	3,25	3,26	3,38
Safety in respect of product (lack of negative interactions)	4,22	4,1	3,87	4,05	3,77
Effectiveness of product protection during exploitation	3,87	3,64	3,74	3,56	3,55
Effectiveness of product protection during logistic processes	3,71	3,28	3,61	3,30	3,48

Source: author's work.

as: ergonomics, weight and shape. Certain difference in evaluation of packaging feature importance hierarchy is noticeable in dependence of packaging material kind. First in a rank of features, lack of negative interactions with food products was very important or important for about 80% of respondents. Analysis of received evaluation revealed larger sensitiveness of woman than men in scope of packaging features essentials for health and life safety. Moreover, it is worth to notice that even more than 20% of indications above-mentioned feature were described as not important, very hardly important, less important and average important and even 45% of these indications considered safety of applying different types of packaging. This result shows the necessity of education actions intensiveness.

Research in a field of life and health safety concerned also recognition of consumer opinion on the topic of danger packaging features set in a case of packaged food products. Dangerous food products packaging according to consumers opinion is characterized mainly by presence of negative interactions with content (35% of indications), lack of efficient product protection during exploitation (20%) and lack of construction tightness (14%). In a smaller range it is characterized by sharp edges causing cuts (7%) and not properly selected packaging material and shape causing lack of stability of packaging during utilization (6%) (Lisińska-Kuśnierz, 2010).

CONCLUSION

In literature and legislation regulations there is lack of safety packaging definition. Only safety product introducing on the market has its definition. Adopting packaging as a product inherently related to packaged product (meeting strictly fixed functions) it is possible to propose safety packaging definition.

As a safety packaging can be described every packaging, which under normal or reasonably foreseeable conditions of use, including duration of packaging utilization, and also regarding packaging type and packaged product type, does not present any risk or only the minimum risks compatible with the product's use, considered as acceptable and consistent with a high level of protection for the safety and health of persons.

In result of presented deliberation and analysis of conducted research it should be stated that aspires of packaging producers and consumers expectations towards packaging materials and packaging use for products resolved to launching on the market safe packaging. Polish consumers are characterized by increasing awareness in a field of their right to safety food and safety packaging. However there is still a large part of young and educated consumers, which do not notice importance of packaging safety. That is the reason why intensification of educational activities is needed in this field.

REFERENCES

BRC/IOP. Global Standard for Packaging and Packaging Materials and Best Practice Guidelines Pack. 2008. TSO, Belfast.

Commission Regulation (EC) N. 282/2008 of 27 March 2008 on recycled plastic materials and articles intended to come into contact with foods and amending Regulation (EC) N. 2023/2006, OJ L 86, p. 9.

Commission Regulation (EC) N. 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food, OJ L 135, p. 3.

Commission Regulation (EC) N. 2023/2006 of 22 December 2006 on good manufacturing practice for materials and articles intended to come into contact with food, OJ L 384, p. 75.

ISO 22000. 2005. Food safety management systems - Requirements for any organization in the food chain.

Lee D., Yam K., Piergiovanni L. 2008. "Food Packaging Science and Technology", CRC Press, Boca Raton – New York.

Lisińska-Kuśnierz M. 2010. "Social Aspects of Packaging Science", Publishers of Cracow University of Economics, Cracow.

Market Statistics and Future Trends in Global Packaging. 2008. World Packaging Organization, Formato Design, Brazil.

Position Paper. Packaging and Food Safety. 2009. World Packaging Organization, www.worldpackaging.org.

Regulation N. 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, OJ L 31, p. 1.

Regulation (EC) N. 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC, OJ L 338, p. 4.

POLYOLEFIN FILMS USED IN FOOD SECTOR AGEING PROCESS ON IMPACT OF MICROCLIMATIC FACTORS

M. LISIŃSKA-KUŚNIERZ

Cracow University of Economics, Faculty of Commodity Science, Packaging Department, Rakowicka 27 St., 31-510 Cracow, Poland

corresponding author: liskusm@uek.krakow.pl

ABSTRACT

Polyolefin films used in food products packaging undergo ageing under impact of microclimatic factors occurring while exploitation. The aim of this research was to indicate the range of changes in utility characteristic of packaging film under air temperature and humidity during their storage or storage of packaged products. On the basis of literature study in the research four options of microclimatic conditions were taken under consideration. Microclimatic conditions were created in microclimatic cabinets where two types of three-layered thermo-shrink polyolefin films were storage.

The parameters that characterized utility values were mechanical properties (breaking load and extension). In order to describe influence of microclimatic conditions on the range of changes on films properties the study was conducted for 196 days, tests were made every 28 days.

The analysis of obtained results indicated that microclimatic conditions have influence on mechanical properties of tested films. Mechanical properties, breaking load and extension are characterized by curvilinear dependence from time of microclimatic conditions impact, which most preciously formulate equalities of polynomial function of the fifth grade. On the range of changes greater influence has temperature than humidity of surroundings. In storage conditions of overestimated temperature the mechanical parameters undergo the greatest changes. In conditions of overestimated air humidity changes of quality were also noticed but in minor range.

Research also revealed that assurance of constant, normal climate conditions enable preservation film properties on constant level during seven months of storage.

Key words: mechanical properties of films, microclimate factors, polyolefin films.

INTRODUCTION

Plastic films used in food product packaging process with usage of fill up-packaging machines should meet fixed strength requirements and food contact materials requirements. Packaging film made from plastic, especially polyolefin after their manufacturing undergo ageing process. Adverse changes of utility properties caused by the entirety of physical and chemical transformation taking place in plastic material structure in time function of its storage concerning mainly climate exposures (Hakkarainen *et al.*, 2004). Influence effects of climate factors such as: temperature, solar radiation, humidity and contamination mainly depend on exposure time and type of studied polymer (Chi Ming, 1994).

Research concerning evaluation of plastic films utility value conducted so far mainly concentrate on such aspects as:

- utility value parameter identification of films sampled directly from production line (Vergnaud, 2006; Dostal *et al.*, 2008; Zweiful *et al.*, 2001; Soares, 2004),
- evaluation of utility value on the base of packaging materials strength parameters changes in short-term storage (Christie *et al.*, 1995; Lange, 2000),
- influence evaluation of one climatic factor on the changes of plastic films' properties (Ghosh *et al.*, 2000; Bal *et al.*, 2007).

In literature there is a lack of studies concerning plastic packaging films sensitivity on atmospheric conditions, occurring during storage and exploitation. Moreover technical specifications of packaging materials do not specify microclimatic conditions of storage and exploitation life. Simultaneously packaging films use in food packaging according to Regulation N. 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC should have specified shelf-life and defined conditions which assure during storage proper utility value. Furthermore according to recommendations of the standard ISO 22000 there is a need of continuous monitoring of utility properties characterizing materials intended to come into contact with foodstuff during their storage (ISO 22000, 2005). In connection to above-mentioned the research concerning description of factor determining stability of quality level of plastic films and microclimate requirements concerning their storage in defined time was undertaken.

MATERIALS AND METHODS

Studied materials were two types of multilayer films made from polyolefin OPPPE, first manufactured in Poland marked as A, second in Europe, non-EU country marked as B. Films characterized the same thickness and identical declared purpose.

For influence of temperature and humidity determination on mechanical properties of films, materials were storage in different microclimate conditions. On the basis of literature (ISO 554, 1995; EN ISO 2233, 2000) and economical practice, following options of constant microclimate conditions were defined:

- I option: T=20±1°C, Ø=60±2% (normal atmospheric conditions),
- II option: T=40±1°C, Ø=60±2% (T- overestimate in comparison to option I),
- III option: T=20±1°C, Ø=90±2% (Ø - overestimate in comparison to option I),
- IV option: T=-20±1°C, Ø=40±2% (T and Ø - complied with exploitation of film in frozen food packaging process).

For determining influence of aging time on films under influence of different microclimate factors studied materials were storage under these conditions for 196 days. Research of mechanical properties such as breaking load and extension were conducted in fixed periods of time, every 28 days on tensile testing machine Instron model 4301, separately for along and across films' directions according to EN ISO 527-1.

RESULTS AND CONCLUSIONS

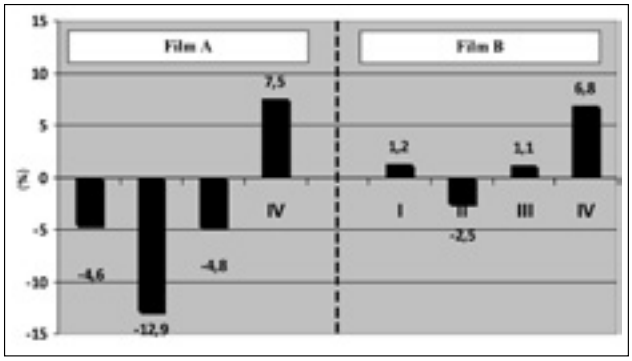


Fig. 1 - The range of extension changes during tensile test of A and B films after 196 days of storage in different microclimate conditions options (source: author's work).
I, II, III, IV - option of microclimate condition.

For determining function describing of changes continuance in time of storage in diverse microclimate conditions options the statistic analysis of obtained results of mechanical properties was conducted. On the basis of this analysis it was stated that changes of breaking load and extension are depended of time and it is possible to model them by a function of fifth grade.

By analyzing obtained results it should be stated that on the effect of different microclimate factors during long-term storage in studied polyolefin films significant changes of mechanical properties were noticed. This changes are more evident in results of extension (fig. 1), which is a parameter more strongly reacting in process of aging than breaking load (fig. 2).

Analysis of dynamic changes ratios studied parameters allowed to notice that range of these changes significantly depends on temperature and in much smaller range on air humidity. Studied parameters systematically changed, especially on the dynamic of changes meaningful influence has overestimated temperature. It was stated also that type of film has influence on range of parameters changes in long-term storage in analogous microclimate conditions.

Obtained results confirmed, that assurance of constant, normal, microclimate conditions makes possible to preserve satisfactory quality level during seven month period of storage. Presented studies are being continuous for determining correlation between mechanical properties changes of plastic films and their physical changes in range of structure changes. Moreover undertaken storage studies for determining border shelf-life and exploitation time of these films.

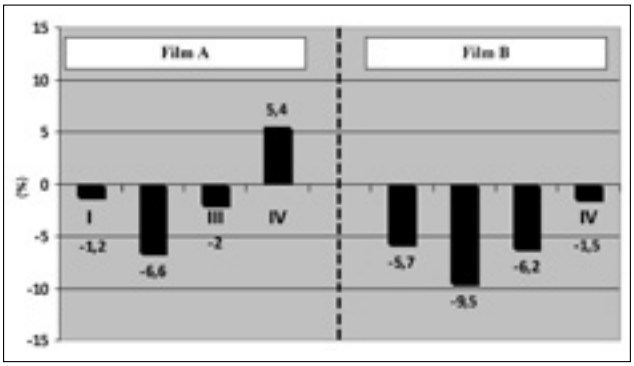


Fig. 2 - The range of breaking load changes during tensile test of A and B films after 196 days of storage in different microclimate conditions options (source: author's work).
I, II, III, IV - option of microclimate condition.

REFERENCES

Bal S., Mahesh D., Seu T., Ray B. 2007. Effect of changing environments and microstructure of HDPE polymer. Journal of Minerals & Materials Characterization & Engineering. 1.

Chi-Ming Ch. 1994. "Polymer Surface Modification and Characterization". Hanser Publishers, New York.

Christie G., Macdiarmid J. 1995. Determination of film requirements and respiratory behavior of fresh produce in modified atmosphere packaging. Postharvest Biology and Technology. 6.

Dostal J., Kasperkova V., Zatloukal M., Muras J., Simek L. 2008. Influence of the repeated extrusion on the degradation of polyethylene. Structural changes in low density polyethylene. European Polymer Journal. 44.

EN ISO 527-1. 1998. Plastics - Determination of tensile properties - Part 1: General principles.

EN ISO 2233. 2000. Packaging - Complete, filled transport packages and unit loads - Conditioning for testing.

Ghosh S., Khastgir D., Bhowmick A., Mukunda P. 2000. Thermal degradation and ageing of segmented polyamides. Polymer Degradation and Stability. 67.

Hakkarainen M., Albertsson A. 2004. Environmental degradation of polyethylene. Advances in Polymer Science. 169.

ISO 554.1995. Standard atmospheres for conditioning and/or testing – Specifications.

ISO 22000. 2005. Food safety management systems - Requirements for any organization in the food chain.

Lange D. 2000. New films technologies for horticultural commodities. Hort Technology. 10.

Regulation N. 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC, OJ L. 338, p. 4.

Soares Joao B. 2004. Polyolefins with long chain branches mode with single-site coordination catalysts: a review of mathematitical model ling techniques for polimer microstructure. Macromolecular Materials and Engineering. 289.

Vergnaud J. 2006. "Assessing Food Safety of Polymer Packaging". Rapra Technology, Shrewsbury.

Zweifel H., Amos St. E. 2001. "Plastics Additives Handbook" 5th ed. Hauser Gardner Publications, Munich.

PREDICTIVE MODELING THE CHANGES OF CRYPTO CLIMATE INSIDE PACKAGING DURING STORAGE

M. UCHEREK

Cracow University of Economics, Faculty of Commodity Science, Packaging Department, Rakowicka 27 St., 31-510 Cracow, Poland

corresponding author: ucherekm@uek.krakow.pl

ABSTRACT

For products packed in modified atmosphere packaging, the important determinant of quality preservation of packed product is mixture of gases in package, which must be selected individually for each packed product. Gas mixture composition during storage period is changing from reaction in product – crypto climate inside the package system, as well as crypto climate inside the package – packaging material – microclimate system. Qualitative parameter, which characterizes mixture of gases in analyzed snacks in MAP is oxygen content in the package.

The aim of this work was to analyze changes of oxygen content in packaging of snacks depending on type of packaging and initial composition of gas mixture in package during storage.

Based on presented considerations it can be concluded that the higher initial oxygen content in packaging the greater amount of oxygen enters in direct reaction to packaged product, accelerating process of auto oxidation of fat in product. But scope and intensity of these changes are lower in case of products packaged in steel cans.

Key words: crypto climate in packaging, modern packaging system, snacks, shelf-life.

INTRODUCTION

Packaging represents one of the most widely spread activities of the modern, sophisticated society: it reflects not only the advacement of knowledge in materials

sciences but also the mastery of technological achievements. Thanks to modern packaging technology, food products can be distributed over a wide geographical area as well over a long period of time without unacceptable loss of quality and within economical constraints (Innovation in food packaging, 2005).

New technologies, such as modified atmosphere packaging have been introduced on a large scale, while consumer demands have driven the market to convenience foods that can be prepared quickly. On the basis of the studies of related publications, and of author's own research completed so far, we can state that the efficacy of the packaging in a mixture of gases depends on a multitude of factors associated both with the product, packaging material, form of the packaging, composition of the gas mixture in the packaging and with the microclimatic conditions of storage, which have to be taken into account all at the same time, in order to ensure tangible benefit both for the producer and distributor, and more importantly, for the consumer (Robertson, 2006; Ucherek, 2004, 2009).

The aim of this work was to analyze changes of oxygen content in packaging of snacks depending on type of packaging and initial composition of gas mixture in package during storage.

MATERIALS AND METHODS

The research focused on snacks packaged in bag with a side fold PET/EVOH-LDPE (mass of 150 g) which were moulded in v.f.f.h and as steel cans (mass of 150 g). Snacks were packed in a MAP. For the purpose of the tests, two variants of the initial composition of modified atmosphere in packing were applied, namely: 0-1,0% O_2 and 1,1-3,0% O_2 (Company Normalization Document, 2005).

Packaging product were storage in standardized conditions (temp. 18°C±1°C, R=75%±2%) (ISO 554, 1995). The storage tests were conducted during 48 weeks on a 4 weekly basis (Steel, 2004). All the packaging units destined for the tests had been controlled for their tightness, using the Package Test System by Skye (Quality estimation of packaging materials and packaging, 2005).

The changes of oxygen content in a package was analyzed. This parameter was measured using an Oxygen Analyzer LC-700 F (Total gas control, 2005).

RESULTS AND CONCLUSIONS

The average values, calculated on the basis of the results showing changes in oxygen content in the package (O_2) depending on package type and the initial variant of gas mixture in the package were used to determine the function mapping the course of these changes during storage. Analysis of results indicated high importance of the impact of storage time in all variants of storage conditions on the O_2 regardless of the form of packaging and the initial oxygen content in the package.

Formal models describing the dependence of O_2=f(t) for all analyzed variants are presented in fig. 1-4. Diversified range and intensity changes of the O_2 content can be seen. They depend on form of packaging design, as well as the initial composition of gas mixture in packaging.

According to this work it can be concluded that the higher initial oxygen content in packaging the greater amount of oxygen enters in direct reaction to packaged product. But scope and intensity of these changes are lower in case of products in cans.

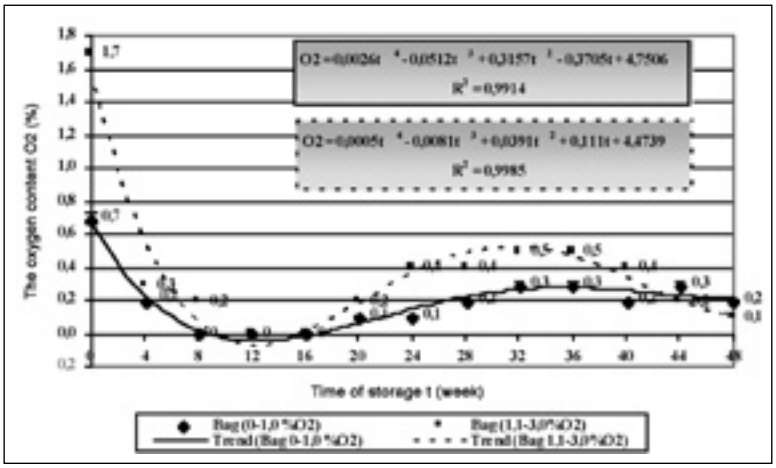


Fig. 1 - The changes of the oxygen content (O_2) in bags with different initial variant of modification of gas mixture in the package during storage in standardized conditions (temp. 18°C±1°C, R=75%±2%), % (source: author's work).

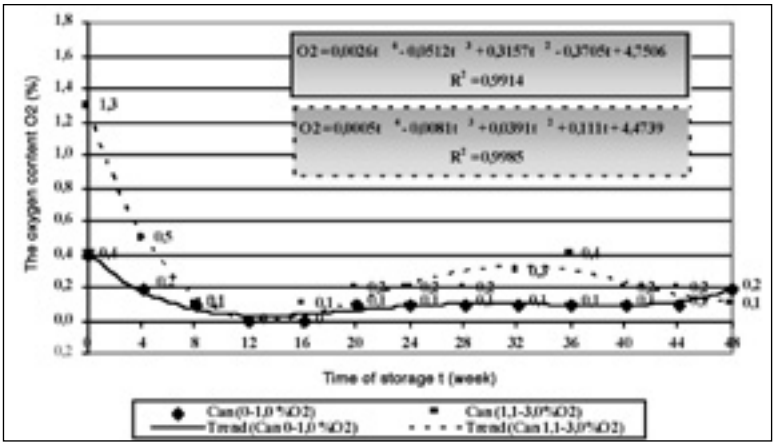


Fig. 2 - The changes of the oxygen content (O_2) in cans with different initial variant of modification of gas mixture in the package during storage in standardized conditions (temp. 18°C±1°C, R=75%±2%), % (source: author's work).

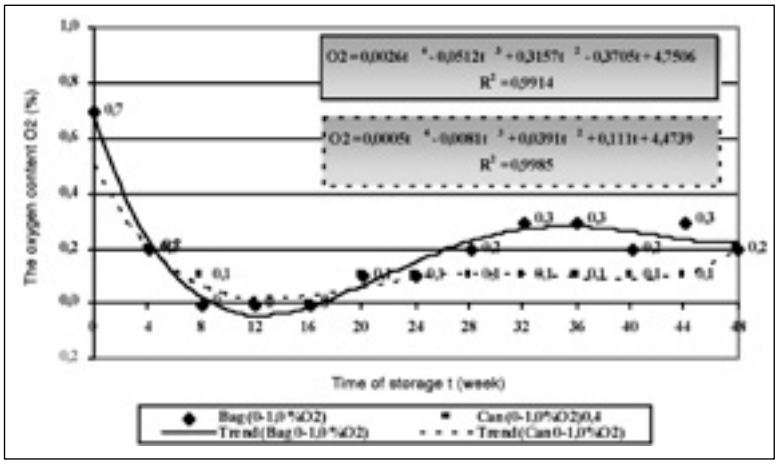


Fig. 3 - The changes of oxygen content (O_2) in different kind of packaging with initial variant of modification of gas mixture in the package: 0-1,0% O_2 during storage in standardized conditions (temp. 18°C± 1°C, R=75%±2%), % (source: author's work).

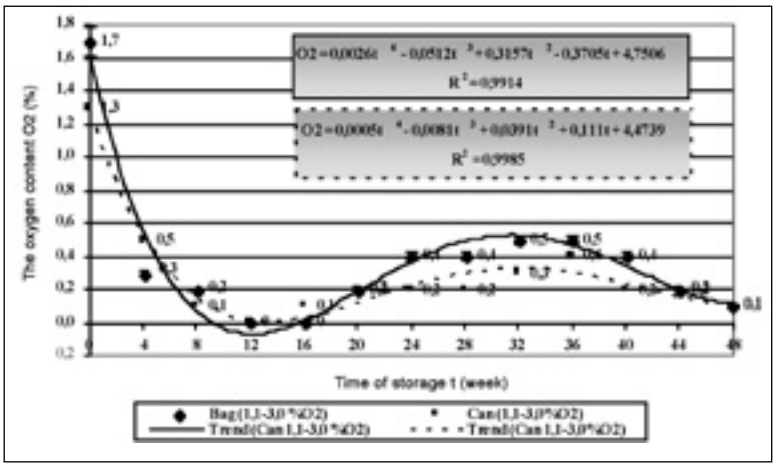


Fig. 4 - The changes of oxygen content (O_2) in different kind of packaging with initial variant of modification of gas mixture in the package: 1,1-3,0% O_2 during storage in standardized conditions (temp. 18°C±1°C, R=75%±2%), % (source: author's work).

It can also be said that the smaller the initial oxygen content in package is, the longer it takes to decline its content to minimum. It should be indicated that there is wide usefulness of developed models of dynamics of oxygen content changes in package depending on interaction time of microclimate factors to perform a comparative assessment of selection of MAP process parameters, expressed primarily by the output composition of gas mixture using various forms of package.

REFERENCES

Company Normalization Document ZDN-05/0/7. 2005. Snacks.

ISO 554. 1995. Standard condition of air-conditioning and/or researches.

Jung H. (Ed.) 2005 "Innovations in Food Packaging", Elsevier Academic Press, Oxford.

Lisińska-Kuśnierz M. (Ed.). 2005 "Quality Estimation of Packaging Materials and Packaging", Publishers of Cracow University of Economics, Cracow.

Robertson L.G. 2006 "Food Packaging Principles and Practice", Marcel Dekker Inc: New York.

Steel R. 2004 "Understanding and Measuring the Shelf-life of Food", CRC, Woodhead Publishing Limited, Cambridge.

Total Gas Control. 2005, PBI Dansensor A/S, Ringsted, Denmark.

Ucherek M. 2004. "An integrated approach to factors affecting the shelf-life of product in modified atmosphere packaging (MAP)", Food Review International, vol. 20: 297-307.

Ucherek M. 2007 "Shelf-life of Foods in Modified Atmosphere Packaging", Publishers of Cracow University of Economics, Cracow.

Ucherek M. 2009. "Testing procedure of shelf-life of product packages in a mixture of gases", Italian Journal of Food Science, Special Issues: 237-241.

SHELF-LIFE STUDY OF OSMO-AIR-DRIED CRISPY APPLE CHIPS IN DIFFERENT PACKAGING SOLUTIONS

S. GOBBI[1]*, S. LIMBO[2], P. LAMIANI[2] and D. TORREGGIANI[1]

[1]Agriculture Research Council-Food Technology Unit (CRA-IAA), Via Venezian 26, 20133 Milano, Italy

[2]Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche (DiSTAM), Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy

*corresponding author: serena.gobbi@unimi.it

ABSTRACT

Considering the extremely low water activity (~0.11) of osmo-air-dried apple chips, the sorption isotherm was studied in the water activity range 0.035-0.842. Different concentrations of Sulphuric Acid were used to create a_w values between 0.035 and 0.182, in order to obtain, in the first part of the isotherm, a more detailed curve than with the only use of the saturated salt solutions. A model aimed to predict the quality decay kinetics of the packaged apple chips was developed, and the influence of the packaging solution on moisture content changes during shelf-life was studied. In particular the research focused on the barrier properties of the package film, that represent the crucial point for the optimization of apple chips shelf-life. An experimental shelf-life simulation was developed in order to confirm the model prediction. Osmo-air-dried and air-dried apple chips were packaged in two different films (OPP 20 μm, WVTR $_{38°C, 90\%RH}$ = 2.505±0.035 g m^{-2} $24h^{-1}$; met PP, WVTR $_{38°C, 90\%RH}$ = 0.50±0.02 g m^{-2} $24h^{-1}$), with an area exposed to the mass flux of 0.024 m^2. Packages were stored at 38°C with 90% RH. At each storage time moisture content, water activity and crispness index were measured.

Key words: sorption isotherm, crispy apple chips, osmo-air-dehydration, shelf-life modelling.

INTRODUCTION

The shelf-life of dried crispy foods is mainly determined by the changes of sensory acceptability, which is strongly related to their texture. Dried apple

chips, manufactured as ready-to-eat crispy snack, are very sensitive to moisture gain and they lose crispness very easily (Konopacka *et al.*, 2002). This phenomenon creates problems in production, packaging and storage, and also limits consumer acceptability of the product. A knowledge of the sorptional equilibrium of dehydrated products, that deteriorate mainly by moisture gain (Kats and Labuza, 1981), is important both for shelf-life predictions and for the determination of moisture content and water activity critical values, above which the product is no longer acceptable to the consumer. The aim of this work was to study the water sorption mechanism of dried apple chips and their behavior during shelf-life in different packaging solutions, evaluating the validity of the predictive model developed.

MATERIALS AND METHODS

Materials. Apples *cv* Golden Delicious were used. Apple discs (20 mm diameter and 5 mm thickness) were obtained from a cylinder cut parallel to the main axis of the fruit, using a cork borer connected with a dynamometer (Instron, mod. 4301, UK), and sliced by parallel blades. Immediately afterwards, the discs were immersed for 120 minutes at 25°C in a concentrate sugar mixture (a_w=0.90) consisting of fructose, sucrose and glucose in the same proportions found in fresh apples (57% fructose, 35% sucrose and 7% glucose). The sugar solution was continuously recirculated through a peristaltic pump. The ratio fruit/solution was 1/3. Air drying was performed at 82°C, up to constant weight, which was achieved when the difference in weight was less than 1 mg/g solids after 90 additional minutes of drying. Not pre-treated apple rings were dried as a control.

Table 1 - Constant humidity solutions used for sorption isotherm determination, and corresponding a_w values.

Solution	a_w
H_2SO_4 75%	0.035
H_2SO_4 70%	0.060
H_2SO_4 65%	0.104
LiCl	0.113
H_2SO_4 60%	0.182
$C_2H_3KO_2$	0.223
$MgCl_2$	0.330
K_2CO_3	0.444
$Mg(NO_3)_2$	0.534
NaCl	0.755
KCl	0.842

Methods. Water activity (a_w) of apple chips was measured by an electronic hygrometer (Aqua Lab. CX-2 – Decagon Devices, Pullman, USA), results are the mean of 4 determinations. Moisture content (m) of dried apples was determined according to Karl Fischer method after extraction in anhydrous methanol (ASTM D 6304-2004 a, 1-procedure A). A Mettler Toledo DL53 Titrator equipped with an electrode Mettler Toledo DM142, was used. Results are the mean of 4 determinations and are expressed as g H_2O/100 g solids. Apple chips mechanical properties were determined using a puncture test (Bourne, 2002), by means of a Zwick Machine (mod. Z005, Zwick Roell, Germany), fitted with a 100 N load cell. One apple disc at a time was placed on a centre hollow support; a cylindrical probe (2 mm diameter) was driven down at a speed of 0.5 mm min^{-1}, until 90% of maximum force was reached. The slope (i.e. the elastic modulus, E) before the first fracturability peak of highest magnitude was considered as a crispness index. Final results are the mean of 10 replicates. Sorption isotherms were studied in the water activity range 0.035-0.842, using the solution indicated in tab. 1 (Lewicki, 1997). Fitting of the experimental data for sorption study of apple chips was carried out by means of Table Curve 2D software. A model aimed to predict the quality decay kinetic of the packaged

apple chips, describing the moisture content changes during time, was used (Del Nobile *et al.*, 2003):

$$dM = \frac{KP_{H_2O}}{l} \cdot A \cdot p_o \cdot [a_w out \quad a_w in] \cdot \frac{1}{W_s} \cdot dt \qquad (1)$$

where: KP = permeability coefficient of the material; l = thickness; A = area exposed to the mass flux; p_0 = water vapour pressure; $a_w out$ = water activity outside the package; $a_w in = f(m)$ = water activity inside the package; W_s = total solids weight of the packaged food. An experimental shelf-life simulation was developed to confirm the model prediction. Osmo-air-dried and air-dried apple chips were packaged in two different films (OPP 20 μm, WVTR $_{38°C,\ 90\%RH}$ = 2.505±0.035 g m^{-2} $24h^{-1}$; met PP, WVTR $_{38°C,\ 90\%RH}$ = 0.50±0.02 g m^{-2} $24h^{-1}$), 20g/pack, with an area exposed to the mass flux of 0.024 m^2. Packages were stored at 38°C with 90% RH. At each storage time, moisture content, water activity and crispness index were measured.

RESULTS AND DISCUSSION

Sorption curves for air dried and osmo-air-dried apple chips were obtained fitting the experimental data by Lewicki equation (Lewicki, 1998). Conditioning a_w values between 0.035 and 0.182 allowed a more detailed curve to be obtained in the first part of the isotherm, very important for this kind of product. Osmo-air-dried and air-dried apple chips showed the same trend, as can be observed in fig. 1. However, the osmo-air-drying process allowed the production of crispier apple chips, which remained significantly crispier than the air-dried ones for about 15 days of shelf-life when packaged in the metalized film (fig. 2). The significant texture differences between osmo-air-dried and air-dried samples flattened during shelf-life in both experiments. A good correlation was observed between crispness coefficient and moisture content during shelf-life (fig. 3), and osmo-air-dried apple chips showed to be more hygroscopic than the air-dried ones, due to their higher sugar content. Fig. 4 shows predicted and experimental values of moisture content during shelf-life; the critical moisture content (m_c) is also indicated and it was graphically derived

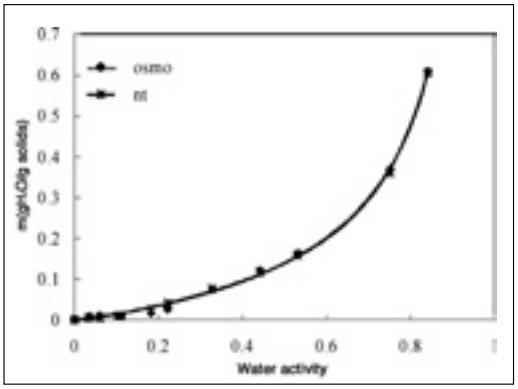


Fig. 1 - Experimental sorption isotherms for air dried (nt) and osmo-air-dried (osmo) apple chips.

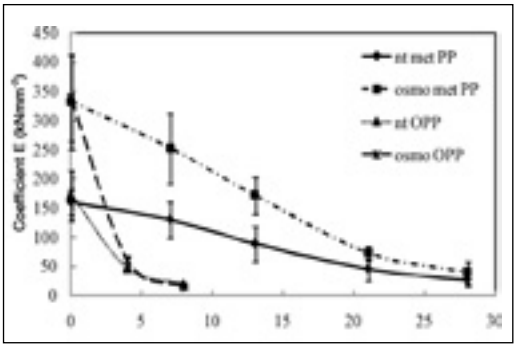


Fig. 2 - Crispness index (kN mm^{-2}) values of air dried (nt) and osmo-air-dried (osmo) apple chips during storage in the two different films (met PP and OPP).

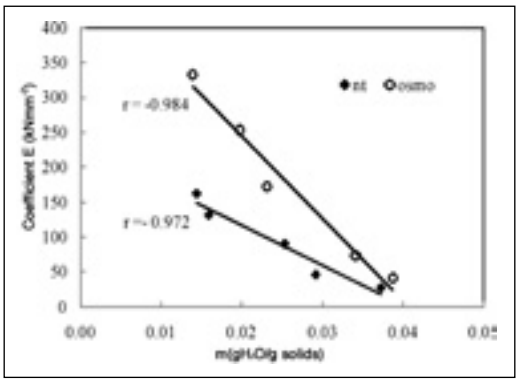


Fig. 3 - Relationship between Crispness index and moisture content values of air dried (nt) and osmo-air-dried (osmo) apple chips packaged in the met PP film.

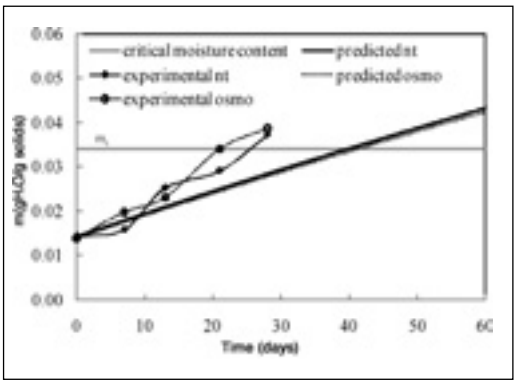


Fig. 4 - Predicted and experimental moisture content values during shelf-life of air-dreid (nt) and osmo-air-dried (osmo) apple chips.

from the isotherms in correspondence to the critical a_w values set in a previous study (Gobbi *et al.*, 2009). The model overestimated of about 40% the shelf-life of the apple chips packaged in both films. The experimental shelf-life was shorter for the osmo-air-dried apple chips than for the air-dried ones, but the difference was not significant.

CONCLUSIONS

In the osmo-air-drying process a compromise should be reached between the crispness optimization and the sugar enrichment, which leads to a more moisture-sensitive product. Further studies should be done in order to preserve for a longer shelf-life period the crispness improvement achieved in the osmo-air-drying process; the degree of metallization of the film, essential for such an hygroscopic product, should be evaluated.

REFERENCES

Bourne M.C. (2002). Food texture and viscosity-concept and measurement. 2nd Edition; Academic Press, San Diego, ISBN:0-12-119062-5.

Del Nobile M.A., Buonocore G.G., Limbo S., Fava P. (2003). Shelf-life prediction of cereal-based dry foods packed in moisture-sensitive films. Journal of Food Science, 68(4): 1292-1300.

Gobbi S., Limbo S., Farris S., Lamiani P., Torreggiani D. (2009). Processing and shelf-life optimization of osmo-air-dried crispy apple rings. Italian Journal of Food Science, Special issue, 66-70.

Katz E.E., Labuza T.P. (1981). Effect of water activity on the sensory crispness and mechanical deformation of snack food products. Journal of Food Science, 46: 403-409.

Konopacka D., Plocharski W., Beveridge T. (2002). Water sorption and crispness of fat-free apple chips. Food Chemistry and Toxicology, 67(1): 87-92.

Lewicki P. (1997). Water sorption isotherms and their estimation in food model mechanical mixtures. Journal of Food Engineering, 32(1): 47-68.

Lewicki P. (1998). A three parameters equation for food moisture sorption isotherms. Journal of Food Process Engineering, 21, 127-144.

SPOILAGE POTENTIAL OF H_2S PRODUCING BACTERIA IN SEAFOOD

A. SERIO, G.C. FUSELLA and A. PAPARELLA

Department of Food Science, University of Teramo, Via C.R. Lerici 1, 64023 Mosciano Stazione, TE, Italy

ABSTRACT

The growth of spoilage organisms is a very important factor determining seafood shelf-life. For this reason, the aim of this work was the characterization of some Specific Spoilage Organisms producing H_2S, isolated from raw tuna and swordfish. Their spoilage potential was estimated by evaluating the production of spoilage markers, such as trimethylamine (TMA), hydrogen sulfide and biogenic amines.

By means of 16S rDNA sequencing, the isolates were identified as 16 *Shewanella baltica*, 1 *Shewanella putrefaciens*, 1 *Shewanella* spp. and 5 *Serratia* spp.

All the strains were able to grow at temperatures ranging from 4° to 11°C or in presence of 6% NaCl at 25°C in 48 hours, showing a perfect physiological adaptation to the microenvironment of seafood.

Regarding the production of specific spoilage markers, all the *Shewanella* spp. produced hydrogen sulfide starting from cysteine, sodium thiosulfate or both compounds, and were strong producers of trimethylamine. All the isolates showed decarboxylating activity on amino acids such as phenylalanine, ornithyne, lysine and tyrosine, and therefore had the capability to form biogenic amines.

The isolates, and especially those belonging to the genus *Shewanella*, showed a great spoilage potential.

Key words: H_2S producing bacteria, Seafood, *Shewanella*, Spoilage.

INTRODUCTION

Microbial spoilage in foods is a topic of global interest, considering that about 25% of all food produced is lost owing to microbial activity (Gram and Dalgaard, 2002). In particular, fresh seafoods are highly susceptible to spoilage, which can be caused by both chemical reactions and microbial growth. Therefore, the growth of Specific Spoilage Organisms (SSOs) is a fundamental factor determining seafood shelf-life (Gram and Dalgaard, 2002) and the SSOs detection is undoubtedly very important for shelf-life prediction.

The aim of this work was the characterization of the spoilage potential of some SSOs isolated from raw tuna (9 isolates) and swordfish (14 isolates). In particular, the production of spoilage markers, such as trimethylamine, hydrogen sulfide and biogenic amines was evaluated.

MATERIALS AND METHODS

Twenty-three colonies were isolated from red tuna and sword fish, coming from fishing zone FAO 37, as presumptive H_2S producers, being dark on Iron Agar (Oxoid, Basingstoke, UK). *Shewanella putrefaciens* ATCC 49138 was used as reference strain for all the tests.

For DNA extraction, overnight cultures, grown in TSB (Tryptone Soy Broth, Oxoid, Basingstoke, UK) modified with 0.5% NaCl (TSBm) at 25°C, were washed twice in sterile saline solution. Cells were lysed with 200 µL of lysis buffer (0.05 mM NaOH, 0.25% w/v SDS) at room temperature for 15 min and then centrifuged at 13,000 rpm for 10 min. The supernatants were purified with immunoaffinity column GFX™ PCR DNA and Gel Band Purification Kit (Amersham Biosciences, USA), and used as template for 16S rRNA amplification, according to Marchesi *et al.* (1998).

The isolates were identified by means of 16S rRNA gene sequencing and the resulting sequences were compared with those in the database of the National Center for Biotechnology Information (NCBI), by means of Basic Local Alignment Search Tool (BLAST) program.

Overnight cultures were inoculated (1% v/v) in TSBm and incubated up to 60 days at 4°, 8°, 11°, 20° and 25°C, to evaluate the growth. In the same way, the isolates (1% v/v) were inoculated in TSBm, added with 4, 6 and 8% NaCl and were incubated at 25°C for 7 days, in order to determine their resistance to salt.

The production of hydrogen sulphide from different sulphur compounds (sodium thiosulphate, L-cysteine, or both) was evaluated according to Gram *et al.* (1987).

Amino acid decarboxylating activity was evaluated on tryptophan, phenylalanine, lysine, tyrosine, ornithine and hystidine, by means of Difco Decarboxylase base Moeller (Becton Dickinsons, Buccinasco, Italy).

RESULTS AND CONCLUSIONS

The isolates were identified as 16 *Shewanella baltica* and 1 *Shewanella putrefaciens.* Despite the high percentage of homology (99-100%) with the sequences in the database, it was not possible to attribute the species for 1 *Shewanella* spp. and 5 *Serratia* spp.

All the *Serratia* spp. were isolated from swordfish, while the *Shewanella* isolates were equally distributed in both swordfish and tuna.

Regarding physiological characterization, all the isolates were able to grow at temperatures between 4° and 25°C in 24-48 hours, or in presence of salt concentration up to 6% at 25°C in 48 hours. These results were in accordance with literature, since both *Shewanella baltica* and *S. putrefaciens* are psychrotrophic bacteria (Vogel *et al.*, 2005), able to grow even on ice-stored seafood. Also microorganisms belonging to the genus *Serratia* are commonly observed in water and can also be found in seafood (Houda *et al.*, 2007).

The production of spoilage markers, such as trimethylamine (TMA), hydrogen sulfide and biogenic amines was studied. Trimethylamine is a pungent volatile amine that is usually associated with the typical "fishy" odor of spoling seafood. Also H_2S is consid-

Table 1 - Amino acid decarboxylating activity of the isolates (TRP = tryptophan, PHA = phenylalanine, HIS = histidine, ORN = ornithine, LYS = lysine, TYR = tyrosine).
Results are expressed as number of positive isolates.

		Amino acids					
Number of isolates	Species	TRP	PHA	HIS	ORN	LYS	TYR
ATCC	*S. putrefaciens*	0	1	1	1	1	1
16	*S. baltica*	0	16	0	16	16	16
1	*S. putrefaciens*	0	1	1	1	1	1
1	*Shewanella* spp.	0	1	0	1	1	1
5	*Serratia* spp.	0	5	4	5	5	5

ered a specific microbial spoilage index, being produced from cystein located in fish muscles (López-Caballero *et al.*, 2001). Biogenic amines are a large group of naturally occurring biologically active compounds, most of which act as neurotransmitters; they can be naturally present in some foods, including fish from the family Scombridae, or can be produced by microorganisms which decarboxylate free amino acids.

Our results showed that all the *Shewanella* isolates, independently of the species, produced H_2S in 2-5 days starting from cysteine, sodium thiosulfate or both compounds, and were strong TMA producers, confirming the role of *Shewanella* spp. as spoilage bacteria (Dalgaard, 1995). On the contrary, the isolates belonging to the genus *Serratia* produced TMA but not H_2S. Data regarding the amino acid decarboxylating activity are reported in tab. 1. All the isolates showed decarboxylating activity on the tested amino acids, with the exception of tryptophan, and therefore showed the capability to form biogenic amines.

In conclusion, the isolates, and especially those belonging to the genus *Shewanella*, showed a perfect adaptation to seafood microenvironment and a great spoilage potential.

On the basis of our data, we believe that the detection and characterization of H_2S producing bacteria, showing a psychrotrophic behavior and the ability to adapt to hostile environments, ought to be considered as a primary tool to predict the shelf-life of seafoods.

REFERENCES

Dalgaard P. 1995. Qualitative and quantitative characterization of spoilage bacteria from packed fish. Int. J. Food Microbiol. 26: 319.

Gram L., Trolle G., Huss H.H. 1987. Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. Int. J. Food Microbiol. 4: 65.

Gram L., Dalgaard P. 2002. Fish spoilage bacteria – problems and solution. Curr. Opin. Biotech. 13: 262.

Houda M.B., Sivertsuik K., Lunestad B.T., Lorentzen G., Rosnes J.T. 2007. Characterisation of the dominant bacterial population in modified atmosphere packaged farmed halibut (*Hippoglossus hippoglossus*) based on 16S rDNA-DGGE. Food Microbiol. 24: 362.

López-Caballero M.E., Sànchez-Fernandez J.A., Moral A. 2001. Growth and metabolic activity of *Shewanella putrefaciens* maintained under different CO_2 and O_2 concentrations. Int. J. Food Microbiol. 64: 277.

Marchesi J.R., Sato T., Weightman A.J., Martin T.A., Fry J.C., Hiom S.J., Wade W.G. 1998. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding 16S rRNA. Appl. Environ. Microbiol. 64: 795.

Vogel B.F., Venkateswaran K., Satomi M., Gram L. 2005. Identification of *Shewanella baltica* as the most important H_2S producing species during iced storage of Danish marine fish. Appl. Environm. Microbiol. 71: 6689.

MOISTURE EFFECTS ON WATER VAPOR PERMEABILITY OF POLYLACTIDE FILMS

FEI LI, LAURA INTROZZI, STEFANO FARRIS and LUCIANO PIERGIOVANNI

Department of Food Science and Microbiology,
Packaging Laboratory, University of Milan,
Via Celoria 2, 20133 Milano, Italy

ABSTRACT

The moisture barrier properties of polylactide films were investigated by studying both the moisture sorption and the water vapor transmission of a commercial film. The first part of this research focused on the moisture sorption of the PLA film showing that it was not linear with the relative humidity, in the range between 11 and 86%. In the second part of this work, the influence of relative humidity on the water vapor transmission rate (WVTR) was investigated at 38°C by two different methods. According to the results, the WVTR values varied linearly with relative humidity differences on the two sides of the film, from 10 to 90%. Therefore, it was concluded that the absorbed moisture did not affect the water vapor permeability.

Key words: Poly lactic acid (PLA), relative humidity (RH), moisture sorption, water vapor transmission rate (WVTR).

INTRODUCTION

Although there is a growing interest in bio-based polymers, it is still difficult to achieve final materials with mechanical and barrier properties comparable with conventional synthetic polymers (Cairncross *et al.*, 2006). Three main biopolymers are currently on the market: polyhydroxyalkanoates, polylactides (PLA) and starch-based polymers (Anon, 2007). Various PLA polymers are used as packaging materials and most of the their final properties are influenced by the

molecular mass, the proportion between L and D-lactide and the additives used in the formula. It is also well known that the water-polymer interactions can affect significantly both the permeability and the mechanical properties of hydrophilic materials such as PLA. The overall performance of PLA-based packaging materials can strongly be affected by even small amounts of moisture absorbed, although in published papers some disagreement about the moisture transport mechanism appeared (Auras *et al.*, 2004). The aim of this work was to study the moisture effects on the water vapor permeability of a heat-sealable PLA film, currently on the market. To this purpose, moisture sorption and WVTR measurements were carried out at 38°C, between 11 and 86% RH, the latter being performed by two different methods.

MATERIALS AND METHODS

A 40 μm thick, three layers (two external sealable layers and a not-sealable core, see fig. 1), transparent PLA film (Polyfilms, BIO121) was used throughout the work.

According to the technical information provided by Polyfilms, the heat seal strength was ≥2 N/15mm in the range 80°-130°C, the surface energy was ≥37 mN/m, haze and gloss were, 3 and 100%, respectively. To evaluate the moisture sorption, saturated salt solutions of LiCl, $MgCl_2$, $[Mg(NO_3)_2 \cdot 2H_2O]$, NaCl, KCl, and KNO_3 were used to provide RH values of 11, 23, 32, 44, 52, 66, 75, 86 and 92%, respectively. All chemicals were analytical-grade and obtained by Fluka. Each of the aforementioned saturated solution was put inside a plastic box containing the PLA specimens (4x40 cm), folded 12 times along the longer side. The instrument "Zero volta ioner" (Semtronics Co. USA) was used to remove dust and the electric charges on the surface of PLA film. The initial weight of the PLA specimens was measured after a drying step in a vacuum oven at 23°C for 24 h. The samples were weighed until equilibrium (±0.1 mg), and the final moisture content was determined by weight difference (Denver Instrument TB-224, Italy). The WVTR measurements, according to the first procedure (bag method), were carried out at 38°C, in the boxes with aforementioned saturated solutions. Bags of the PLA films were obtained from two square films, heat-sealed on three sides, filled with $CaCl_2$, in order to assume negligible the water vapor pressure inside the bags and finally sealed on the fourth side. The filled bags were dried in vacuum desiccators at 23°C, then weighed and placed in plastic boxes at different RH values and their weights measured every 2 days. The weights of the bags, recorded during the experiment, were corrected for the moisture absorbed in the PLA film as determined by the sorption isotherm. In the second procedure, the permeability was measured by a permeability equipment (Extra solution - Multiperm, Italy), working according to the iso-static principle, at 38°C and under driving forces in the range 10-90% RH.

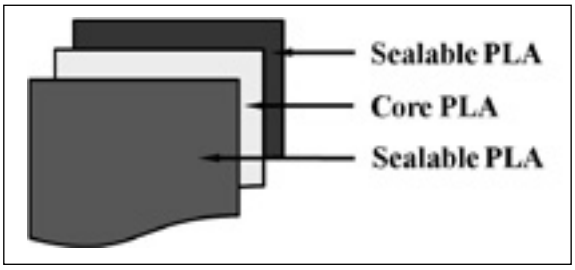


Fig. 1 - Three layers of PLA provided by Polyfilms.

RESULTS AND DISCUSSION

Moisture sorption

Fig. 2 displays the moisture sorption isotherm of a PLA film. The relationship between relative humidity and moisture sorption is not linear but shows a curvilinear shape as many hygroscopic materials. Although it is known that some PLA might degrade during the tests, this degradation should have effect on water sorption only under high RH and temperature values, so it was considered negligible during our experiments (Cairncross *et al.*, 2006).

Water vapor transmission (WVTR)

The values of WVTR versus RH obtained by the 'bag method' and the "instrumental method" are presented in fig. 3. The inset of the figure shows that 2, 4 and 6 days moisture sorption at 11 and 86% RH, for the 'bag method', is fairly linear with time, having high determination coefficient (data not shown). This means that $CaCl_2$ is active during the whole experiment and the driving force remains constant. In order to assess if the two methods were able to provide the same information, the statistical significance of the difference between the slopes of the two linear regression lines was measured by a Student's t test, computed as the difference between the two slopes divided by the standard error of the difference between the slopes. No difference was detected at a significance level $\alpha = 0.01$, so a common regression line for both the WVTR methods was plot and shown in fig. 3 with the statistical table. Clearly, both the gravimetric (bag method) and the instrumental methods, gave WVTR values related to the driving force (ΔRH) according to a linear relationship. Therefore, it can be concluded that the different amounts of water absorbed by this PLA film at the various relative humidity, (which are not proportional as demonstrated by the isotherm experiment) do not affect the proportionality of the water transmission to the RH, in the range considered and at 38°C.

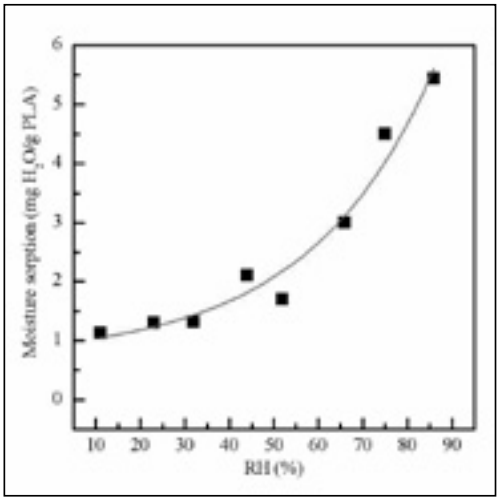


Fig. 2 - Moisture sorption for PLA at 38°C.

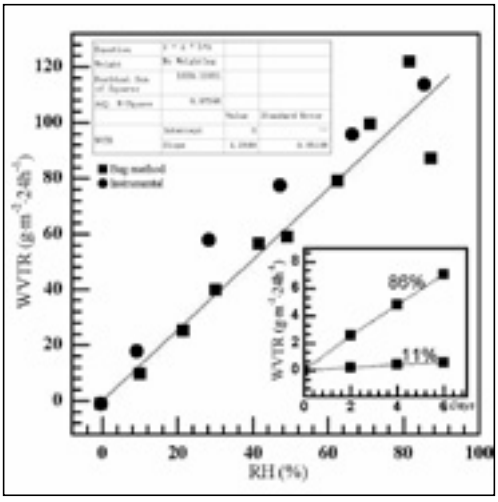


Fig. 3 - WVTR for PLA at 38°C and from 11 to 86% RH by 'bag method' and by instrumental permeability equipment.

CONCLUSIONS

The tested PLA film can absorb measurable amounts of moisture at 38°C, according to the relative humidity values. The water vapor transmission rates, measured with two different methods, increased linearly with the driving forces, demonstrating that the moisture absorbed did not seem to affect the proportionality of the water vapor diffusion.

REFERENCES

Anon, 2007. Biopolymers. Coating International. 40 (4): 7-8.

Auras R., Harte B., Selke S., 2004. Effect of water on the oxygen barrier properties of Poly(ethyleneterephtalate) and Polylactide Films. Journal of Applied polymer science. 92: 1790-1803.

Cairncross R.A., Becker J.G., Ramaswamy S., *et al.* 2006. Moisture Sorption, Transport, and Hydrolytic Degradation in Polylactide. Applied Biochemistry and Biotechnology. 129-132: 774-785.

Finito di stampare
novembre 2011

TipoLitografia Giuseppini - Pinerolo (TO), Italy

ISSN 1120-1770