GSICA
THE ITALIAN SCIENTIFIC GROUP
OF FOOD PACKAGING

The Korean Society of Food
Science and Nutrition

SHELF LIFE INTERNATIONAL MEETING

SLIM 2012

Shelf-life International Meeting

Changwon, May 30 - June 1 2012

Edited by

Dong Sun Lee & Gi Hyung Ryu

Special Issue
of Volume XXIV (4) 2012 of

ITALIAN JOURNAL
OF
FOOD SCIENCE
Rivista italiana di scienza degli alimenti

CHIRIOTTI EDITORI

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In cooperation with

KFN
THE KOREAN SOCIETY
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This Special Issue of the Italian Journal of Food Science collects the presentations given at the "SLIM 2012, Shelf Life International Meeting" organized by the Italian Scientific Group of Food Packaging (GSICA) and Korean Society of Food Science and Nutrition (KFN), held at Changwon, South Korea on May 30-June 1 2012.
These papers were reviewed by the Scientific Committee of SLIM 2012 before their presentation but they did not undergo the conventional reviewing system of the Italian Journal of Food Science.

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Reviews and surveys on specific topics relevant to the advance of the Mediterranean food industry are particularly welcome.

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INTRODUCTION

This volume collects the contributions to 5th Shelf Life International Meeting (SLIM 2012), which was held in Changwon, South Korea in a period of 30th May-June 1st, 2012. SLIM 2012 is a continued version of Shelf Life International Meeting, a unique conference dealing with shelf life of the package foods.

The conference has been organized and hosted by Korean Society of Food Science and Nutrition (KFN) in collaboration with Italian Scientific Group of Food Packaging (GSICA). SLIM 2012 being the first version of this conference outside Europe was attended by 175 scientists coming from all over the world. The participants from Asia including Korea, Thailand, China, Japan and Singapore are more than 80%. Compared to the former SLIM versions, the number of European participants has been reduced probably due to the long distance between Korea and Europe. This would be understood as an attribute of international meeting which rotates the conference place all around world each time. Shelf Life International Meeting now reaches the true international conference whose place goes around the continents.

This volume reports research reports presented as oral and poster papers. The papers were given in three sessions related to shelf life and packaging:

Shelf Life Modeling and Food Qualities: Shelf Life and quality decay of food and beverages, protective properties of packaging materials, kinetics of food quality degradation, predictive microbiology.

New Technologies for Shelf Life Extension: New materials, active and intelligent packaging, new food processing technologies, new food preservatives, new techniques for risk reduction, sustainable packaging.

Shelf Life Testing and Functional Food Properties: Non-invasive analytical techniques, freshness indicators and quality indexes, performance of packaging materials, risk management.

The aim of SLIM 2012 was to provide an international forum for presenting current development works and future directions in shelf life research and applications for the packaged food products. Because prediction, testing and extension of shelf life require multi-disciplinary approach, scientists and technologists in diverse areas were invited to the meeting,

and in-depth discussion in open and free atmosphere was encouraged following the tradition of SLIM. We are sure that the conference structure, the diversity of the attendees and the selected contributions from both industry and academia contributed significantly to identifying the problems and promoting scientific discussions and further collaborations among the shelf life researchers or stakeholders.

As a subset of SLIM 2012, Food Packaging Safety Workshop has been organized in cooperation with Korea Food and Drug Administration. Global issues on food packaging regulation in viewpoints of Korea, China, Japan and Europe were handled in the workshop with attendance of Korean regulatory officers. Direction of harmonized packaging regulation was discussed and became clearer with thanks to this workshop.

Award for excellent poster papers were given to 12 presentations with certificate. This activity of poster awarding worked to give positive encouragement to young scientists as well as a good opportunity of scientific information exchange.

During the gala dinner, the attendees could experience Korean culture of dances and songs building international friendship. The next version of SLIM 2014 was announced to be held in New Jersey, USA in collaboration of Rutgers University. We hope to see you in SLIM 2014.

Dong Sun Lee & Gi Hyung Ryu

SESSION I

"Shelf Life Modeling and Food Qualities"

OVERALL STATUS OF SHELF LIFE STUDIES

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ABSTRACT

While it would be of great interest to know the status of shelf life studies in industry, such data is sadly not available. Therefore, this paper must of necessity confine itself to the status of shelf life studies that are published in the scientific literature. Regrettably, many published studies are irreproducible because key data are lacking or incomplete. This is as much an indictment of the journal referees as it is of the individual authors. This paper reviews recent published shelf life studies and highlights the important details that must be included so they can be reproduced and their findings applied by a wider audience. To preserve anonymity, the journal names and authors are omitted. Although it is unfortunate that so much published research on shelf life is not reproducible, it is also a waste of millions of dollars of research funding and time. In most cases, the missing details could have been easily supplied if requested.

Key words: Shelf life, package dimensions, surface area, volume, degree of filling.

INTRODUCTION

Despite its importance, there is no simple, generally accepted definition of shelf life in the food technology literature. In ASTM E2454, sensory shelf life is described as “the time period that a product may be stored before reaching its end point” and defines the end point as “the point at which a product no longer meets predetermined criteria as defined by test data (for example, discrimination, descriptive or affective, or a combination thereof).” A broader definition is that “shelf life is the

duration of that period between the packing of a product and the end of consumer quality as determined by the percentage of consumers who are displeased by the product." In most cases, packaging has a strong influence on the shelf life of a food. The objective is to provide just enough protection to ensure that the food maintains its acceptability until the end of its desired shelf life. This requires detailed knowledge and understanding of the food, its packaging and the environment to which it will be exposed prior to consumption.

DETERMINATION OF SHELF LIFE

Shelf life can be determined from two sides: the product side or the consumer side. Determining shelf life from the product side implies investigating the deterioration of the product as a function of time and several models are available to assist in the determination. Alternatively, determining shelf life from the consumer side implies asking consumers to accept or reject food which has been stored for various lengths of time without normally specifying the reason for acceptance or rejection.

When shelf life is determined from the product side, sensory evaluation of the food is likely to be used either alone or in combination with instrumental or chemical analyses to determine the quality of the product. Many sensory test methodologies are available and can be classified into either analytical tests or hedonic tests. One of the problems with published shelf life studies is that insufficient details are given about the nature, experience and repeatability of the sensory panels employed.

When determining shelf life from the consumer side, consumer dissatisfaction can be related to the survival function, and models applying survival analysis to the sensory shelf life of foods have been published. Because quality changes in foods are very complex, it is not always possible to make accurate predictions of shelf life based on a mechanistic insight. In such situations, it is necessary to resort to a statistical description so that the mean time to failure and its standard deviation can be accurately estimated, and the probability of future failures predicted.

OVERALL STATUS OF SHELF LIFE STUDIES

So what is the overall status of shelf life studies? A book published in May 2012 entitled *Shelf Life Assessment of Food* and edited by Professor Nicoli may well provide the answer to this question. An earlier book entitled *Food Packaging and Shelf Life* also provides some answers. While it would be of great interest to know the status of shelf life studies in industry, such data is sadly not available. Therefore, this paper must of necessity confine itself to the status of shelf life studies that are published in the scientific literature and some recent studies are reviewed later.

KEY INFORMATION FOR SHELF LIFE STUDIES

Package Dimensions

The dimensions of the package for a given weight of food can have a significant influence on shelf life because the surface area influences permeation and the effect of exposure to light. Although a spherical shape will minimize the surface area of the package (and thus the quantity of moisture or O_2 that will permeate the package wall),

it is not a practical shape for commercial use, and in practice most packages tend to be rectangular or cylindrical. In comparing the surface areas for a range of different package shapes all having the same volume (~450 mL), the surface area of a cylinder is 16% greater, a cube 24% greater, a tetrahedron 49% greater, a rectangular shape 58% greater, and a thin rectangular shape 246% greater compared with the surface area of a sphere which is obviously not a commercially-viable shape. Extremely thin packages have a much greater surface area:volume ratio and thus require a plastic with better barrier properties to get the same shelf life than if the same quantity of product were packaged in a thicker format. Regrettably, many papers omit the dimensions of the package and therefore the research is irreproducible.

Modified Atmosphere Packaging (MAP)

The inhibitory effect of CO_2 on many spoilage bacteria is proportional to the amount of dissolved CO_2 in the product. Thus the effectiveness of MA packaging is generally determined by the amount of available CO_2 that can dissolve into the food, and is a function of the partial pressure of CO_2 inside the package and the degree of filling (DoF) (i.e., volume of product vs. volume of package (mL/mL)). The amount of gas dissolved in a product at equilibrium is proportional to the partial pressure in the atmosphere surrounding the product according to Henry's law. Various reports have shown the relationship between Henry's law and packaging variables such as temperature, gas composition and DoF on the amount of dissolved CO_2 in the product. However, many of the publications on MAP do not state the DoF or the amount of dissolved CO_2, and this makes comparison between different studies difficult and replication impossible.

Effect of Light

Determining the effect of light on the shelf life of foods is a difficult experimental area. The major problem seems to be ensuring that all the packages have been exposed to an even and consistent light source. Clearly, the surface area of the package in relation to its volume is crucial in any interpretation of the results.

Recently, Manzocco *et al.* (2012) showed that shelf life estimation of photosensitive foods (specifically sunflower and soybean oils) under actual or accelerated conditions cannot be correctly determined if the effect of light is not taken into account. They presented a model that predicted the shelf life based on changes in both light intensity and temperature, although the effect of temperature as an accelerating factor was quite limited. It would be of great interest if this research was expanded to include all the common packaging materials used to pack vegetable oils. Again, unless the surface area of the package exposed to light is specified, then the research will not be able to be repeated.

SHELF LIFE STUDIES OF EVOO

There have been many shelf life studies on vegetable oils and in particular extra virgin olive oil (EVOO). These oils are sensitive to both light and oxygen and thus their shelf life is very dependent on the barrier properties of the packaging. As is the case with many foods today, the traditional glass and metal packaging is being replaced by plastics which do not provide the same shelf life. Cecchi *et al.* (2009) critically reviewed the literature results concerning the packaging of olive oil in glass or PET bottles (both of which are used commercially for this purpose) and their conclusions provide a salutary message to researchers (and journal referees)

in the shelf life area. From the analysis of the cited literature, it was clear that the reliability of PET bottles as olive oil containers still needs to be demonstrated, primarily because of inconsistent results.

Their major criticisms concerned the fact that important properties of the PET bottle (O_2 permeability and thickness) were very seldom declared, and most experimental designs were performed using drinking water PET bottles with variable thickness and composition. In their view, the low self-consistency of literature results was also probably related to the use of different oxidation markers, and dissimilar methods to predict the shelf life by different research groups. Since olive oil is not a standardized reference material, they suggested that future experimental designs should make use of the same olive oil for all experiments or should carefully declare the initial O_2, antioxidant and pro-oxidant contents, which are widely known to influence olive oil oxidation during storage. They concluded that detailed, comprehensive and standardized experimental studies on the shelf live of olive oil packed in PET bottles should be encouraged.

EXAMPLES OF PUBLISHED SHELF LIFE STUDIES

To exemplify the overall status of shelf life studies, examples of recent shelf life studies published in the literature will be critically reviewed and their reproducibility estimated. To preserve anonymity, the journal names and authors are not given. However, the examples are drawn from 18 of the top peer-reviewed food science and technology journals.

A paper on the effects of MAP with gas mixtures of either CO_2 and Ar, or CO_2 and N_2, on the quality of pork sausages during refrigerated storage contained no details about the packaging materials, their dimensions or DoF and therefore the results cannot be replicated. A paper on MAP of fresh-cut pears gave the DoF but not the package dimensions and therefore the results cannot be replicated. A paper on the effect of MAP on the quality of Mozzarella contained details about the DoF but not the package dimensions. Although the WVTR and OTR were given, no temperature or humidity was specified. Thus the results cannot be replicated. A paper published on the effect of MAP on the shelf life of salami contained the package dimensions but no details about the DoF. Although the WVTR and OTR were given, no temperature or humidity was specified. Thus the results cannot be replicated.

A paper on the influence of storage temperature on the shelf life of fresh-cut strawberries stored under high-O_2 atmospheres contained details about the DoF but package dimensions were not given. The OTR and CDTR were given for the PP film at 23°C but not at the temperature of storage (4°C). Thus the results cannot be replicated. A paper on the shelf life of cherries under MAP contained details about the package dimensions but not the DoF. The OTR and CDTR were given at 23°C and WVTR at 38°C for the three films but not at the temperature of storage (0°C). The results cannot be replicated.

In a paper that evaluated the use of a chlorine dioxide release system in combination with MAP to control the growth of *S. Typhimurium* and *L. monocytogenes* on raw chicken breast during refrigerated storage, no package (tray) dimensions or details about the DoF were given. The WVTR, CDTR and OTR of trays and lidstock were not given, and neither was their construction. Thus the results cannot be replicated. A paper on the control of *S. Typhimurium* in chicken breast by irradiation and MAP (vacuum packaging or high CO_2 + CO) contained no details

about the pouch dimensions or composition but gave the DoF. The OTR of "high barrier" pouches was given at 23°C but not at the temperature of storage (0°C). It was claimed that the pouches were "essentially impermeable to CO_2 and CO" but no CDTR was provided. Thus the results cannot be replicated.

A paper on the effect of CO on colour stability of beef steaks stored at 1°C contained no details about the pouch dimensions or composition or the DoF. The gas barrier properties of the pouches were not given and therefore the results cannot be replicated. A paper on the effects of vacuum packaging and wrapping with an edible film on the shelf life of fish at 4°C contained no package dimensions and no WVTR or OTR of the "impermeable polyethylene bags." Therefore the results cannot be replicated.

A paper reported the effects of active and MA packaging as well as packaging materials on the quality retention of dark chocolate with hazelnuts. The dimensions of the plastic pouches were not specified, but their OTR at 25°C was. For a given pouch material, the shelf life was independent of the storage atmosphere (N_2 or vacuum). A similar paper from the same authors but focussing on almonds was published in another journal and suffered from the same omissions.

A paper on the effect of packaging on the shelf life of cauliflower stored at low temperature contained no package dimensions. Although the HDPE and LDPE bags were perforated, no details of perforation diameter or WVTR and OTR were given. Therefore the results cannot be replicated.

A paper on the effect of cartons (3 types) and PET bottles on the quality of mandarin juice stored at 4°C for up to 90 days concluded that deterioration was triggered by the rise in O_2 in the headspace of the packages. However, no details about the surface area:volume ratio of the packages or their OTRs were given so study cannot be replicated.

A paper on the effects of packaging materials on the shelf life of shelled walnuts stored in the dark for up to 12 months at 10, 20 and 30°C gave the OTRs of the two pouches. However, no details about the dimensions of the pouches were provided, thus making the results of limited value as study cannot be replicated

A paper on quality changes in EVOO stored in PET bottles with and without an O_2 scavenger stored in the dark and under diffuse light (details not provided) at 20-22°C for up to 13 months provided no details about the surface area:volume ratio of the PET bottles or their OTRs, thus making the results of limited value as the study cannot be replicated. A paper on quality changes in EVOO stored in PET, PVC and glass bottles at 15, 30 and 40°C under fluorescent light or in the dark for 12 months gave the OTRs of the plastic bottles but provided no details about the surface area:volume ratio of the bottles, thus making the results of limited value as the study cannot be replicated. A recent paper examined changes in the chemical composition and sensory characteristics of EVOO resulting from prolonged storage at different temperatures in various containers. The results showed a gradual loss in quality during storage, and the container types related to this loss followed the order: PET bottles >glass bottles>Tetra-Brik®. Although all container types had the same surface area exposed to light and air, regrettably no details about the three packages were provided, making it impossible to replicate this study.

A paper compared the shelf life of blackberries stored at 3°C and 85% RH for 3 weeks in OPLA and OPS containers with snap-fit lids. Although the WVTR (38°C and 100% RH), OTR and CDTR (23°C and 0% RH) were given, the surface area:volume ratio of the containers was not specified, thus making the results of limited value as the study cannot be replicated.

In a paper in which whole wheat bread was stored at 22°C for up to 5 weeks in LDPE bags "with high O_2 permeability," enrichment of bread with α-tocopherol resulted in higher degrees of rancid aroma and flavour. However, no details about the dimensions of the bags or their barrier properties were provided, thus making the results of limited value as the study cannot be replicated. A paper on the shelf life of whole wheat bread stored for up to 5 weeks at room temperature (unspecified) provided no details about the dimensions of the bags or their barrier properties, thus making the results of limited value as the study cannot be replicated.

A paper on the effect of light on the quality of juices in PET bottles contained no details on the surface area:volume ratio of the bottles. A bottle rotation system was used to minimize variability in light or UV exposure and temperature but insufficient details were provided to enable replication. Another paper reported the effects of MAP, O_2 absorbers, ethanol emitters and fluorescent light on 150 g of cheese packed in 500 mL plastic pouches with headspaces of 300-350 mL. The DoF and pouch dimensions were not given, and there was no indication of the surface area exposed to light. Therefore it is impossible to replicate this study.

In a further example of the difficulties of accounting for all the variables that affect the shelf life of foods, the effect of the light barrier properties of three different packaging films on the photo-oxidation and shelf life of commercial cookies containing 23.5% fat stored at 40°C under UV-light was reported. However, there were large differences in the OTRs of the three films (the OTR of the best was 25 times that of the poorest) that would have had a significant influence on shelf life, in addition to the effect of the different light transmission properties of the three films which varied by a factor of 12. Therefore, it is not possible to draw any conclusions from their results.

CONCLUSIONS

As this brief review has shown, too many published papers on shelf life contain insufficient details to enable the experiments to be replicated by others. In particular, important details related to package dimensions, surface area:volume ratios and the DoF for MAP are lacking. One of the well-established principles of scientific publishing is that others should be able to reproduce the work. The examples cited indicate that all the major food journals have permitted publication of irreproducible results and seem unaware of the need to include these details. While this is obviously unintentional, it reflects badly on the journals, their editors and reviewers. Although it is unfortunate that so much published research on shelf life is not reproducible, it is also a waste of millions of dollars of research funding and time. In most cases, the missing details could have been easily supplied if requested.

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PACKAGING AND SHELF LIFE OF FERMENTED FOODS

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ABSTRACT

Food Packaging scientists are almost exclusively concerned with the negative roles that microorganisms play in foods. The threats for food safety and the risks of precocious spoilage are the main microbial issues they have to face daily, driving their efforts in selecting the best packaging material and good packaging technologies for specific foods and beverages. However, it cannot be neglected that there is an enormous variety of foods where microbial activities are essential features of their production, the main responsible of their sensorial and nutritional value, as well as the determinant of their potential durability. Fermented foods are known and used world widely but in this lecture the focus will be on some Mediterranean fermented foods only. For some of these fermented foods (red wine, fish and fresh cheese) the most recent achievements, obtained by our Department in this special field of research, are shortly presented.

Key words: Fermented foods, shelf life, safety, packaging.

INTRODUCTION

Microbial activities are essential features in the production of a huge variety of foods, and food scientists call these foods *Fermented* or *Microbial Foods*. Nowadays the field of applied biology that uses living organisms to produce or modify food products is currently considered a relevant part of Biotechnology, and Packaging Science should paid much more attention than ever before on this field, for different reasons. One important motive, which here is not dealt with, is that the use of bio-resources and bio-processes in novel packaging materials production is gaining more and more interest; a second key reason, totally consistent with this lecture, is the special care that should be taken to these particular foods in order

to preserve their outstanding value and to extend possibly their commercial lives. According to the most common definition, *Fermented Foods* (FF) *are foods produced or preserved by the action of microorganisms*. The definition, typically, refers to the fermentation of sugars to alcohol (by yeast) and to other fermentation processes which involve the use of bacteria (mainly lactic acid bacteria); several benefits are generally associated to FF consumption because it is known that they enrich the diet through the development of a diversity of aromas, they preserve the food through lactic acid, alcohol, and acetic acid production, they enrich the biological value of food with essential amino acids, fatty acids, and vitamins accumulation and eliminating, in special cases, possible present anti-nutrients; last but not least, they decrease cooking time and energy consumption also.

Actually what comes out from these general assumptions is not at all the truth, because fermentation can take place and proceed even without living cells and, actually, in 1907 Eduard Buchner from the University of Berlin, received the Nobel Prize in Chemistry for his biochemical researches and his discovery of cell-free fermentation. Moreover, not always all the advantages mentioned are effective and sometime even some risks have been noted in consuming fermented foods. Actually, risks of botulism for improperly fermented animal products, as well as accumulation of biogenic amines in fermented foods are well known. Alaska, for instance, has more cases of botulism than any other state in the United States of America, due to the traditional Eskimo practice of allowing whole fish to ferment for a long time before being consumed. This risk is even exacerbated when an air-tight container is used because the bacteria thrive in the anaerobic conditions created by the package.

HISTORICAL BACKGROUND

Fermented foods are produced and consumed everywhere, in all the countries of the planet. The FF consumption is probably higher in East and Southeast Asia, at least as far as vegetable fermented foods are concerned, but is also strong in Africa, Americas, Middle East, Europe and Oceania. The almost ubiquitary presence of FF in all the countries does not mean they are similarly constituted and/or manufactured. On the contrary a broad diversity (we can really say *biodiversity* in this case) distinguishes these products for composition, manufacture characteristics and properties. FF are manufactured using meat or fish, like salami in south Europe or the fermented herring in the north Europe; a lot of fermented products origin from milk, especially in Mediterranean area but also in Eastern Europe where probably the Kefir and Yogurt production started in very ancient time. Largely spread all around the entire world are also several kinds of fermented beverages: beers and wines above all, at least in terms of worldwide production.

Investigating them and their peculiarities, we just write down in scientific language what has been discovered before mankind had any understanding of microbiology. They are an emblematic case of products that anticipated the knowledge: even several thousands of years ago (before any basic knowledge of fermentation process had been gained), it was gradually learned that the conversion of some food products by means of a natural transformation (fermentation) is an effective means of preservation. Really, we can conclude that fermentation is the most ancient way of shelf life extension, discovered and developed when the shelves were not been in use yet.

The earliest evidence of winemaking, which is one of the most ancient biotechnology the men ever used, dates from 8000 years ago, in Georgia, in the Caucasus

area and 7000 year-old jars containing the remains of wine have been excavated in Iran. For sure we cannot know who first invented the first FF, but looking for a name to be cited about them we cannot neglect the name of Louis Pasteur. A worldwide well known French scientist (1822-1895), he was a chemist, even if his best achievements have been in the fields of Biology, Food Technology and Medicine.

We are in debt to Pasteur for several different things, many of them are well related to this topic. For instance, we learned from him to talk about *microbiology* in Food Science, instead of using the term *bacteriology* proposed and used by other scientists like Robert Koch (1843-1910). Pasteur's doctoral thesis on crystallography demonstrated for the first time the existence of chiral molecules and he did this investigating the grapes fermentation in wine making. We are in debt to him for new cures, both for animals and humans and very famous are his works against rabies and puerperal fever. However, he is best known to the general public for inventing a method to stop milk from causing sickness, a process that came to be called *pasteurization* and that is also applied after a fermentation process, in order to produce more stable products. Less known but definitely much more related to the topic of this lecture is the work he made on *carbonic maceration* a winemaking technique, in which whole grapes are fermented in a carbon dioxide rich environment prior to crushing. During carbonic maceration, an anaerobic environment is created by pumping carbon dioxide into a sealed container filled with whole grape clusters. The results of the carbonic maceration are a brilliant red wine production, the maintaining of the original sweetness of the fresh fruit and the selection of proper yeasts. The roles of the gases and the container in this wine making technology, well understood by Louis Pasteur, really represent an anticipation of Modified Atmosphere Packaging and Active Packaging technologies.

OLD AND NEW RESEARCH IN THE FIELD

It's easy to demonstrate that packaging has and always had a very important role in food preservation, even when fermented foods are concerned. However in its very long story, packaging changed its role and importance quite frequently. Starting from the most ancient time, the very first function of packaging (or better, the function of what we can consider a packaging prototype) may be assimilated to an hideaway: objects used to hide and protect foods, intended as essential and valuable products, against predators and enemies. Following the human beings evolution the functions of containing and transporting became more and more important and the need of protecting perishable products became a must when the distance of trading grew. In modern society the hedonistic role of packaging is well known and the definition of silent seller for packaging is worldwide used but, probably, the most intriguing change in packaging role and functions is the very recent shift from a passive to real active role in protecting and presenting foods. This fundamental change took place when it became clearly possible to manage and to control the properties of the packaging materials and the performance of the packages.

However this is not just a today achievement, because about 60 years ago, Dr. Charles Robert Oswin, a British scientist working in British Cellophane Ltd, wrote a fundamental book (nowadays almost lost or forgotten), focusing on the essential role of packaging materials performance (Oswin, 1954). The title of this book (Protective Wrappings) is something particular, being protective wrapping what we call today flexible packaging. Even if published more than 60 years ago, it was facing more or less the same packaging problems as we do today: e.g. high barrier

and breathable materials were something to be achieved, and minimally processed vegetable was a big deal and a big potential business (Fig. 1).

Oswin has been the first packaging scientist who put oxygen permeability versus water vapor permeability, getting a single coordinate value to be used as a guide for shelf life optimization. He proposed this theoretical optimization through the so called Oswin's windows and in Fig. 2 we can see one of these windows, the one built up for a fermented meat product. The not masked areas of the diagram in the figure focus the only values of oxygen and water vapor permeability which should be compatible with the maximum shelf life of the product and the letters correspond to specific packaging materials; the window also emphasizes the risks of quality decay due to wrong choices of packaging materials. It's real a pity that Robert Oswin couldn't contribute to the modern packaging era and that he had to deal only with waxed papers, PVDC coated cellophanes and simple polyolefin films. Actually, in the 50s the flexible packaging was quite different from the one

Fig. 1 - Pictures from 1954 Oswin's book regarding the main problems of those days: barrier materials, minimally processed vegetables, breathable films.

we know today and Oswin couldn't know the entire story; in particular the active packaging concept was totally unknown and, in fact, we must wait till the late 80s or better the 90s to read something really pertinent to the shift of the packaging role from passive to active. Packaging is defined as active when it performs some desired role other than to provide an inert barrier between the product and the outside environment (Yam, 2009).

But once again, what seems to be totally new is somehow a heritage, a legacy of the past. The reference is to very old containers, the earthenware potteries that can act as active packaging indeed (Park and Lee, 2010). Earthenware container that has not been fired to the point of vitrification is slightly porous and coarser than stoneware and porcelain; during the firing, the fine particles covering the surface fuse into an amorphous, glasslike

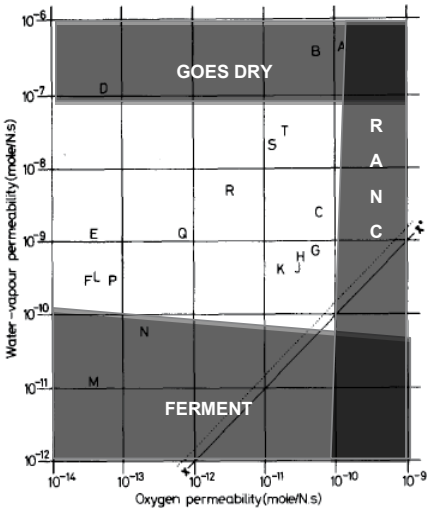

Fig. 2 - The Oswin's windows for a fermented sausage.

layer, sealing the pores of the clay body but very likely also changing the surface properties of the materials and, maybe, inducing a weak infrared emission that can be relevant to some phenomena or process. These containers, whose permeability seems to be modulated by the manufacturing process, are thought to be about 9,000 years old and they are still widely used in the 21st century.

The short presentation of examples of research carried out on FF in the DeFENS (Department of Food, Environmental and Nutritional Sciences) starts from the use of earthenware containers in wine making and re-fining (Piergiovanni *et al.*, 2010).

The process of wine making is mainly based on the alcoholic fermentation of grape must by means of yeasts, especially *Saccharomices cerevisiae*, which transform glucose and fructose present in the fruits, in ethanol, glycerol and several minor, but very important, byproducts. The process of the red wine making is also characterized by a second fermentation which occurs later, reducing the acid level (by the transformation of malic acid in lactic acid), increasing the taste body by production of dextrans and glucans, increasing the flavor complexity (buttery, nutty, honey vanilla, leather, spices). At least three genera of Lactic acid bacteria (*Lactobacillus*, *Pediococcus*, *Oenococcus*) can be responsible for the Malo-Lactic fermentation. In the red winemaking process a third fundamental physical-chemical phenomenon is *maceration*, already investigated by L. Pasteur as we have seen before. This is the process where the phenolic materials of the grape (tannins, anthocyanins and flavor compounds) are leached from the grape skins, seeds and stems into the must. Maceration is also the process by which the red wine receives its peculiar color. In the production of white wines, grape is pressed, then all solid materials are removed before antocyanins and tannins are released in the must.

The very very ancient way of making, storing and delivering wines was always supported by earthenware containers. Therefore, we evaluated the chemical and sensory differences between a 2 years old red wine produced and refined in earthenware jars and the same wine obtained by means of the current technology in a concrete tank. Significant differences between the two wines are expressed by the color parameters and the levels of anthocyanin fractions as Table 1 shows. All of such data show a higher amount of anthocyanin-tannin polymers. The oxygen supplied to the wine by the earthenware jars certainly allowed proanthocyanidins to effectively polymerize both with themselves and with the anthocyanins as shown by the vanillin index to the proanthocyanidins ratio. Such a behavior can

Table 1 - Chemical parameters of the same wine, manufactured and refined in different containers.

	Red wine in earthenware jars	Red wine in concrete tank
Anthocyanins $_{(mg/L \pm 10\%)}$	24.2	34.6
Colour indices:		
Intensity ($A_{420\ nm+520\ nm+620\ nm} \pm 0.01$)	0.69	0.61
hue $_{(A420\ nm\ /\ A520\ nm \pm 0.01)}$	0.83	0.86
Coloured anthocyanins $_{(A520\ nm \pm 0.05)}$		
wine	0.335	0.297
wine + SO_2	0.288	0.219
wine + HCl (pH 1)	0.339	0.319
Vanilline index $_{(A500\ nm \pm 10\%)}$	0.322	0.278
Proanthocyanidins $_{(mg/L \pm 10\%)}$	1640	1280
Vanillin index/proanthocyanidins	0.00020	0.00022

be explained only if the polymerization phenomena were promoted by an effective oxygen transfer in the jar. Both the sensorial evaluations and the analytical determinations performed on the two wines demonstrated that the ancient way of using earthenware jars for grapes fermentation and wine re-fining is still adequate to provide a marketable red wine. Moreover, noticeable differences were observed between the two wines that is reasonable to correlate to the specific performance of the clay tanks used, in terms of oxygen permeability, heat insulation and, possibly, of microorganisms selection: a selection very likely mediated by the specific surface properties of the earthenware.

At the DeFENS, fermented foods are also the object of microbiological researches, focused on fundamental issues like safety and nutrition, as the next examples shortly show. As just underlined, Malo-Lactic fermentation is a fundamental step in red wine making but it is problematic in cold regions. Genetic aspects and phenotypic traits of thirty six *Oenococcus oeni* strains, were investigated over three consecutive years. Micro-vinification experiments allowed the selection of strains with potential oenological performances and an interesting capability to grow in cold conditions was confirmed (Vigentini *et al.*, 2009). The research also permitted to identify some *Oenococcus oeni* strains (see Fig. 3) able to form high level of biogenic amines phenylethylamine (up to 47 mg/L) and tyramine (up to 36 mg/L), both in cultural broth and in wine, which for this reason pose some concerns in enological applications.

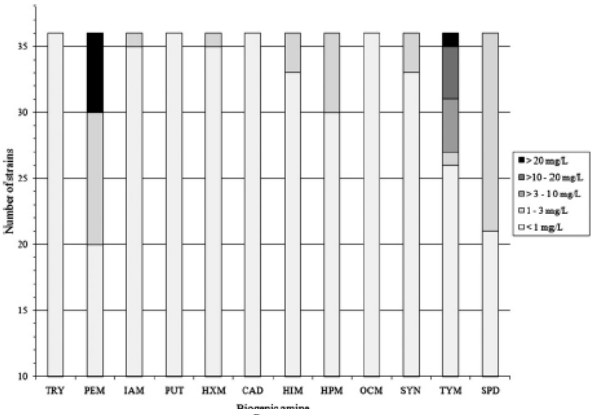

Fig. 3 - Production of biogenic amines by *O. oeni.* Phenylethylamine (PEM) and tyramin (TYM) were quite high in some strains.

Again about possible presence of biogenic amines in fermented foods, the microbiologists of our Department developed quantitative PCR assay (qPCR) for the specific detection of *Morganella morganii*, a fish pathogen responsible for the Histamine Fish Poisoning (Ferrario *et al.*, 2012). Specific PCR products were identified by melting curve analysis (see Fig. 4), and a reproducible distinct melting point (Tm) of 84°C.

Standard curves were constructed to investigate the linearity and the sensitivity of the method, leading to a highly specific and rapid assay for the

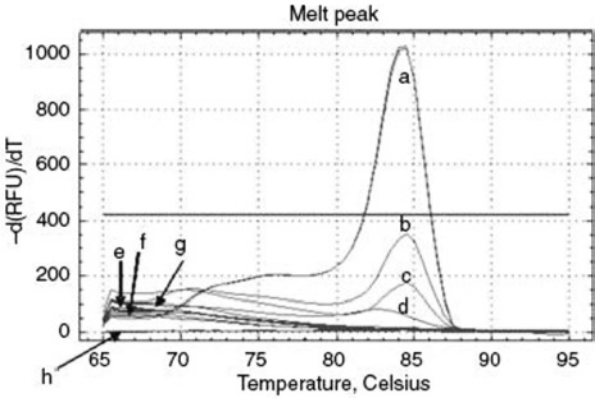

Fig. 4 - Specific PCR products were identified by melting curve analysis, and a distinct melting point of 84°C.

detection of *M. morganii* in tuna fish samples. In the last example proposed (Cocolin *et al*, 2007), two strains of *Enterococcus faecium*, M241 and M249, isolated from goat milk, were studied for their capability to produce antibacterial compounds. Goat milk is used in the preparation of traditional fermented fresh cheeses. It was determined that the bacteriocins produced by both strains were active towards *Listeria monocytogenes* and *Clostridium butyricum*, and they did not have any activity with respect to other species of lactic acid bacteria. Enterocins A and B were targeted by polymerase chain reaction (PCR) and sequenced, after cloning, in both strains. The bacteriocins contained in the cell free supernatants were stable when subjected to treatments at high and low temperatures or with lipase, catalase and a-amylase.

Lastly, a co-culture experiment with *L. monocytogenes* in skimmed milk was also performed showing that in presence of the *E. faecium* strains, the pathogen showed a delay in the growth of about 6 hours and it reached a maximum counts of about two orders of magnitude lower with respect to the control as Fig. 5 shows.

CONCLUSION

These results suggest the possibility to use the strains studied as starter cultures to enhance food safety of dairy products and, more in general, all these examples witnesses how the research in shelf life, in packaging and in fermented foods can be strictly correlated and mutually beneficial.

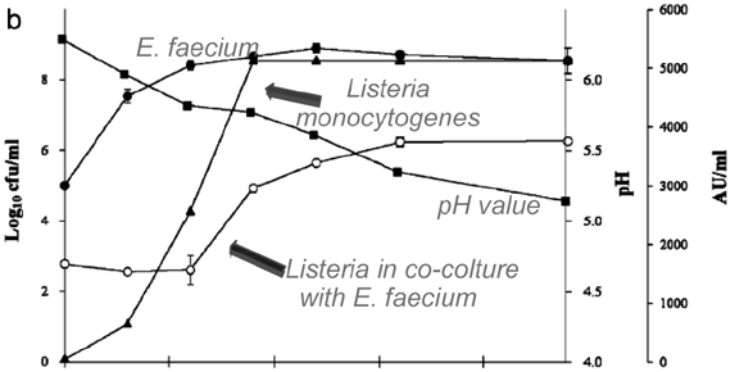

Fig. 5 - Growth curves and acidification trends obtained from the control and the co-culture experiments.

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PREDICTIVE SHELF LIFE OF GREEN OAK BY IMAGE ANALYSIS

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ABSTRACT

Relationship between rate of gas exchange and rate of quality loss for prediction the shelf life of Green oak has been studied in this research. Green oak was washed, cut, and centrifuged for 1 minute by manual salad spinner to remove excess water and packed in LLDPE plastic bag. Three MAP conditions of O_2:CO_2:N_2 equal to 1:5:94, 5:5:90, and 10:5:85 were studied and all packages were stored at temperature of 5°C. The image of Green oak samples was taken 7 times over 20 days and analyzed for area of irregular color appearing on each bag of samples with self-developed image analysis program. The samples on same day were subjected to visual evaluation for scaling the acceptability by trained panellist. Results showed that irregular color area of Green oak greater than 9% was defined as the unacceptable criteria and the color deterioration rate was found to follow the modified 1st-order reaction rate. In addition, respiration rates of cut green oak under the three MAP conditions were analyzed from the rate of gas exchange data. All cases of the respiration rate data of Green oak were obeyed the enzyme kinetic rate form proposed by Uchino. The rates of respiration were varied dependently on the initial filling gas compositions. Simplified form of the enzyme kinetic respiration rate was superimposed with the form of modified 1st-order reaction rate used for modelling the color deterioration rate, so we proposed that shelf life prediction could be obtained directly by the respiration rate model via the correlation between respiration and color deterioration. Studied results also confirmed that the image analysis program and respiration rate measurement were crucial tools for modelling the shelf life of vegetable salad product.

Key words: Image analysis, kinetic rate, shelf life, color deterioration, vegetable.

INTRODUCTION

Nowadays, modified atmosphere packaging (MAP) is widely used in freesh vegetable and fruit industry to preserve their freshness and prolong the product shelflife. By means of controlling the respiration rate of fresh products together with cold storage temperature, deterioration rate of the product quality could be delayed effectively in various kinds of veggies and fruits resulting in longer shelf life. However, designing of MAP to prolong the shelf life of each type of veggie products requires the knowledge of its respiration rate, rate of quality deterioration, and packaging material properties. Without the first two factors knowledge, industry practices commonly design the MAP for fresh veggies and fruit based on experimental data only. To accurately predict the product shelf life packed in MAP, mathematical correllation of the respiration rate model and the rate of quality deterioration is indeed a significant information. Even though, various mathematical models for respiration rate and quality deterioration rate of fresh vegetables and fruits were proposed as nicely reviewing by Fonseca *et al.* (2002). Among the respiration models, the Michaelis-Menten type models or enzyme kinetic rate models have been applied to describe the relation between the respiration rate and factors affecting it such as gas compositions and storage time for many vegetable and fruit varieties. Lee *et al.*(1991) applied the Michaelis-Menten-type model to calculate the respiration rate of blueberry effecting by gas composion inside the package while Uchino *et al.* (2004) developed temperature and time dependent respiration models for eggplant, asparagus and broccoli base on enzyme kinetic rate model. The effect of temperature on the respiration rate and quality change could be described by Arrhenius relation.

Since the rates of some quality deterioration such as color change and loss of vitamin are also governed by enzymatic reaction, the correlation, if any, between the respiration rate and rate of quality deterioration of fresh veggies and fruits may definitely be useful for predicting the shelf life. Therefore, the purpose of this study was to predict the shelf life of green oak packed in MAP and to describe the mathematical relation between the kinetic rate of its respiration and the rate of quality loss due to color change.

MATERIALS AND METHODS

Sample preparation Fresh Green oak were bought from a local shop in Suranaree University of Technology, Thailand. Green oak were cut, washed, centrifuged for 1 minute by manual salad spinner to remove excess water and 40 g of shredded green oak were packed in double bags made of linear low density polyethylene (LLDPE). The double bags were used to ensure that the changing of gas concentrations in each set of initial filling composition were not at the same level throughout 20 days, so effect of MAP conditions on the respiration rate and color deterioration was truly justified. All bags were initially filled with a specific gas composition of O_2:CO_2:N_2 and stored at temperature of 5°C. Three sets of O_2:CO_2:N_2 composition were at 1:5:94, 5:5:90, and 10:5:85.

Image acquisition In order to determine the deterioration rate of the sample color, green oak samples were photographed 7 times over 20 days at a resolution of 1024 x 786 pixels by digital camera (FUJIFLIM, FinePix S1500) under illumina-

tion of D_{65} lamp positioning at 20 cm above the sample and all images were saved in the RGB color space with a file formats of JPEG.

Digital image analysis for determination of color deterioration rate

Every image was analyzed for irregular-color area by using self-developed computing code that was capable of integrating for the area having specific H° values. In this case, the irregular color was assigned by two ranges of H° value shown in Table 1.

Table 1 - H° ranges of irregular colors from digital image of Green oak samples.

Ranges	H°	
	Lower limit	Upper limit
1	35	80
2	95	100

The remaining area of regular color was used for modeling the deterioration rate of sample color. Kinetic model coefficients were obtained by a non-linear regression method using SigmaPlot version 10 (Systat Software, Inc., USA).

Respiration rate measurement Gas sample in the headspace of each sample bag was taken every 4 days to analyze for O_2 and CO_2 concentration using GC-TCD (GC-2010, Shimudzu, Kyoto, Japan). Then, the enzyme kinetic respiration rate as a function of storage time, Eq. 2 was modeled and reported in term of O_2 consumption and CO_2 production rates. Here, the respiration rate was presumed to be governed by the enzyme kinetic rate model similar to the one reported by Uchino (2004) and model coefficients were obtained by non-linear regression method using SigmaPlot version 10 (Systat Software, Inc., USA). Model fitness was indicated by R-square.

Correlation between the kinetic rates of respiration and color deterioration The relation between the respiration rate and rate of color deterioration could be described by comparing the kinetic rate constants of both reactions. Transformation of the respiration rate described by Eq. 2, which contains 3 types of kinetic rate constants, to the modified 1st-order rate of color deterioration is needed prior to the comparison of the kinetic rate constant. It should be noted here that the enzyme kinetic respiration model in the form of Eq. 2 could be actually written in 3 parameters consisting of only a kinetic rate constant and two reaction constants.

RESULTS AND CONCLUSION

Fresh fruits and vegetables maintain metabolic activities such as the respiration and photosynthesis after harvest until senescense state. The process of respiration can be represented by a simplified chemical reaction (Lee *et al.*, 1991)

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + Energy \quad (1)$$

Respiration process may be somehow pictured as an enzyme kinetic governing rate as widely reported for many kinds of fruits and veggies (Andrich *et al.*, 1991; Lee *et al.*, 1991; Peppelenbos and van't Leven, 1996). In general, two mathematical forms of enzyme kinetic respiration rate models were written namely for rates of

CO_2 production and O_2 consumption as a function both gas concentrations inside the package and as a function eznzyme-substrate, rate constant, and time. The latter form explicitly indicated that both rates of CO_2 production and O_2 consumption were changed as a function of storage time and remaining O_2 in the package. Therefore, shelf life prediction was easily deduced by this particular form of respiration model. Hence, in modeling the respiration rate, we chose to describe the rates of CO_2 production and O_2 consumption by applying a form of enzyme kinetic respiration rate reported by Uchino *et al.* (2004). The enzyme kinetic respiration rate model can be expressed as follows:

$$R = k\left\{\frac{k_s}{k} - \left(\frac{k_s}{k_d} - [ES]_0\right)e^{(-k_d t)}\right\} \quad (2)$$

where R is the respiration rate (% by mol.kg^{-1} .day^{-1}), k is the rate constant of respiration (day^{-1}), t is the time (day), $[ES]_0$ is the initial enzyme-substrate concentration which is assumed equal to initial concentration of O_2 in the bag (% by mol. kg^{-1}) and k_S and k_d are rate constants for synthesis and decomposition of enzyme-substrate (% by mol.kg^{-1} .day^{-1}), (day^{-1}), respectively. The Eq. 2, R, was used to predict both the rate of O_2 consumption (R_o) and rate of CO_2 production (R_c) for this research. The results are shown in Fig. 1 and all parameters for the R_o and R_c models were summarized in Tables 2 and 3, respectively.

In this research, the enzyme kinetic respiration rate models, O_2 consumption rate and CO_2 production rate, in the form of Eq. 2 was transformed to the modified 1st-order reaction rate. The model can be expressed as follows:

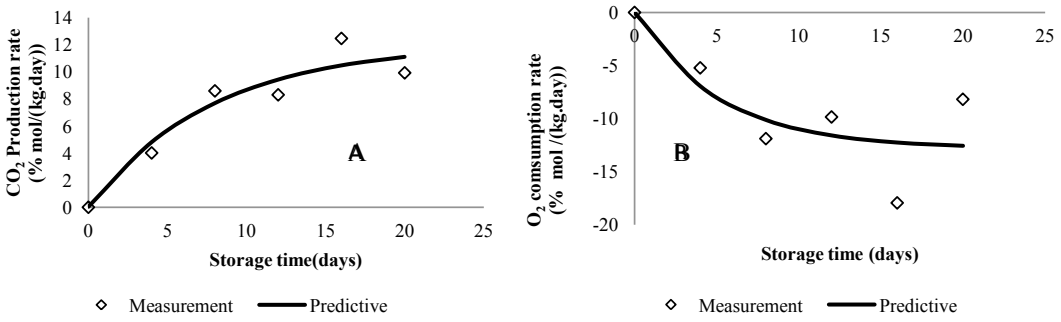

Fig. 1 - Experimental data and predictive model obtained from Eq. 2 of CO_2 production (A) and O_2 consumption (B) of Green oak at MAP condition 1:5:94 (O_2:CO_2:N_2) and at 5°C.

Table 2 - Model parameters of eq.(2) for prediction of O_2 consumption rate of Green oak at 5°C.

MAP condition (O_2:CO_2:N_2) (% by volume)	k(days^{-1})	k_s(%mol.kg^{-1}.(days^{-1})	k_d(%mol.kg^{-1}.(days^{-1})	R^2
1:5:94	-0.0012	2063.9447	0.1947	0.6719
5:5:90	-0.0120	367.4467	0.3193	0.8307
10:5:85	-0.0040	4407.6137	1.1131	0.9581

Table 3 - Model parameters of eq.(2) for prediction of CO_2 production rate of Green oak 5°C.

MAP condition (O_2:CO_2:N_2) (% by volume)	k(days^{-1})	k_s(%mol.kg^{-1}.(days^{-1})	k_o(%mol.kg^{-1}.(days^{-1})	R^2
1:5:94	0.0007	2063.4406	0.1263	0.9194
5:5:90	0.0047	362.0072	0.1499	0.9570
10:5:85	0.0047	362.0072	0.1499	0.9850

$$R_o = a_o e^{(-k_o t)} + b_o \qquad (3) \qquad\qquad R_c = a_c e^{(k_c t)} + b_c \qquad (4)$$

where R_o is O_2 consumption rate at any time (% by mol. kg^{-1}.day^{-1}), R_c is CO_2 production rate (% by mol .kg^{-1}.day^{-1}), t is the time (day), k_o is the rate constant of O_2 consumption rate, k_c is the rate constant of CO_2 production rate (day^{-1}), a_o b_o, a_c, and $b_c$are constants for O_2 consumption rate and CO_2 production rate(% by mol. kg^{-1}.day^{-1}).

For example, the parameters of O_2 consumption rate and CO_2 production rate for Green oak sample, packed under MAP condition of O_2:CO_2:N_2=1:5:94 and stored at 5°C, were k_o = 0.2000, a_o = 13.2180, b_o = –12.8160,k_c = 0.1310, and b_c = 11.9870. The profiles of O_2 consumption rate and CO_2 production rate obtained by Eqs. 3 and 4 were superimposed with the forms of R_o and R_c models based on Eq. 2 (profile not shown here). On the other hand, the O_2 consumption rate and CO_2 production rate of other types of vegetables, which are governed by enzyme kinetic respiration rate, may be directly modeled with the modified 1st-order reaction rate in order to get one kinetic rate constant.

In addition, we found that the rate of color deterioration (remaining area of regular color) was agreed well wtih the modified first order reaction rate. The model can be expressed as follows:

$$A = a_A e^{(k_A t)} + b_A \qquad (5)$$

where A is remaining area of regular color (%), k_A is the rate constant of color change (day^{-1}), t is the time (days), a_A,and b_A are constants (%). For example, percent remaining area of regular color of Green oak stored under MAP condition of O_2:CO_2:N_2=1:5:94 and at 5°C was presented in Fig. 2, and the parameters for rate of color deterioration was summarized in Table 4.

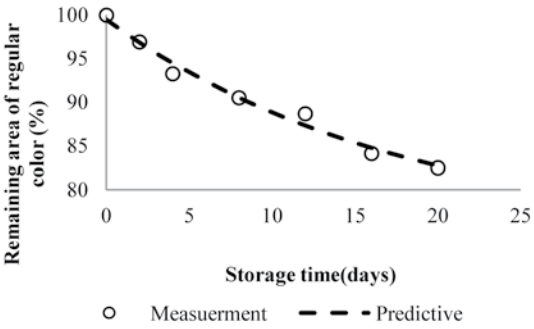

Fig. 2 - Remaining area of regular color of Green oak at MAP condition 1:5:94(O_2:CO_2:N_2).

Table 4 - Model parameter for predicting the remaining area of regular color of Eq. 5.

a_A (%)	k_A (days^{-1})	b_A	R^2
25.1410	0.0550	74.3090	0.9830

By comparison of the reaction rate constants (k-value), we found that the rate constnat of O_2 consumption rate and the rate constant of CO_2 production of Green oak, stored under MAP condition of O_2:CO_2:N_2=1:5:94 and at 5°C, was higher than the rate constant

of color (area of regular color) deterioration about 3.6 and 2.4, respectively. Once the ratio of rate constants between quality deterioration rate and respiration rate was defined, shelf life predictive model could be drawn directly from the respiration rate model. Anyhow, this propose concept still needs for further validation since the enzyme kinetic rate governing pathway for each reaction, which are not thoroughly understood, may be much different theoretically. To find a correlation of quality deterioration rate and respiration rate for practical usage was proved possible in this work, further studies were aimed at the effect of temperature, MAP conditions, and varieties of vegetables and fruits.

ACKNOWLEDGEMENTS

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EFFECT OF TEMPERATURE ON LIPID OXIDATION KINETICS OF TUNA OIL TWO-LAYERS EMULSION DURING STORAGE

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ABSTRACT

This research is aimed to determine the influence of storage temperature (5, 10, 15 and 25°C) on the oxidation rate that occurs during storage of fish oil multilayer emulsion. The tuna oil-in-water two-layers emulsions containing 13 wt% tuna oil, 2.6 wt% lecithin and 0.52 wt% chitosan were prepared. Lipid oxidation was evaluated by measuring lipid hydroperoxides (LHs) and thiobarbituric acid reactive substances (TBARs). The pH of emulsion during storage was also measured. The kinetic equations for evaluation of lipid oxidation respected to zero-order at studied temperatures with R^2 higher than 0.95. The rate constants were temperature dependent according to the Arrhenius equation with activation energy values of 83.2 and 44.9 kJ mol^{-1} for LHs and TBARs, respectively. It is of considerable interest that the lipid oxidation of fish oil emulsion as well as fish oil enriched products could be controlled by storage temperature.

Key words: Storage temperature, lipid oxidation, tuna oil, multilayer emulsion.

INTRODUCTION

Long-chain n-3 fatty acids are beneficial for health as proven to prevent several diseases, mainly cardiovascular disease (CVD). These fatty acids are naturally present

in fish oils (e.g. tuna oil). As a result, fish oil has a great potential to be used as functional food ingredients (Kolanowski and Laufenberg, 2006). Even with a great benefit for health, fish oils have their limitation to be used as food ingredient due to its oxidative stability problem and its unique fishy odor and taste (Jacobsen *et al.*, 2008). However, modern food technology makes it possible to fortify various food products with fish oils, especially in the form of fish oil emulsion (Jacobsen, 2008). Nevertheless, there are some concerns that the oxidative stability of fish oil enriched food products may be mainly affected by the instability of fish oil emulsion itself during storage. Therefore, the effect of storage temperature (5, 10, 15 and 25°C) on the oxidation rate of fish oil multilayer emulsion during storage was investigated in order to better understand and be able to control the quality of fish oil enriched products.

MATERIALS AND METHODS

Tuna oil-in-water two-layers emulsions containing 13 wt% tuna oil, 2.6 wt% lecithin and 0.52 wt% chitosan were prepared using layer-by-layer deposition technique (Ogawa *et al.*, 2003). A concentrated one-layer tuna oil in-water emulsion stabilized by lecithin (20 wt% oil, 4 wt% lecithin) was made by mixing 20 wt% tuna oil and 80 wt% lecithin solution (100 mM acetate buffer, pH 3.0). This mixture was then blended using a high-speed blender. Then, the coarse emulsion was three passed through a two-stage high-pressure valve homogenizer at 5,000 psi. This one-layer emulsion was diluted with aqueous chitosan solutions by mixing with a high-speed stirrer for 15 min to form a two-layers emulsion (13 wt% tuna oil, 2.6 wt% lecithin, and 0.52 wt% chitosan). Any flocs formed in this emulsion were disrupted by passing through a high-pressure valve homogenizer at a pressure of 4000 psi.

To monitor lipid oxidation during storage, emulsions (35 mL) were placed in lightly sealed screw-cap test tubes at different temperatures (5, 10, 15 and 25°C) in the dark for 28 days. Lipid oxidation was evaluated by measuring lipid hydroperoxides (LHs) and thiobarbituric acid reactive substances (TBARs). The pH of emulsion during storage was also measured.

RESULTS AND CONCLUSION

The results indicated that the pH of emulsion was dropped over the storage period at all storage temperatures (data not shown). The higher is the storage temperature, the more is the liberation of free fatty acid from tuna oil. Assuming that decreased in pH may be related to a progressive of triglyceride hydrolysis with caused an increased concentration of free fatty acids. Moreover, the observed decrease in emulsions may be attributed to the formation of several short chain aliphatic acids resulting from lipid oxidation (Steger and Muhlebach, 1998; Tamilvanan *et al.*, 2010).

Fig. 1 shows the increase of oxidative deterioration of tuna oil-in-water emulsions with increasing temperature. More specifically, a linear increase of LHs (1A) and TBARs (1B) with oxidation time was observed at all processing temperatures ($P \leq 0.05$). However, the determination of TBARs indicated that secondary oxidation products were at very low levels under our experimental conditions. The kinetic equations for evaluation of lipid oxidation can be described as zero-order at studied temperatures with R^2 higher than 0.95.

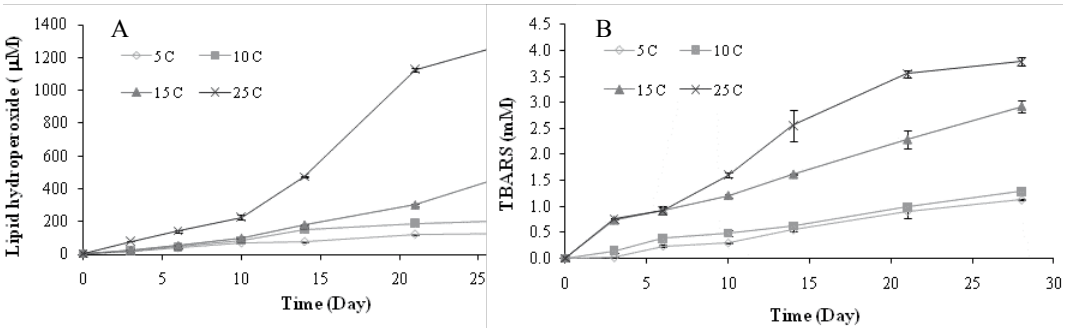

Fig. 1 - Formation of lipid hydroperoxide (A) and TBARs (B) in emulsions during storage at different temperatures.

Table 1 - The rate constants (k, day^{-1}) and correlation coefficients (R^2) of lipid hydroperoxides (LHs) and thiobarbituric acid-reactive substances (TBARs) during oxidation of 13% tuna oil-in-water two-layers emulsion stored at different temperatures.

Storage temperature (°C)	Lipid hydroperoxides (LHs)		Thiobarbituric acid-reactive substances (TBARs)	
	k	R^2	k	R^2
5	4.775	0.967	0.043	0.984
10	8.136	0.959	0.045	0.992
15	18.091	0.956	0.097	0.982
25	51.165	0.945	0.142	0.960

The LHs formation rate (k_{LH}) was 4.775, 8.136, 18.091 and 51.165 µM day^{-1} for storage temperature of 5, 10, 15, and 25°C, respectively. For TBARs, the development rate constant (k_{TBARs}) was 0.043, 0.045, 0.097 and 0.142 mM day^{-1} for storage temperature of 5, 10, 15, and 25°C, respectively (Table 1). By studying the rates of lipid oxidation as a function of temperature, we found that an increasing rate of oxidation can be observed as temperature increases for either LHs or TBARs. LHs, oxidation primary products, are formed during the propagation stage of oxidation through reactions of the free radicals that have been generated at the initiation stage with polyunsaturated fatty acids. Tuna oil is rich in polyunsaturated fatty acids, e.g., the oil used in our experiments has an EPA and DHA content of 33.2%. A linear increase of primary product values has also been observed by other researchers in oxidation of emulsions (Calligaris *et al.*, 2007: Dimakou *et al.*, 2007).

The rate constants of LHs and TBARs accumulation increased with temperature, following the Arrhenius equation as presented in Fig. 2. Plotting the logarithmic of rate constant values (ln k) of LHs and TBARs accumulation versus inverse of absolute temperature (1/T) indicated that the Arrhenius equation is followed with a good correlation coefficient higher than 0.9. Using these regression parameters, the activation energies, and Q10 numbers for the formation reaction of the LHs and TBARs were calculated. The magnitude of the temperature effect on the oxidation rate of the tuna oil emulsion was evidenced by the Q10 numbers. In general, a higher Q10 number implies that a smaller temperature change is needed to induce a certain change in the rate of lipid oxidation. For this experiment, the Q10 number

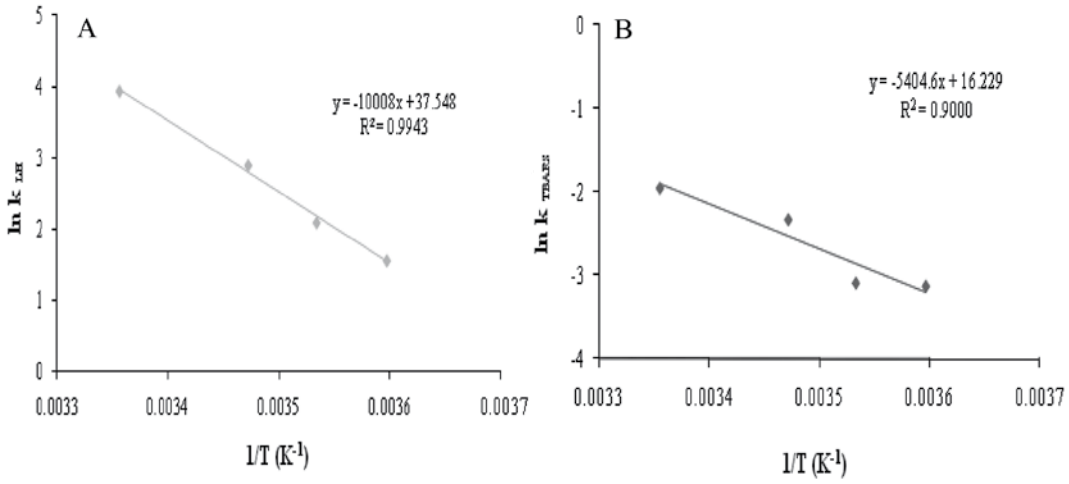

Fig. 2 - Zero order rate constants of lipid hydroperoxide (A) and TBARS (B) formation as a function of temperature.

for LHs and TBARs was 3.35 and 1.92, respectively (data not shown). From these results we concluded that the formation of LHs was more sensitive to temperature change than TBARs formation. The activation energy (Ea) was found equal to 83.2 and 44.9 kJ mol^{-1} for LHs and TBARs, respectively. These values are in agreement with literature data reporting typical Ea values for lipid oxidation ranging from 24 to 240 kJ mol^{-1} (Dimakou *et al.*, 2007). From these results, it is of considerable interest that the lipid oxidation of fish oil emulsion as well as fish oil enriched products could be controlled by changing storage temperature.

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THE QUALITY CHANGES OF IN-PACKAGE FRESH-CUT MANGOSTEEN

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ABSTRACT

Fresh-cut produce is a rapidly growing product due to the convenience and fresh-like quality. The development of new treatments to minimize the impact of processing operations while preserving the original fresh-like characteristics of the fruit is highly needed. Therefore this work was aimed to study some of the physical, chemical, microbiological and sensory qualities changes during storage of in-package fresh-cut mangosteen, queen of tropical fruit. Mangosteen fruits (*Garcinia mangostana*, L.), partially ripe, yellow-green with pink spots-skinned were prepared into fresh-cut form, packed in PP tray overlaid with OPP/LLDPE film. Samples were stored at 5 °C and 85% RH and analyzed at the beginning for respiration rate and ethylene production. Then after 3, 6, 9, 12 and 15 days of storage, in-package gas composition, physical, chemical, microbiological as well as sensory qualities were determined. The initial respiration rate of fresh-cut mangosteen was 10.7 ml CO_2 kg^{-1} h^{-1} and declined rapidly within 7 h then gradually decreased and relatively stable until the end of storage. There were relative high C_2H_4 and CO_2 production at the beginning and then decreased. Atmospheric composition and ethylene inside package did not reached equilibrium because the transmission rate of the film may not fit to seal the trays. Flesh firmness decreased as the storage time increased and correlated with the increased weight loss. The change of browning index (BI) value was quite low, showing that low temperature storage plays an important role as anti-browning

function, due to the inhibition activity of PPO enzyme. At the end of storage, the fresh-cut mangosteen became darker with lower L* and higher a* values. Acetaldehyde and ethanol content increased slightly after cutting and then rose sharply in the last few days of storage. All of tested microorganism (total viable count, yeast & mold, *E. coli* and *Salmonella* sp.) populations slightly increased throughout the storage although levels were deemed acceptable. The intensity scores of browning, off-odor, texture and overall acceptability were different between the different storage times.

Key words: Fresh-cut fruit, mangosteen, in-package fresh-cut mangosteen, quality changes.

INTRODUCTION

Fresh-cut produce is a rapidly growing segment in the food service and retail markets. It is new form of product intended to meet the consumer desires for convenience and fresh-like quality. The development of new treatments that minimize the impact of processing operations while preserving the original fresh-like characteristics of the fruit, as well as facilitating improvements in the distribution of refrigerated products are crucial to satisfy the demand for ready-to-use or ready-to-eat products. Peeling, coring and slicing operation are critical because they limit the shelf life of fruit products due to physiological stresses caused by physical damage or wounding. The respiration of fresh-cut is greater than that of the intact fruit, whereas ethylene production has been shown to be notably induced within a few minutes of processing. These responses increase biochemical reactions related to changes in color, flavor, texture and nutritional quality. Likewise, browning or changes in tissue appearance caused by pectinolytic breakdown can also lead to spoilage.

There is a relatively little information regarding quality chances of fresh-cut mangosteen, therefore the aims of this work were to study the some of the physical, chemical and microbiological changes during storage of in-package fresh-cut mangosteem packed and sealed under film lid.

MATERIALS AND METHODS

Mangosteen fruits (*Garcinia mangostana*, L.), partially ripe, yellow-green with pink spots-skinned with a mean weight of 120±5 g were prepared into fresh-cut form in cleaned minimal-processing room at 30 °C. The flesh of mangosteen were dipped in cold water (10 °C) for 5 min, dried and packed in PP tray and OPP/LLDPE film lid. Samples were stored at 5 °C with 85% RH and analyzed at the beginning of the experiments for respiration rate and ethylene production and after 3, 6, 9, 12 and 15 days of storage for in-package gas composition (Agar *et al.*, 1999), physical (firmness, weight loss, browning index (Supapvanich *et al.*, 2011) and color, chemical (acetaldehyde and ethanol content modified from Gonzalez-Aguilar *et al.*, 2008), microbiological (TVC, yeast & mold, *E.coli* and *Salmonella* sp. followed BAM, 2001) as well as sensory qualities (browning, off-odor, texture and overall acceptability).

RESULTS AND CONCLUSION

Fresh-cut mangosteen showed high initial respiration (10.7 ml CO_2 kg^{-1} h^{-1}) rate and declined rapidly within 7 hours (Fig. 1). Thereafter, it gradually decreased and relatively stable at 1 ml CO_2 kg^{-1} h^{-1} until the end of storage. The high initial respiration rate is likely due to the wound response by the effect of cutting, which is in agreement with the findings in melon (Aguayo *et al.*, 2004). The ethylene production was low. There was a relative high C_2H_4 at the beginning of the experiment and then decreased afterward, the same trend was found in the CO_2 production. Atmospheric composition and ethylene inside package were not reached equilibrium because film lid used in this study may have high gas transmission rate.

The firmness decreased concurrently with the storage time and correlated with the increase in weight loss (Fig. 2). The change of browning index (BI) value was quite low (Table 1). It is possible that low temperature (5 °C) storage condition plays an important role in terms of anti-browning function, due to the inhibition activity of PPO enzyme. At the end of storage, the fresh-cut surfaces became darker with lower L* values and higher a* values.

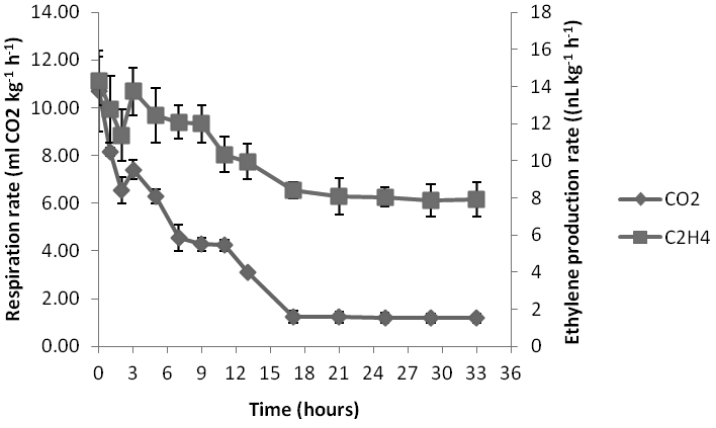

Fig. 1 - Respiration and ethylene production rate of fresh-cut mangosteen.

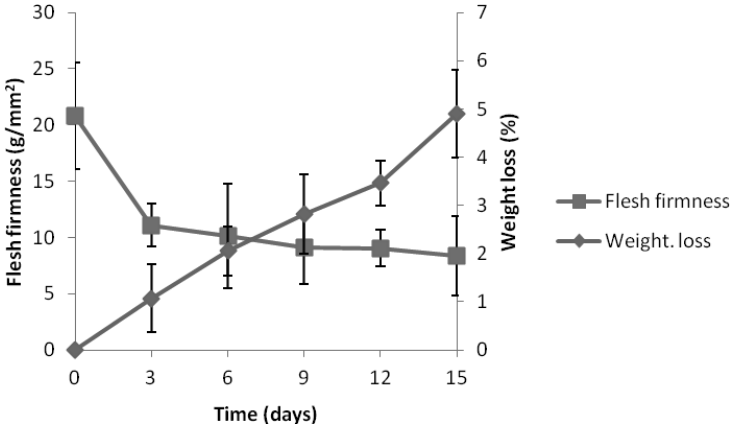

Fig. 2 - Flesh firmness and weight loss of fresh-cut mangosteen during storage.

Acetaldehyde and ethanol content in fresh-cut mangosteen increased slowly during 12 days storage at 5 °C and then drastically increased afterwards (Fig. 3). It appears that volatile accumulation could not be related to an in-package atmosphere. The O_2 and CO_2 contents observed in the package were sufficient to cause anaerobic respiration. However, the increase of acetaldehyde and ethanol was observed after 12 day storage. It is well known that acetaldehyde and ethanol normally accumulate during maturation of fruit. It is possible that the gradually increase of the accumulation observed in the package, could be more related to the ripening process than to the atmosphere during storage.

Table 1 - Browning index (A_{420}) and color of fresh-cut mangosteen during storage.

Color	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
BI (A_{420})	0.0364± 0.0011a*	0.0424± 0.0015b	0.0419± 0.0009b	0.0416± 0.0009c	0.0424± 0.0011b	0.0422± 0.0009 b
L*	85.07±2.24 a	83.83±3.45 b	81.68±3.57 bc	80.81±4.59 c	78.53±3.26 d	75.26±5.05 c
a*	0.24±0.77 a	1.13±0.87 b	1.29±1.23 b	2.16±1.27 c	2.60±1.36cd	2.68±1.45d
b*	14.51±2.23 a	13.59±2.44 b	13.06±2.13 b	11.96±1.79 cd	11.16±2.77c	12.26±2.22d
C	.53±2.24 a	13.66±2.50 b	13.17±2.21 b	11.51±2.90 c	12.20±1.90 cd	12.60±2.40d
H°	0.23±1.53 a	1.41±0.49 b	1.14±0.96 c	1.35±0.09bd	1.31±0.51d	1.36±0.09d

*Mean± SD from 3 replications followed by the same letter within row are not significantly different (P>0.05)

It was observed that all of microorganisms (total viable count, yeast & mold, *E. coli* and *Salmonella* sp.) seem to be stable throughout the storage and did not reach the microbial limit. The amount of microorganism increased very slowly, probably due to the high content of organic acid in mangosteen that gave low pH values (pH 2-3) and they were kept in cold temperature. Whereas the intensity scores of browning, off-odor, texture and overall acceptability (OVA) were different between the different storage times. The OVA score correlated well with other sensorial attributes which demonstrated that the acceptability scores of fresh-cut mangosteen were up to 7 days of storage.

According to the results of this study, fresh-cut mangosteen evidently suffered from undesirable changes. In addition, the film used in this study had high O_2

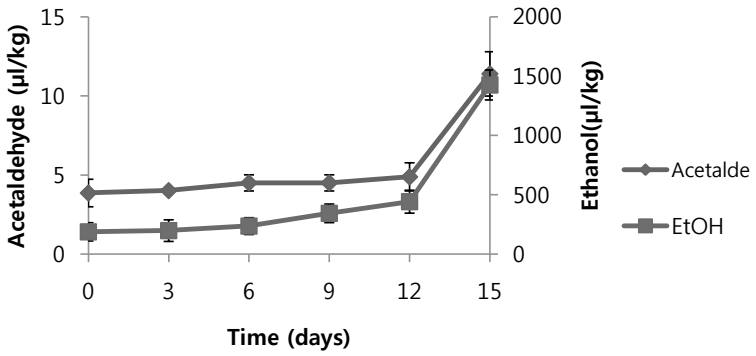

Fig. 3 - Acetaldehyde and ethanol contents of fresh-cut mangosteen during storage.

and CO_2 transmission rates and acted as a bad barrier to gas exchange resulting in undesirable alterations in product quality. Therefore more promising films are likely desirable for the packaging to improve the shelf-life and safety of packed fresh-cut mangosteen.

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THE ROLE OF SHELF LIFE IN THE INTRODUCTION OF WILD DECORATIVE PLANTS INTO VASES

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ABSTRACT

The objective of this work is indeed to estimate if the attitudes of consumers towards the *shelf life* is one of relevant elements that may entail the introduction of native plants in the end markets to be allocated in a pot; or rather the existence of market opportunities for the companies that intend to follow this strategy of differentiation of productions. The approach that has driven the research proved to be a useful tool to analyze the attitude of the consumers towards the main commodity characteristics of the product which play the main role in the selection of native ornamental potted plant.

Key words: Role, shelf life, decorative plants, vases

INTRODUCTION

The division of decorative plants for several years now has shown signs of energy and quite intense productive transformation in relation to the rather profound modification of the increasingly diversified and personalized consumption patterns. The stimuli coming from the question has posed and still poses to Italian floriculture the issue of broadening and diversifying its product offerings, aiming principally at the development of wild plants of the cultivating tradition and existing cultures of the Mediterranean. Still, in other words, the market assimilates to these latest products, native species in other geographic settings in which the climate is similar to that of the Mediterranean; consequently, they each become counted conventionally into the category of "Mediterranean plants". Whichever the correct commercial definition is of the species that are

counted, from our point of observation, the cited category, introducing new needs that the common production and mass does not appear able to satisfy, would become propulsive for new and old consumers. What is interesting to our purposes is the degree of involvement of the consumer for Mediterranean plants, in the moment of purchase, understood as both consideration of the store and behavior inside the place of sale. In this context, the study puts forward the objective of assessing the attitudes of consumers towards new products and the advantages compared to alternatives ("conventional" plants) existing in the place of sale, where the diverse environmental conditions - spatial types (décor, lighting, noise level, space) and social types (advice, courtesy, competence of the sales staff) - entail the different situations of involvement of the consumer. In this paper, the first results are reported, which were realized by the study in question, which indeed requires further consideration of broadening and deepening levels of investigation.

MATERIALS AND METHODS

In this work, we have tried to analyze which are the factors that influence the choice towards Mediterranean plants, or rather that entail the involvement of the consumer. In general, among the principal factors that can influence the diverse degree of involvement of the consumer, here the attention is focused on the "social-organizational" and "socio-demographic" factors and on the factors related to the purchasing situation (De Luca P, 2003). In this latter respect, a screening was done to see if against a different degree of involvement by the consumer the importance attributed to the atmosphere of the place of sale could also vary. As briefly highlighted, an exploratory study was developed with the objective of studying the phenomenon and of deepening the relations among the variables that define the involvement of the purchaser and those relative to the atmosphere of the store. The survey was based on direct interviews through the administration of a special questionnaire structured and adapted to the statistic method of conjoint analysis, therefore, composed of a series of descriptive forms of specific characteristic profiles of the sector examined. The surveys were conducted in several traditional and modern commercial retail outlets to a random group of people composed of 120 units; the latter 80% of these women represented were distributed into different age classes, in particular, 4.2% from 18 to 25 years old, 36.7% from 26 to 45 years old, 38.3% from 46 to 60 years old, and, finally, 20.8% over 60 years old (list following):

Sample distribution of consumers by age and sex

Age classes	Men		Women		Total	
	N.	%	N.	%	N.	%
18 - 25 y/o	-	*0.0*	5	*5.2*	5	*4.2*
26 - 45 y/o	8	*33.3*	36	*37.5*	44	*36.7*
46 – 60 y/o	5	*20.8*	41	*42.7*	46	*38.3*
60+ y/o	11	*45.8*	14	*14.6*	25	*20.8*
Total	24	*100.0*	96	*100.0*	120	*100.0*

Therefore, the survey deals with adult consumers predominantly middle-aged women with an average-to-high level of education and an average-to-low family income who stated that they regularly purchase decorative house-plants. The data calculations made it possible to have some information on the questions underlined above, through the application of statistical tools aimed at the determination of the frequency or the intensity of the observed phenomenon; in particular, in correspondence of each variable of the phenomenon the relative intensity was defined through simple composition ratios. The variables for the profile examined were placed according to an ordinal scale in which the higher percentage numeric values were transformed into descriptive indicators (in particular: 0 = "indifferent"; 1% - 20% = "low"; 21% - 40% = "medium"; 41% - 60% = "high"; 61% - 80% = "elevated"; 81% -100%= notable).

RESULTS

The research allowed us to achieve interesting results about the attitudes of the consumers in the face of the purchasing of potted decorative plants of species (or range of) newly introduced to the Mediterranean environment. From the data taken from the questionnaire emerge the combined effects on consumers of the principal attributes of the market that characterize the various grand typologies of potted decorative plants (conventional plants, improved plants, original plants); this has allowed us to extrapolate the relative importance of those attributes that create satisfaction (total utility) in the final users of such indoor floral and ornamental products. While aware of the limits that involve the use of conjoint analysis, the results of the research, shown in the following tables, can be considered for identification of distributive profile acts placing the newly introduced potted decorative plants; this also takes into consideration the combined effects of the level of involvement of the consumer and the atmosphere of the store. Atmosphere is understood not only as an aesthetic presentation of the product (visual merchandising) as compared to the parameters on the retention of

Table 1 - Results of the involvement of the consumer in relationship to the nature and intensity of the innovation of product

Indications	Conventional plants (*)	Improved plants (**)	Original plants (***)
I purchase the plants for me	Low	Medium	Elevated
I purchase the plants for my family	Low	High	High
I purchase the plants for friends	Medium	Medium	High
I spend time to the choice of the plant	Indifferent	Low	Notable
I like to keep updated on the various proposals or offers, I consult specialized journals or articles	Low	Low	Elevated
In the stores I like to look at various types of plants just out of curiosity	Low	High	High
I am always very careful to purchase the proper plant suitable for each occasion	Low	Medium	Elevated

(*) Range of ornamental plants for some time offered on the market.

(**) Range of ornamental plants for some time offered on the market with measures to improve the actual performance and / or perceived (packaging, new type of farming, etc..).

(***) Range of new offer: Mediterranean ornamental plants (autochtonous and not).

Table 2 - Results of the purchasing behaviour for levels of involvement of the consumer and for great characters of the Mediterranean plants

Involvement	Similar plants for bio-morphological characters (*)	Different plants with the same bio-morphological characters (*)	Different plants for bio-morphological characters (*)
Of the product	Indifferent	Low	Elevated
In the choice of the type (**)	Medium	Low	Elevated
In the purchase	High	Low	Medium

(*) Practical indication based on appearance, the shape and characteristics of the plant.
(**)Question independent from group of ornamental plants (shrubs, vines, rock, bulbs, succulents, palms, etc.) pre-chosen by the consumer.

Table 3 - Effects of the "primary" and "secondary" shelf life of the Mediterranean ornamental houseplants in relation to the consumer's in the phases pre-purchase and post-purchase

Phase pre-purchase	Type of plants (*)		Phase post-purchase	Type of plants (*)	
Primary shelf life	Convention	Original	*Secondary shelf life*	Convention	Original
Intensity of colour	Medium	Elevated	Durability	Low	Notable
Vigor of the plant	Low	Notable	Loss and/or color changing	High	High
Aesthetics aspects	High	High	Absorbing foreign odors	Medium	Elevated
Time care	Low	Low	Facility of allocation	Medium	Medium
Persistence of the flowers on the branches	Low	Notable	Persistence of the leaves on the branches	High	High

(*) See Table n. 1.

specific characteristic merchandise by type of ornamental plant (extended shelf-life) but also as an image and business ethics, quality of service (customer service), etc.

Table 4 - Results of the buying behavior of consumers of ornamental mediterranean houseplants

Indications	Houseplant	Balcony plant	Garden plants	Officeplant	Green fronds	Flowering
Planning time of purchase	High	Medium	Medium	Low	High	Medium
Time and effort of searching	Notable	High	Medium/High	Low	Medium	Medium
Comparisons between florist	Notable	Notable	Medium	Low	High	Medium/High
Level of expense	Notable	High	Medium	Low	High	Medium
Frequency of purchase	Long-term	Seasonal	Annual	Long-term	High	Annual
Buying habits	*Speciality*	*Speciality*	*Shopping*	*Convenience*	*Shopping*	*Convenience*

Indications of the buying habits of the number table 4

Indications	Convenience	Shopping good	Speciality good
Purchase	Frequent	Moderate	Not frequent
Cost	Limited	Limited High	High
Rationality	Impulsive	Rational	Much rational
Time of choice	Reduced	Relevant	Relevant
Information	Minimal	Conducted	Supported

CONCLUSION

The study conducted indicates good inclination of the consumers to purchase new native, potted, Mediterranean plants. The need which affects consumer behavior becomes especially crucial in the pre-purchase phase. In this latest phase, the store carries out the most important strategic function: adequate “shelf-life primary” appropriate environmental “atmosphere”, appropriate skills of those involved, etc. Although the results of the present research have made it possible to provide useful guidance about the involvement behavior of the consumer of floral-ornamental

Table 5 - Results of the consumers' involvement in relation to major physical and social factors that characterize the atmosphere of the store (*)

Indications	Physical dimension		Dimension	
	experiential	functional	social	olfactory
Suitable style and designer for the furnishings	High			
The atmosphere is pleasant	High			
There is some background music	Indifferent			
Appropriate level of assortment		High		
Adjusted disposition of the plants on the shelves		Medium		
Suitable indicators for the identification of plants with greater facility		Low		
Proper illumination for the performances		Medium		
Suitable spaces, facility of movement		Low		
Courtesy of the sales staff			High	
Competence of sales staff			High	
Advices of sales staff			Low	
The store is characterized by the nice smelling fragrance				High

(*) It should be noted that for this profile, the surveys were conducted predominantly in traditional stores, interesting however

products in regards to new products in the decorative Mediterranean plant category, further research would still be needed, especially with the expansion of the territorial bases upon which the continuation of the same research would be based.

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THE MARKET FOR ALTERNATIVE SPECIES OF SHRIMP PROCESSED TO INCREASE THE SHELF-LIFE

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ABSTRACT

A reason that has put at the center of attention the crustaceans has been the growing demand for fast food products to which these fishes are particularly suitable. This paper proposes for the first time the use of the species of shrimp *Parapeneus longirostris* of the Mediterranean sea to realize precooked trays of V range and analyze the evolution of the process of melanosis with respect to commercial products in general coming from the Atlantic Ocean.

Key words: *Parapeneus longirostris*, shelf-life (SL), market, PPO, precooked.

INTRODUCTION

In recent years, eating habits have privileged the "pharmafood" products to prevent any diseases. Today the world production of shellfish has increased, recording since the 1980s, an unprecedented boom. This highlights also the dependence of European Mediterranean States from third countries, in particular in other geographical areas, although crustaceans have minor impact on the level of Community trade. Since under this trade category, the total production of shrimp from fish in the Mediterranean Sea (especially those of Italy) proved to be insufficient for a increasing Community market demand. It is of some interest to understand the trends in trade flows in the Mediterranean area, in which the greater supply of such products (fresh and/or "processed") for the Community markets takes place

and the *Parapenaeus longirostris* feeds traffic flow represented mostly by "transformed" (frozen) product, and the corresponding data that track the trend will be examined here using the international official statistical source of FAO. The aim of the working group is to contribute to the development of Mediterranean fisheries through research and technology transfer of new high-value production processes aimed at sustainable exploitation of fisheries resources and the maximization of the value of the fish. This paper proposes for the first time the use of the species of shrimp *Parapeneus longirostris* of the Mediterranean and analyze the evolution of the process of melanosis with respect to commercial products in general coming from the Atlantic Ocean. The enzyme polyphenol oxidase (PPO) is responsible for the process of melanosis in shrimp extracts of *Parapeneus longirostris*. Numerous studies aim to reduce the effects of melanosis using a wide range oftreatments inhibitor (Buta *et al.*, 2001; Liao *et al.*,1988; Chinivasagam *et al.*, 1998). It is shown that the effectiveness of the treatments is influenced by the type of the species of the crustacean and the stage of intermuta to which they face.

MATERIAL AND METHODS

The *Parapenaeus longirostris* was caught with bottom trawls on sandy-muddy bottoms at depths between 50-500 meters of the Tyrrhenian coast. Shrimp were selected with a size of 16-17 cm, very clear color tending to pink-orange and were discarded those who had trauma from capture. Each sample had a very pronounced rostrum with seven dorsal teeth. The enzymatic analysis included the determination of tyrosinase spectrophotometrically in according to the method proposed by Leonard *et al.* (1985). The enzymatic activity was expressed as the change in absorbance at 490 nm per minute per mg protein. The market analysis of *Parapenaeus longirostris* (frozen), and corresponding data that trace the trend will be examined here using the official statistical source FAO International.

RESULTS AND DISCUSSION

Fig. 1-a shows the enzymatic activity of tyrosinase in commercial samples ofshrimp currently available on the local market of the Mediterranean. The heat treatment (blancing) suffered by the minimally processed product with Tropical Shrimp has completely inhibited the enzyme activities differently to those obtained

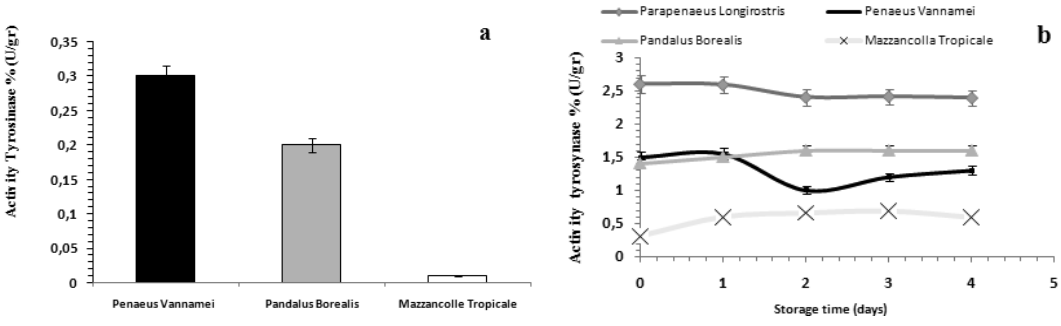

Fig. 1 - Activity of tyrosinase in commercial samples (a) and on different species during the freezing (b).

with *Penaeus vannamei* and *Pandalus borealis*. In order to analyzethe products currently available on the market trends have been studied regardingthe traffic streams of the crustaceans. In the Mediterranean basin is realized intensive trade in frozen Crustaceans, largely as a result of trends that characterize the group "shrimps and prawns".

In reality, according to the FAO trade consists in mainly movements in import, so much so that the trade balance of all countries bordering the Mediterranean Sea, was negative; as it is also shown in the balance normalized. This negative balance, however, in the evolution intervened in the last decade, as can be observed from Table 1, appears to be increasing by +37% considering all Crustaceans, +46% examining the group the "shrimps and prawns". From the examination of the exposed data, the consumption of "shrimps and prawns" frozen in the Mediterranean would be realized therefore with product of other origin, for the most oceanic. From market analysis can be seen that however, for certain species belonging to this large group of crustaceans, the production obtained in the Mediterranean, would feed streams of traffic over the same area of the Mediterranean. In this situation the species *Parapenaus longirostris* is found, in fact the amount of frozen product prevails on its trade balance, a situation that occurred mostly during the decade examined, in fact, against a steady increase in the product realized there is a loss in the trade balance. In particular, *Parapenaus longirostris* captures in Mediterranean Sea, always with reference to FAO statistic, is mainly used fresh, de facto,

Table 1 - Development of trade Crustaceans, frozen, and frozen shrimps and prawns in the countries of the Mediterranean Sea.

Commodity	**2001 - 2003 Thousand ton**	**2004 - 2006 Thousand ton**	**2007 - 2009 Thousand ton**
		Import	
Crustaceans (a)	391.874	467.267	526.661
	100	119	134
Shrimps, prawns (b)	256.778	322.292	362.354
	100	126	141
(b)/(a)*100	65.5	69.0	68.8
		Export	
Crustaceans (a)	71.367	75.465	88.682
	100	106	124
Shrimps, prawns (b)	45.183	46.995	54.445
	100	104	121
(b)/(a)*100	63.3	62.3	61.4
		Trade balance	
Crustaceans	- 320.507	- 391.802	- 437.979
	100	122	137
Shrimps, prawns	- 211.595	- 275.297	- 307.909
	100	130	146
		Balance Normalized (%)	
Crustaceans	-69.2	-72.2	-71.2
Shrimps, prawns	-70.1	-74.5	-73.9

Table 2 - Distribution of production Parapenaus longirostris in the countries of the Mediterranean Sea (our calculations based on FAO statistic).

Commodity	2001 - 2003 Thousand ton	2004 - 2006 Thousand ton	2007 - 2009 Thousand ton
Capture production	30.722	40.479	42.637
	100	*132*	*139*
Frozen production	15.139	14.728	17.851
	100	*132*	*139*
frozen/captured (%)	49.3	36.4	41.9
Import	15.716	13.457	11.091
	100	*86*	*71*
Export	3.493	3.077	1.881
	100	*88*	*54*
Trade balance	- 12.223	- 10.379	- 9.210
	100	*85*	*75*
Balance Normalized (%)	-63.6	-62.8	-71.0

only 40% is the share of the production of frozen product. Indeed, as shown in Table 2 between the 2001/2003 and 2007/2009, the catches of 30.7 thousand tons, coming to 42.6 thousand tons are brought, the frozen product frozen from 15.1 thousand to 17.9 thousand tons. At the same time period, the trade balance gradually decreases from 12.2 to 9.2 thousand tons.

In Fig. 1-b shows the relative activity of tyrosinase minimally treated with 4 different fish species. The tyrosinase showed a continuing increase in the species Pandalus Borealis until the third day of storage that we set as the limit of shell-life. The activity of tyrosinase Parapeus Longiristris, even high than the other species, has been maintained constant in time. It is observed that the main alterations detected in the trays minimally processed are related to the chromatic variations and loss of texture or juiciness. The main issues related to engineering and manufacturing technique are related to tuning and optimization of blancing and portioning of shrimp. The technology used in this preliminary study does not significantly impede the physiological processes of melanosis previously identified as the main qualitative issues. In conclusion, from the data obtained, the species Parapeneus Longirostris could be used to obtain minimally processed and pre-cooked shrimp products able to increase the shelf-life (SL) as an alternative to commercial products.

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THE IMPLICATIONS ON THE "SHELF LIFE" OF INTERNATIONAL TRADE FLOWS OF SHRIMP IN ITALY

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ABSTRACT

In the fish market we observe increasing substitution processes of fresh fish: in fact, heat-treated fish (*frozen and non-frozen*) is preferred to fresh fish, with very low substitutability between them, especially in the case of shrimp. The Italian food industry uses this product primarily for processing, to obtain pre-cooked ready products, which have a different "shelf-life" depending on whether the imported raw material is frozen or not (*frozen and non-frozen*). In fact, trays of shrimp, non-frozen, pre-cooked, packaged in controlled atmosphere, have a shelf life reduced to only 3 days at 4°C and have an apparent phenomenon of melanosis in the outer part of the cuticle. Instead, regarding pre-cooked frozen shrimp (about 6 months), the "shelf-life" appears to be greater as the freezing process reduces the alteration of shellfish, although this involves an obvious loss of the nutritional component of the fish product. To meet the domestic demand of different types of merchandise aforesaid, Italy is dependent on the importation of foreign shrimp, especially from countries where production is carried out in oceanic waters. The present work aims to capture the trends in imports of shrimp in Italy and to identify current and future trends of these trade flows. The data are taken from the official statistics of the International "United Nations Commodity Trade Statistics Database".

Key words: Shrimp, frozen, non-frozen, shelf-life, imports.

INTRODUCTION

1. This work analyzes, by resorting to the official international statistics, the data of trade between Italy and the outside in the period of 1999-2010, pay-

ing careful attention to the variables, also the "normalized balance". The analysis shows that the flow of goods comes mainly from South America, from the Asian continent, and from countries of the Mediterranean and Central America. The levels of import prices are decidedly high and increasing, except, in part, the fish products from Asia. It adds, as a further negative effect, the need to resort to chemical treatments such as, for example, the use of metabisulfite, in order to increase imports. This work, therefore, calls for a reflection in order to increase local production and to promote imports where the advantage price and quality is better. In Italy the levels of import prices appear even higher, except the fish products from Asia.

2. One of the main problems that greatly influences the marketing of crayfish/shrimps is the phenomenon of melanosis, i.e. their browning due to tyrosinase (E.C. 1.14.18.1). Numerous studies aim to reduce the effects of melanosis using a wide range of restrictive treatments (Buta e Moline, 2001; Walker, 1977). It is scientifically proven that the effectiveness of treatment is influenced by the type of species of the crustacean and the phase of intermuta to which they should face (Mendes R., 2002). Because of this, Italy imports, in a substantial manner, the crayfish/shrimps from countries outside the European Union, with negative effects on the profitability of domestic stocks.

MATERIALS AND METHODS

1. For the accomplishment of the present job, references were made to the international official statistic "Un Comtrade". The relevant data of the methodology refer to the quantities and the correspondent values of the commercial exchanges of shrimp between Italy and foreign countries, between 1999-2010. Particularly, the acquisition of the data has concerned the universe of the countries of the World with which Italy carries out the tides of traffic of shrimp. The collected raw data have been submitted to special elaborations in order to then be assembled for Continental areas, that are Asia, South America, North America, the basin of the Mediterranean Sea and Europe (European Union), in which, in fact, the principal competitors of Italy are found. The annual data have been translated in triennial averages of the periods 1999-2001, 2002-2004, 2005-2007, 2008-2010, determining their intervened evolution, also through the use of simple index numbers, with reference to the quantities and to the considerable monetary values. Another elaboration has concerned the determination of the normalized balance, that is "the ratio, expressed as a percentage, between the current balance and the sum of exports and imports. Its value varies between -100, in the case the country is uniquely importing, and + 100, in the case in which the country is solely an exporter; instead, if the balance is equal to the normalized sale it is equal to 0" (ISTAT).

2. The species of fish analyzed were the *Parapenaeus longirostris*, *Pandalus borealis*, *Penaeus vannamei*, and tropical shrimp. On each sample enzymatic analyses were made that focused on the determination of the tyrosinase spectrophotometrically, by recording the formation of dopacromo by L-dihydroxyphenylalanine (L-DOPA) according to the method proposed by Leonard *et al.* (1985). The enzymatic activity was expressed as the change in absorbance at 490 nm per minute per mg protein.

RESULTS AND CONCLUSIONS

1. The mass of the collected and elaborate data has allowed us to underline the strong dependence of Italy from foreign countries for the shrimp which has produced a commercial deficit that in the last decade has increased and almost doubled from -227 million dollars to -404 million dollars, because of the entity of the imports, that, from 1999-2001, have gone from 38,810 tons to 61,699 tons in the last triennium of 2008-2010, the exports being poorly meaningful, that besides, in the same temporal arc considered, those exports appear with a quantity that poorly varies, that is around 2,500 tons, as seen in Table 1. The entity of the commercial deficit is determined primarily by the flows of commodity that originate out of the European Union. In fact, the prevailing weight is given by the rest of the world with 71.7% of the interested quantities in the first three years reaching up to 74.1% in the latest examined; also in terms of monetary value, the participation appears to be more elevated, from 76.5% to 79.2%, while the remaining corresponding rates point out the importance of the imports from the countries that are members of the European union. In this last geographical area, as reported in Table 1, the totality of the exports of Italy is shown, above all in Spain, with which commercial exchanges of a certain entity of shrimp is shown. However, European trade of shrimps is demonstrated, to a large extent, in regime of re-export and/or re-distribution. Whichever the conditions of realization of the interchanges may be, Italy is confirmed as a strong importer of shrimp, in fact, also the normalized sales show a timid modification.

Table 1 - Developments the trade of Italy with shrimp and prawns (*).

Continents	1999-2001				2002-2004				2005-2007				2008-2010			
	tons	%	000 $	%	tons	%	000 $	%	tons	%	000 $	%	tons	%	000 $	%
World																
Export	2,527	*100.0*	19,471	*100.0*	2,329	*100.0*	26,793	*100.0*	2,262	*100.0*	32,951	*100.0*	2,552	*100.0*	30,483	*100.0*
Import	38,819	*100.0*	246,732	*100.0*	44,204	*100.0*	295,973	*100.0*	60,103	*100.0*	411,404	*100.0*	61,699	*100.0*	434,157	*100.0*
Balance of trade	-36,292	*100.0*	-227,260	*100.0*	- 41,875	*100.0*	-269,179	*100.0*	- 57,840	*100.0*	-378,452	*100.0*	- 59,147	*100.0*	-403,674	*100.0*
Balance normalized			-85				-83				-85				-87	
Europe (UE)																
Export	2,465	*97.5*	19,179	*98.5*	2,226	*95.6*	26,192	*97.8*	2,038	*90.1*	31,796	*96.5*	2,277	*89.2*	28,778	*94.4*
Import	10,983	*28.3*	62,003	*25.1*	13,764	*31.1*	96,851	*32.7*	15,082	*25.1*	114,603	*27.9*	13,705	*22.2*	112,620	*25.9*
Balance of trade	-8,519		-42,823		-11,538		- 70,658		- 13.044		- 82,807		- 11,428		- 83,841	
Balance normalized			-53				-58				-57				-59	
Continents Other																
Export	62	*2.5*	292	*1.5*	103	*4.4*	601	*2.2*	224	*9.9*	1,156	*3.5*	275	*10.8*	1,705	*5.6*
Import	27,835	*71.7*	184,729	*74.9*	30,440	*68.9*	199,122	*67.3*	45,021	*74.9*	296,800	*72.1*	47,994	*77.8*	321,538	*74.1*
Balance of trade	-27,773		-184,437		-30,337		-198,521		- 44,797		-295,645		- 47,719		-319,833	
Balance normalized			-100				-99				-99				-99	

(*) Our calculations based on UN COMTRADE

Considering the imports of the other geographical areas of the planet, Fig. 1 puts South America in first position given that from here, Italy, with reference to 2008-2010, imports almost 2/3 of the shrimp of the rest of the world; succeeding them is the Asian continent with little over 30% and then, at a notable distance, are third party countries of the basin of the Mediterranean and Central America. This classification has not suffered, in truth, during the considered decade, any modification, although Asia and the basin of the Mediterranean Sea have also reduced their importance in terms of monetary values. Of a certain interest is the distribution of the international prices of the shrimps realized in the course of the decade with imports in Italy from the principal exporting

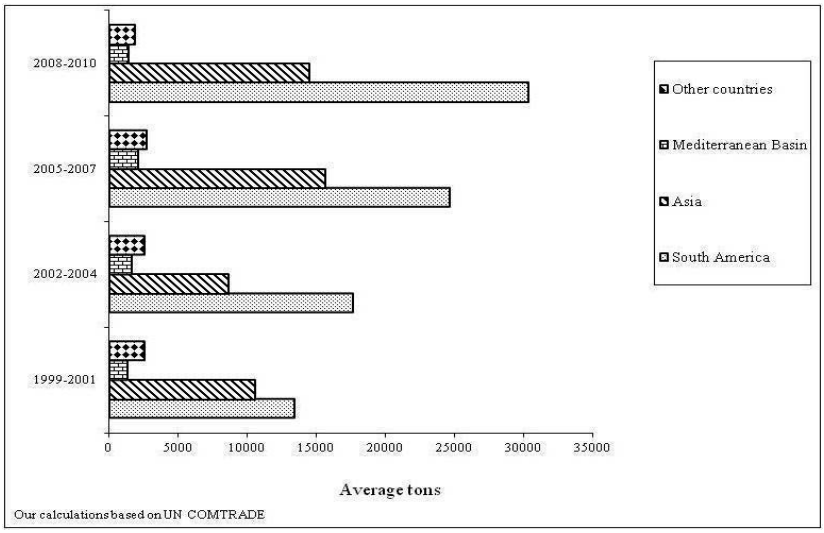

Fig. 1 – Developments the imports of shrimps and prawns in Italy.

Table 2 - The average prices to the importation in Italy (*).

Countries	1999 - 2001		2002 - 2004		2005 - 2007		2008 - 2010	
	$	Index	$	Index	$	Index	$	Index
			Europe					
Belgium	4.929	**100**	5.690	**115**	5.627	**114**	5.337	**108**
Denmark	3.424	**100**	4.905	**143**	6.564	**192**	7.635	**223**
France	8.956	**100**	9.088	**101**	9.568	**107**	8.936	**100**
Netherlands	5.964	**100**	5.078	**85**	5.011	**84**	5.983	**100**
Spain	7.982	**100**	10.071	**126**	10.229	**128**	10.048	**126**
United Kingdom	4.426	**100**	4.335	**98**	5.305	**120**	6.473	**146**
			Asia					
China	4.121	**100**	7.209	**175**	3.599	**87**	3.967	**96**
India	4.787	**100**	4.271	**89**	4.655	**97**	4.729	**99**
Indonesia	6.518	**100**	5.081	**78**	6.429	**99**	4.839	**74**
Malaysia	5.190	**100**	4.696	**90**	5.113	**99**	3.877	**75**
Rep.di Korea	3.211	**100**	4.913	**153**	11.317	**352**	3.535	**110**
Thailand	5.646	**100**	5.219	**92**	6.614	**117**	6.533	**116**
Viet Nam	5.206	**100**	4.869	**94**	5.368	**103**	4.745	**91**
			Mediterranean Basin					
Tunisia	9.440	**100**	10.317	**109**	11.548	**122**	14.263	**151**
Turkey	4.493	**100**	4.420	**98**	7.297	**162**	8.102	**180**
			South America					
Argentine	8.001	**100**	8.631	**108**	9.867	**123**	8.974	**112**
Ecuador	7.339	**100**	5.597	**76**	5.907	**80**	6.455	**88**
Venezuela	10.382	**100**	9.970	**96**	9.702	**93**	11.217	**108**
			Central America					
Mexico	-	-	9.250	**100**	12.833	**139**	7.936	**86**
Panama	-	-	5.293	**100**	6.112	**115**	6.158	**116**
Australia	6.235	**100**	12.791	**205**	15.788	**253**	16.309	**262**

(*) Our calculations based on UN COMTRADE

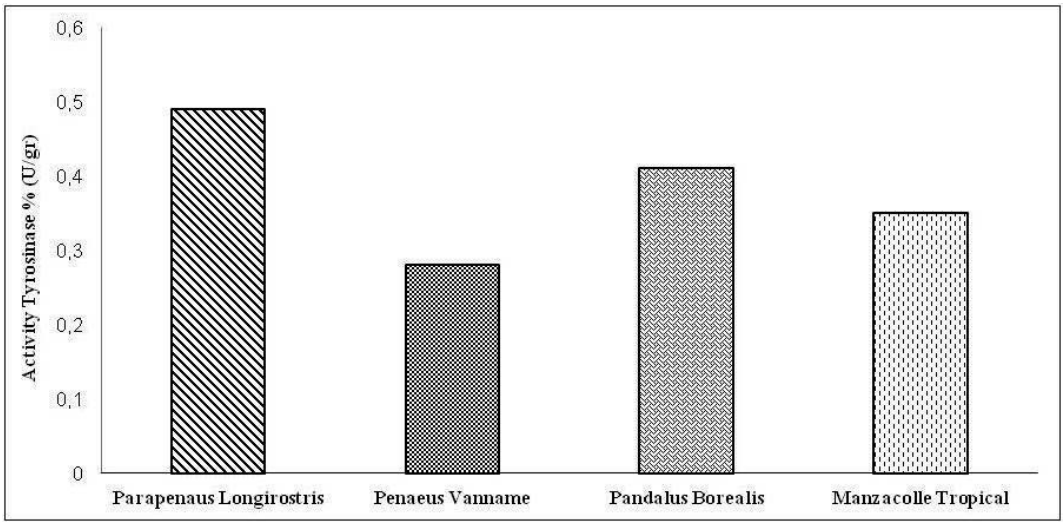

Fig. 2 - Activity tyrosinase related to the raw material shrimp.

countries of the various continents. In this way, Table 2 allows us to observe the varied variability that is recorded passing from one country to another, also inside the same continent. However, levels of prices in many cases are in increase; instead, quotations in rebate are observed especially in correspondence to countries of Asia, where the levels of the prices in importation are very low compared to the ones recorded in other countries (above all Australia, Tunisia, Venezuela and Spain). All of this involves negative effects on the profitability of the national fish enterprises, as also on the loss of ability to differentiate the national productions, therefore on the quality perceived by the consumers.

2. Fig. 2 shows, for each sample of enzymatic characterizations, individually *Parapenaeus longirostris* species, *Pandalus borealis*, *Penaeus vannamei*, and tropical shrimp. The *Parapenaeus longirostris* shows the highest initial activity of tyrosinase. The activity was quite comparable in the shrimp *Penaeus vannamei* and Manzacolle Tropical. Its value exceeds by about one third those of other species of shrimp used in the following work. It stands at 0.49 U/g differently from what can be seen in other types of shrimp. The sample of *Pandalus borealis* has presented the lowest initial tyrosinase activity. Despite the value of the tyrosinase activity being high compared to other species, this could be used as an alternative to the trade of domestic products.

3. According to the results of enzymatic tests on the above species of shrimps, it's considered appropriate to increase the traffic flow of pandalidae shrimps (*Pandalus borealis*), because they're commonly found in Italian fish trade too. On the basis of the available data from FAO, the Italian imports of pandalidae shrimps are made with processed products (frozen or not): during the 2000s, the frozen product has actually helved (from about 4,000 tons to just 2,000 tons), the not frozen product has maintained likely constant quantities (i.e., around a thousand tons). Whatever is the imported type, therefore, is desirable an increase of the relevant flows from producing countries (Canada above all).

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THE BIOLOGICAL CONTROL OF FUNGI FROM WOODEN CULTURAL PROPERTIES

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ABSTRACT

In this study, there has been investigated the decontamination of Korean cultural artifacts by gamma irradiation. *Tyromyces* and *Coriolus* were isolated from naturally contaminated wooden artifacts stored in the warehouse of a forest museum. The identification was conducted using 18S rRNA sequence analysis. The two isolated fungi were cultivated and treated in a ^{60}Co irradiation unit with doses up to 20 kGy. *Tyromyces* and *Coriolus* were inactivated at a dose of 5 kGy. These results demonstrate the effectiveness of gamma irradiation for the recovery of damaged wooden artifacts, which will facilitate the preservation of important historical materials.

Key words: Gamma irradiation, *Tyromyces*, *Coriolus*, biological control.

INTRODUCTION

Cultural artifacts made of wood are vulnerable to biodegradation by fungi. Fungi can hydrolyze a wide variety of polymers, including cellulose and hemicellulose. Under environmental conditions favourable to their growth, cellulolytic fungi can destroy wooden materials in a short period of time. Methyl bromide has previously been used as a fumigation agent to control the fungal biodegradation of wooden cultural artifacts. However, methyl bromide was banned in 1992 based on the Montreal Protocol, due to its toxicity to humans and the environment (Da Silva *et al.*, 2006). As a substitute for chemical treatment, gamma irradiation methods have been developed for the preservation of organic materials. Gamma irradiation

is a well-known treatment for the preservation of food and sterilization of medical devices, and has been used for theses purpose for the last 50 years. Although several recent studies have described the application of gamma irradiation to control fungal growth on paper and wood products, the optimal dose of radiation for the wood products has not been specifically identified (Hanus, 1985). Thus, in this study, fungi were isolated from Korean cultural artifacts stored in a museum, and identified using 18S rRNA sequencing. Experiments were conducted to determine the irradiation dose required to inactivate these fungi.

MATERIALS AND METHODS

The cultural artifacts used in this study were traditional Korean agricultural artifacts including *Hongdukkae* (the wooden roller used in smoothing cloth) and *Holtae* (tools stripping off the grains), which have been stored in the warehouse of Wando Forest Museum (Wando, South Korea).

Strains were isolated from different positions on each artifact by using a sterilized cotton bud, and subsequently incubated on a PDA medium (potato dextrose agar; Difco Laboratories, Sparks, MD, USA) at 25°C. To identify the fungi on the artifacts, method based on DNA sequencing analysis was used in this study. Genomic DNA was isolated from the fungi by using a preparation kit (MP Biomedicals LLC, Irvine, CA, USA). To obtain 18S rDNA, PCR was performed with the 18S rRNA gene primers of ITS (Internal Transcribed Spacers)1 (TCC GTA GGT GAA CCT GCG G) and ITS4 (TCC TCC GCT TAT TGA TAT GC) (Korabecna *et al.*, 2003). The gene sequence of the PCR products was analyzed by comparison with previously reported 18S rDNA sequences from the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Fungi were irradiated by a ^{60}cobalt irradiator (point source, AECL, IR-79, Nordion, Canada) with doses of 0, 1, 5, 10, and 20 kGy for inactivation. The temperature of the irradiation room was controlled at 22 ± 2°C with a relative humidity of 50 ± 5%. The source strength was approximately 11.1 PBq with a dose rate of 10 kGy/h at the location of the sample. Dosimetry was carried out using alanine dosimeters (Bruker Instruments, Rheinstetten, Germany) and measured with a Bruker EMS 104 EPR Analyzer. The actual doses were within 2% of the target dose.

To assess the radiosensitivity of isolated fungi, fungi were inoculated on plastic Petri dishes with MA2% (Malt Extract Agar 2%; Difco Laboratories) and irradiated. After irradiation, the plates were incubated at 25°C for 7 days. The controls were also cultivated on MA2% and were maintained in the same conditions as the treated fungi. The colony diameter was measured to verify fungal growth comparing to the controls. The fungal inactivation was reconfirmed with the inoculation of the irradiated fungi into Yeast Malt Extract Broth (Difco Laboratories) and the survival was recorded over a 10-day period at 25°C.

RESULTS AND CONCLUSION

In this study, Korean agricultural artifacts, including *Hongdukkae* (a wooden roller used for smoothing fabrics) and *Holtae* (a tool for stripping grains), were tested for fungal contamination. These historical implements were used in the late 19th century and are currently stored in a forest museum (Wando Forest Museum,

Wando, South Korea). Several fungi were isolated from the artifacts; 2 most abundant strains were cultivated and their genomic DNA was prepared for sequence analysis. From the 18S rDNA sequence analyzed, the isolates were identified as species of the genera *Tyromyces* and *Coriolus*. *Tyromyces* and *Coriolus* are known to degrade cellulose and hemicellulose in wood (Xu and Goodell, 2001; Tsujiyama *et al.*, 1998). These fungi, capable of breaking down fibers via the activity of several cellulase enzymes and of reducing sugars in wood fibers, were shown to have contaminated the cultural artifacts used in this study, despite storage of the artifacts under controlled museum conditions. Although the storage conditions did not stimulate fungal growth, long-term storage allows for biodegradation in the absence of active treatment. Countermeasures aimed at controlling biodegradation are, therefore, necessary for the preservation of such cultural artifacts.

To assess the radiosensitivity of these isolated fungi, the cultivated fungi were exposed to gamma irradiation. Growth of *Coriolus* and *Tyromyces* was measured after gamma irradiation at different doses. As shown in Fig. 1, fungal growth was inhibited by gamma irradiation. At a dose of 1 kGy, growth of *Coriolus* was significantly inhibited compared to the non-irradiated control. At a dose of 5 kGy, *Coriolus* was completely inactivated. The inhibition of *Tyromyces* growth was considerably more marked compared with *Coriolus* at 1 kGy. As shown in Fig. 2, non-irradiated *Coriolus* and *Tyromyces* grew well, but after irradiation at 1 kGy, fungal growth was retarded and strains irradiated at doses above 5 kGy did not show any growth on the plate.

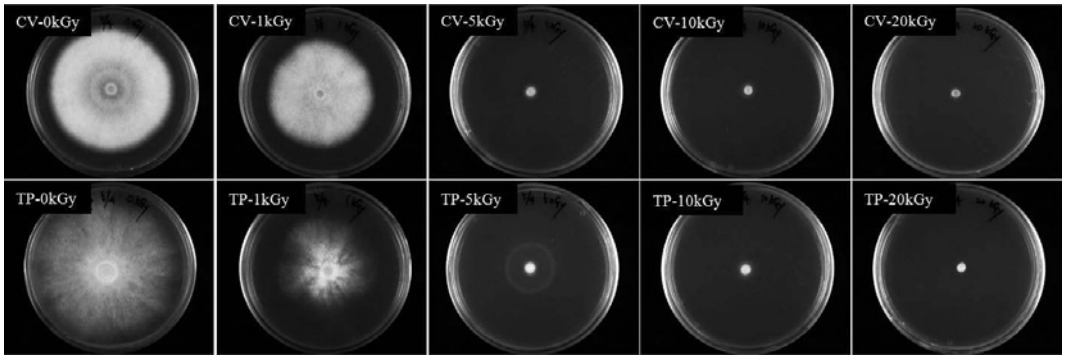

Fig. 1 - Effect of irradiation on *Coriolus* (upper line) and *Tyromyces* (lower line) with doses of 0, 1, 5, 10, and 20 kGy.

Gamma rays are used extensively for sterilizing microflora and for killing insects, particularly on organic materials. A dose of at least 500 Gy is required to kill larvae and to prevent the emergence of adult insects. Fungi are less sensitive to ionizing radiation than insects, and different strains show different levels of sensitivity. Generally, most fungi are inactivated by a dose of 10 kGy. Blank and Corrigan (1995) investigated the resistance of *Penicillium* and *Aspergillus* spores to gamma rays, and reported that radiation doses of 0.236–0.416 and 0.209–0.319 kGy, respectively, were necessary to inactivate 90% of the initial cells. In a study investigating the use of gamma rays for controlling the biodegradation of cultural artifacts, a gamma irradiation dose of 6–15 kGy successfully inactivated fungi found in various materials, including paper, wood, and clothes (Da Silva *et al.*, 2006). In the case of waterlogged archaeological wood, a dose of 10 kGy was sufficient to inactivate all marine and terrestrial fungi (Pointing *et al.*, 1998).

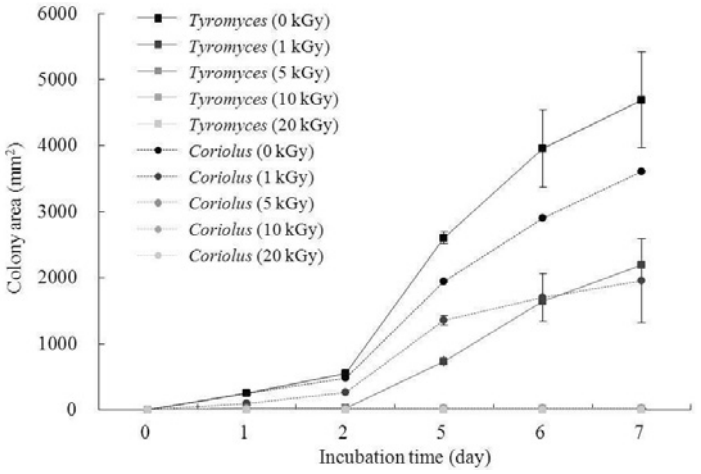

Fig. 2 - Growth of *Coriolus* (circles) and *Tyromyces* (square) irradiated with doses of 0, 1, 5, 10, and 20 kGy on MA2% plates.

These results confirmed that radiation treatment is extremely efficient for the control of fungi. Recently, Choi *et al.* (2012) reported that gamma irradiation up to an absorbed dose of 50 kGy did not cause any deleterious effects on paper. With its effectiveness at decontamination, its high penetration depth, and lack of mechanical damage to the objects on which it is used, irradiation technology can be utilized for the preservation of cultural material.

ACKNOWLEDGEMENTS

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MODELING THE GROWTH AND ENTEROTOXIN PRODUCTION OF *BACILLUS CEREUS* ON GARAETTEOK AS A FUNCTION OF TEMPERATURE AND RELATIVE HUMIDITY

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ABSTRACT

The objectives of this work were to model the growth and enterotoxin production of *Bacillus cereus* on garaetteok as a function of temperature and relative humidity during the storage period. Growth data of *B. cereus* in garaetteok under different temperature and relative humidities were collected and fitted into the modified Gompertz model. The generated growth parameters including growth rate and lag time were used to estimate the secondary models using polynomial equation. Additional experiments not included in the development of models were conducted for model validation. *B. cereus* enterotoxin was measured with *Bacillus* diarrhoeal enterotoxin visual immunoassay kit at the sampling time which was set to be 24 h considering the shelf life of garaetteok at room temperature. The enterotoxin production data for each treatment were analysed using the logistic procedure. The polynomial equations for growth rate and lag time of *B. cereus* were developed with high coefficients of determination. The validation results indicated that the

developed models could describe the growth data of *B. cereus* on garaetteok well. The estimated logistic regression model described the probabilities of *B. cereus* enterotoxin production on garaetteok as a function of temperature and relative humidity and identified the temperature and RH limits for enterotoxin production of *B. cereus*. The percent concordance index of the probability mode was 94.3%, indicating a high degree of agreement between the model predictions and observed responses.

Key words: *Bacillus cereus*, predictive microbiology, temperature, relative humidity, enterotoxin.

INTRODUCTION

Rice cake is one of the most popular of the Korean traditional foods. Many types of rice cake can be prepared, via the use of different ingredients and different manufacturing processes. Garaetteok, a very popular traditional rice cake in Korea, is made by pounding steamed rice flour and forming into a long white cylinder shape. The major ingredients of the rice cake are rice flour and water and it causes rapid starch retrogradation during storage. Korean traditional rice cakes are generally packaged by linear low-density polyethylene after steamingand cooling at room temperature, and then distributed throughout markets (Lee *et al.*, 2011). *Salmonella* spp., *Staphylococcus aureus*, and *Bacillus cereus* are the major microbiological hazards of cereal grains and related products (FDA, 2001). It was reported that 40% of 136 rice and processed products were contaminated with *B. cereus*in Korea (Jang *et al.*, 2006). Total 27 outbreaks of foodborne illnesses associated with *B. cereus* were reported to the Korea Food and Drug Administration from 2001 to 2008. The number of *B. cereus* illnesses is largely underestimated since the symptoms of the emetic illness are similar to those caused by *Staphylococcus aureus* poisoning, and the diarrheal illness symptomsare similar to those of *Clostridium perfringens* type-A food poisoning (Stenfors *et al.*, 2008). The growth and enterotoxin production of *B. cereus* on garaetteok as a function of temperature and relative humidity during the storage period were investigated.

MATERIALS AND METHODS

Mixed culture of *B. cereus* strains (JNHE36, ATCC 14579 and F4810/72) were used and inoculated on the surface of ice cake (garaetteok,about 3 mm inthickness) samples purchased from a local supermarket in Chuncheon, Korea. The initial pathogen level was approximately 3.0 log CFU/g. Inoculated samples with *B. cereus* were put into Petri dish and packaged using polyethylene (PE) film and then transferred into a closed environmental chamber (BF-600GC, BioFree, Seoul, Korea) which was used to simulate the storage temperature and relative humidity. Then, tested samples were exposed on sterile aluminum foil and were stored at 15, 25, 35 and 45°C with different relative humidities (50 and 80%). Sampling was generally carried out for enumeration based on the designed intervals, depending on different incubation temperatures. Enumeration was conducted at the same time through spread plate method. The population of *B. cereus* was enumerated

by plating on MYP (Mannitol-Egg Yolk-Polymyxin) Agar (Difco) supplemented with Egg Yolk Enrichment 50% and Antimicrobic Vial P before 24 h incubation at 37°C. For toxin detection, after stored for 24 h, sampling was generally carried out for the toxin detection using *Bacillus* diarrhoeal enterotoxin visual immunoassay kit (BDE VIA, Tecra®, Bioenterprises Pvt. Ltd., Roseville, New South Wales, Australia). Means of cell populations from each treatment were calculated from three replications.

The collected growth data at each combined condition were fitted into the modified Gompertz model to estimate the growth rate (GR: log CFU/h) and lag time (LT: h), and a secondary model was established for obtained GRs using polynomial equation. Then, a validation step was carried out through external validation using independent data sets for selected conditions, which were not used for model development. The coefficient of determination (R^2), bias factor (B_f), accuracy factor (A_f), and % standard error of prediction (%*SEP*) were employed to evaluate the fit quality of each model. The probability of positive toxin production of NHE enterotoxin (numbers of samples with toxin positive growth divided by the numbers of total sample tested) for each treatment as a function of the storage temperature and relative humidity were analyzed using the logistic procedure with backward selection of SAS 9.2 for Windows (SAS Institute Inc., Cary,NC) for fitting the model. The initial model for fitting had the following form:

$$\text{logit}\ (p) = a_0 + a_1 \cdot T + a_2 \cdot RH + a_3 \cdot T \cdot RH + a_4 \cdot T^2 + a_5 \cdot RH^2 \qquad (1)$$

where p is the probability of growth, a_0-a_5 are the coefficients to be estimated, T is the temperature, RH is relative humidity. Once the models were selected for rice cake, the toxin production probabilities (p) of *B. cereus* on rice cake were calculated using the following formula:

$$\text{logit}\ (p) = \ln\left(\frac{p}{1\text{-}p}\right) \qquad (2)$$

in which the probability of growth is $p = 1/(1 + e^{-\text{logit}(p)})$.

RESULTS AND CONCLUSION

Development of primary models

According to experimental observations, no detectable *B. cereus* was found on uninoculated rice cake samples (control). *B. cereus* did not grow well on rice cake stored at 5 and 10°C (data not shown) with different relative humidities. Declined *B. cereus* cells were observed at 5°C. In contrast, no growth was observed at 10°C for up to 7 days storage period. The reason may be because the target samples were different food matrixes and *B. cereus*is particularly sensitive to nutrient depletion. The data at these lower incubationtemperatures (5 and 10°C) were not included in the model development and validation. The experimental data of *B. cereus* on the inoculated rice cake samples at each combined condition of temperature (15, 25, 35 and 45°C) and relative humidity (50 and 80%) were collected and fitted into the modified Gompertz model. The results indicated that the modified Gompertz modelw as capable of producing a good fit (R^2>0.98) for the growth curves. The generated growth parameters including GR and LT were shown in Table 1. Based on the combined consideration of the results shown in Table 1, it can be seen that

a higher temperature and RH lead to a higher final cell population of *B. cereus* at different relative humidities conditions. The LT values obtained at 35 and 45°C were shorter than those at 15°C. Similar results were reported in previous work (Wang and Oh, 2012).

Table 1 - Growth parameters (growth rate and lag time) of B. cereus on garaetteok at different experimental conditions.

Humidity (%)	Growth rate (log CFU/h)				Lag time (h)			
	45 °C	35°C	25°C	15 °C	45°C	35 °C	25°C	15°C
80	0.47	0.44	0.18	0.071	3.81	2.96	5.84	10.3
50	0.45	0.41	0.17	0.068	3.61	3.51	6.16	10.6

Development of secondary model

In order to determine the combined effect of temperature and relative humidity on the growth of *B. cereus* on rice cake, the secondary model was established based on the GR and LT values (Table 1) using polynomial equations. Therefore, the secondary polynomial equations for the GR and LT of *B. cereus* on rice cake were determined as follow:

$$GR = -0.2149+0.02325*T-0.1932*RH+0.00237*T*RH-0.000176*T^2+0.1344*RH^2$$
$$LT = 19.944-0.99612*T+9.9238*RH+0.04233*T*RH+0.01231*T^2-9.2324*RH^2$$

where T is temperature and RH is relative humidity.

The coefficientof determination (R^2) estimated from the process of model establishment is an overall measure of the goodness-of-fit of the obtained models which is able to measure how well the model describes the growth parameters. R^2value of the secondary model was 0.940 for GR, while 0.995 for LT. The predictive ability and performance of prediction of the developed models should be evaluated through validation steps.

Validation of predictive model

External validation was conducted using experimental data sets not used for the model development. The $B_f$and $A_f$values were calculated to evaluate the performance of the developed model. The B_f values of the predictive models for GR and LT were 1.056 and 0.993, respectively. The A_f values for GR and LR were 1.056 and 1.067, respectively. In the present study, the obtained B_f and A_f values should be regarded as acceptable according the criteria reported in previous publications (Ross, 1999; Ross *et al.*, 2000). %*SEP* values of predictive models for GR and LT on rice cake were 4.78 and 6.55, respectively. Compared with the results reported in this study, both values proved the acceptable goodness of the proposed models.

Development of *B. cereus*boundary model of toxin production

The numbers of samples with growth and toxin production for each treatment are listed in Table 2. Among the treatments tested, 2 treatments had no toxin production in all 12 samples, 6 treatments had toxin production in from 1 to 11 samples, and 4 treatments had toxin production in all 12 samples.

Table 2 - Treatments for examining the probability of toxin production of B. cereus on rice cake, and the number of samples with positive growth (probability of growth) in each treatment of 12 samples*, and the observed and predicted toxin production probabilities.

Treatment	Temperature (°C)	RH (%)	Countsof *B. cereus*** (log CFU/g)	Toxin production samples	Observed *p*	Predicted *p*
1	15	80	3.85	0	0.00	0.02
2	20	80	5.64	3	0.25	0.17
3	25	80	6.56	7	0.58	0.63
4	30	80	7.39	11	0.92	0.93
5	35	80	8.61	12	1.00	0.99
6	40	80	7.71	12	1.00	1.00
7	15	50	3.57	0	0.00	0.01
8	20	50	5.60	1	0.08	0.09
9	25	50	6.32	6	0.50	0.46
10	30	50	7.24	10	0.83	0.88
11	35	50	8.28	12	1.00	0.98
12	40	50	7.55	12	1.00	1.00

*Sampling time: 24 h; **Bacteria counts of *B. cereus* at sampling time.

Comparing the observed and predicted values, it was observed that the predictions underestimated the probabilities for treatment 3 and 9; production was observed in both of these treatments, predicted to be likely production by the model. Overall, the observed and predicted probabilities of the other 10 treatments were in agreement, indicating that the obtained boundary model closely described the overall production or no production of *B. cereus* diarrhoeal enterotoxin on garaetteok during storage at different temperatures for 24 h.

The parameters estimated for the logistic regression model describing the probabilities of enterotoxin production of *B. cereus* on garaetteok as a function of temperature and relative humidity are shown in Table 17.The resulting logistic regression model was shown as follows:

$$\text{logit}(p) = -11.9606 + 0.4242 \cdot T + 2.3640 \cdot RH$$

As shown in Table 3, temperature term was significant ($p<0.05$) for the *B. cereus* enterotoxin production on garaetteok, while the quadratic terms of T, RH and the interaction of T·RH, were not significant ($p>0.05$) and had been removed. Temperature is much more influential on the toxin production of *B. cereus* on garaetteok

Table 3 - Parameter estimates of the logistic regression*.

Parameter	Estimate	Standard error	Pr >ChiSq
Intercept	-11.9606	2.4289	<0.0001
Temperature	0.4242	0.0725	<0.0001
RH	2.3640	2.0228	0.2425

*Association of predicted probabilities and observed responses: percent concordant, 94.3; percent discordant, 2.9; and percent tied, 2.8.

than the ambient relative humidity changes within the limits of the experimental design. The percent concordance index of the model was 94.3%, indicating a high degree of agreement between the model predictions and observed responses.

In conclusion, the obtained models for *B. cereus* growth and enterotoxin production were qualified to provide reliable information for controlling the storage conditions of temperature and relative humidity in microbiological risk assessment of *B. cereus* on garaetteok in Korea.

ACKNOWLEDGEMENTS

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SESSION II

"New Technologies for Shelf Life Extension"

COMPATIBILITY OF CHLORINE DIOXIDE AS ANTIMICROBIAL GAS FOR FOOD PACKAGING APPLICATION

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ABSTRACT

The consumers' healthier trend towards ready-to-eat (RTE) minimally processed food products like fresh-cut fruits and RTE leafy green salads also increase the risks of foodborne outbreak since the food will be consumed without any further processing or with minimal preparation. Active packaging, more specifically, antimicrobial packaging systems is one of the safety strategies that could be applied to such products. Chlorine dioxide (ClO_2), a strong oxidizing agent, is among the promising antimicrobial gases many researchers considering using in vapor-phase decontamination of food products. The compatibility of ClO_2 as antimicrobial gas for food packaging was reviewed in this work.

Key words: Antimicrobial packaging, chemical residue, chlorine dioxide; *Escherichia coli* O157:H7, polymeric material.

CURRENT STATUS

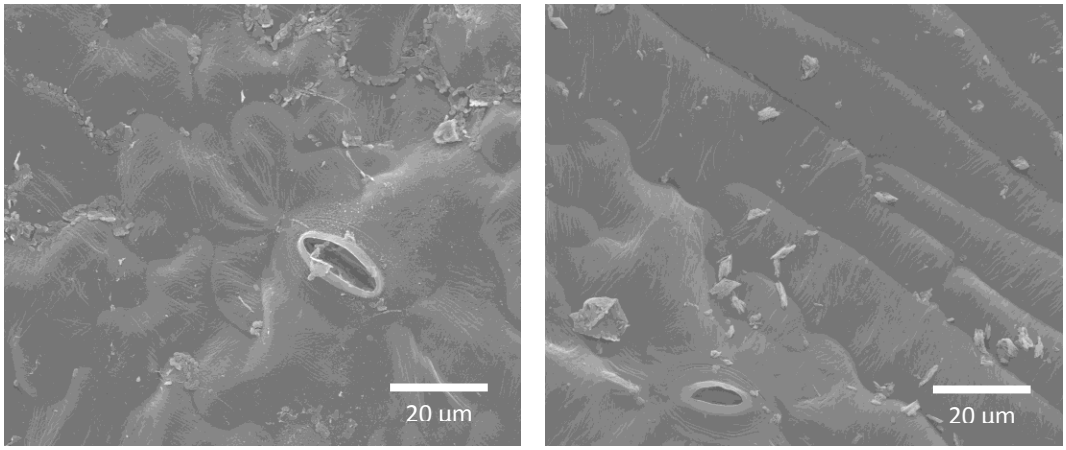
Currently, ClO_2 is mainly used as a bleaching agent in paper and pulp production, and sanitizing agent in portable water and waste water production. In limited applications, ClO_2 solution has been commercially used as a cleaning agent in food production, especially for fruit and vegetable-related products. The applications of its gas form; during post-harvest storage and in the processing line, as well as, inside the product/package system, have been a subject of many research studies since the gas phase improves its accessibility to hard-to-reach areas.(Netramai *et al.*, 2012) For ClO_2 gas to be selected as antimicrobial gas in commercial packaging

system for foods, the following information are necessary; its antimicrobial efficacy, its compatibility with packaging materials and the data on possible chemical residues left on the treated food surfaces.

Chlorine dioxide is known to disinfect microorganism by oxidation, mainly, through the one-electron transfer mechanism, in which the compound itself is reduced to chlorite (ClO_2^-). It has demonstrated broad antimicrobial efficiency against many pathogenic and spoilage microorganisms, such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. It was reported to have significant antimicrobial affects against biofilms of some important microorganisms as well. (Kreske *et al.*, 2006; Netramai *et al.*, 2012; USEPA, 1999) (Fig. 1).

$$ClO_{2(aq)} + e^- \rightarrow ClO_2^- \qquad E^\circ = 0.954V$$

In rare occasions, changes in sensorial attributes of food products were observed after subjected the foods to ClO_2-treatments, for example, off-flavors in fat-containing meat and discolorations in leafy green produce (Netramai *et al.*, 2012).



(a) (b)

Fig. 1 - Biofilm of *E. coli* O157:H7 on (a) un-treated Romaine lettuce surface; and (b) lettuce surface after batch treatment of 0.2 mgClO_2/L for 30 min (Netramai, 2011).

A few works studied the effects of ClO_2 gas on the properties of packaging materials, particularly, polymeric material. In their separate works Ozen (2000) and Netramai (2011) assessed ClO_2-barrier properties of some commonly used polymeric packaging materials for food products using quasi-isostatic- and isostatic techniques, respectively. Having permeability coefficient (*P*) for ClO_2 gas ranged between $< 7.36 \times 10^{-19}$ to 5.40×10^{-17} $kgClO_2 \cdot m \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$, biaxially oriented poly(propylene) (BOPP), poly(ethylene terephthalate) (PET), poly(lactic acid) (PLA) and nylon are regarded as having high barrier to ClO_2 gas, while polystyrene (PS), poly(vinyl chloride) (PVC) and polyethylene (PE), with *P* for ClO_2 gas ranged between 2.35×10^{-16} to 9.68×10^{-16} $kgClO_2 \cdot m \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$, could be classified as having low barrier to ClO_2.(Netramai, 2011; Ozen, 2000) Polymers with different barrier properties could be chosen for different applications involving ClO_2 gas, for example, materials with high ClO_2-barrier could be assigned as barrier-layer in packaging structure where majority of ClO_2 gas should

stay in the package headspace to decontaminate the content inside; or in designing ClO_2 gas delivery system, the material for such system could be selected according to their *P* values to provide specific rate of gas delivery.

Long-term exposure to ClO_2 gas (batch treatment of 10 mgClO_2/L for 14 d) significantly changed some polymers' barrier and mechanical properties, as well as, altered permselectivity () ratio of ClO_2-treated PE, PS, PET, and nylon films by up to 46.8% (Table 1). The stable permselectivity value of polymeric packaging material is crucial when its applications for respiring products is concerned.(Netramai *et al.*, 2010) Note that the ClO_2 concentration applied was higher than what typically used on food products. However, the manufacturers should still consider the possibility that some important properties of polymeric packaging materials might be altered after subjected to ClO_2 treatment, especially where long-term exposure to ClO_2 gas is expected, for example, when the packaging system is designed to deliver ClO_2 in a small dose, for a long period of time; or if the packaging system is designed to be reused for the same application as in the case of distribution packaging for fresh produce. Additional case-by-case study is recommended if mentioned polymers are to be selected for such applications.

A brief study on the influences of packaging design on antimicrobial effectiveness of ClO_2 gas, against *E. coli* O157:H7, inoculated onto fresh-cut Romaine lettuce, was reported. The results indicated that traveling distance of the gas to the target surfaces and the gas releasing area are significantly correlated to the antimicrobial activity of ClO_2 gas, specifically, for flexible packaging system of fresh-cut Romaine lettuce.

Table 1 - Barrier to O_2 and CO_2 of selected polymeric materials exposed to 10mg ClO_2/L (Netramai, 2011).

Material	Treatment time, day	$P \times 10^{-18}$, $kg \cdot m \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$		$P_{CO_2}^*/P_{O_2}^*$	% Change
		$P_{CO_2}^*$	$P_{O_2}^*$		
HDPE	0	58.3 ± 8.08[a]	9.26 ± 1.24[a]	6.30±0.32[a]	
	14	77.6± 0.40[b]	8.40 ± 0.29[a]	9.25±0.28[b]	+ 46.8
PET	0	1.54 ± 0.04[a]	0.22 ± 0.01[a]	6.88±0.13[a]	
	14	1.53 ± 0.04[a]	0.26 ± 0.04[a]	5.85±0.65[b]	- 14.9

*different superscript letters indicates statistically differences between means of the same material type, at α of 0.05.

For ClO_2 gas to be recognized as an effective and safe choice for sanitation of food products, both qualitative and quantitative information of chemical residues left on ClO_2-treated surface need to be considered. So far, the identified chemical residues left, after ClO_2-treatments are ClO_2, chlorite (ClO_2^-) and chloride (Cl^-), with ClO_2^- as a major by-product. The type and amount of chemical residues left depended on type of ClO_2-delivery system, ClO_2 concentration, treatment time and temperature, as well as, the type of food product (Netramai *et al.*, 2011; Trinetta *et al.*, 2011).

FUTURE DIRECTIONS

Chlorine dioxide showed its potentials as antimicrobial gas in packaging system for several food products. Still, additional studies to obtain crucial data, such as the effects of ClO_2 treatment on the food products' sensorial properties, the effects of storage temperature on ClO_2-barrier properties of packaging material, impacts of

ClO_2 exposure on packaging materials and decomposition mechanism of chemical residues left on the ClO_2-treated food surface, are necessary for it to be used more effectively and safely.

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EVALUATION OF α-TOCOPHEROL STABILITY DURING THE PROCESSING OF ACTIVE PACKAGING FILMS

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ABSTRACT

Addition of antioxidants (AO) to polyolefins is a common practice during film's manufacture because they protect the polymers from degradation. For this reason, part of the antioxidant is lost because of its ability to function as a free-radical scavenger. One of the most common synthetic antioxidants is butylated hydroxytoluene (BHT), however, nowadays there is an increased interest in replacing synthetic by natural ones. α-tocopherol is one of the most interesting natural AO, which can also be used to protect packaged foods from oxidation during storage. In this work, active films with different α-tocopherol and BHT contents were prepared through a two-step process (mixing and extrusion) with the aim of determining the amount of α-tocopherol and BHT in the film after processing, in order to optimize α-tocopherol quantity for further use as an antioxidant for the stabilization of food packaged products.

Key words: Active packaging, antioxidant, processing.

INTRODUCTION

The use of active packaging in contact with food is an alternative to the addition of high levels of antioxidants (AOs) directly to the food. The package can

act as a reservoir in order to maintain a constant concentration of the additive in the food during storage. This beneficial role of antioxidants in packaging films has increased the research regarding the manufacture of antioxidant-added films and their utilization in various food-packaging applications. Addition of antioxidants to polyolefins, such as low density polyethylene, is a common practice during film manufacture because they are also used to protect polyolefins from degradation. In fact, during plastic processing, the carbon–carbon bonds in the polymer break because shear stresses and mechanically induced chain scission and initiation of alkyl radicals and alkyl peroxyl radicals occur. Thus, part of the antioxidant is lost because of its ability to function as a free-radical scavenger. Also, it may be lost to the environment because of its high volatility at processing temperatures. The most common antioxidants are synthetic such as Irganox 1010, Irganox 1076 and BHT. However, nowadays there is an increased interest on replacing synthetic by natural antioxidants as stabilizers for polymers due to the fact that they are biologically degradable in nature and *in vivo*, which makes them attractive to be used to protect packaged foods from oxidation during storage. Therefore, for the above mentioned reasons, in order to ensure the extension of the shelf-life of food susceptible to deterioration by oxidation, active packaging films must be formulated with a higher antioxidant concentration than common polyolefin films.

In this work, active films with α-tocopherol and BHT were prepared through a two-step process (mixing and extrusion casting) with the aim to determine the amount of α-tocopherol and BHT in the film after the processing, in order to optimize its quantity for further use as an antioxidant for the stabilization of food packaged products.

MATERIALS AND METHODS

The active films were obtained loading two different antioxidants into an unstabilized LDPE matrix (Polimeri Europa, Italy): the natural α-tocopherol (Sigma-Aldrich, Italy) and the synthetic BHT (Sigma-Aldrich, Spain)). They were loaded at the concentrations of 1% w/w. The obtained active films were labeled as LDPE/1TOC, LDPE/1BHT and LDPE/0.5TOC/0.5BHT.

Film preparation The active films were prepared through a three-step process. LDPE pellets and α-tocopherol were fed into a mixer (HAAKE Rheocord 9000), using a speed of rotation set to 20 rpm, at T=140°C for 6 min. The mixed matter was afterwards collected, pressed using a COLLIN P300P press and then pellettized. A twin co-rotating screw extruder (Thermoprism) was used (L/D=24). The temperature profile from the feeding zone to the die was 135-150°C. The extruded film was pressed with a calender into a thin film (150 micron)

Determination of α-tocopherol in the active films Different kinds of extraction techniques can be used to extract the antioxidant from the polymer matrix. In this work, the following procedure has been used: 0.5 g of film was extracted with 20 mL of acetonitrile (ACN) for 24 h. Extracts were filtered and injected into an Ultra performance Liquid Chromatograph (UPLC®) with diode array detection (DAD). A second extraction was carried out under the same conditions with the same film. A UPLC BEH (5 x 2.1 mm, 1.7 μm) column was used at 30°C. A

gradient elution method was employed: between 0 min to 1.5 min, gradient elution from 10:90 water/ACN to 100% ACN, followed by isocratic elution of 100% ACN. The total run time of each analysis was 5 min. The flow rate was 0.3 ml/min and the injection volume was 10 µl. α-tocopherol was detected at 295 nm and BHT at 277 nm.

Oxygen induction time (OIT) A Differential Scanning Calorimetry (DSC) was used to measure the OIT of the investigated films in an oxygen atmosphere with a flow rate of 80 mL/min at 180°C. OIT is the time interval to onset of exothermic oxidation of the film and represents the efficiency of the phenolic antioxidant to reduce the oxidative decomposition of the polymer. The OIT values are determined as the intersections between the baseline and the tangent to the curve.

Oxygen permeability Oxygen permeability was determined by means of Ox-Tran (Mocon, Model 2/20). Samples with a surface area of 5 cm^2 were tested at 23°C. The oxygen permeability of the investigated film was determined setting the relative humidity (RH) at the downstream and upstream side of the film at 50%. Each test was made in duplicate.

RESULTS AND CONCLUSIONS

The amount of α-tocopherol determined in the active films using the procedure previously described is reported in Table 1. Results show that, due to its volatility, films LDPE/1TOC lost a high amount of α-tocopherol during processing. On the contrary, for films prepared with both α-tocopherol and BHT, the loss of natural AO was significantly lower. In fact, the residual amount of α-tocopherol after processing was 73% of the initial amount in the case of the plastic film LDPE/0.5TOC/0.5BHT. This means that BHT protects α-tocopherol from degradation. Indeed the amount of BHT found in the LDPE/1BHT films is higher than the amount found for LDPE/0.5TOC/0.5BHT films.

Table 1 - Concentration of active films in regards to TOC and BHT.

Samples	TOC/BHT *Initial amount (ppm)*	TOC/BHT *Extracted from the film (ppm)*
LDPE/1TOC	10000/0	2535/0
LDPE/1BHT	0/10000	0/4083
LDPE/0.5TOC/0.5BHT	5000/5000	3666/1137

Regarding the oxygen induction time of the investigated materials, results show that α-tocopherol efficiency is higher than that of BHT (Fig.1). In fact, despite the lower α-tocopherol residual content in the film compared to residual BHT content, OIT values of LDPE/1TOC films are similar to those of LDPE/1BHT. This is most likely due to the formation of α-tocopherol oxidation products which also exhibit antioxidant properties. Moreover, the combined effect of TOC and BHT shows a higher efficiency in the film stabilization. As confirmed by the chromatographic results, the use of BHT prevents the degradation of α-tocopherol, thus increasing its residual amount in the film after the two-step process.

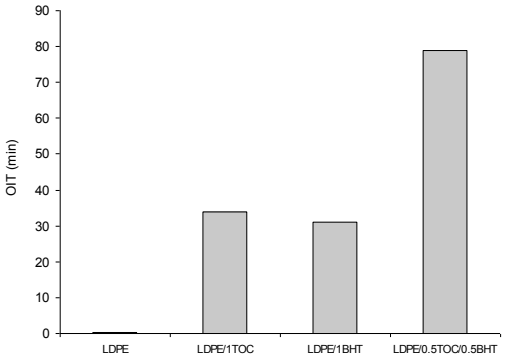

Fig. 1 - OIT values of the investigated films and pellets.

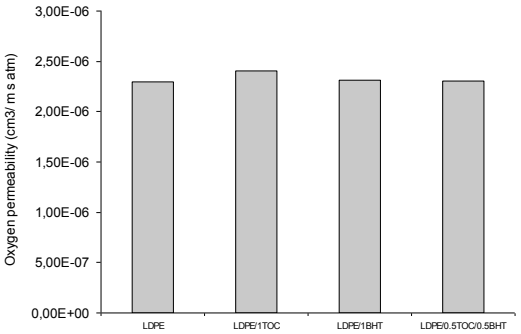

Fig. 2 - Oxygen permeability of the investigated films obtained using an OxTran at 23°C and 50% RH.

As far as oxygen barrier properties are concerned, no significant differences are observed among the neat LDPE and the active films (Fig.2). This can be related to the similar degree of cristallinity observed for these films (data not shown). These data provide a positive result since an increase in the oxygen transmission rate could result in an acceleration in the oxidation of the packaged product and, thus, counteract the positive shelf-life extension effect due to the presence of the residual antioxidant into the active film.

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QUALITY PRESERVATION OF SWEET PERSIMMON BY USING MASTER PACKAGING SYSTEM

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ABSTRACT

A persimmon master packaging system consisting of 8 inner individual primary packages (3 fruit bag each of 30 μm thick microperforated oriented polypropylene (OPP) bag with 3 or 5 microperforations of 59 μm) and outer secondary package (50 μm thick low density polyethylene (LDPE) film liner bag) was designed to allow separation of individual packages from the secondary package at retail display of higher temperature. The master package was placed in the corrugated box, stored for 160 days at 0°C and periodically opened to take out and store individual primary package units further for 5-10 days at 10°C as simulated retail display. The developed system was compared to 5 kg bulk package of 50 μm LDPE film bag and individual fruit package of microperforated 30 μm OPP film in modifying package atmosphere and preserving fruit quality. Individual packages inside master package attained the equilibrated package atmosphere after 62 days at 0°C: O_2 concentration of 0.5% and CO_2 concentration of 11.3% for inner packages with 3 microperforations; O_2 concentration of 0.5% and CO_2 concentration of 9.4% for inner packages with 5 microperforations. In master package system removal of outer liner (secondary) packaging and transfer of individual unit (primary) packages to 10°C resulted in higher CO_2 accumulation (15.8-22.9%) inside the package and slight increase of O_2 concentration (0.7-2.6%). This package atmosphere range high in CO_2 concentration seemed to be still in beneficial range. Overall benefits of master packaging system were reduced weight loss, reduced physiological injury particularly in flesh softening and surface blackening, and better retention in firmness and ascorbic acid, and delayed color change during the chilled storage and/or the display condition.

Key words: Master packaging, bulk package, microperforation, temperature change, gas composition.

INTRODUCTION

Concept of master packaging consisting of double layers of gas barrier was found previously to be effective to handle with temperature-variant regimes in persimmon distribution (Jeong *et al.*, 2012). This study designed master packaging system for individual package of 3 fruits in a secondary master packaging and tested their effectiveness depending on the design variable, which is the microperforation number.

MATERIALS AND METHODS

As a control treatment, bulk packages of about 5 kg, which are widely used for long distance transportation and marketing, were fabricated. Twenty four fruits were wrapped with 60 μm thick polyethylene (PE) film (78 x 77 cm) whose opening was clipped tightly by mechanical tie (1.2 mm of thickness). The fruits in the bag were aligned in two layers between which a corrugated board (31 x 40.5 cm) was placed for stacking stability and partial stress buffering. As a master packaging concept trial, 8 individual persimmon packages of 30 μm thick oriented polypropylene (OPP) film (30 x 15 cm) were placed in the same corrugated box instead of naked fruits. Each individual package contained 3 persimmon fruits placed on the polystyrene tray (25 x 10 cm). The OPP film bag of individual package was with three or five microperforations of 59±29 μm diameter made manually by a fine metal needle of 50 μm in diameter. Apart from treatments of master packaging system, the individual OPP film bags with 3 microperforations and without secondary liner package were also submitted to the storage and simulated distribution tests as an independent package treatment. The prepared packages were stored under the condition of -1±1°C and relative humidity (RH) of 70%. The bulk and master packages stored for some period of time have been dismantled to separate individual fruits or packages, which were moved to the simulated retail shelf condition of 10°C and RH of 30%, and then stored for 5 or 10 more days. The secondary or primary packages taken out from the storage and/or simulated display were measured in O_2 and CO_2 concentration of the headspace. The fruits from the packages were subjected to the quality measurement in weight loss, visual physiological injury of flesh softening, skin blackening and browning, firmness, ascorbic acid content, and surface color.

RESULTS AND DISCUSSION

When compared to two other packaging treatments (individual microperforated package and bulk package), two layers of gas barrier (microperforated OPP and LDPE liner) toward the fruit in the master packaging system showed greater accumulation of CO_2 and higher depletion of O_2 inside the individual packages (Fig. 1). With this phenomenal effect, master packaging system at 0°C seemed to provide MA condition helpful for persimmon preservation; generally MA condition of 1-3% O_2 and 4-7% CO_2 is known as optimal for persimmon preservation. Effects of film and seal higher CO_2 concentration with O_2 concentration above 0.5% has been reported to be beneficial elsewhere. Bulk package may be seen to have package atmosphere close to the optimal window, while individual microperforated package created too high O_2 concentration detrimental to the persimmon causing surface blackening. In master package system removal of outer liner (secondary) packaging

and transfer of individual unit (primary) packages to 10°C resulted in higher CO_2 accumulation (15.8-22.9%) inside the package and slight increase of O_2 concentration (0.7-2.6%) (Fig. 1). Individual microperforated package not in master package also experienced the similar change of atmosphere.

The highest moisture loss of the persimmon in bulk package during storage at 0°C (1.1% at 160 days) was observed probably due to the least moisture vapor barrier of this packaging (Fig. 2). Master packaging systems with two different microperforations on inner package showed negligible weight loss during storage at 0°C (0.4% at 160 days). Even the individual packages without outer liner bag gave little weight loss during storage at 0°C. The individual packages separated from the master package gave little weight loss under simulated display condition at 10°C (<0.2% in 10 days). The individual microperforated package without outer liner bag also showed little weight loss during the simulated distribution at 10°C.

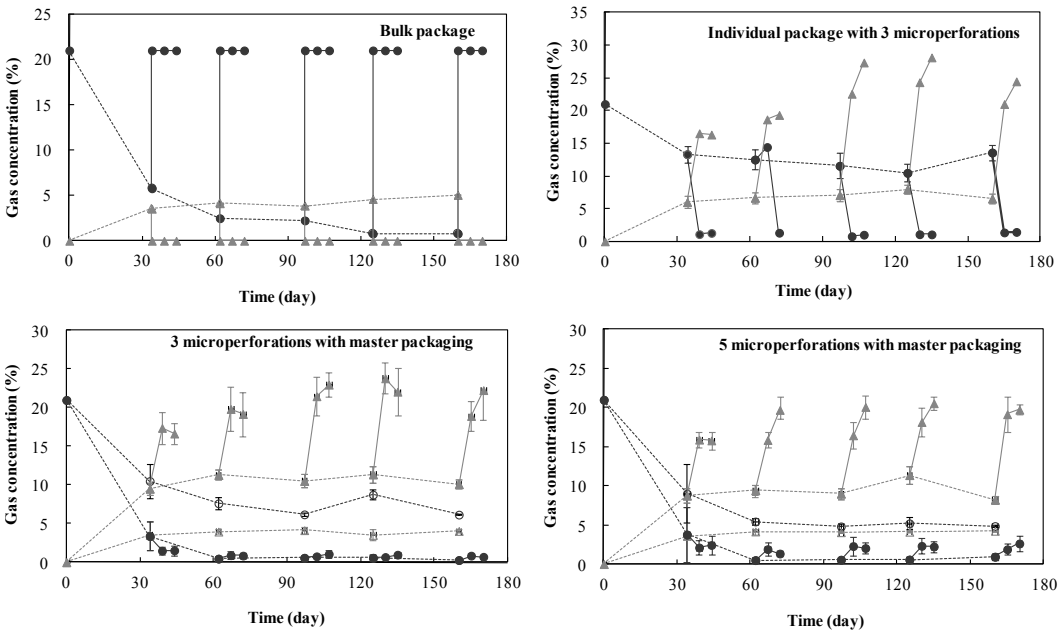

Fig. 1 - Gas composition changes of each level packages of 'Fuyu' persimmon during the chilled storage (0°C) and simulated retail display (10°C). Dotted line is for the chilled storage and solid line for the simulated retail display. Vertical bars are the standard deviations. ○: O_2 concentration inside master package; △: CO_2 concentration inside master package; ●: O_2 concentration around fruit; ▲: CO_2 concentration around fruit.

During the storage at 0°C, bulk package resulted in significantly high occurrence of skin blackening (Fig. 3). Bulk package started to have occurrence of fresh softening symptoms at 62 days compared to other packages and particularly had higher occurrence of fresh softening symptoms after the transfer of fruits to 10°C. Considering that bulk packaging maintained MA close to optimal condition, this high softening might be related to the high weight loss observed in this treatment. Individual microperforated package without master package also had pretty good preservation of visual quality comparable to packages in master packaging system.

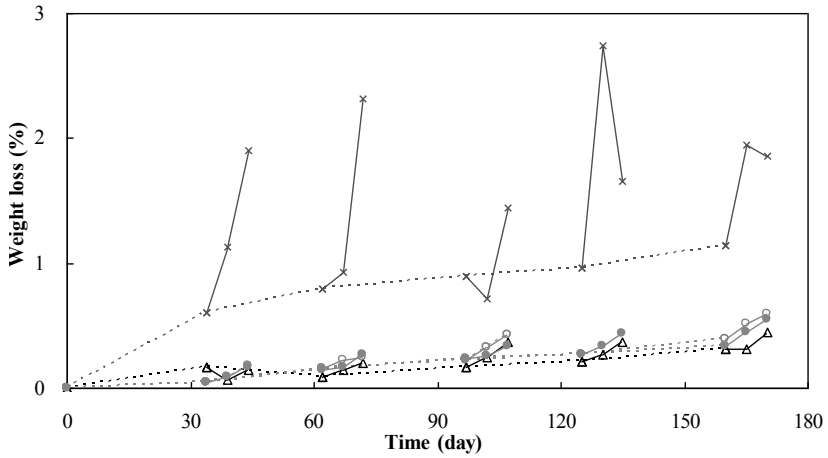

Fig. 2 - Weight loss of the persimmon during the chilled storage (0°C) and simulated retail display (10°C). Dotted line is for the chilled storage and solid line for the simulated display. ×: bulk package; △: individual package with 3 microperforations; ○: 3 microperforations with master packaging system; ●: 5 microperforations with master packaging system.

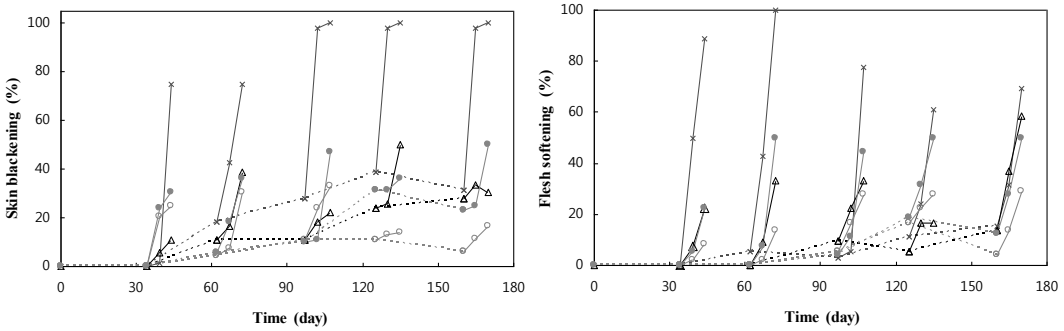

Fig. 3 - Physiological injuries of the persimmon during the chilled storage (0°C) and simulated retail display (10°C). Dotted line is for the chilled storage and solid line for the simulated display. ×: bulk package; △: individual package with 3 microperforations; ○: 3 microperforations with master package system; ●: 5 microperforations with master package system.

While MA of 10.5-13.6% O_2 and 6.0-7.9% CO_2 in the individual microperforated package at the stage of 0°C storage was very different from that of inner packs in the master packaging system, its package atmosphere in the simulated distribution was not so different from that of the package with 3 microperforations which had been placed in master package. Transfer of the individual naked fruits to 10°C after opening the bulk package resulted in increase of all the physiological disorders (Fig. 3). When compared to bulk package of 5 kg unit, master packaging system gave only milder increase in physiological injuries at 10°C, particularly for flesh softening and surface blackening. Clearly there are benefits of master packaging system in preserving the fruit quality (lower occurrence of physiological disorders), which would have been achieved by MA around the fruit being maintained through chilled storage (0°C) and simulated display (10°C).

Firmness of fruit in all treatment packages was generally preserved well during

storage of 160 days at 0°C (data omitted). Transfer of fruits to display condition of 10 °C with opening master or bulk package caused drastic changes in firmness. The impact of the transfer was greater with bulk package than master packaging system particularly after 10 more days at 10°C. Master packaging systems could retain higher ascorbic acid than bulk package and individual microperforated package without master package during the storage at 0°C (data omitted). The benefits of the master packaging systems could be still maintained even after the transfer of the individual packages or fruits to 10°C.

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PRESERVATION OF *PARAPENAEUS LONGIROSTRIS* FROM POLYPHENOL OXIDASE ACTIVITY ASSAYED *IN VITRO* WITH SOME MELANOSIS INHIBITORS

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ABSTRACT

Pink shrimp is an abundant crustacean widespread in Mediterranean Sea, which is strongly affected by oxidation due to polyphenol oxidase (PPO, EC 1.14.18.1), an enzyme producing pigments responsible for the undesirable dark color (melanosis). Sulphites and resorcinol derivatives are the most common and effective additives used to prevent melanosis in crustaceans. However, after having discovered that these antioxidants were related to allergic reactions in some consumers, several researches were carried out with alternative compounds. Several methods such as the addition of antioxidants and the exclusion of oxygen, as well as thermal processing were employed to inhibit the enzymatic reactions in pink shrimp. This work aimed to assessing the efficacy *in vitro* of some natural anti-browning agents (alginic, ascorbic, benzoic, caffeic, citric, ferulic and glutamic acid) in minimally processed pink shrimp provided from Port of Catania (Sicily, Italy). The crustaceans were washed, allowed to dry in order to remove the water in excess present in surface, and packed in ordinary atmosphere bags (polyethylene terephthalate, PET), covered by a double barrier film and chilled. The enzymatic activity was inhibited by addition of each anti-browning agents and the greater reduction of PPO was observed in the enzymatic assay containing alginic acid, the most effective for melanosis prevention. The effectiveness of the anti-browning treatments decreased with the following order: alginic, ferulic, glutamic, citric, ascorbic, benzoic, and caffeic acid. Data suggested some industrial applications, *e.g.* the addition of one or more of these inhibitors into fish packages, as alternatives to sulphites and resorcinol derivatives.

Key words: Pink shrimp, antibrowning, alginic, L-ascorbic, benzoic, caffeic, citric, ferulic, L-glutamic acids.

INTRODUCTION

Pink shrimp are abundant crustaceans belonging to *Parapenaeus longirostris* (Lucas, 1846) species, widespread in Mediterranean Sea. They are largely consumed in many recipes as fresh or cooked fish and, more recently, as shelled and packaged fish product, a rising commercial area in the food industry, favored by the consumers who look for products that require a minimum preparation time. The most important issue occurring in pink shrimp during post-mortem storage is the oxidation due to 'tyrosinase', producing pigments responsible for its undesirable dark colour (melanosis). In detail, the two copper atoms within the active site of tyrosinase enzyme interact with dioxygen to form a highly reactive chemical intermediate that then oxidizes the substrate (Prasad *et al.*, 2009). The activity of tyrosinase is related to catechol oxidase, a class of copper oxidase. Tyrosinase and cathecol oxidase are collectively termed polyphenol oxidase, therefore it would be more properly use this nomenclature (PPO, EC 1.14.18.1).

Sulphites and resorcinol derivatives (e.g. 4-hexylresorcinol) are the most common and effective additives used to prevent melanosis in crustaceans (Montero *et al.*, 2006). However, after having discovered that these antioxidants were related to allergic reactions (bronchial asthma, nausea, abdominal pain, blue lips, nails and skin) in some consumers, several researches were carried out with alternative compounds. Several methods such as the addition of antioxidants and the exclusion of oxygen, as well as thermal processing were employed to inhibit the enzymatic reactions in pink shrimp. This work aimed to assessing the efficacy *in vitro* of some natural anti-browning agents (alginic, L-ascorbic, benzoic, caffeic, citric, ferulic and L-glutamic acid) in minimally processed pink shrimp provided from Port of Catania (Sicily, Italy).

MATERIAL AND METHODS

The pink shrimp (*Parapenaeus longirostris* Lucas, 1846) were purchased from Port of Catania (Sicily, Italy). The crustaceans were kept in ice with a shrimp/ice ratio of 1:2 (w/w) and transported to the DISPA laboratory within 1.5 h. Upon arrival, shrimp were washed in cold tap water, air-dried to remove the water in excess present in surface, peeled of cephalothoraxes and randomly packed in ordinary atmosphere bags (polyethylene terephthalate, PET) with following dimensions, length 18 cm, width 13 cm, height 5 cm, and containing an average of 500 g of shrimps. Each bag was covered by a double barrier 'anti-fog' film (Melinex 850, Italy - O_2 permeability: 35 cc/m^2/24h, CO_2 permeability: 135.8 cc/m^2/24h; H_2O permeability: 15g/m^2/24h), and chilled for 24 h at -18 ± 0.5 °C. The next day, the peeled shrimp were homogenized with an Ultraturrax T25 (Janke & Kunkel, Germany) for 5 min in an iced bath, minimizing light and oxygen exposition by wrapping the samples with aluminum foil. The enzyme extraction and the spectrophometric assay were carried out according to the method of Simpson *et al.* (1987) with some modifications. The potential melanosis inhibitors, alginic acid L-ascorbic acid (Sigma, ≥99.0% purity), benzoic acid (Sigma-Aldrich, ≥99.5% purity), caffeic acid (Sigma-Aldrich, ≥95.0%), citric acid (Sigma-Aldrich, ≥99.5% purity), ferulic acid (Aldrich, ≥99% purity), L-glutamic acid (Aldrich, ≥98.5% purity) at same concentrations (1%) were individually mixed with crude PPO extract to obtain the final concentrations of 0.5% (w/v). This reaction mixture was incubated for 30 min at room temperature. Then, the

assay buffer was added. To initiate the reaction, pre-incubated enzyme substrate was added. The control was run in the same manner, except that extracting buffer was used instead of the melanosis inhibitors. One unit of oxidase activity was defined as an increase in the absorbance by 0.001 at 475 nm at 25 ± 0.5 °C. All measurements were conducted in triplicate. Data were statistically analysed by the analysis of variance (ANOVA) using the Statistical Analysis System (SAS Ver. 9.0). Differences among means were evaluated for significance using the Duncan Multiple Range Test (DMRT) at $p \leq 0.05$.

RESULTS AND CONCLUSION

The enzymatic activity was inhibited by addition of each anti-browning agent (alginic, L-ascorbic, benzoic, caffeic, citric, ferulic and L-glutamic acid) and according to ANOVA statistical analysis, data were statistically different ($p \geq 0.05$). In order to better evidence the enzymatic changes in comparison to the untreated control, the results were graphically represented as relative activity, by fixing equal to 100 the initial enzyme activity (Fig. 1). The greatest oxidase reduction was observed in the enzymatic assay containing alginic acid, the most effective for melanosis prevention. The effectiveness of the anti-browning treatments decreased with the following order: alginic (-49.6%), ferulic (-48.0%), glutamic (-41.0%), citric (-34.1%), L-ascorbic (-28.2%), benzoic (-24.7%), and caffeic acid (-13.7%). These results were confirmed by positive changes of the chromatic parameters in a parallel test of shrimp submitted to a dipping treatments for 3 min at 4.0 °C with same final concentration (0.5%) of each potential melanosis inhibitors (data not shown). Data suggested some industrial applications, e.g. the addition of one or more of these

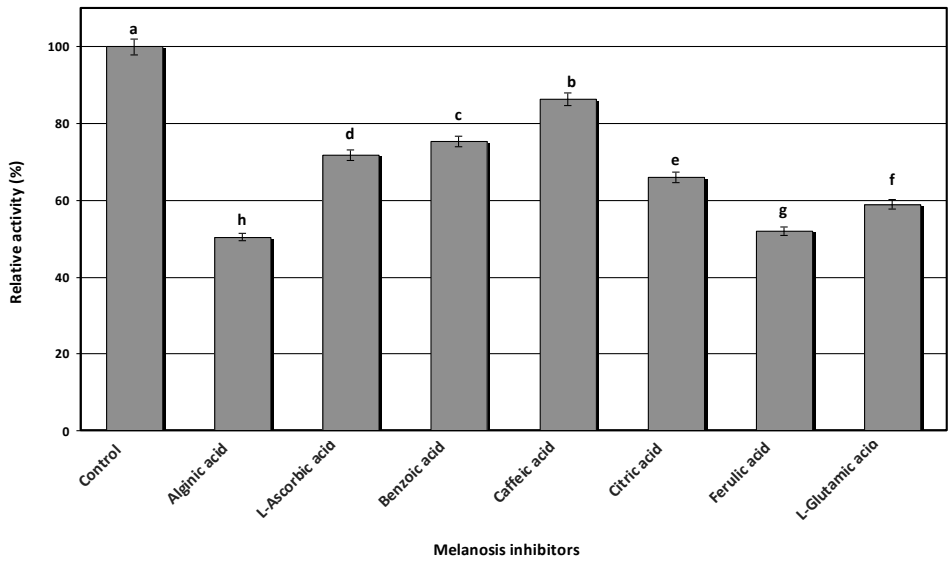

Fig. 1 - Effect of anti-browning treatments on the inhibition of PPO enzyme from minimally processed pink shrimp. Bars represent the standard deviation from triplicate determinations. The different letters on the bars indicate significant differences ($p \geq 0.05$).

melanosis inhibitors into crustacean packages, as alternatives to sulphites and resorcinol derivatives.

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POLYPHENOL OXIDASE CHANGE IN READY-TO-USE MARINATES ANCHOVIES AND SARDINES

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ABSTRACT

Blue-fish is particularly abundant in the Ionian Sea and generally consumed as fresh, canned or salted product. According to the amount of anchovies and sardines available, there is a possibility of developing new products. One possible commercial alternative is the production of marinated ready-to-use blue-fish. Nevertheless, polyphenol oxidase (PPO, EC 1.14.18.1) is an enzyme producing pigments responsible for the undesirable dark colors which spoil these products. This research aimed at assessing the efficacy as anti-browning agents of citric acid and marination in ready-to-use anchovy and sardine fillets compared to the untreated controls throughout a 5-days chilling storage. The results showed that no significant differences ($p \geq 0.05$) were noticed in gas concentration (O_2 and CO_2) inside the bags during storage. Each anti-browning treatment (citric acid or acidification and marination) produced a significant ($p \leq 0.05$) PPO inhibition. The greatest enzymatic reduction as compared to the untreated control samples was observed in marinated anchovies stored up to 4 days. The effectiveness of the preservation treatment with citric acid was confirmed in terms of color parameters, whereas the addition of sunflower oil in marinated blue-fish 'masked' their chromatic changes during storage. Data suggested industrial applications, *e.g.* the addition of one or more PPO inhibitors into blue-fish packages.

Key words: Anchovies, sardines, browning, polyphenol oxidase, marination, chilling storage.

INTRODUCTION

The Mediterranean Sea is one of the largest reserves of marine and coastal biodiversity, with 28% of its species being endemic and with 7.5% of the marine

fauna and 18% of the marine flora to be found in the world. In particular, anchovy (*Engraulis engrasicolus* Linneus, 1758) and sardine (*Sardina pilchardus* Walbaum 1792) are particularly abundant in the Ionian Sea facing Catania (Sicily, Italy). This blue-fish is generally consumed as fresh, canned or salted. According to the amount of anchovies and sardines available there is a possibility of developing new products. One possible alternative is the production of marinated ready-to-use blue-fish. They consist of fresh, frozen or salted fish or portions of fish processed by treatment with edible acids and salt, and placed in brines, sauces, creams or oils (marinade). Marinates are semi-preserves; organic acids, usually acetic or citric and salt, are added to retard the spoiling action of bacteria and enzymes, resulting in a product with a characteristic flavour and an extended but limited shelf life. The acidic conditions of marination inhibits tissue oxidation caused by polyphenol oxidase (PPO, EC 1.14.18.1), an enzyme producing pigments responsible for the undesirable dark colors which spoil products. This work aimed at assessing the efficacy of citric acid and marination as anti-browning agents in ready-to-use anchovies and sardines compared to the untreated control samples.

MATERIALS AND METHODS

Fish preparation The blue-fish species (anchovies and sardines) was caught in the Ionian Sea in June 2011 and provided from a local market of Catania in Sicily (Italy). Approximately 10 kg of each species was collected. Anchovies and sardines had an average weight of 20.8 g (min 18.74 g – max 25.8 g) and 26.9 g (min 23.4 g – max 33.1 g), respectively. All fish samples were washed, dried to remove water in excess, filleted, treated with anti-browning (dipping with citric acid 2% for 3 min, or acidification and marination with sunflower oil up to cover the fillets with addition of NaCl, 0.5 g inside each bag), packed in ordinary atmosphere bags (PET, 9.55 x 9.55 x 4.94 cm), covered by a double barrier film (Melinex 850, Italy) and stored at 4 °C up to day 5.

Gas concentration (O_2 and CO_2) The analysis was carried out by monitoring gas concentration (PBI Dansensor, Denmark) inside the packages.

Polyphenol oxidase The activity (PPO) was determined spectrophotometrically (Cary IE-100 UV-VIS, Varian, Palo Alto, CA, USA) at 505 nm using a specific procedure based on the method proposed by Spagna *et al.* (2005). One unit of PPO activity (U) was defined as the amount of enzyme which produces an increase in absorbance of 0.001 per min at 25 ± 0.5 °C under the experimental conditions. Results are expressed as U g^{-1} fillet weight.

Color measurements According to CIE Lab standard (1986), L* (lightness), a* (green-red shade) and b* (blue-yellow shade) parameters were recorded using a compact tristimulus chromameter (N-3000, Nippon Denshoku Ind. C. Ltd, Tokyo, Japan).

Statistical analysis. All measurements were conducted in triplicate (n=3). Data were statistically evaluated by the analysis of variance (ANOVA) using the Statistical Analysis System (SAS Ver. 9.0). Differences among means were evaluated for significance using the Duncan Multiple Range Test (DMRT) at $p \leq 0.05$.

RESULTS AND CONCLUSION

Gas concentration (O_2 and CO_2) The results of Dansensor monitoring showed that no significant differences ($p \geq 0.05$) were noticed in gas concentration (O_2 and CO_2) inside the bags during chilling storage. The range of values was, O_2=20.05±0.22% and CO_2=0.70±6.46% for all samples.

Polyphenol oxidase According to ANOVA statistical analysis the anti-browning treatments (citric acid or acidification and marination) produced a significant ($p \leq 0.05$) PPO inhibition (Table 1).

Table 1 - Effect of anti-browning treatments on the inhibition of polyphenol oxidase.

PPO	Anchovies			Sardines		
	Control	Citric acid	Marination	Control	Citric acid	Marination
0		70.89 c (untreated)			50.24 d (untreated)	
1	77.23 b	71.11 c	57.82 e	58.18 b	50.57 d	29.00 g
2	84.01 a	38.20 f	32.06 g	61.10 a	53.96 c	15.64 h
3	64.56 d	22.98 h	15.95 i	54.02 c	44.12 f	13.77 i
4	58.14 e	16.08 i	7.02 l	47.13 e	28.75 g	11.02 l

The greatest enzymatic reduction as compared to the untreated control was observed in marinated anchovies stored up to day 4. In particular, anchovies showed a higher initial PPO activity in comparison with that of sardines (ca. 40%). In both blue-fish species, the two control samples noticed an initial increase in activity until day 2, then it decreased up to day 4. In anchovies treated with citric acid the enzyme activity remained unchanged at day 1, decreasing suddenly afterwards, whilst in sardines PPO increased up to day 2 and then declined gradually. In marinated anchovies and sardines PPO decrease was significant ($p \leq 0.05$) already at day 1, with a more effective inhibition of the enzymatic activity in anchovies. Although the initial PPO activity in sardines was lower than that in anchovies, the values of residual activity of sardines at day 4 was higher than anchovies, probably due to a larger surface contact area of the sardines fillets compared to the anchovies ones, with a relatively lower efficiency of the anti-browning treatments. The effectiveness of the preservation treatment with citric acid was confirmed in terms of color variations.

Color measurements Fresh anchovies and sardines showed (Table 2) L* values not significantly different ($p \geq 0.05$). In both control fish samples a decrease of the parameters L*, a* and b* was noticed during chilling storage, confirming respectively, loss of the lightness as well as the pinkish color typical of freshly caught fish, accompanied by a slight browning. In treated products the parameter L* tended to increase, whilst the parameter a* declined to values significantly different ($p \leq 0.05$) in comparison to the respective controls. In particular, at day 4 in anchovies and sardines dipped with citric acid, the values of a* were lower compared to the control samples, with consequently discoloration of the fillets. Fish treated both with citric acid and marination, showed values of a* lowered towards negative values, probably due to the chlorophyll note of the sunflower oil inside the packages. In fillets

Table 2 - Changes of color parameters in control and treated anchovy and sardine fillets.

Anchovies	Control			Citric acid			Marination		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
0	53.41 b	6.64 a	9.57 c	(untreated)					
1	52.81 c	5.98 b	8.71 d	55.44 a	5.74 b	7.96 de	56.86 a	5.71 b	13.22 b
4	42.79 e	5.17 b	7.27 e	50.15 d	4.06 c	10.04 c	53.57 b	-1.43 d	18.91 a
Sardines	**Control**			**Citric acid**			**Marination**		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
0	52.67 b	5.08 a	9.11 c	(untreated)					
1	51.08 c	5.06 a	8.77 c	52.94 b	4.82 a	9.48 c	52.91 b	3.41 b	12.37 b
4	45.48 d	4.76 a	6.96 d	54.17 b	3.85 b	11.57 b	56.34 a	-2.81 c	19.06 a

treated with citric acid the parameter b* decreased in both species confirming the effectiveness of dipping against browning phenomena, whereas the addition of oil in marinades blue-fish 'masked' their color changes.

Certainly, some additional enzymatic reactions (calpain, cathepsin and calpastatin activities) or non-enzymatic reactions, and interactions occurred inside the packaging. Data suggested industrial applications, e.g. the addition of one or more PPO inhibitors into blue-fish packages.

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THE IMPORTANCE OF INFORMATION TO CONSUMERS IN THE NEW EU REGULATORY LANDSCAPE AND THE "SHELF LIFE"

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ABSTRACT

This paper discusses the changes made by the European Union (EU) on the provision of food information pointing out that, compared to the past, the matter is now regulated according to a discipline in more rigid and restrictive manner creating a platform of greater security for the health of consumers in the food field, covering the required elements which must appear on the label (standards addressed by business operators). The EU has done so in order to put the consumers inexperienced or non-technical subject, in a position to easily understand the information when they need to manage their food choices on the shelf without fear of falling into error. In this sense, this work aims to highlight how, through the regulatory aspects such as the languages to use, simple and immediate indication of nutritional factors and health risk as well as the most important determinants for personal food choices, the consumer has made free to be able to make autonomous choices.

Key words: Regulations, labeling, nutritional, market, shelf-life.

INTRODUCTION

In recent years it has spread a greater general awareness of the importance of a balanced diet. The problems connected with the recruitment of specific nutrients including saturated fats, sugars or sodium were already taken into account by the European Commission White Paper in considering the health problems that arise as overweight and even obesity. To ensure nutrition information can be accessed by all consumers and then easily and clearly understandable, the European institutions have intervened with a regulatory plan to provide the consumer a basis for making informed choices, by regulating the form and content of information

that are on labels so that everyone can make informed choices according to their personal needs and diets.

COMPARISON OF EU REGULATIONS

To satisfy the new needs has been adopted as a new Regulation, precisely, the Regulation (EC) No. 1169/2011 on the provision of food information to consumers and on food labelling requirements. The Regulation has been published on 22 November 2011 in the EU Official Journal and the purpose is to help citizens to find a balance among their health, well-being, social and economic interests giving them a high level of protection on nutrition labelling for foodstuffs. In fact, in order to achieve a high level of health protection for consumers it is necessary to ensure the "right to information". The label is the means by which information is transmitted to consumers and it is for this reason that the new legislation has focused on it by specifying precisely what information the label should contain and how they should be indicated. It should be noted that there were already some laws which protected the area, for this reason, before moving on to detail the discipline, we want to discuss innovative aspects compared to the previous legislation. The Regulation (EC) No. 1169/2011 repeals some other dispositions including EC Directive 90/496/EEC on nutrition labelling and EC Directive 2000/13/EC on food labelling since its aim is to combine, modify, simplify and clarify current legislation on labelling laying down new methods of presentation and advertising of foodstuffs to be sure that food information can be understood by the final consumer. In other words new text consolidates and updates two areas of the legislation with respect to labelling: the general labelling of foodstuffs which is regulated by directive 2000/13/CE and nutritional labelling contained as objective of directive 90/496/EEC. Compared with the past, the new Regulation improves requirements on food allergens, vegetable, imitation foods and its intents to extend the mandatory country of origin labelling to other products. About allergens, the new statement requires that those elements must be separated from the rest of the list of ingredients, so they could easily individualize costumers. Moreover, in case ingredients or processing aids used in the food originate from a substance causing allergies or intolerance, those must appear on the label. The same is for non-prepacked foods sold to the final consumers. The abuse of certain foods that contain certain elements can endanger human health. In order to make consumers more aware if it contains such elements in it when buying a product, some rules have been laid down: nutritional information is compulsorily introduced for the majority of processed foods and the elements which must be declared and displayed in the same visual field are as follows: the energy value, fats, saturated fats, carbohydrates, sugars, proteins and salt; Moreover, information relating to the energy value may be repeated in the main visual field alone or with the quantities of fats, saturated fats, sugars and salt, and it must be used the form "per 100 g or per 100 ml" to better permit the comparison of products. Another important aspect is about trans fatty acids and it is taken into consideration in the Regulation 1169/2011 where it is written that the European Commission shall submit a report, within a 3-year period, that may be accompanied by a legislative proposal. Before the new regulation, the information on the energy content as well as amounts of fat, saturates, carbohydrates, sugar, protein and salt were discretionary "unless a nutrition-related claim was made concerning the food" but now this requirement will become mandatory.

CURRENT EU REGULATION

The new rules concern the mandatory nutrition labelling, legibility, distance selling and engineered nanomaterials in food; moreover, the new Regulation improves requirements on food allergens, vegetable, imitation foods and its intents to extend the mandatory country of origin labelling to other products, and these dispositions apply to all the activities of food business operators at all stages of the food chain and to all foods intended for the final consumer, including foods delivered by mass caterers, and foods intended for supply to mass caterers. The food business operator responsible for compliance with the food labelling rules is the operator under whose name or business the food is marketed, or if established outside the EU, the importer into the EU market (paragraph 3). For example always considering the aim of the Regulation not to mislead consumers it should be clear label to indicate the presence of an ingredient that has been substituted (e.g. imitation foods); to indicate the consumer that a particular product has been defrosted when such condition will have an effect on the safety, taste and physical quality of the product; and to indicate the specific vegetable origin of vegetable oils. Country of origin and the origin of main ingredients will have to be given if different from where the final product is made. Allergen information will have to be provided on all foods (whether sold prepacked or loose). For prepacked foods, the allergens will have to be highlighted on the ingredient list. Drinks with high caffeine content will have to be additionally labelled as not recommended for children, or pregnant and breastfeeding women, with the actual caffeine content quoted. Meat and fish products that look like a cut, joint or slice and contain more than 5% added water will have to show this in the name of the food.

Table 1 - List of Mandatory elements contained in articole 9

The name of the food.
The list of ingredients.
Any ingredient derived from a substance causing allergies or intolerances even if in an altered form.
The quantity of certain ingredients or categories of ingredients.
The net quantity of the food.
The date of minimum durability or the ‘use by’ date.
If it is necessary any special storage conditions and/or conditions of use.
The name or business name and address of the food business operator.
The country of origin or place of provenance; instructions for use where it is necessary for an appropriate use of the food.
With respect to beverages containing more than 1,2 % by volume of alcohol, the actual alcoholic strength by volume.
A nutrition declaration.

Table 2 - The principles governing mandatory food information contained in article 4

Information on the identity and composition, properties or other characteristics of the food.
Information on the protection of consumers’ health and the safe use of a food. In particular: compositional attributes that may be harmful to the health of certain groups of consumers.
The health impact, including the risks and consequences related to harmful and hazardous consumption of a food.
Information on nutritional characteristics so as to enable consumers, including those with special dietary requirements, to make informed choices.

Another aspect of warranty is given by the prevision of a consultation of the European Food Safety Authority in the case of any Union measure about food information law which can have an effect on public health shall be adopted can-

not be adopted without. Consistent with the purpose of clarity and simplicity and directness of the comprehensibility of directions on the label, the Regulation has been involved in two other elements: what language to use and the measures:

- the language to be easily understood by the consumers of the Member States where a food is marketed. Member States may dictate that the information shall be given in one or more languages from among the official languages of the Union;

- as regards the measure (presentation of information) the nutrition labelling must be provided in a tabular form (or linear format due to) on the packing and in the same field of vision (except to foods in small packaging) and for that aim it sets the minimum font size that may be used on food labels. However, in the case of distance selling the mandatory food information must be given before the purchase is concluded.

The Regulation will apply from 13 December 2014, (but there are some few exceptions like the nutrition declaration which will apply from 13 December 2016) since the commission has recognized that the business operators will need a significant period of time to adapt their products to the new legislation.

CONCLUSIONS

The new Regulation (EU) on the provision of food information is not limited to laying down stricter conditions but it establishes that information will be presented in a comprehensible way by all consumers even the inexperienced, by regulating other aspects of the language, metrology, etc. Only in this way it is possible to achieve the goal of warranty and freedom of consumers at the same time:

- warranty because, if the label is the only means of communication of information of food that consumers have when buying a product, it is clear that the latter can be protected only by regulating the obligations and prohibitions method of transmission;

- freedom, because by providing the guarantees that were just mentioned, the result is that consumers are independent in choosing of food since they can compare themselves, directly from the shelf, the directions on the label without the help of any experts, knowing that thanks to the new discipline they cannot be misled by unfair information.

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PROLONGING STORAGE LIFE OF ORCHID FLOWERS USING ACTIVE PACKAGING

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ABSTRACT

Thailand is the world's largest exporter of tropical orchid flowers. However, orchid flowers are fragile and sensitive to ethylene that has a major role in the senescence of most orchid flowers. Ethylene-induced symptoms include flower discoloration, premature wilting and flower fall. In addition, increasing ethylene gas concentration inside the flower packages during storage can cause premature senescence. This research was thus aimed to extend storage life of orchid flowers by using active packaging based on porous clay coated paper. The porous clay has high surface area with uniform and specific pore sizes and could be used as an ethylene scavenger. The developed active paper was used to keep orchid flowers, in comparison with those kept in regular packaging (control samples). Changes in orchid qualities were monitored throughout the storage period at 12°C and 85% until the flowers perished. The results showed that the active packaging could adsorb ethylene inside flower packages and thus prolong the storage life of orchid flowers.

Key words: Active packaging, ethylene, orchid flowers, storage life.

INTRODUCTION

Orchid flowers e.g. genera Dendrobium are fragile and sensitive to ethylene. The end of short life of cut orchids is associated with climacteric rises in ethylene production and respiration. Ethylene has a major role in the senescence of most orchid flowers. Ethylene-induced symptoms include flower discoloration and premature wilting and flower fall. In addition, increasing ethylene gas concentration inside the flower packages during shipment can cause premature senescence (Almasi *et al.*, 2012). Therefore the development of technology and packaging material which can prolong storage life of Thai orchid flowers effectively is needed. Porous clay has high surface area with specific pore sizes and thus can be used as an ethylene scavenger. This research aimed to develop active packaging from paper coated with porous clay that can effectively absorb ethylene to prolong the storage life of orchid flowers.

MATERIALS AND METHODS

Active packaging used in this research was prepared by coating porous clay on whilte Kraft paper. The porous clay was prepared according to the method described by Srithammaraj, Magaraphan, & Manuspiya (2012). Orchid flowers (*Dendrobium spp.*) were provided by Siam Taiyo Farm Co., Ltd, Thailand. Orchid flowers (LL size) were harvested when at least 4 flowers per spike are open. The orchid flower spikes used in this study were harvested on the same day, had similar maturation and were free from defect. Each orchid sample was prepared as individual spikes with about 12-20 flowers. The orchid flowers were separated into 2 sample groups. The first sample group was used as control. The control samples were wrapped with white Kraft paper. For the second sample group, the flowers were wrapped with porous clay coated Kraft paper. The orchid flowers were then packed into a carton (60x20x20 cm^3) and stored in a temperature control cabinet at 12 °C and 85% relative humidity. The qualities of all samples were monitored weekly until orchid flowers senescence.

The quality of orchid flowers during storage at 12°C and 85% RH was evaluated. The scores of deterioration were evaluated based on flower discoloration and premature wilting and flower fall. The deterioration scores were given as 1 (no deterioration) to 5 (extreme deterioration); 1 means no deterioration; 2 means very slight deterioration (withering, falling, or discoloring <5%); 3 means slight deterioration (withering, falling, or discoloring = 5%); 4 means moderate deterioration (withering, falling, or discoloring >5% but <25% of bouquet); 5 means high deterioration (withering, falling, or discoloring >25%). The flowers were unacceptable when the average score was higher than 2.

RESULTS AND CONCLUSION

In this study, the porous clay coated paper was prepared and used as active packaging for storing orchid flowers and compared with conventional packaging. The changes in qualities of orchid in both packaging conditions during storage at 12°C and humidity 85% were monitored weekly and the result is shown in Fig. 1. The results showed that the deterioration scores of control orchid flowers increased rapidly and orchid flowers became unacceptable (deterioration score >2) after one

week storage. On the other hand, orchid flowers wrapped with porous clay coated paper had deterioration scores lower than 2 for up to 4 weeks.

Increasing ethylene gas concentration inside the flower packages during shipment can cause premature senescence. In addition, ethylene-induced symptoms include flower discoloration and premature wilting and flower fall. According to Lerslerwong and Ketsa (2008), yellowing, drooping, and epinasty of florets are major observable signs of senescence of orchid flowers. This ethylene-induced deterioration and discoloration could be due to an increase of vacuole's pH or enzyme effects such as polyphenol oxidase and peroxidase on senescing flowers as reported by Almazi *et al.* (2012).

Orchid flowers in control packages showed signs of water loss and wilting. These are most likely to be attributed to the higher rate of respiration, evaporation and transpiration in the perianth. This suggests that early senescence and wilting is correlated to high level of ethylene concentration. The effects of ethylene on senescence of *Dendrobium* 'Khao Sanan' were also observed by Lerslerwong and Ketsa (2008).

When the florets start to senesce, their color became darker due to browning process. This suggests that the browning of perianth was induced by a loss of water through the sepal and petal via transpiration. These results are in good agreement with those of Ketsa and Rugkong (1999) who found that exogenous ethylene resulted in autocatalytic increase in endogenous ethylene of the cut *Dendrobium* 'Pompadour' flowers.

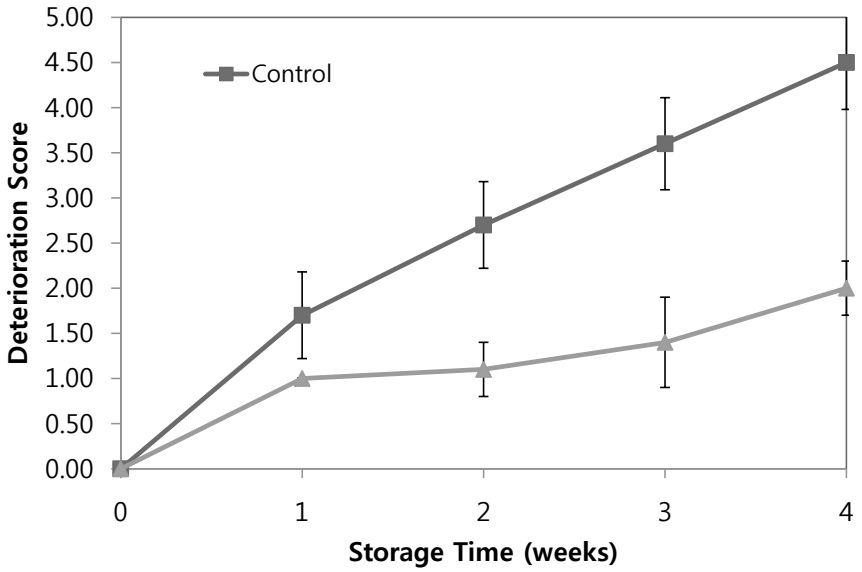

Fig. 1 - Deterioration scores (flower discoloration and premature wilting and flower fall) of orchid flowers during storage. 1 = no deterioration; 2 = very slight deterioration; 3 = slight deterioration; 4 = moderate deterioration; 5 = high deterioration.

When compared to control samples, flowers packaged in porous clay coated paper had significantly lower rate of senescence. The porous clay coated paper could slow down the senescence process of orchid flowers. This could be because the ability of porous clay coated paper could adsorb ethylene inside the flower pack-

ages. Our preliminary studies indicated that porous clay coated paper packaging could absorb ethylene which is the cause of changing in orchid flower qualities. Because of its high surface area with uniform and specific pore sizes, porous clay can be used as an ethylene scavenger.

The results suggested that porous clay coated paper was able to adsorb ethylene. The porous clay coated paper could extend the storage life of orchid cut flower up to 4 weeks, while the orchid flowers in conventional packaging had storage life of less than 2 weeks. Therefore, we can conclude that due to its ethylene adsorption efficacy, porous clay coated paper could extend storage life of orchid cut flowers better than conventional packaging. The porous clay coated paper used in this research has potential to be used as active packaging for prolonging the storage life of Thai orchid flowers.

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ANTIMICROBIAL EFFECT OF MYROBALAN (*TERMINALIA CHEBULA*) ETHANOLIC EXTRACT AGAINST FOODBORNE MICROORGANISMS

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ABSTRACT

Myrobalan (*Terminalia chebula*) is known well for against microorganisms. The antimicrobial properties of different concentrations of ethanolic extracts of myrobalan (EEM) against foodborne microorganisms were evaluated. Myrobalan was extracted by using different concentrations of ethanol containing 70 and 95% ethanol (v/v). The antimicrobial activity of EEM against 14 strains of foodborne microorganisms were determined by minimum inhibitory concentrations (MIC) using agar dilution method. The gallic acid content of EEM from different concentrations of ethanol was analyzed by high performance liquid chromatography (HPLC). Results showed MIC values of EEM against foodborne microorganisms obtained by 70 and 95% ethanol ranged from 3.0-8.0 and 3.0-7.0 mg/ml, respectively. Gram-positive and Gram-negative bacteria were more resistant to EEM than yeast except for *Vibrio parahaemolyticus*. Among Gram-positive bacteria, lactic acid bacteria were more resistant to EEM than other Gram-positive bacteria. From HPLC spectrum, gallic acid was the main active compounds presented in EEM. It can be concluded that EEM has significant antimicrobial potential against foodborne microorganisms. Thus, EEM has a potential to use as natural antimicrobials for chemical substances replacement which is beneficial to food preservative purpose.

Key words: Antimicrobial activity, ethanolic extract, foodborne microorganisms, MIC, myrobalan, *Terminalia chebula*.

INTRODUCTION

Myrobalan is a native herb in the family Combretaceae. It has been extensively used in industrials including dietary supplements, nutraceuticals and functional foods. Myrobalan contains several constituents like tannins, flavonoids, sterols, amino acids, fructose, resin and fixed oils. Gallic acid, which is hydrolysable tannin, is an important constituent of myrobalan fruit and it has been reported the antimicrobial activity against bacteria, fungi and virus (Kannan *et al.*, 2009; Ashwini *et al.*, 2011). Moreover, it is also found to have antioxidant, anticancer, anti-inflammatory and anti-diabetic activities (Ashwini *et al.*, 2011). However, there is lack of evidence of its antimicrobial activity against food microorganisms. Therefore, the objective of this study was to investigate antimicrobial properties of myrobalan, in different solvents, against foodborne microorganisms and analyze gallic acid from myrobalan extract.

MATERIALS AND METHODS

Microbial strains and inoculum preparation A total of 14 strains of foodborne microorganisms were used. They were cultured in 10 ml of broth at 37°C for 2 successive 24 h and 18 h transfers before used, except *Bacillus cereus* TISTR 687, and *Zygosaccharomyces bailii* BCC 12666 incubated at 30°C. Tryptic soy broth (TSB; Merck, Germany) was used for *B. cereus*, *Staphylococcus aureus* TISTR 1466, *Escherichia coli* TISTR 780, *Salmonella enterica* serotype Typhimurium ATCC 13311 and Weltevreden DMST 15677; TSB supplement with 0.6% yeast extract was used for *Listeria monocytogenes* strains 101, 108, 310, Scott A and V7; TSB and 3% NaCl was used for *Vibrio parahaemolyticus* BCC 24339; de Man, Rogosa and Sharpe (MRS) broth was used for *Lactobacillus plantarum* TISTR 844 and 850; and Yeast Malt (YM) broth was used for *Z. bailii*.

Preparation of ethanolic extract of myrobalan (EEM) Dried myrobalan from Nakhon Pathom province were obtained from Heaven Herb Co., Ltd. (Pathumthani, Thailand). Extraction method of myrobalan was modified from Malekzadeh *et al.* (2001). Myrobalan was mixed with ethanol (70 and 95% v/v) to obtain 10% (w/v) ethanolic extract of myrobalan (EEM). The ethanol was not evaporated.

Determination of Minimum inhibitory concentration (MICs) MICs were performed by agar dilution method as modified from Schwalbe and others (2007). Maximum concentration of EEM in the agar was 8.0 mg/ml.

Determination of gallic acid by high performance liquid chromatography (HPLC) analysis Analysis of gallic acid was carried out by HPLC using reverse-phase HiQsil C18HS culumn (4.6 × 250 mm) at 30°C. Injection volume was 20 µl. The mobile phase was acetonitrile and 0.1% trifluoroacetic acid (7:93) at 0.6 ml/min. The wavelength selected for analysis was 280 nm.

RESULTS AND DISCUSSION

Both EEM, obtained from 70 and 95% (v/v) ethanol, showed significant antimicrobial activity against all tested strains (Table 1). Their MICs ranged from

3.0-8.0 mg/ml. The MICs of EEM obtained by 95% ethanol against Gram-positive bacteria, including *B. cereus*, *L. monocytogenes* and *St. aureus* ranged from 4.0-6.0 mg/ml, of which EEM obtained by 70% ethanol ranged from 5.0-7.0 mg/ml; whereas those MICs against *Lb. plantarum* were 7.0 and 8.0 mg/ml, respectively. For Gram-negative bacteria showed MIC values of EEM obtained by 95 and 70% ethanol ranged from 3.0-5.0 mg/ml and 3.0-6.0 mg/ml, respectively; while MICs against yeast namely *Z. bailii* was 4.0 mg/ml for EEM obtained by both 95 and 70% ethanol. Comparing between concentrations of solvent, the inhibitory effect of EEM obtained by 95% ethanol was stronger than EEM obtained by 70% ethanol, these phenomena may be due to higher active substances of myrobalan dissolved in 95% ethanol. Besides, the ethanol at the concentration used as solvent did not have inhibitory effect to tested microorganisms (data not shown).

From HPLC spectrum, gallic acid was the main active compounds presented in EEM (Fig. 1 and Table 2). A correlation among antimicrobial activity and content of gallic acid found that EEM obtained by 95% ethanol presented higher content of gallic acid and stronger antimicrobial effect than those of 70% ethanol. These results supported that antimicrobial activity of EEM was due to gallic acid content. Similar to our results, it has been revealed that ethanolic extract of *T. chebula* exhibited antibacterial activity against methicillin-resistant *St. aureus*, *S.* Typhimurium SSFP 4S, *St. epidermidis* MTCC 3615, *St. aureus* ATCC 25923, *B. subtilis* MTCC 441 and *P. aeruginosa* ATCC 27853 (Sato *et al.*, 1997; Kannan, 2009). The active compounds responsible for antibacterial activity were gallic acid and its ethyl es-

Table 1 - MICs of EEM determined by agar dilution method.

Microorganism	MICs of EEM (mg/ml)	
	EEM obtained by 70% ethanol	EEM obtained by 95% ethanol
Gram-positive bacteria		
B. cereus 687	5.00	4.00
L. monocytogenes 101	5.00	5.00
L. monocytogenes 108	5.00	5.00
L. monocytogenes 310	5.00	5.00
L. monocytogenes Scott A	5.00	5.00
L. monocytogenes V7	5.00	5.00
St. aureus 1466	7.00	6.00
Lactic acid bacteria		
Lb. plantarum 844	8.00	7.00
Lb. plantarum 850	8.00	7.00
Gram-negative bacteria		
E. coli 780	6.00	5.00
S. typhimurium 13311	6.00	5.00
S. weltevreden 15677	6.00	5.00
V. parahaemolyticus 24339	3.00	3.00
Yeast		
Z. bailii 12999	4.00	4.00

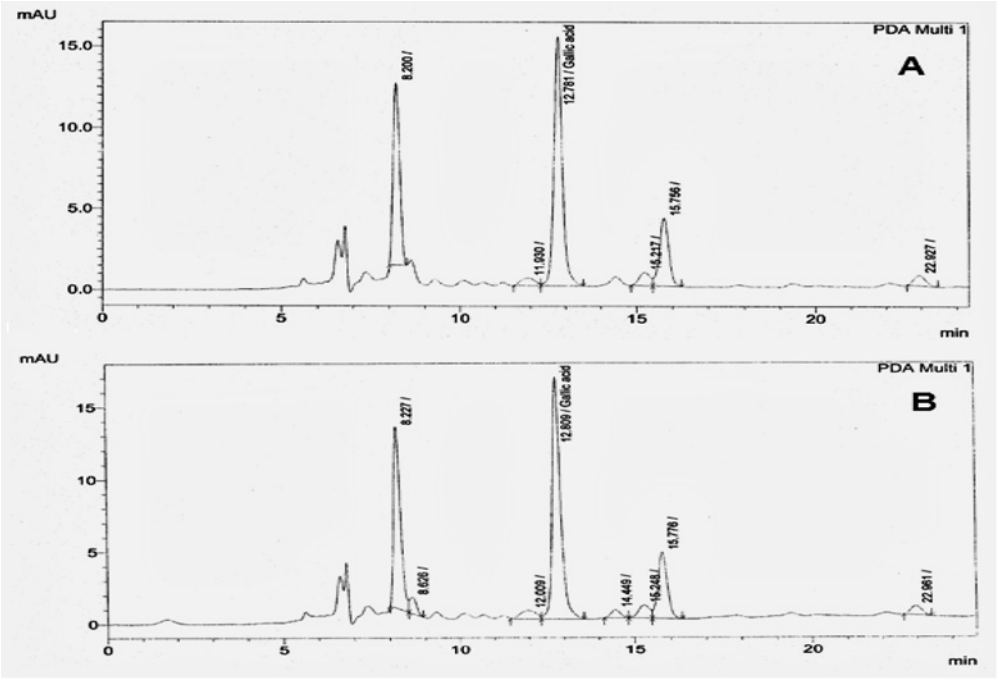

Fig. 1 - Content of gallic acid of EEM in different solvents, 70% ethanol (A) and 95% ethanol (B) analyzed by HPLC.

Table 2 - Content of gallic acid in EEM by HPLC.

Percentage of ethanol in EEM	Content of gallic acid of EEM (mg/ml)
70%	1.53
95%	1.69

ter (Sato *et al.*, 1997). Furthermore, Chung *et al.* (1998) reported that tannin may induce complexation with enzymes or substrates which consequently led to inhibit many microbial enzymes in raw culture filtrates or in purified forms, and as well as tannin toxicity may relate to its action on the membrane of the microorganisms.

CONCLUSION

Considering the results, it can be concluded that EEM had significant antimicrobial potential against foodborne microorganisms including bacteria and yeast, but the effect is species dependent. Most strains were susceptible to EEM obtained by 95% ethanol more than EEM obtained by 70% ethanol due to higher content of gallic acid, an important constituent of myrobalan. Thus, EEM has a potential to use as natural antimicrobials for chemical substances replacement which beneficial to food preservative purpose and shelf life extension.

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PRELIMINARY ANTIMICROBIAL CASING INCORPORATED WITH CHITOSAN BY VACUUM IMPREGNATION

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ABSTRACT

The objective of this research was to produce the antimicrobial casing by delivered chitosan into cellulose casing using vacuum impregnation, and then the penetration of chitosan and antimicrobial activity of the impregnated casing were investigated. Cellulose casing was impregnated with chitosan solution 1%, v/v. The impregnated cellulose casing (IMC) were dried in hot air oven. The cross sections of casings (10 μm thickness) were analyzed by Fourier transform infrared (FTIR) microspectroscopy. The antimicrobial activity of IMC was examined against *Listeria monocytogenes* Scott A. The amount of bacteria was examined using the spread plate method. According to FTIR microspectroscopy part, the spectrum of chitosan displayed primary and secondary amides. The IMC related to higher intensity of amides than the untreated casing, those amide bands including 3190-3170 cm^{-1} indicating the symmetric stretching of primary amide NH_2, the peak at 1680-1660 cm^{-1} indicating that primary amide C=O were stretching and also 1680-1640 cm^{-1} describing the stretching of secondary amide C=O. This result indicated the penetration of chitosan into cellulose casing. Associated with the part of antimicrobial activity, IMC could reduce microbial growth and decrease number of *L. monocytogenes* Scott A by 1.2 logs at 24 h, comparing to control. In conclusion, the impregnation technique can deliver chitosan into cellulose casing leading to antimicrobial casing production. However, these results were an initial step toward development of optimum impregnation condition.

Key words: Antimicrobial activity, casing, chitosan, FTIR-microspectroscopy, impregnation, *Listeria monocytogenes* Scott A.

INTRODUCTION

Chitosan is a deacetylated of chitin which found mainly in natural sources such as crustaceans, fungi and insects. It is a copolymer of *N*-acetyl-d-glucosamine and d-glucosamine in various ratios. The glucosamine moieties in chitosan carry free amine groups that are protonated in an acidic environment, the amount and the positions of the glucosamine determine the charge and the charge-distribution in the chitosan molecule (Raafat *et al.,* 2010). The positive charges of chitosan would interfere with the negatively charged residues on the microbial cell surface, causing the membrane leakage (Kong *et al.,* 2010). Thus, chitosan has potential for a wide range of food applications due to its broad spectrum antimicrobial activity (Friedman *et al.,* 2010)

Vacuum impregnation (VI) is considered as a useful technique to quickly introduce external liquids into the porous structure of animal and plant tissue due to the action of hydrodynamic mechanisms promoted by pressure change (Zhao *et al.,* 2004). The operation is carried out in two steps after product immersion in a chamber containing the liquid phase. Pressures are imposed in a solid-liquid system (vacuum step) followed by the restoration of atmospheric pressure. During the vacuum step, the internal gas in the product pores of the product is expanded and partially flows out. All of these are coupled with the capillary penetration as the function of interfacial tension of the liquid and the diameter of pores. In the atmospheric step, the residual gas is compressed, and the external liquid flows into the pores due to the function of the compression ratio (Fito *et al.,* 1994). The purpose of this study was to produce antimicrobial casing against *L. monocytogenes* Scott A by using vacuum impregnation technique as to deliver chitosan into cellulose casing and investigate penetration ability of chitosan including its antimicrobial activity.

MATERIALS AND METHODS

Preparation of IMC VI chitosan (low molecular weight, 22-218 KDa; Sigma-Aldrich, USA) solutions at a concentration of 1% (w/v) in 1% (v/v) acetic acid were prepared. The cellulose casing tubes were cut into pieces of 2 cm in length and immersed in chitosan solution 30 ml. The operation was carried out in two steps. First step, vacuum pressure (-680 mmHg) was imposed to the system for 30 minute. After that, atmospheric pressure (760 mmHg) was restored in the chamber for 5 second. Then samples were consecutively left immersed for 10 minutes at atmospheric pressure. The impregnated cellulose casing (IMC) was dried in hot air oven.

FTIR microspectroscopy analysis IMC samples were embedded into paraffin basin and then cut by microtome. The cross section of casings (10 μm thickness) were put on CaF_2 window and analyzed by FTIR microspectroscopy (Tensor27; Bruker, Germany). FTIR spectra and contour mapping were recorded in a spectral range of 4000 - 700 cm^{-1} with a resolution of 4 cm^{-1}; 128 scans.

Antilisterial activity of IMC Antilisterial activity analysis of IMC was adapted from Han *et al.* (2007). For the liquid culture test, the IMC was cut into 50x70 mm rectangles using aseptic technique. Four film rectangles were immersed in a sterilized flask containing 40 ml Müller-Hinton broth (Merck, Germany) supplemented with 0.6% yeast extract and inoculated with *L. monocytogenes* Scott A (10^5 CFU/ml), and then incubated at 37 °C with agitation 120 rpm. To obtain microbial growth profiles, the sample was taken at 0, 3, 6, 12, 18 and 24 h, spreaded on a trypticase soy agar

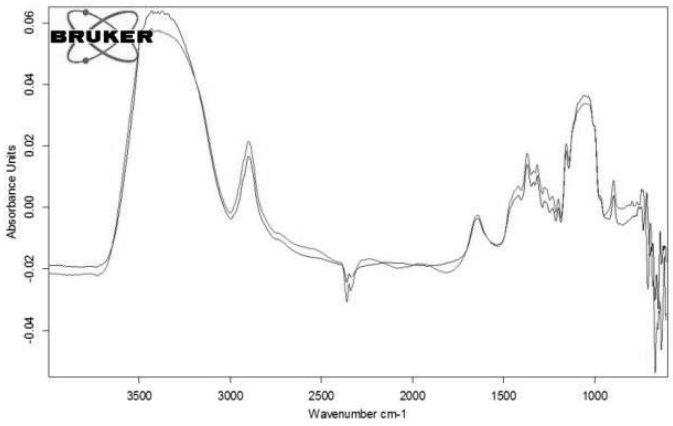


Fig. 1 - The FTIR spectra of all casings.

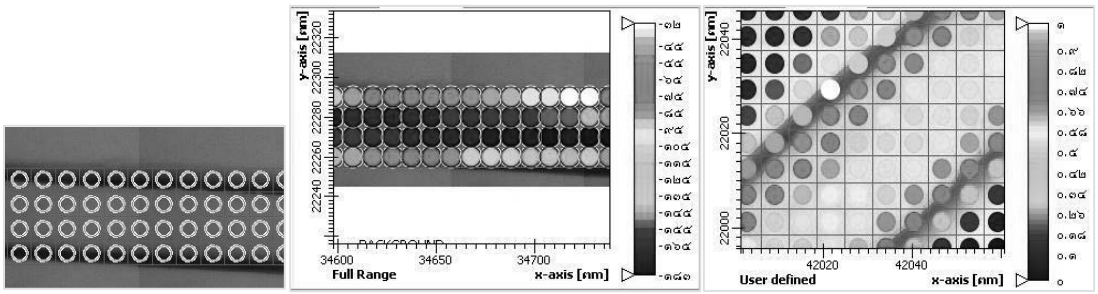
(Merck) supplemented with 0.6% yeast extract and then overlaid with modified *Listeria* selective agar (Oxoid, England). The plates were incubated at 37 °C for 24 h.

RESULTS AND DISCUSSION

Characterization of the casing The FTIR spectra of IMC and untreated casing (non-IMC) are shown in Fig. 1. Both casings showed a few difference on its spectra and in this study we focused on the intensities of primary and secondary amide which are the basic characteristics of chitosan FTIR spectrum. IMC related to higher intensity of amides than non-IMC (Table 1), those amide bands including 3190-3170 cm^{-1} indicating primary amide NH_2 symmetric stretching, 1680-1660 cm^{-1} indicating primary amide C=O stretching and 1680-1640 cm^{-1} indicating secondary amide C=O stretching. For contour mapping shown only at primary amide (1680-1660 cm^{-1}), IMC had more penetration of chitosan than non-IMC (Fig. 2). In the core region of IMC, it shows that there is a higher intensity of primary amide than non-IMC (blue and white color mean lowest and highest intensity primary amide, respectively).

Table 1 - Intensity of primary and secondary amide casing.

Casing	Intensity of primary amide		
	3190 – 3170 cm^{-1}	1680 – 1660 cm^{-1}	1680 – 1640 cm^{-1}
non-IMC	0.1275[a]	0.03550[a]	0.56025[a]
IMC	0.2500[b]	0.06625[b]	0.73650[b]
Mean value in each column are significantly different ($p < 0.05$).			



(A) (B) (C)

Fig. 2 - The contour mapping of all casings evaluated by FTIR microspectroscopy; (A) Area of casing which evaluated by FTIR microspectroscopy, Spectral intensity of a primary amide(1680-1660 cm^{-1}), (B) non-IMC and (C) IMC.

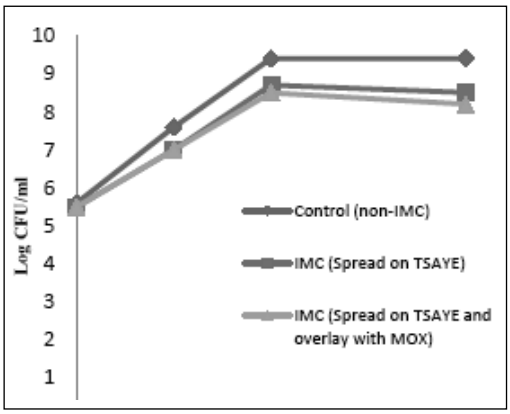


Fig. 3 - Number of L. monocytogenes Scott A in MHBYE enumerated on TSAYE and TSAYE overlay with MOX during 24.

Moreover, IMC had more distributions of primary amide than non-IMC where its distribution was only at the border region (Fig. 2B-2C). These results indicated VI technique promoted the penetration of chitosan into cellulose casing.

Antilisterial activity and relationships of VI technique Antilisterial activity of non-IMC and IMC was shown in Fig. 3. For non-IMC, the results showed it had no antimicrobial activity while IMC effectively reduced the growth of *L. monocytogenes* Scott A overtime by 0.9-1.2 log after 24 h. The antilisterial efficacy of IMC occurred from chitosan which was impregnated into cellulose casing between VI processes. When vacuum pressure (-680 mmHg, 30 min) was promoted, the expansion and outflow of internal gas in the product makes the product pore release its native liquid. At atmospheric pressure (760 mmHg, 10 min), it has greatly reduced the volume in the gas remaining in the pores (Fito *et al.*, 2001), and thus to the subsequent influx of chitosan solution into the cellulose casing.

CONCLUSION

The impregnation technique can deliver chitosan into cellulose casing which lead to antimicrobial casing production. However, these results were an initial step toward development of optimum impregnation condition. It is necessary to demonstrate the optimum condition for vacuum impregnation and investigate the maximum penetration level of chitosan to the casing, in consequence to antimicrobial effectiveness.

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ANTIMICROBIAL ACTIVITY OF CHITOSAN AND CARBOXYMETHYL CHITOSAN FROM DIFFERENT TYPES AND SOURCES OF CHITOSAN

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ABSTRACT

The aim of this study was to determine the antimicrobial activity of chitosans and carboxymethyl chitosan (CMCH) from two molecular sizes (polymer and oligomer) and three sources (shrimp, crab and squid). The CMCH were synthesized *via* carboxymethylation method with monochloroacetic acid in isopropyl alcohol under alkaline condition. The antibacterial activity of chitosan and CMCH were examined against sixteen of food borne pathogenic bacteria and two strains of lactic acid bacteria (LAB). The minimum inhibitory concentration (MIC) of chitosans and CMCH were examined by agar dilution method and the minimum bactericidal concentration (MBC) values were determined by broth dilution method. The results showed that all types of chitosans were generally more effective antibacterial than CMCH. The MIC of chitosans ranged from 0.008-0.15% and MBC ranged from 0.01-0.18%. LAB was the most sensitive strain to the chitosan groups (MIC = 0.008%, MBC =

0.01%). In contrast, all CMCH groups had no effect against foodborne pathogenic bacteria (MIC and MBC were higher than 1%) except for LAB which was inhibited by oligomeric CMCH, in which those of the MIC and MBC values were less than chitosan oligomer at 45-56.25 times and 25 times, respectively (MIC and MBC of chitosan crab oligomer = 0.008-0.01% and 0.02%, respectively). These observations suggested that the antibacterial activity of chitosan and CMCH depends on the chitosan sources, molecular size and the target microorganism. Therefore, application of chitosan and CMCH must be chosen selectively in order to control the target foodborne pathogens.

Key words: Antimicrobial activity, carboxymethylchitosan, chitosan, foodborne pathogens, oligomer, polymer.

INTRODUCTION

Chitosan, a linear polysaccharide consisting of (1,4)-linked 2-amino-deoxy-β-D-glucan, is a deacetylated derivative of chitin which is a predominantly component exoskeleton of crustacean. Chitosan has broad spectrum antimicrobial activity against bacteria, yeast and molds that leads to attract great consideration from researchers to study about its antimicrobial property (Kong *et al.,* 2010). However, the solubility of the chitosan is limited to moderately in acid aqueous solution (pH above 6.5) which is a drawback for potential application. Thus, chemical modification was used to improving solubility of chitosan in water. An important method of chemical modification is carboxymethylation (Anitha *et al.,* 2009). Carboxymethyl chitosan (CMCH) is not only soluble in water, but has unique chemical, physical and biological properties such as high viscosity, large hydrodynamic volume, low toxicity, biocompatibility and film, gel-forming capabilities (Chen *et al.,* 2003). Since, antimicrobial mechanism of chitosan has suggested that the positive charge forms of ammonia group which is attached to the phosphoryl group of phospholipids at the bacterial cell membrane leading to proliferation of the microbial cell (Hagiwara *et al.,* 1999; Sukmark *et al.,* 2011). While CMCH possesses negative charges on the carboxyl groups, it would be interesting to investigate the antimicrobial activity of CMCH. Then, the aim of this study was to prepare chemically modified CMCH from two molecular sizes (polymer and oligomer) and three sources (shrimp, crab and squid) of chitosan and compare the antimicrobial activity of these chitosan derivatives and native chitosan.

MATERIAL AND METHOD

Preparation of CMCH Chitosans (Ta Ming Enterprises Co., Ltd, Thailand) were characterized from three different sources (shrimp, crab crust and squid pen), with each source having two molecular sizes (oligomer and polymer). CMCH was prepared by following Tantala *et al.* (2012).

Preparation of test microorganisms Sixteen of bacterial strains were tested. Each strain was grown in 10 ml of suitable broth at 37°C for 2 successive 24 h and 18 h transfers before used except for *B. cereus* were incubated at 30°C.

Preparation of chitosan and CMCH solution Chitosan solutions were prepared

at a concentration 1% (w/v) in 1% (v/v) acetic acid while, CMCH solutions were prepared at a concentration 2% (w/v) in 100 ml of deionized water.

Determination of minimum inhibitory concentration (MIC) by agar dilution method A number of test tubes, each containing 5 ml of melt agar at double strength concentration, was mixed with chitosan, CMCH solution and distilled water at pH 6.3 to give a final volume 10 ml. One microliter of each bacterium culture at the cell concentration about 7 log CFU/ml weas dropped on the agar. Then, the agar was incubated at 37°C except for *B.cereus* incubated at 30°C. MIC value was determined as the lowest concentration of antimicrobial agent required to inhibit the visible growth of the bacterium after incubation for 24 h.

Determination of minimum bactericidal concentration (MBC) by broth dilution method Test tubes containing 5 ml of broth at double strength concentration was mixed with tested bacterium (4–5log CFU/ml), chitosan or CMCH solution and distilled water at pH 6.3 to give a final volume 10 ml. The test tubes were incubated. Enumerations of bacteria were examined using the spread plate technique. MBC value was determined as the lowest concentration of antimicrobial required to diminish more than 99.9% of the initial bacterial amount at 24 h.

RESULTS AND DISCUSSION

MIC and MBC values of chitosan and CMCH All types of chitosans generally showed stronger antibacterial effect than CMCH. The MIC (Table 1) and MBC (Table 2) values of chitosan and oligomeric CMCH, ranging from 0.008 % to above 1.00%,

Table 1 - Minimum inhibitory concentration (% v/v) of chitosan and oligomeric CMCH.
Microbial strains Chitosan types (%v/v)

Microbial strains	Chitosan types (%v/v)								
	Polymeric chitosan			Oligomeric chitosan			Oligomeric CMCH		
	Shrimp	Crab	Squid	Shrimp	Crab	Squid	Shrimp	Crab	Squid
Gram-negative bacteria									
Sal. weltevreden DMST 15677	0.15	0.15	0.15	0.15	0.15	0.15	>1.00	>1.00	>1.00
Sal. typhimurium ATCC 13311	0.08	0.08	0.08	0.09	0.10	0.10	>1.00	>1.00	>1.00
E .coli TISTR 780	0.08	0.07	0.06	0.06	0.07	0.07	>1.00	>1.00	>1.00
V. parahaemolyticus BCC24339	0.05	0.05	0.04	0.05	0.07	0.03	>1.00	>1.00	>1.00
V. parahaemolyticus TDH330	0.05	0.05	0.03	0.05	0.07	0.03	>1.00	>1.00	>1.00
V. parahaemolyticus TRH015	0.05	0.05	0.07	0.05	0.07	0.06	>1.00	>1.00	>1.00
Gram-positive bacteria									
B. cereus TISTR 687	0.07	0.06	0.05	0.07	0.07	0.07	>1.00	>1.00	>1.00
S. aureus TISTR 1466	0.07	0.06	0.06	0.07	0.06	0.06	>1.00	>1.00	>1.00
L. monocytogenes 101	0.06	0.05	0.06	0.06	0.06	0.05	>1.00	>1.00	>1.00
L. monocytogenes 108	0.06	0.05	0.05	0.06	0.06	0.04	>1.00	>1.00	>1.00
L. monocytogenes 310	0.06	0.05	0.06	0.06	0.06	0.06	>1.00	>1.00	>1.00
L. monocytogenes Scott A	0.06	0.05	0.07	0.06	0.06	0.06	>1.00	>1.00	>1.00
L. monocytogenes V7	0.06	0.05	0.07	0.06	0.06	0.06	>1.00	>1.00	>1.00
L. innocua DMST 9011	0.04	0.06	0.05	0.04	0.04	0.05	>1.00	>1.00	>1.00
Lac. plantarum TISTR 844	0.008	0.01	0.01	0.009	0.01	0.008	0.55	0.45	0.70
Lac. plantarum TISTR 850	0.01	0.02	0.008	0.01	0.008	0.009	0.55	0.45	0.70

Table 2 - Minimum bactericidal concentration (% v/v) of chitosan and oligomeric CMCH.

Microbial strains	Chitosan types (%v/v)								
	Polymeric chitosan			Oligomeric chitosan			Oligomeric CMCH		
	Shrimp	Crab	Squid	Shrimp	Crab	Squid	Shrimp	Crab	Squid
Gram-negative bacteria									
Sal. weltevreden DMST 15677	0.18	0.18	0.18	0.18	0.18	0.18	>1.00	>1.00	>1.00
Sal. typhimurium ATCC 13311	0.10	0.11	0.10	0.10	0.09	0.12	>1.00	>1.00	>1.00
E. coli TISTR 780	0.09	0.08	0.08	0.09	0.10	0.09	>1.00	>1.00	>1.00
V. parahaemolyticus BCC24339	0.06	0.07	0.05	0.07	0.06	0.06	>1.00	>1.00	>1.00
V. parahaemolyticus TDH330	0.06	0.05	0.06	0.05	0.04	0.06	>1.00	>1.00	>1.00
V. parahaemolyticus TRH015	0.07	0.07	0.09	0.07	0.09	0.08	>1.00	>1.00	>1.00
Gram-positive bacteria									
B. cereus TISTR 687	0.08	0.08	0.08	0.08	0.08	0.08	>1.00	>1.00	>1.00
S. aureus TISTR 1466	0.08	0.09	0.09	0.09	0.07	0.09	>1.00	>1.00	>1.00
L. monocytogenes 101	0.07	0.07	0.08	0.07	0.07	0.07	>1.00	>1.00	>1.00
L. monocytogenes 108	0.08	0.06	0.06	0.08	0.08	0.06	>1.00	>1.00	>1.00
L. monocytogenes 310	0.07	0.06	0.07	0.07	0.07	0.08	>1.00	>1.00	>1.00
L. monocytogenes Scott A	0.07	0.07	0.08	0.07	0.07	0.07	>1.00	>1.00	>1.00
L. monocytogenes V7	0.07	0.06	0.08	0.07	0.07	0.07	>1.00	>1.00	>1.00
L. innocua DMST 9011	0.05	0.07	0.06	0.05	0.05	0.08	>1.00	>1.00	>1.00
*Lac. plantarum T*ISTR 844	0.02	0.02	0.02	0.02	0.02	0.01	0.60	0.50	0.80
Lac. plantarum TISTR 850	0.03	0.03	0.02	0.04	0.02	0.02	0.65	0.50	0.80

slightly differed with the tested bacteria and types of chitosan. Between groups of the bacterial tested, *Lac. plantarum* was the most sensitive strain to the chitosan groups (MIC = 0.008%, MBC = 0.01%) while *Sal.* Weltevreden was the most resistant strain to all groups of chitosan (MIC = 0.15%, MBC = 0.18%). Chitosan showed the stronger antibacterial effects against Gram positive bacteria than for Gram negative bacteria, as observed by Jeon *et al.* (2001), No *et al.* (2002) and Sukmark *et al.* (2011). This phenomenon was due to the complex structure of Gram negative bacteria cell wall, leading to a difficulty for foreign molecules to come into the cell (Xie *et al.*, 2002). However, the mechanism of chitosan and chitosan derivatives as an antimicrobial compound is not well understood. All oligomeric CMCH groups had no effect against foodborne bacteria (MIC and MBC were higher than 1%) except for *Lac. plantarum*. Among the CMCH groups, CMCH oligomer from crab showed the best antilactobacillus activity (MIC = 0.45%, MBC = 0.50%), but their MIC and MBC values were higher than chitosan oligomer by 45-56.25 times and 25 times, respectively (MIC and MBC of chitosan crab oligomer = 0.008-0.01% and 0.02%, respectively).

According to Qin *et al.* (2006) reported that the water soluble chitosan had no antimicrobial activity unlike its parent chitosan which is dissolved in acidic solution. It would be expected that when chitosan was dissolved in acid, it forms an ammonia group which is attached to the phosphoryl group of phospholipids at the bacterial cell membrane. In addition, it causes a hole in the cell membrane leading to cytoplasmic leakage and causing cell death (Raafat *et al.*, 2008; Sukmark *et al.*, 2011). While CMCH possesses negative charges on the carboxyl groups, the adsorp-

tion and binding of ionic group of bacterial cell wall are not effective to explain its poor antibacterial activity (Xie *et al.,* 2002).

CONCLUSION

This study has demonstrated that the water soluble chitosans which synthesized *via* carboxymethylation method showed less antibacterial activity than their parent chitosans. The MIC and MBC of chitosan and CMCH depend on the chitosan sources, molecular size, characteristic and the target microorganism. Therefore, application of chitosan and CMCH must be chosen selectively in order to control the target foodborne pathogens. Furthermore, the MIC and MBC values of the chitosan and CMCH investigated can be applied in the food for various purposes such as a natural food preservative to extend the shelf life of food.

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PHOTOCATALYTIC ACTIVITY OF PLA/TIO_2 NANOCOMPOSITES AND TIO_2-ACTIVE MULTILAYERED HYBRID COATINGS

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ABSTRACT

Poly(lactic acid) (PLA) has attracted increasing interests due to its biodegradability, compostable property, good mechanical properties and processability. The incorporation of nanometer sized inorganic particles, such as nano-titanium dioxide (TiO_2), within PLA matrix or hybrid coating prepared by sol-gel approach represents a valuable solution to confer new functionalities such as photocatalytic activity. TiO_2-based nanocomposites can have potential applications in the food packaging industry due to their antimicrobial activity. In this study, films of PLA functionalized with TiO_2 nanoparticles, prepared both by direct melt processing and by depositing the active hybrid coating, were characterized and their photocatalytic activity was investigated. It has been proven that the functionalization of TiO_2 particles with 1H,1H,2H,2H-perfluorooctyltriethoxysilane enhances the photocatalytic activity of deposited multilayered coating. In particular, after 10 h of UV-irradiation, only the nanocomposites loaded with 10 wt% of TiO_2 particles was able to reach a decolorization degree of 80%, whereas for multilayered hybrid coatings the same decolorization degree was obtained for a TiO_2 percentage content of approximately 0.002 wt%. This result can be ascribed to a better dispersion and to a higher TiO_2 concentration on the surface of the film.

Key words: Photocatalytic activity, TiO_2, PLA, coating.

INTRODUCTION

Poly(lactic acid) (PLA), which is made from renewable agricultural sources, has attracted an increasing interest because of its biodegradability, biocompatibility, compostable property, nontoxicity and processability. It has been considered as a promising alternative to petrochemical- based polymers for packaging and biomedical application. However, the low deformation at break, higher cost, poor flexibility, impact resistance, and gas barrier properties of PLA limit its practical applications. The incorporation of nanometer-sized inorganic particles (e.g., layered silicate, carbon nanotubes, nanotitanium dioxide etc.) within PLA matrix as well as the deposition of functional hybrid coating prepared by sol-gel approach represents a valuable solution to improve various practical properties and to confer new functionalities such as photocatalytic activity (Luo *et al.*, 2009). TiO_2-based nanocomposites can have potential applications in the food packaging industry due to their antimicrobial activity. In this study, PLA/TiO_2 composite films were prepared by direct melt processing and their photocatalytic activity was investigated. A big challenge in the development of TiO_2-based materials is the need to enhance their photocatalytic activity, which is strictly related to the exposed surface of titania particles at the coating/air interface. A possible approach to reach this goal is the surface fluorination of TiO_2 particles; in the literature, in fact, it has already been reported the tendency of fluorine containing groups to migrate onto the film/air interface (Schmidt *et al.*, 2006). For this reason a multilayered coating was produced by depositing on PLA substrate a first protective coating consisting of crosslinked chitosan-silica (CS-3-Glycidoxypropyltriethoxysilane (GOTMS)) hybrid obtained by sol-gel method and an upper layer containing functionalized and unfunctionalized TiO_2 nanoparticles dispersed in the same hybrid matrix. The photocatalitic activity of the obtained active coatings was investigated and compared with that of melt-processed TiO_2 nanocomposites.

MATERIALS AND METHODS

Preparation of PLA/TiO_2 nanocomposites obtained by melt processing

PLA/TiO_2 composite films were prepared by direct melt processing following the procedure reported by Zhu *et al.* (2011). TiO_2 (Degussa P-25, powder form) and NatureWorks™ Poly(L-lactide) polymer 2002D PLA (pellet form) were first mixed at 180°C and at 60 rpm for 5 min in an internal mixer (Rheomix1 600 Haake, Germany) with a volumetric capacity of 50 cm^3, and then the following procedure was used to obtain nanocomposite films containing 2, 5 and 10 wt% of TiO_2 particles. After mixing and homogenization, slabs with thickness of 1 mm were prepared by using a Collin P300P press at a temperature of 180°C and at a pressure of 50 bar for 3 min, and subsequently cooled to 30°C under pressure. The slabs were then granulated by a pelletizer to obtain a material suitable for extrusion. Nanocomposite films with thickness of 100–200 micron were produced using a co-rotating twin-screw extruder PRISM EUROLAB 16 (Thermo Electron Corporation, UK) equipped with a sheet die of width 10 cm. The blends were fed with a single-screw volumetric feeder. The obtained films were thermoformed using a Collin P300P press at 140°C and 150 bar for 3 min, and cooled down for 7 min at 10°C/min and 10 bar.

Preparation of TiO_2-based active multilayered coatings

Active multilayered coatings were produced by following the procedure reported by Zhu *et al.* (2012) as briefly reported.

A certain amount of P90 TiO_2 particles were dispersed in anhydrous toluene under ultrasonic vibrations (MISONIX ultrasonic liquid processors, USA.) at room temperature for 10 min. 30 wt% of 1H,1H,2H,2H-perfluorooctyltriethoxysilane (FTS) coupling agent was added. Subsequently, the TiO_2 slurry was filtered, dried and then purified and the surface fluorinated TiO2 particles (named F–P90) were finally obtained by removing the solvent under vacuum in an oven at 50 °C overnight.

Chitosan was dissolved in an acetic acid aqueous solution (1% v/v) to form a chitosan solution of 2 wt%. The prehydrolysis solution of GOTMS was prepared by mixing 3.198 g of H_2O, 3.1074 g of ethanol and 7 g of GOTMS. The mixture was stirred and then cooled to room temperature. Subsequently, colloidal dispersion was mixed with the chitosan. The hybrid sols were diluted by adding ethanol (30 wt% on the total weight of chitosan and GOTMS mixture) and then applied onto circular PLA membranes using a spin coater (CEE Cost Effective Equipment 100, USA, 2000 rpm, 25 r s–1, 90 s). The coated PLA membranes were dried first at room temperature for 2 h and then at 50 °C for 24 h. A certain amount of photocatalysts (P90 and F–P90) was dispersed in 2-butanon under ultrasonic treatment in an ice bath. Subsequently, known amount of the photocatalysts dispersion was added in the sols of tetraethoxysilane (TEOS) and GOTMS, precursors of the siloxane matrix and then subject to ultrasonic irradiation in an ice bath. The application of the photocatalytic coating on the intermediate layer made of chitosan and GOTMS deposited onto PLA (named CS-GOTMS/PLA) was carried out by spin coating. The final samples were coded as P90/CS-GOTMS/PLA and F–P90/CS-GOTMS/PLA, respectively, for films containing unfluorinated and surface-fluorinated TiO_2 particles.

Determination of photocatalytic activity

An aqueous solution of methyl orange (MeO) was used as a model contaminant for studying photocatalytic activities of the multilayer coatings deposited on the PLA films. Photocatalysis experiments were carried out by using self-regulating UV irradiation instrument (Zhu *et al.*, 2011). A quartz pan containing both 10 mL of a 5 ppm MeO solution and the film (5 cm diameter) was placed under the UV lamps. The intensity of the radiation reaching the solution surface was 3.4 mW cm^{-2} and was detected by a GOLDILUX GPR-1 radiometer equipped with a UV-A probe. The change in the concentration of MeO was monitored by measuring the absorbance at λ_{max} = 464 nm with a SCINCO S-3100 UV–vis spectrophotometer. The extent of MeO degradation or decolorization degree was calculated by using the following equation:

$$\eta = (A_0 - A_t)x100/A_0$$

where A_t is the absorbance of the MeO solution at reaction time t, and A_0 is the absorbance of the initial 5 ppm MeO solution.

Results and Conclusion

The photocatalytic activity of the nanocomposites obtained by melt processing depends on the content of TiO_2 and on the adopted procedure. In particular, the higher activity was exhibited by the nanocomposites containing 10 wt% of TiO_2 obtained by melt extrusion and thermoforming. Fig. 1 shows that the decolorization ratios of MeO in contact with the investigated nanocomposite at UV-irradiation time equal to 5 and 10 hr are 37 and 80%, respectively. This is mainly due to presence of a higher amount of nanoparticles and to their distribution within the film. As

discussed by Zhu *et al.* (2012), when most TiO_2 particles distribute in the central position but not on the surface of the film, the photo-generated reactive radicals are difficult to diffuse to the bulk solution and react with MeO within their lifetime, which results in low activity of the films.

For this reason and in order to improve the effectiveness of the photocatalytic activity, the concept of a multilayered coating was proposed. Furthermore, to enhance the concentration of TiO_2 particles on the surface, the particles were functionalized with 1H,1H,2H,2H-perfluorooctyltriethoxysilane. The material was produced by depositing on PLA substrate a first protective coating consisting of crosslinked chitosan-silica (CS/GOTMS) hybrid obtained by sol-gel method and an upper layer containing functionalized and unfunctionalized TiO_2 nanoparticles dispersed in the same hybrid matrix. In Fig.1 the decolorization ratio of MeO in contact with the obtained active multilayered coating after 5 and 10 h of UV-irradiation is also reported. Results show that the decolorization degrees of MeO for P90/CS-GOTMS/PLA and F–P90/CS-GOTMS/PLA films were 58 and 80%, respectively. This indicates that the hybrid multilayer coatings are active and able to degrade MeO under UV irradiation. Moreover, F–P90/CS-GOTMS/PLA films exhibit a photoactivity higher than P90/CS-GOTMS/PLA films due to the better surface distribution of TiO_2 particles determined by the fluorination of TiO_2 surface (Fig. 2). During the drying of the wet films, the F–P90 particles move to the interface between the coating and air and gather homogeneously at the surface of the multilayer coating. Thus, the photo-generated reactive radicals are easy to come in contact and react with MeO within their lifetime resulting in the higher activity of the films.

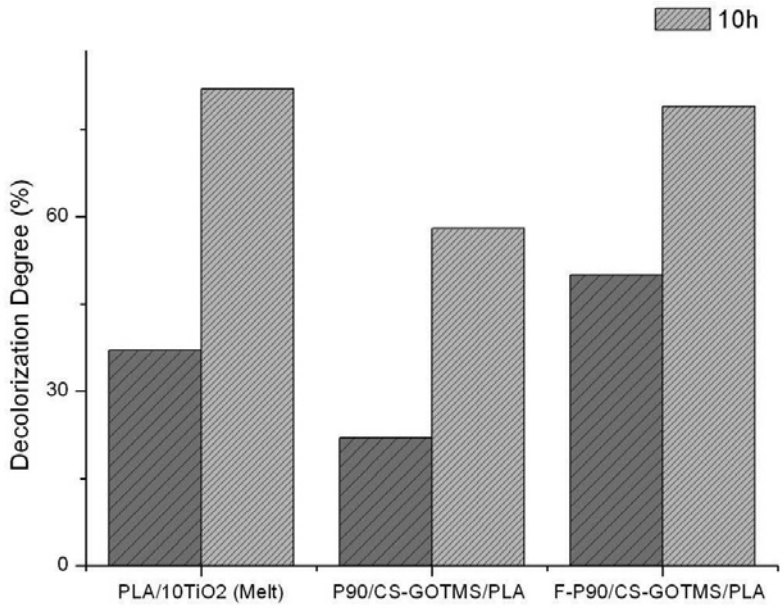

Fig. 1 - Decolorization degree of MeO after 5 and 10 hours of UV irradiation in photocatalytic experiments performed using PLA/(10%)TiO_2 composite films and multilayer coated PLA containing neat (P90/CS-GOTMS/PLA) and functionalized TiO_2 particles (F–P90/CS-GOTMS/PLA).

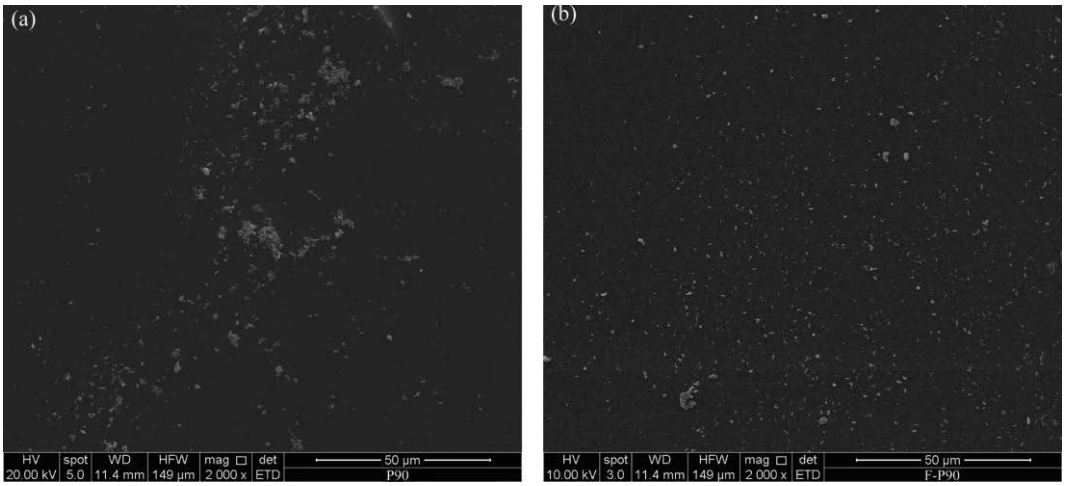

Fig. 2 - SEM micrographs for the surface (a) P90/CS-GOTMS/PLA and (b) F-P90/CS-GOTMS/PLA membranes.

In conclusion, it can be highlighted that among the melt processed films only that one loaded with 10 wt% of TiO_2 particles is able to reach a decolorization degree of 80%, whereas the same decolorization degree has been easily achieved by the developed multilayer photoactive films containing a very low TiO_2 percentage content such as, approximately, 0.002 wt%. This result confirms the validity of the proposed approach.

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ANTIBACTERIAL PROPERTY OF GINSENG EXTRACT/ALGINATE FILMS

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ABSTRACT

In this study, the extruded ginseng was processed in a co-rotating intermeshing twin screw extruder with feed moisture of 20% and barrel temperature of 115 and 130°C. The antibacterial properties of ginseng extract/alginate blend films on three selected gram-positive foodborne bacteria (*Staphylococcus epidermidis*, *Bacillus subtilis* and *Listeria monocytogenes*) have been determined via optical density (OD) method. It can be seen that ginseng extract/alginate blend films exhibited significant antibacterial activities compared to the pure alginate film. The alginate film blended with extruded ginseng extract at 115°C extrusion temperature (EX-115) had strong inhibition against *Bacillus subtilis* and *Listeria monocytogenes*. In *Staphylococcus epidermidis*, the OD was highly decreased with alginate film blended with white ginseng extract (WG). The types of ginseng caused a small change in thermal behavior of the films when tested using thermogravimetric analysis (TGA). The TGA curves of ginseng extract/alginate blend films indicate one reaction stage, while the pure alginate film degrades in two steps. Furthermore, the quality of extruded ginseng was also confirmed by HPLC analysis of individual ginsenosides of the ginseng extract samples. The lowest yields of total and individual ginsenosides were gained with WG. Increasing barrel temperature from 115 to 130°C resulted in a significant decrease in the quantities of ginsenoside in extrudate samples.

Key words: Alginate film, extruded ginseng extract, ginsenosides, gram-positive foodborne bacteria, thermogravimetric analysis.

INTRODUCTION

Active constituents in ginseng (ginsenosides, acidic polysaccharides polyacetylenic alcohol and flavonoid) have found to show antibacterial, antioxidant and anticancer properties (Shibata 2001; Bae *et al.*, 2002; Benny and Vanitha, 2004). Red

ginseng has demonstrated more effective on biological properties than white ginseng (Do *et al.*, 1993; Kim *et al.*, 2000). Recently, ginseng through extrusion process has become an attractive food additive due to its high crude saponin, ginsenoside and phenolic contents (Ha *et al.*, 2005; Ryu, 2006). The thermal and chemical reactions that occur during extrusion change the rheological properties of the raw food material as well as functional properties of extruded food products. Moreover, the production of biodegradable and edible film from carbohydrate adds value to low cost raw material and can play an important role in food preservative (Ave'rous *et al.*, 2001), which alginate could be considered as a promising candidate. Thus, the aim of this research was to fabricate extruded ginseng blended with alginate film and to characterize its thermal, functional and anti-gram positive foodborne bacteria properties.

MATERIALS AND METHODS

Extrusion cooking

Extrusion experiments on ginseng powder were carried out in co-rotating intermeshing twin screw extruder. A die with three holes, each hole being 3 mm in diameter, was used to produce extrudates. The screw speed and feed rate were kept constant at 250 rpm and 140 g/min, respectively. Moisture content of sample was adjusted to 20% before process. The barrel temperature was maintained at 115 and 130°C. The extrudates were dried in air oven at 50°C for 8 h and then packed into sealed plastic bag.

Film preparation

Sodium alginate (2 g) and calcium chloride (0.01 g) were dispersed into 100 ml of distilled water. The solution was heated at 80°C under high speed stirring. The 60% ethanolic ginseng extracts were incorporated into the alginate film solution at final concentrations of 0.5 g/ml of filming solution. The cast solution was then allowed to dry in air at 40°C for 24 h. After drying, 45 ml of 2% $CaCl_2$ solution was poured onto the dried alginate filmfor 30 s and re-dried again in an oven at 40°C for 4-6 h. The films were peeled off and kept in a desiccators for 2 days.

Antibacterial assessment of ginseng/alginate films

A 0.1 g of dry film was added into 10 ml of Mueller Hinton broth. Then, the bacteria (0.1 ml) were transferred into a sample tube and incubated at 37°C for 24 h. During the incubation, the turbidity of the medium was measured at 600 nm every 4 h. All of operations were done in aseptic technique.

Thermogravimetric analysis (TGA)

Thermogravimetric measurement was carried out on a TGA-DSC instrument (Model SDT Q600, USA). TGA scans were done from 5 to 350°C at the heating rate of 10 °C/min under an inert nitrogen atmosphere.

High performance liquid chromatographic analysis of individual ginsenosides

The individual components of saponins ginsenosides were quantitatively determined by HPLC on a C18 column with a mobile phase of acetonitrile/water (80/20, v/v), flow rate of 1 ml/min, UV detector at wavelengths 203 nm and injection vol-

ume of 20 µl. The content of ginsenosides were calculated by peak height or peak area measurements using a calibration curve generated by injection of six major ginsenoside standards, Rg_1, Re, Rb_1, Rc, Rb_2 and Rd.

RESULTS AND DISCUSSION

Fig. 1 demonstrated the curve of optical density (OD) versus culture time for the ginseng extract/alginate blend films against three selected gram-positive foodborne bacteria. For *Bacillus subtilis*, OD of extract/alginate blend films was much lower when compare to pure alginate films. After 12 h, the growth rate of *Listeria monocytogenes* incubated with all ginseng extract/alginate blend films was decreased, which indicating mode of action of ginseng extract may be induction cell lysis as for *Listeria monocytogenes*. The weight loss at around 150-245°C was a decomposition of the ginseng extract/alginate blend films as shown in Fig. 2. The alginate film blended with WG gave lower wt% loss than that with EX-115 and extruded ginseng extract at 130°C extrusion temperature (EX-130). Table 1 was a summary of the HPLC results of the six major ginsenosides. Contents of ginsenoside-Rg_1, Re, Rb_1, Rc, Rb_2 and Rd were increased in heat treatment by extrusion process. The ginsenosides-Rg_1 from EX-130 was decreased markedly at about 1.5 times compared to EX-115.

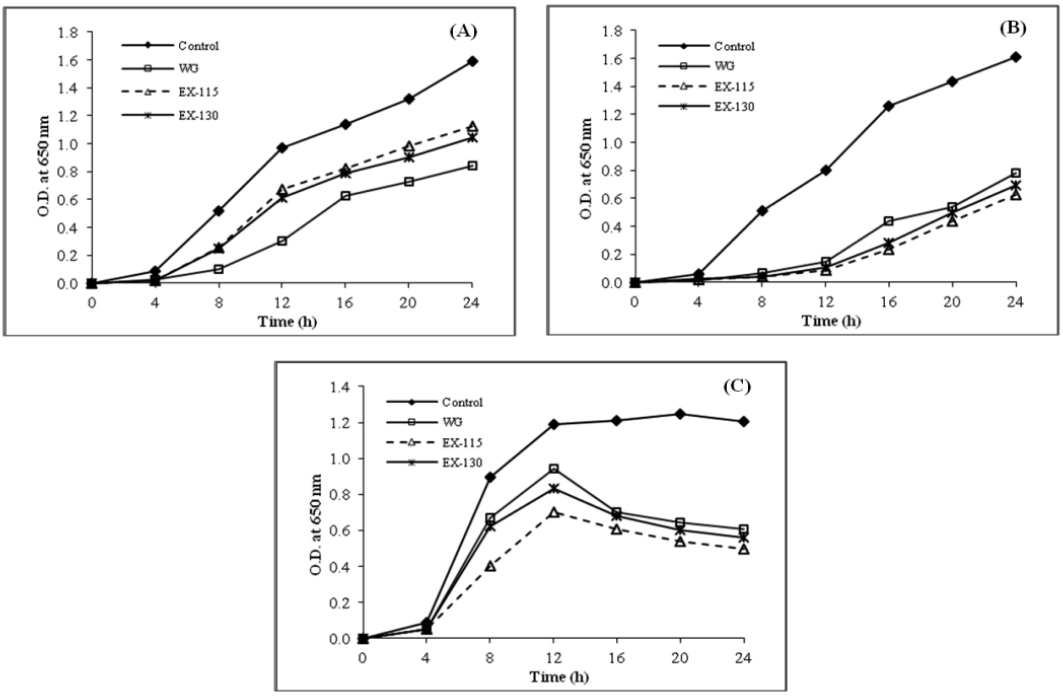

Fig. 1 - The changes of anti-gram positive foodborne bacteria activities of ginseng extract/alginate blend films. (A): *Staphylococcus epidermidis*, (B): *Bacillus subtilis*, (C): *Listeria monocytogenes*.

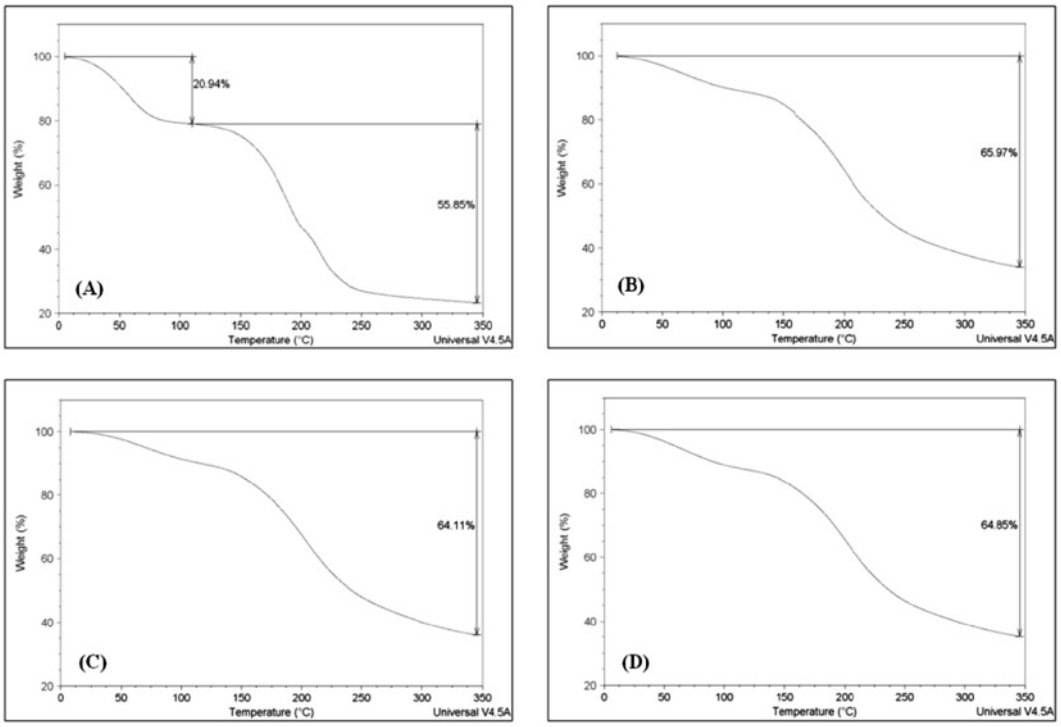

Fig. 2 - The TGA curves of the pure and ginseng extract/alginate blend films. (A): pure alginate film, (B): alginate film blended with white ginseng extract, (C): alginate film blended with extruded ginseng extract at 115°C extrusion temperature, (D): alginate film blended with extruded ginseng extract at 130°C extrusion temperature.

Table 1 - Contents of six ginsenosides in white and extruded ginseng extracts.

	Ginsenoside composition (mg/g extract)						
Samples	Rg_1	Re	Rb_1	Rc	Rb_2	Rd	Total
WG	1.5	0.9	0.7	1.3	0.5	0.2	5.1
Ex-115	6.1	3.5	3.3	3.8	2.4	2.0	21.1
Ex-130	4.2	2.5	2.6	2.9	1.8	1.5	15.5

These results suggest that incorporating ginseng extracts into edible films can be used to control food pathogens and improve shelf life in food system. The antibacterial activity of alginate film enhanced by blending with ginseng extracts. The alginate film blended with ginseng extract shows better thermal stability than that of pure alginate film.

ACKNOWLEDGEMENTS

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MECHANICAL AND WATER BARRIER PROPERTIES OF MULTICOMPONENT (AGAR/CARRAGEENAN/KONJAC) HYDROGEL FILMS

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ABSTRACT

Multicomponent hydrogel films composed of agar, κ-carrageenan, konjac powder, and nanoclays (Cloisite Na^+, Cloisite 30B) were prepared and the effect of clays on the mechanical and water barrier properties such as water vapor permeability (WVP), water contact angle (CA), water solubility (WS), swelling ratio (SR), water vapor uptake ratio (WVUR) were determined. The tensile strength (TS) of the nanocomposite films (51.9±2.2 and 48.0±1.7 MPa for Cloisite Na^+ and Cloisite 30B nanocomposite films, respectively) was comparable with the control agar/κ-carrageenan/konjac films (49.2±0.7 MPa). The WVP and WS of both nanocomposite films decreased significantly ($p<0.05$) without sacrificing the swelling ratio. The multicomponent hydrogel films can hold water more than 43 fold of its weight and they have almost six times higher water vapor adsorption capacity than silica gel.

Key words: Agar, carrageenan, konjac, multicomponent film, hydrogel, water adsorption.

INTRODUCTION

Biopolymers produced from various natural resources, such as starch, cellulose, and proteins, have been considered as attractive alternatives for non-biodegradable petroleum-based plastics since they are abundant, renewable, inexpensive, environmentally friendly, and biodegradable. However, there are some limitations to the commercial use of biopolymer films due to their poor mechanical properties and high sensitivity to moisture. Various efforts have been made to overcome these problems and to improve the property of biopolymer-

based films through not only physical, chemical, or enzymatic treatments, but also blending with hydrophobic additives or other biopolymers. Recently, nanocomposite technology, compositing biopolymer with layered silicate clay materials such as montmorillonite, has been tested to improve film properties (Mittal, 2011; Rhim and Ng, 2007).

The main objective of this study was to prepare blending films with biopolymers, such as agar, κ-carrageenan, and konjac powder, and nanoclays (Cloisite Na^+, Cloisite 30B) through the solvent casting method to improve mechanical and water resistant properties of the blend film.

MATERIALS AND METHODS

Materials Food grade agar and κ–carrageenan, and konjac powder were obtained from Fine Agar Agar Co., Ltd. (Damyang, Jeonnam, Korea), Hankook Carragen (Whasoon, Jeonnam, Korea), and Miryang Agaragar Co., Ltd. (Milyang, Kyungnam, Korea). Cloisite Na^+, Cloisite 30B were purchased from Southern Clay (Gonzales, TX, USA).

Preparation of films Two grams of each of agar, carrageenan, and konjac powder were dissolved in a constantly stirred mixture of distilled water (200 mL) and glycerol (3.0 g) with heating above 90°C for 20 min. The dissolved film solution was cast onto a leveled glass plate coated with Teflon layer (Cole-Parmer Instrument Co., Chicago, IL, USA) and dried for about 24 h at room temperature, and then peeled from the casting surface.

Agar/κ-carrageenan/konjac/clay (Cloisite Na^+ and Cloisite 30B) nanocomposite films were prepared by using the solution casting method according to the method of Rhim *et al.* (2011).

X-ray diffraction (XRD) pattern The XRD patterns were taken using a PANalytical Xpert pro MRD diffractometer (Amsterdam, Netherland), operated at 40kV and 30mA, equipped with Cu Kα radiation at a wavelength 0f 0.1546 nm and a curved graphite crystal monochromator (Rhim *et al.*, 2011).

Tensile property Tensile strength (TS) and elongation at break (E) of the films preconditioned at a humidity chamber (25°C, 50% RH) for at least 48 h was determined using a Instron Universal Testing Machine following an ASTM D 882-88.

Water vapor permeability (WVP) WVP ($g \times m/m^2 \times s \times Pa$) of blend and nanocomposite films was determined by the WVP cup method (Gennadios *et al.*, 1994).

Water contact angle (CA) The CA of water in air on the film surface was measured using a CA analyzer (model Phenix 150, Surface Electro Optics Co., Ltd., Kunpo, Korea) after a water drop of ca. 10 μL was placed on the surface of film using a microsyringe (Rhim *et al.*, 2006).

Water resistance Water solubility (WS) of films was determined as the percentage of soluble matter to initial dry matter of film sample, swelling ratio (SR) of films was determined gravimetrically after immersion into distilled water, and water vapor uptake ratio (WVUR) of the films was determined as weight increase after adsorption of water vapor of films placed in a humidity controlled glass bottle at 25°C, 94% RH for 24 h (Rhim *et al.*, 2011).

Statistical analysis Statistics on a completely randomized design were determined using the General Linear Models (GLM) procedure in the SAS program. The significance of each mean property value was determined ($p<0.05$) with the Duncan's multiple range test.

RESULTS AND DISCUSSION

XRD pattern The XRD result (Fig. 1) exhibits that the diffraction peaks of pristine clays (Cloisite Na^+ and Cloisite 30B) were shifted to the lower angle from 8.53 and 4.87° to 2.45 and 1.99° for Cloisite Na^+ and Cloisite 30B, respectively. This indicates both clays were intercalated from 1.45 and 1.81 nm to 2.45 and 1.99 nm, respectively, after the formation of nanocomposite with the biopolymer blend.

Tensile properties Table 1 shows the results of TS and E for the biopolymer blend and nanocomposite films. The TS of both Cloisite Na^+ and Cloisite 30B incorporated Nanocomposite films were comparable to the control agar/κ-carrageenan/konjac blend films, however, the E of nanocomposite films increased significantly (p<0.05).

Water vapor permeability (WVP) and water contact angle (CA) The WVP and the CA values of the films are also shown in Table 1. The WVP of both clay nanocomposite films decreased significantly (p<0.05) compared with the control film, which was probably attributed to the tortuous pathway for the diffusion of water vapor developed in the nanocomposite biopolymer films. The CA, which is one of the ba-

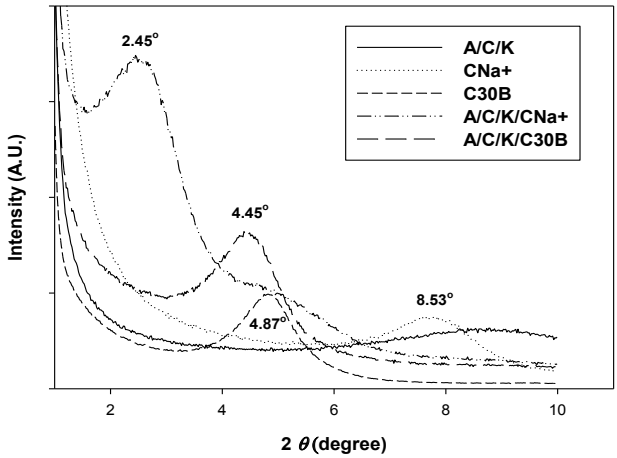

Fig. 1 - XRD patterns of two different type of nanoclays and agar/κ-carrageenan/konjac blend film and their nanocomposite films. (A/C/K: agar/carrageenan/konjac blend film; CNa^+: Cloisite Na^+; C30B: Cloisite 30B; A/C/K/CNa^+, A/C/K/C30B: biopolymer blend and clay nanocomposite films).

Table 1 - Tensile strength (TS), elongation at break (E), water vapor permeability (WVP), and water contact angle (CA) of agar/κ-carrageenan/konjac blend films[1)].

Film[2)]	Thickness (μm)	TS (MPa)	E (%)	WVP ($\times10^{-9}$g.m/m^2.Pa.s)	CA (deg.)
ACK	55.4±1.1^a	49.2±0.7^a	10.8±0.4^a	1.79±0.21^b	48.4±0.9^a
ACK/CNa^+	57.8±1.4^a	51.9±2.2^b	12.9±1.0^{ab}	1.59±0.14^a	56.3±0.6b
ACK/C30B	57.6±1.4^a	48.0±1.6^a	20.6±1.8^c	1.63±0.40^a	59.5±0.8c

[1)]Each value is the mean of three replicates with the standard deviation. Any two means in the same column followed by the same letter are not significantly (p>0.05) different by Duncan's multiple range test. [2)]ACK: agar/carrageenan/konjac blend film; CNa^+: Cloisite Na^+; C30B: Cloisite 30B.

sic wetting properties of packaging materials indicating hydrophilic/hydrophobic properties of the film surface, of the nanocomposite films increased.

Water solubility (WS) and swelling ratio (SR), water vapor uptake ratio (WVUR) The WS, a measure of resistance of film against water, of the control film was 30.9±1.0% (Table 2), and it decreased significantly (p<0.05) after blended with nanoclays. The SR, a measure of water holding capacity of film, of the control film was 4348.8±25.3%, and it was not affected appreciably when incorporated with both of the clays. The WVUR showed the similar trend as the SR.

Table 2 - Water solubility (WS), swelling ratio (SR), and water vapor uptake ratio (WVUR at 25°C for 24 h) of agar/κ-carrageenan/konjac blend films[1)] .

Film	MC (%, w.b.)	WS (%)	SR (%)	WVUR (%, 24 h)
ACK	18.3±0.7[a]	30.9±1.0[b]	4348.8±25.3[a]	156.7±1.1[c]
ACK/CNa$^+$	21.8±0.3[b]	29.5±3.2[a]	4345.3±28.9[a]	150.7±0.7[a]
ACK/C30B	21.8±0.7[b]	29.3±1.3[a]	4516.3±12.0[b]	152.4±0.9[b]

[1)]The same as in Table 1.

Water vapor adsorption capacity Water vapor adsorption capacity of agar/carrageenan/konjac blend film was tested at different temperatures and RH (5, 25, and 40°C, and 96, 94, and 89% RH, respectively) with comparing with silica gel, a representative water vapor adsorbent (Fig. 2). As expected, water vapor adsorption increased with increase in temperature in both the biopolymer blend film and silica gel. The water vapor adsorption capacity of the biopolymer blend films was 6-10 times greater than that of the silica gel. This result indicates that the biopolymer blend film can be properly used as a biodegradable water vapor adsorbent instead of silica gel.

Conclusion The agar/κ-carrageenan/konjac blend film exhibited high water holding and water vapor adsorption capacity. This property of the biopolymer blend films has high potential for the application in the packaging of highly moisture foods or foods contacted with high RH condition to keep the food quality and to extend the shelf-life of the foods.

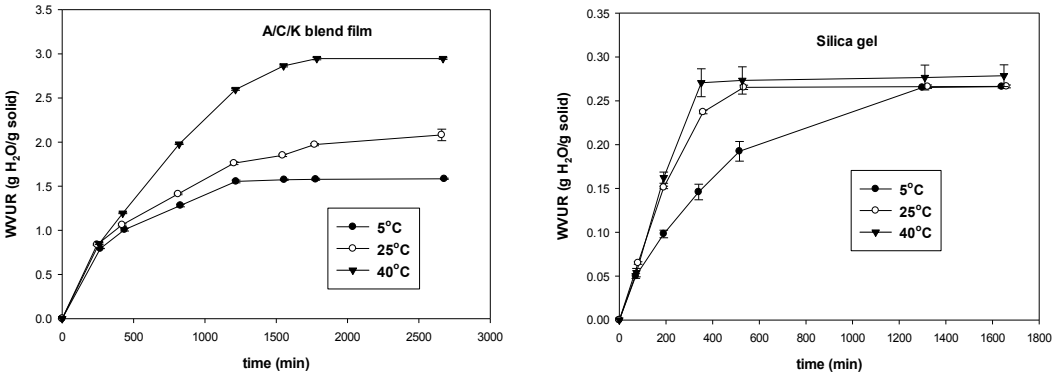

Fig. 2 - Change in water vapor uptake ratio of biopolymer blend film and silica gel at different temperatures.

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RADIATION PRESERVATION OF PORK PATTIES WITH ADDITIVE *UNDARIA* POLYSACCHARIDES

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ABSTRACT

This study was conducted to examine the effectiveness of adding polysaccharides extracted from *Undaria* to pork patties in enhancing preservation by irradiation. The combination of gamma irradiation and the addition of *Undaria* polysaccharides was shown to be more effective for microbiological control than gamma irradiation alone. Pork patties to which *Undaria* polysaccharides were added showed lower lipid oxidation, and inhibition against lipid oxidation caused by gamma-irradiation. These results suggest that gamma irradiation in combination with *Undaria* polysaccharide treatment has a positive effect on microbial stability and quality of the pork patty.

Key words: Gamma irradiation, *Undaria* polysaccharies, pork patty, microbial safety.

INTRODUCTION

The demand for ready-to-eat meat products is gradually increasing due to their convenience. Ready-to-eat meat products are manufactured, distributed, and sold as frozen items (< -10°C). Despite freezing, hygienic quality can occasionally be threatened by the growth of food-borne pathogens such as *Listeria monocytogenes*. Numerous researchers have reported that gamma irradiation at low doses (< 10 kGy) kills most microorganisms without causing deterioration of food quality (Diehl, 1990; Thayer, 1994). However, several adverse effects caused by ionizing radiation, including lipid oxidation and softening of meat, have prevented this technology from being extended to preservation of meat. Use of antioxidants has been applied to prevent oxidation in some products (Lee *et al.*, 1999). Synthesized antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxy-

anisole (BHA) have been produced economically, but safety problems have been reported when using high doses of these chemicals. Recently, natural antioxidants have been used as a safer alternatives, to scavenge free radicals and to inhibit lipid oxidation.

Undaria is one of the most common edible brown seaweeds, and grows at the bottom of the eulittoral and at the top of the sublittoral zones. *Undaria* has been consumed as a popular dish in Korea and Japan for many years, and has recently attracted the attention of Western culture as a health food items. Studies of *Undaria* extract have shown that it contains substantial quantities of polysaccharides, including laminarin and fucoidan (Rioux *et al.*, 2007). These polysaccharides are known to have biological activities including antioxidant, anticoagulant, antihypertensive, and immunomodulating effects (Smit, 2004). The objective of this study was to evaluate the combined effects of gamma irradiation and addition of polysaccharide extract from *Undaria* on microbial safety and quality of ready-to-eat pork patties.

MATERIALS AND METHODS

Undaria was collected from Wando, South Korea. After collection, it was washed several times with cold water and kept at -4°C until its use.

Polysaccharide was extracted following the methods of Rioux (2007). *Undaria* was extracted with 85% ethanol solution at 70°C for 6 hrs. The residue was extracted with 2% $CaCl_2$ solution and filtered with Whatman filter paper. The obtained polysaccharides were dialyzed and freeze-dried.

Pork patties were made of chilled pork, pork back fat, and other ingredients. The patties were cooked in preheated cooker (NU-VU ES-3 cooker, Food Service System Menominee, USA) at 95°C for 30 min. The cooked patties were cooled in air for 1 hr. The packaging material was the oxygen-impermeable nylon bag (2 mL O_2/m^2/24 hr at 0°C, 0.09 mm thickness; Sunkyung Ltd., Seoul, South Korea). The packs were sealed and transferred to a refrigerator and stored.

Gamma irradiation was carried out in a ^{60}Co irradiator (point source, ACEL, IR-79, MDS Nordion International Co. Ltd, Ottawa, Ontario, Canada) with doses of 0, 2, 4, 6, and 8 kGy. The source strength was approximately 11.1 PBq, and the dose rate was 10 kGy/h. The irradiation room temperature was 22°C. Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), calibrated against an international standard set by the International Atomic Energy Agency (Vienna, Austria).

For microbial analysis, samples were diluted with sterilized saline solution (9 g NaCl/L water, 9 mL) by mixing for 10 s at 3000 rpm (Maxi Mix II, Thermolyne, Dubuque, IA). Total aerobic bacteria, and yeast and mold counts were obtained from a plate count agar (Difco Laboratories, Detroit, MI) and a potato dextrose agar (Difco Laboratories), respectively. Experiments with each microbial culture were independently conducted by triplicate.

Lipid oxidation was measured by 2-thiobarbituric acid reactive substances (TBARS) method following Jo and Ahn (2000). Pork patty (5 g) and 15 mL of deionized distilled water were homogenized with 50 μL BHA (7.2%) for 15 sec. 2 mL of the patty homogenate was transferred to a disposable test tube and then 4 mL of thiobarbituric acid/trichloroacetic acid (20 mM TBA in 15% trichloroacetic acid) solution was added. The mixture was blended and incubated in a boiling water bath for 15 min. The sample was cooled in cold water for 10 min, and then centrifuged

for 15 min at 2,500 g at 4°C. Absorbance was measured at 532 nm and the lipid oxidation was reported as mg malondialdehyde/kg meat.

RESULTS AND CONCLUSION

The effect of the combined treatment on the microbial safety of pork patties is shown in Table 1. By using a gamma irradiation at the dose of 2 kGy, the number of colony forming units (CFU) in pork patties decreased from 3.48 to 3.07 log CFU/g. When *Undaria* polysaccharides were added, the initial microbial concentration in the patties was decreased to 3.17 log CFU/g, and no viable colonies were detected at an absorbed dose of 2 kGy. These results suggest that the lower irradiation doses could be used to kill pathogenic organisms in meats, with the addition of the *Undaria* polysaccharides.

To investigate the effect of *Undaria* polysaccharides on lipid oxidation caused by gamma irradiation, TBARS (2-thiobarbituric acid reactive substances) measure-

Table 1 - Effect of combined treatment on the microbial concentration of pork patties.

Irradiation dose (kGy)	CFU, patties without added *Undaria* polysaccharides (log CFU/g)	CFU, patties with added *Undaria* polysaccharides (log CFU/g))
0	3.48[a]	3.17[b]
2	3.07[a]	ND*
4	ND	ND
6	ND	ND

[a-b]in the same row indicates that values are significantly different ($p < 0.05$).
*Viable colonies were not observed at the detection limit (< 10^2 CFU/g).

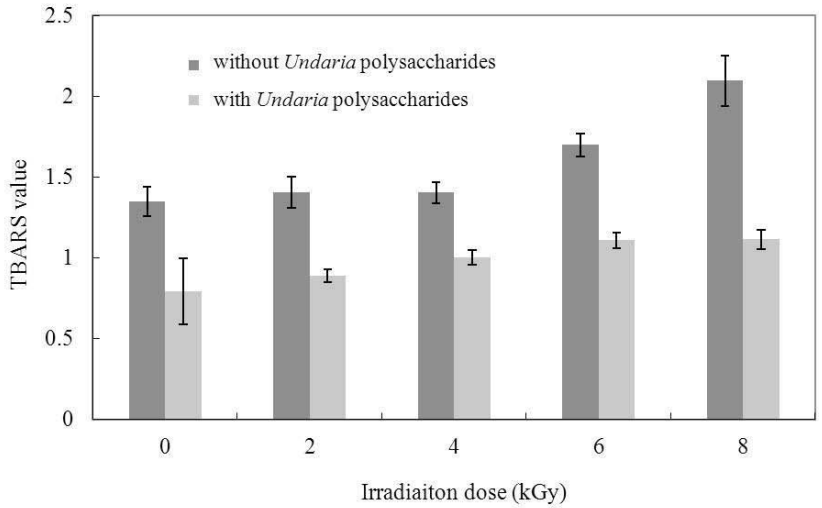

Fig. 1 - TBARS values of pork patties with and without Undaria polysaccharides after gamma irradiation.

ments were carried out (Fig. 1). The TBARS value increased from 1.35 to 2.10 at the absorbed dose of 8 kGy. In general, it is known that irradiation induces lipid oxidation, and that oxidation progresses during storage (Byun *et al.*, 1999). However, when *Undaria* polysaccharides were added at a concentration of 1%, the TBARS value was 0.79 in non-irradiated samples. When patties were irradiated at a dose of 8 kGy, the TBARS value was only 1.11. These results show that pork patties to which *Undaria* polysaccharides were added experienced lower levels of irradiation-induced lipid oxidation compared to patties without *Undaria* polysaccharides.

These results suggest that addition of *Undaria* polysaccharide extract can improve the microbial safety of meat products, and it decrease lipid oxidation caused by irradiation.

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EFFECT OF IRRADIATIONS ON *HIZIKIA FUSIFORMIS* COOKING JUICE

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ABSTRACT

Recently, *Hizikia fusiformis* cooking juice, the by-product of the drying process of *H. fusiformis*, was reported to have various biological activities. Irradiation can be used as an effective technique to decontaminate the cooking juice. In this study, we compared the effects of 2 types of radiation: electron beam and gamma rays from ^{60}Co. After irradiation, the number of microorganisms in the cooking juice was considerably reduced, regardless of the radiation source. Gamma irradiation removed the redness and increased the lightness of the cooking juice more effectively than electron beam irradiation did. Our results indicate the differences between the effect of electron beam and gamma rays and may be useful for selecting the proper radiation type for decontamination of food.

Key words: Gamma ray, electron beam, decontamination, cooking juice.

INTRODUCTION

Gamma rays have been used for the decontamination of food and sterilization of medical devices for more than 50 years. A benefit of using gamma rays is their high penetration power. Products can be irradiated in the packaged state and large volumes can be processed. However, the use of radioactive source (cobalt-60 or caesium-137) and long processing time can be hurdles for the commercialization of gamma irradiation. Recent advances in electron-beam technology have made this mode of sterilization a strong competitor to the traditional gamma-processing techniques (Abramyan, 1988). Electron-beam irradiation has many advantages, such as relatively short processing time, in-line processing, high effectiveness, low facility cost, and an increased available energy.

Hizikia fusiformis is one of the most common edible brown seaweeds in Korea and Japan. Because *H. fusiformis* is sold commercially in the dried form, a large amount of cooking juice is produced as a by-product during the drying process. This cooking

juice has been reported to have various biological activities (Oh *et al.*, 2007). However, the high microbial contamination and dark color of the cooking juice render it unsuitable for use in the food, cosmetic, or medicine industries. Recently, studies have shown that gamma irradiation can effectively decontaminate the cooking juice extract and increase its antioxidant properties (Choi *et al.*, 2010). In addition, gamma irradiation can cause discoloration of green tea leaf extracts (Jo *et al.*, 2003).

Therefore, in the present study, we investigated the difference between the effectiveness of electron beam and gamma rays in decontaminating and changing the color of the *H. fusiformis* cooking juice.

MATERIALS AND METHODS

H. fusiformis cooking juice was purchased from Younghae Industry (Yeosoo, Korea). To remove the debris, cooking juice was filtered using Whatman filter paper No. 4 (Whatman International Ltd, Springfield Mill, Kent, U.K.) and stored in a refrigerator (-4°C).

Cooking juice samples in tightly capped bottles (1 L) were irradiated in a ^{60}Co irradiator (point source, ACEL, IR-79, MDS Nordion International Co. Ltd, Ottawa, Ontario, Canada). The source strength was approximately 11.1 PBq. The irradiation room temperature was 22°C. Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany) calibrated against an international standard set by the International Atomic Energy Agency (Vienna, Austria). For electron beam irradiation, the cooking juice packed in polyethylene envelope. The width and length of packed sample were 100 and 200 mm, respectively. The thickness of the packed sample was less than 3 mm because of the low penetration power of the beam. But one can safely use thickness around 30 mm for one-sided irradiation when a 10 MeV electron-beam was used. Irradiation was carried out using a linear electron-accelerator facility (UELV-10-10S accelerator, NII EFA, Moscow, Russia). The irradiation conditions were mean energy of 10 MeV, current of 1 mA and output of 570 kW. The gamma ray and electron beam irradiation facilities were located at the Advanced Radiation Technology Institute, Jeongeup, South Korea. The doses were 0, 0.5, 1, and 2 kGy for microbial tests and 10 and 20 kGy for color changes, respectively.

For microbial test, samples (1 mL) were diluted with sterilized saline solution (9 g NaCl/L water, 9 mL) by mixing for 10 s at 3000 rpm (Maxi Mix II, Thermolyne, Dubuque, IA). Total aerobic bacteria was obtained from a plate count agar (Difco Laboratories, Detroit, MI). The microbiological analysis of the irradiated HCD was carried out immediately after the irradiation. Experiments with each microbial culture were independently conducted by triplicate.

Color change was measured by transferring cooking juice samples into a glass cell (CM A-98, 10 mm in width) with a color difference meter (Spectrophotometer Model CM-3500d, Minolta, Osaka, Japan). The instrument was calibrated with standard black and white tiles before analysis. A large-sized aperture was used, and the measurement was conducted in triplicate with a computerized system using the Spectra Magic Software (Version 2.11, Minolta Cyberchrom Inc., Osaka, Japan).

All of the experiments were carried out in triplicate. One-way analysis of variance was carried out using the SPSS software system. The Duncan's multiple-range test with the significant level of $P < 0.05$ was used to compare the differences among the mean values. Mean values with pooled standard errors of the mean were reported.

RESULTS AND CONCLUSION

Table 1 shows the total counts of aerobic bacteria in the non-irradiated, gamma-irradiated, and electron beam-irradiated cooking juice samples. The microbiological tests were performed immediately after irradiation. Irradiation at doses of 0.5 kGy and higher led to, at least, 2 log cycle count reduction of the analyzed microbiological populations, and irradiation at a dose of 1 kGy resulted in non-detectable counts (less than 100 CFU/mL). No difference was observed between gamma rays and electron beam irradiation in their decontamination effects. In the previous study, similar results were also obtained for fruit juices (Song *et al.*, 2006). In that study, the viable microorganisms in the non-irradiated juice were approximately 10^7 CFU/mL, but irradiation at a dose of 1 kGy decreased the microbial count to 10^4 CFU/mL. In general, the D_{10} values (the dose required to inactivate 90% of a population) of *Escherichia coli* in semi-dried fish was 0.55 kGy and in carrot juice was 0.3 kGy. Environmental factors, especially water content, had an effect on the radiation sensitivity of the microorganisms. When the water content was high, the extent of reduction of microbial population was greater because of the radiolysis effect of water. Our results show that irradiation is a very effective method for the decontamination of *H. fusiformis* cooking juice. Moreover, the microbicidal effect was independent of the source of the radiation.

The changes in the Hunter color values of the cooking juice are shown in Table 2. Although the L*-values increased significantly with the increased in the absorbed

Table 1 - Effect of irradiation on the microbial concentration of cooking drips.

Irradiation dose (kGy)	Viable cell counts after gamma irradiation (log CFU/mL)	Viable cell counts after electron beam irradiation (log CFU/mL)
0	4.23[a]	4.23[a]
0.5	2.07[a]	2.13[a]
1	ND*	ND
2	ND	ND

[a-b]in the same row indicates that values are significantly different ($p < 0.05$).
*Viable colonies were not observed at the detection limit (< 10^2 CFU/g).

Table 2 - Color changes of cooking drips after irradiation.

Irradiation dose (kGy)	L* (Lightness)		a* (Redness)		b* (Yellowness)	
	Gamma ray	Electron beam	Gamma ray	Electron beam	Gamma ray	Electron beam
0	59.79		15.34		51.11	
10	80.13*	73.21	1.71*	2.13	24.91	23.11
2	87.31*	85.21	1.31*	1.72	21.03	21.71

*Two values of gamma-irradiated and beam-irradiated samples are statistically different with $p < 0.05$.

doses, the a*- and b*-values decreased. These data show that the dark color of the cooking juice turned lighter after irradiation. The effect of radiation on the color of the cooking juice differed between the gamma-irradiated and electron beam-irradiated samples. Gamma irradiation is highly effective for color removal. Choi et al (2010) also reported that gamma irradiation effectively removed the color of the extract of cooking juice. Previous studies also show that gamma irradiation increased the Hunter color L*-value of green tea extract (Jo *et al.*, 2003). In these studies, the colored components such as chlorophyll were found to be degraded by gamma rays. In brown seaweeds, such as *Hizikia*, fucoxanthin and chlorophyll derivatives impart the color. Carotenoids in the cooking drips could be degraded by gamma irradiation, resulting in a lighter-colored product (Choi *et al.*, 2010).

These results confirm that radiation treatment is extremely effective for controlling microbial growth and discolorization of *H. fusiformis* cooking juice. Even though gamma rays and electron beams do not differ in their decontamination effect, each radiation source has its own specific uses. Electron-beam irradiation is suitable for continuous irradiation of small, uniform volumes of cooking juice because of the short processing time. However, for the treatment of large volume at a time, gamma irradiation is preferable because of its superior penetration power.

ACKNOWLEDGEMENTS

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EFFECTS OF MODIFIED ATMOSPHERE PACKAGING ON PAPAYA

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ABSTRACT

Papaya (*Carica papaya* L.) has a short shelf life under ambient conditions. The effects of modified atmosphere packaging (MAP) (3% O_2+8% CO_2+89% N_2) on storage quality of fresh papaya with 50% of yellow skin were studied at 13°C. Compared with samples which were stored under ambient conditions and samples packaged in PP bags without MAP (non-MAP), MAP made fruit firmness and water loss of papaya decrease less and skin yellow change slower. MAP protected main nutrient compositions such as the content of total soluble solids (TSS) and vitamin C from losing. MAP decreased the activity of polygalacturonase (PG) and increased the activity of peroxidase (POD) while delayed their peak time. The production rate of malondialdehyde (MDA) was also suppressed. The results showed that the shelf life of papaya under MAP at 13°C was extended from 25 days to 38 days. MAP was effective to prolong papaya's shelf life with maintaining good quality.

Key words: Enzymes, fruit quality, malondialdehyde, modified atmosphere packaging, papaya.

INTRODUCTION

The metabolism of postharvest papaya continues, which causes a 46% loss. So the preservation of papaya is very important. Modified atmosphere packaging (MAP) as a non-pharmaceutical treatment technology can extend the shelf life of most fruits and vegetables.

The enzymes, for example, polygalacturonase (PG) and peroxidase (POD) play important roles in many metabolisms of postharvest fruit ripening process (Wakabayashi *et al.*, 2000, Guan *et al.*, 2000). malondialdehyde (MDA) is the end-product of membrane lipid peroxidation, and its content can reflect the rotten level of the fruit (Saquet *et al.*, 2003). But the researches for changes of enzymes and MDA of papaya are lacking.This study aimed to the effects of MAP on storage quality, the contents of main components, enzymes and MDA of papaya.

MATERIALS AND METHODS

Fruit sample 'Sunrise', a local popular branch of Papayas (Carica papaya L.), was harvested from the orchard at Zhuhai, Guangdong, China, with similar maturity of 50%.

Reagents Gas (N_2, O_2, CO_2), Zhuhai Weiming Development Co., Ltd.; Polyvinylpyrrolidone, AR, Sigma Company; 2,6–dichlorphenol indophenols, Guaiacol, Sodium hydroxide, Ethanol, Ascorbic acid, Oxalic acid, Sodium acetate, Acetic acid, Polyethylene glycol – 6000, 30% Peroxide, Trition X-100, AR, Tianjin Damao Chemical factory.

Experimental Methods Papaya was washed with tap water and stored in the temperature-controlled cabinet (GDJS-100, Guangzhou Chengcheng experimental equipments factory, China) at 13°C with the 90% RH. Samples in atmosphere were stored without packaging. Samples in non-modified atmosphere packaging were packaged directly with polypropylene (PP) bags. And samples in modified atmosphere packaging were packaged in PP boxes which contained O_2, CO_2 and N_2 and the ratio of them was 3:8:89 (v/v).

Skin color, weight loss, firmness, the contents of total soluble solids (TSS), vitamin C (Vc), PG, POD and MDA were determined by the methods introduced by Cao *et al.*(2007).

RESULTS AND CONCLUSIONS

Figs. 1-5 showed the effects of MAP on papaya storage quality. The firmness of papaya had no difference in the first 9 days by the influence of three different environments. As the time went on, MAP showed a clear advantage, and the firmness was maintained well in the 38th day (Fig. 1). Fig. 2 showed the skin color in the first 5 days changed obviously and increased smoothly in MAP. In the first 5 days, the weight loss was very serious under atmosphere storage, but it was stable in MAP, particularly after 10 days (Fig. 3). Figs. 4 and 5 showed the influence of three

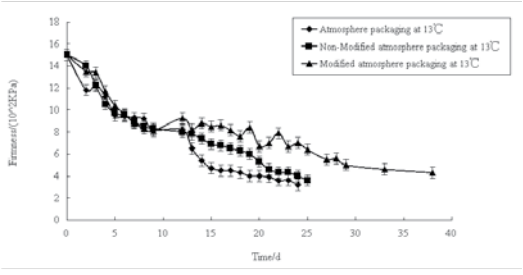

Fig. 1 - Changes of firmness of papaya in different storage environments.

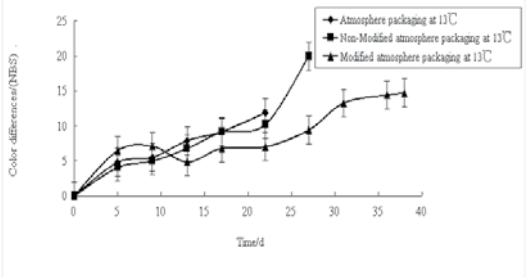

Fig. 2 - Changes of skin color of papaya in different storage environments.

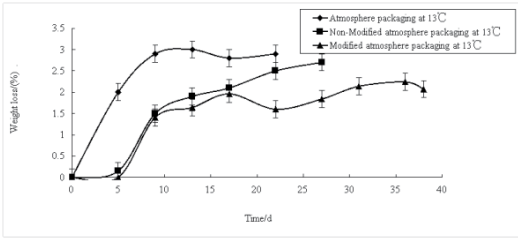

Fig. 3 - Changes of weight loss of papaya in different storage environments.

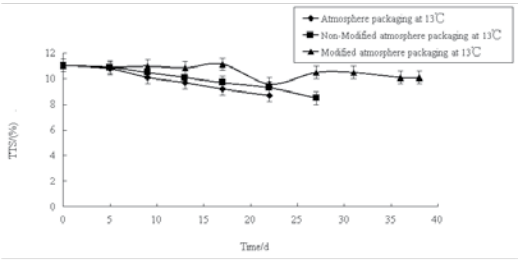

Fig. 4 - Changes of TSS content of papaya in different storage environments.

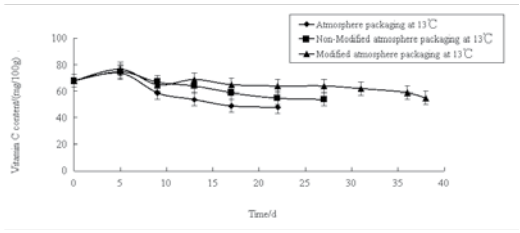

Fig. 5 - Changes of vitamin C content of papaya in different storage environments.

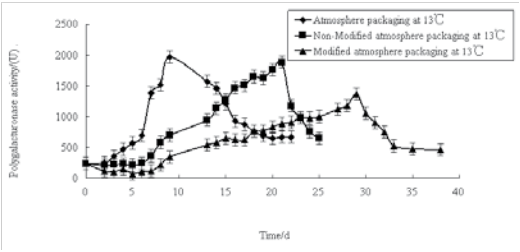

Fig. 6 - Changes of PG of papaya in different storage environments.

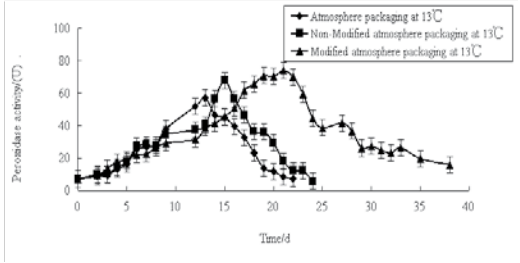

Fig. 7 - Changes of POD of papaya in different storage environments.

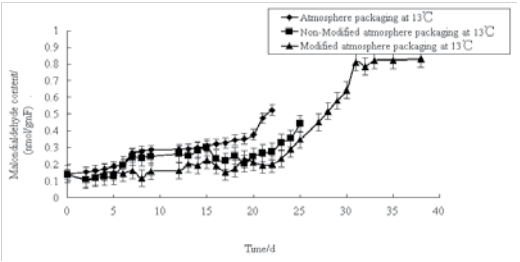

Fig. 8 - Changes of MDA content of papaya in different storage environments.

environments on TSS and Vc had no difference in the first 5 days. The content of TSS and Vc of papaya in MAP had been maintained well for 38 days.

As shown in Figs. 1-5, the storage quality under MAP (3% O_2 + 8% CO_2 + 89% N_2) was better than in atmosphere and non-MAP at 13°C. In atmosphere condition without any packaging, the respiration of papaya was uncontrolled and strong. But when the papaya was packaged with PP bags, the concentration of O_2 decreased and the concentration of CO_2 increased, although the original gas concentrations was the atmosphere. So the respiration of papaya became weakened, and the quality changed slower than those stored in atmosphere. In MAP, the respiration was respressed and the ability of metabolism was reduced due to the low concentration of O_2 and the more high concentration of CO_2. The quality of papaya were well protected.

Figs. 6 and 7 showed that in MAP the activities of two important enzymes during the papaya ripening process were different clearly from those of other storage environments.

Pectinase is one of the main enzymes leading to fruit soften. PG is a part of pectinase. Fig. 6 showed the activities of PG increased firstly and then decreased during the storage. The activity of PG changed slowly in MAP during the storage, and reached maximum on the 29th day. In addition, the maximum of PG in MAP was less than that in atmosphere and non-MAP. The peak time of PG was postponed from the 9th day (non-MAP) to the 29th day (MAP), meanwhile its activity was declined by approximate 25%.

The H_2O_2 is harmful for plant cell during the papaya ripening process. POD is an important ubiquitous oxidoreductase in fruits and vegetables. It can catalyze H_2O_2 oxidation. Then the phenols become quinines and the cells are protected. Fig. 7 showed the activity of POD increased firstly and then decreased. The time reaching the maximum was the 13th day in atmosphere, 15th day in non-MAP, and the 21th day in MAP. And the activity of PG in MAP showed well on the 33th day.

The content of MDA reflects the level of membrane lipid peroxidation. MDA is harmful for the membrane and organelles, and changes the conformations of protein and nucleic acid (Saquet *et al.*, 2003). During the storage process of the 6th day to the 22th day, there was little change of MDA in MAP (Fig. 8). In other words, the content of MDA produced by degradation was little, and the quality of papaya maintained well.

Above all, MAP was effective to prolong papaya's shelf life with maintaining good quality.

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CHARCOAL POWDER POLYMERS FOR FUNCTIONAL FOOD PACKAGING

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ABSTRACT

Charcoal has been widely used in various commercial products such as catalyst, medicine, cosmetic, and filter due to its multifunctional physiochemical properties including porous structure, gas adsorption, dehumidification, mineral release, and antibacterial effect. In this study, we established a manufacturing process of charcoal plastic chips which are used as starting polymeric materials for the production of plastic wares. In order to manufacture the charcoal plastic chips, we combined charcoal powders and conventional polymer materials including PE, PP, PVC, and PET. Using these complex materials, several types of plastic wares were fabricated for the package and storage of foods. By culturing bacteria with charcoal plastic chips, we found that these package materials exerted an antibacterial activity as charcoal particles killed harmful bacteria. Analysis of surface topology using SEM revealed the protrusion of charcoal particles with various range of size from 10 to 1000 nm, which enabled bacterial cells to be killed during the culture. These results strongly suggest that the polymers containing charcoal particles have a great potential to be applied for the food package and storage by killing harmful bacteria or inhibiting the growth of foodborne bacteria.

Key words: Charcoal, antibacterial effect, food packaging, plastic chip.

INTRODUCTION

Authentic physicochemical characteristics of charcoal have been studied focusing on gas adsorption, dehumidification, mineral release, and anti-microorganism (Chyka and Seger, 1997; Gaudreault, 2005). By carbonization of wood at high temperature (800°C), charcoal contains micro- and nano-size mesoporous structure with large surface area (1000 ~ 2000 m^2/g). Using these properties, charcoal products are applied for many industrial areas including food, cosmetic, environment, and catalyst.

For the purpose of extended shelf life of foods, packaging materials require several physical and chemical properties such as exclusion of external gas or liquid, suppression of moisture loss from content, and maintenance of food quality against temperature change. Petrochemical plastics including PE, PP, PVC and PET have been widely used for food package due to their properties of matter and economical efficiency. In this study, food packaging materials containing charcoal powders were fabricated, and their antibacterial activity was assessed by film-adhesion method.

MATERIALS AND METHODS

Charcoal powders were prepared by ball milling of oak wood charcoal pyrolized at 1400°C for 6 hours. After removing harmful gas from charcoal, micro size powders separated by 1000-mesh nylon paper were heated and combined with plastic resin by 0.5 ~ 5% w/w ratio under vacuum condition (Fig. 1). In this work, high density polyethylene (HDPE) with 0.5% charcoal was used as a polymer material to fabricate plastic bags for food.

Particle size analyser (LS 13320, Beckman Coulter) was used to measure the sizes of charcoal powders. The sizes of charcoal particles on the HDPE bag were measured by taking pictures of plastic samples under SEM (scanning electron microscope, S-3500N, Hitachi) and light microscope (Imager A2, Carl Zeiss) (Cha *et al.*, 2012). Distribution of powder size was statistically analyzed from 15 microscopic pictures of sample (140 × 105 μm).

Two different bacterial strains, *Escherichia coli* (ATCC 8739) and *Staphylococcus aureus* (ATCC 6538) were used to investigate the antibacterial effect of charcoal polymer by film-adhesion test (Kim and Kwak, 2008). After 24 hour incubation of bacterial cells covered with films made of HDPE with or without charcoal powders, 5 μL of each culture broth was diluted and spread on LB agar plates. Cell concentration was calculated by scoring the number of colonies formed on the agar plates.

RESULTS AND CONCLUSION

After the second step of manufacturing process (Fig. 1), ground charcoal powder with small size was harvested using 1000-mesh nylon filter (Fig. 2a). Before combining powder with polymer, the size of charcoal was measured using DLS (dynamic light scattering). As shown in Fig. 2b, the range of particle diameter was from 0.7 to 20 μm. Charcoal powders with diameter from 3 to 15 μm exist as major population (≤ 70%). However, the distribution of charcoal powder sizes shifted

Fig. 1 - Manufacturing process of charcoal powder plastic chips and packaging wares. Oak wood was pyrolized in a kiln at 1400°C for 6 hours. Charcoal powder was produced by grinding and ball milling. Plastic resin (HDPE) was combined with charcoal powder in vacuum condition with heat treatment. Plastic chips (0.5% w/w charcoal in HDPE) were used to fabricate plastic wares (zipper bag) for foods.

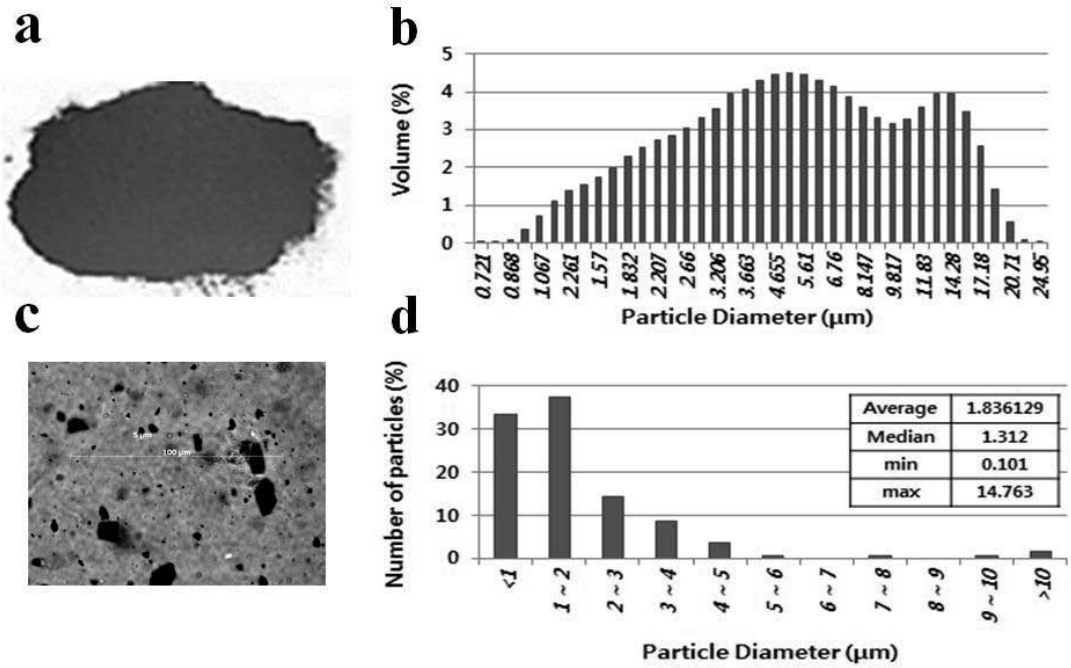

Fig. 2 - Analysis of charcoal powder size. (a) Charcoal powders after filtering using nylon filter for the manufacture of plastic chips; (b) Size distribution of charcoal powder before combining with plastic resin; (c) Microscopic image of charcoal particles in plastic (HDPE) bag film; (d) Size distribution of charcoal particles in polymer (HDPE) bag film.

to the smaller size after fabrication process for plastic bags. By the observation of charcoal particles in HDPE bags under microscope (Fig. 2c), we found that the powders with diameter less than 2 μm were mainly incorporated into the plastic wares (≤ 70%, Fig. 2d).

These results demonstrate that the larger size particles have been removed or changed into smaller size ones during the combining of charcoal powder with plastic polymer. Since charcoal powder was exposed to a condition with heat and high pressure, particles were able to be degraded and then incorporated into polymer mixture.

Many studies about food packaging have recently been focused on anti-microorganisms through nanotechnology (McCurdy, 2006; Sorrentino *et al.*, 2007). Inorganic nanoparticles including silver, titanium, and zinc exerted antibacterial activity when they were supplemented into the culture vessels for bacterial cells (Kim and Kwak, 2008). Since HDPE films also contain nano-size particles, antibacterial effect of charcoal power polymer was investigated using two different foodborne bacteria, *E. coli* and *S. aureus*. As shown in Fig. 3, the growth of bacterial cells was significantly inhibited in both of two strains by covering with HDPE films containing 0.5% charcoal powder. Above 90% of *E. coli* and *S. aureus* cells were killed in 24 hours, while cells under the film without charcoal were grown in the same time period.

Antibacterial mechanism of charcoal particles has not been fully understood. Given that manufactured nanoparticles kill bacterial cells by disintegration of cell wall or membrane, charcoal particles with nano-size are able to make damages on the plasma membrane of two foodborne bacteria. However, in our case, another different mechanism might exist behind the antibacterial effect because the par-

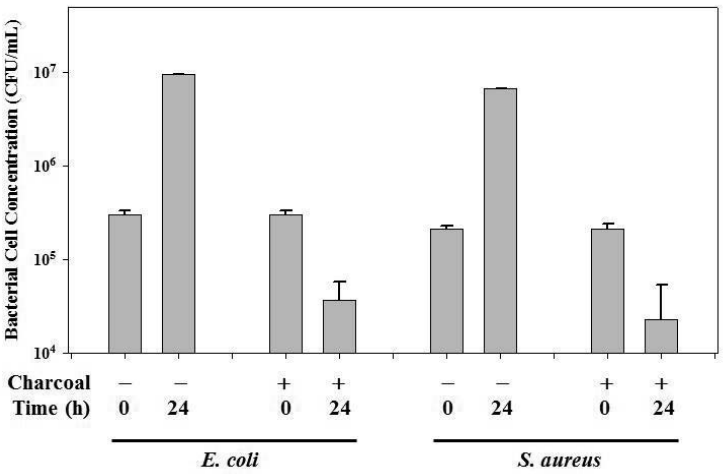

Fig. 3 - Antibacterial effect of charcoal polymer. After two foodborne bacterial strains (*E. coli* and *S. aureus*) were incubated on agar plates covered with HDPE films with or without charcoal for 24 hours, the number of colonies generated from each culture broth was scored on a new agar plate. Error bars indicate standard deviations.

ticles were fixed on a surface or inner space of plastic resin. We have also found the bacteria killing effect when the plastic chips mixed with charcoal powder were supplemented into bacterial cell culture medium.

Results demonstrate that polymers incorporated with charcoal powder are very useful materials for food packaging with antibacterial activity. Since the charcoal content ratio from 0.5-5% (w/w) did not change the physical property of polymer including HDPE, charcoal plastics have a great potential for functional packaging materials to extend shelf life of foods by killing foodborne bacteria.

ACKNOWLEDGEMENTS

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NEW ACTIVE PACKAGING MATERIALS BASED ON PROPOLIS

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ABSTRACT

The use of propolis as an active compound, successfully exploited primarily in the pharmaceutical field, was intended in this work to set up natural packaging materials. To achieve this goal, coating techniques were used for the deposition of active layers on plastic films and cellulosic sheets. The stability of the so obtained active coatings was evaluated according to the polyphenols degradation over time.
Key words: Active packaging, antioxidant activity, coating, propolis.

INTRODUCTION

Propolis, a natural resinous substance collected by honeybees, is well-known for its several properties such as antimicrobial (Katircioglu and Mercan, 2006), antioxidant (Jasprica *et al.*, 2007) and anti-inflammatory (Burdock, 1998). These properties are due to its unique composition: propolis is composed by a pool of molecules that act synergistically. It usually contains resins (50%), flavonoids and phenolic acids, essential oils (10%), pollen (5%) and various inorganic compounds (5%) such as Fe and Zn, vitamins (B_1, B_2, B_3 and B_6), fatty acids, esters, quinones, steroids and sugars as well (Pastor et al, 2010). Recent studies have paved the way for potential applications of propolis also within the food and food packaging fields, e.g. to control primary factors of antimicrobial degradation and oxidation (Pastor *et al.*, 2011; Mascheroni *et al.*, 2010). The use of propolis as an active compound could

be helpful to extend the shelf life of perishable foods. Towards this goal, innovative packaging characterized by 'active features' provided by propolis were developed.

MATERIALS AND METHODS

Three active coatings were prepared according with the following different formulations: a) hydroalcoholic (60 wt% ethanol) solution of propolis (81.5%), gelatin (10%), glycerol (7.5%) and lipids (1%); b) hydroalcoholic (60 wt% ethanol) solution of propolis (96.5%) and cellulose nanocrystals (3.5%) and c) hydroalcoholic (60 wt% ethanol) solution of propolis.

The coating technique used is described hereinafter: an aliquot of the a) and b) solutions were deposited separately by roll-coating onto the corona-treated side of 12 ± 0.5 μm thick poly(ethylene terephthalate) (PET) films (Toray Saehan, Kyungbuk, South Korea) and another aliquot of the c) solution onto a commercial paper samples by use of an automatic applicator (ref 1137, Sheen Instruments, Kingston, UK) equipped with a steel horizontal wire-wound rod, at a constant speed of 150 $mm \cdot s^{-1}$, according to ASTM D823-07 – Practice C. Coatings were firstly dried using a constant and perpendicular flux of mild air (25.0 ± 0.3°C) at a distance of 40 cm from the applicator for 2 min, followed by overnight storage at room temperature.

Finally, the samples were stored in darkness at room temperature for two months in air and in 100% nitrogen conditions. Both antioxidant activity and polyphenols concentration were assessed by means of the DPPH$^{\bullet}$ assay and HPLC method, respectively, at regular time spans (10, 30, and 45 days).

For the determination of the antioxidant activity of the samples, a UV-Vis spectrophotometer Lambda 25 (PerkinElmer, USA) was used. The decrease in DPPH$^{\bullet}$ concentration was measured by monitoring the decrease in the absorbance continuously at 517 nm during 30 min. Aliquots of 40 μL of each sample extracted with methanol and 2.96 mL of the DPPH$^{\bullet}$ 90 μM solution were placed in each test tube in triplicate. A blank with methanol and the DPPH$^{\bullet}$ solution was included.

Polyphenols were extracted with methanol and detected using a HPLC-LC Module I plus (Waters Corporation, Milford, Massachusetts, USA) system equipped with a Waters Symmetry C18 Column, W486 Tunable Absorbance Detector as UV detector and a W715 Ultra Wisp autoinjector. Quantification was performed by integration of peak areas, with reference to calibrations done while using known amounts of pure compounds. The gradient profile was formed using solvent A (Water plus 0.1% trifluoracetic acid) and solvent B (Acetonitrile plus 0.1% trifluoracetic acid) in the following program: the initial proportion of B (20%) remained for the first six minutes, increased from 20% to 30% over the next 4 min, increased to 40% over the next 30 min, increased to 60% over the next 5 min, increased to 100% over the next 5 min, returned to the initial conditions over the next 3 min and finally remained at the initial conditions for five minutes. The flow rate was 1.2 ml/min, and the column temperature was 30°C.

RESULTS AND CONCLUSIONS

The analyses revealed the same results for plastic films coated with gelatin and cellulose nanocrystals: no significant degradation of polyphenols occurred when propolis was loaded as coating on commercial plastic films (PET) and stored in air

and in 100% nitrogen conditions for 45 days in darkness at room temperature (Table 1). In the case of commercial paper coated with propolis (Table 1) there was found a degradation of 50% of polyphenols. The correlation between the concentration of polyphenols that remain stable and the antioxidant activity (evaluated by means of a linear regression) (Fig. 1) resulted in good linearity with a R^2 coefficient higher than 0.92. Therefore, it can be stated that polyphenols are responsible for the antioxidant activity of this coating with propolis. Moreover, no differences in losses between samples stored in air and in 100% nitrogen conditions (Table 1) apparently indicate that the oxygen is not the primary factor of polyphenols degradation for these active materials.

Table 1 - Concentration of polyphenols (mg polyphenols/g dry coating) in coatings with gelatin or cellulose nanocrystals onto PET or commercial paper samples and stored in air or in 100% nitrogen conditions.

t (days)	1	2	3	4	5
0	222 ± 7	222 ± 7	270 ± 19	270 ± 19	238 ± 16
10	249 ± 6	221 ± 23	263 ± 1	279 ± 22	230 ± 8
30	238 ± 6	252 ± 12	294 ± 14	279 ± 6	162 ± 10
45	219 ± 18	228 ± 24	283 ± 0	273 ± 12	119 ± 8

1: coating with gelatin onto PET and stored in air; 2: coating with gelatin onto PET and stored in 100% nitrogen conditions; 3: coating with cellulose nanocrystals onto PET and stored in air; 4: coating with cellulose nanocrystals onto PET and stored in 100% nitrogen conditions; 5: coating onto commercial paper samples in air and in 100% nitrogen conditions.

These results confirm that propolis can be used successfully in a coating of gelatin or cellulose nanocrystals onto plastic films, whereas coating of propolis directly on cellulosic materials is not suitable as a packaging material because of lack of stability of the polyphenols fraction. In fact, in PET coatings there were no

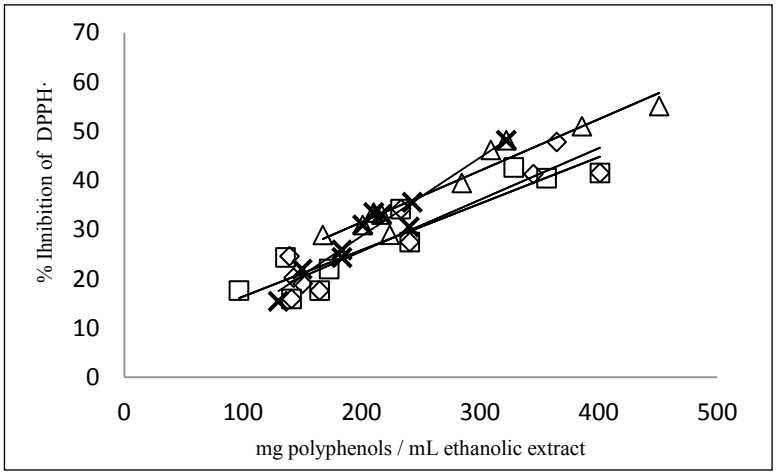

Fig. 1 - Relation between % inhibition of DPPH• and the concentration of polyphenols in coating with gelatin or cellulose nanocrystals onto PET and stored in air or in 100% nitrogen conditions. □ Coating with gelatin onto PET and stored in air; Δ coating with gelatin onto PET and stored in 100% nitrogen conditions; ◊ coating with cellulose nanocrystals onto PET and stored in air; × coating with cellulose nanocrystals onto PET and stored in 100% nitrogen conditions.

significant losses of polyphenols, while in paper coating samples high degradation of polyphenols occurred. The main reason could lie in the interaction between polyphenols and the chemical compounds of the paper sheets.

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OLIVE MILL WASTEWATER EXTRACTS EXERT *IN VITRO* ANTIMICROBIAL ACTIVITY AGAINST COMMON MEAT SPOILING AND PATHOGENIC BACTERIA

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ABSTRACT

Polyphenol mixtures obtained from olive mill wastewater were tested for their activity against spoiling and pathogenic bacteria commonly isolated from meat products, such as *Brochothrix thermosphacta, Listeria monocytogenes, Salmonella enterica* subsp. Derby and *Staphylococcus aureus.* Minimal Inhibitory Concentrations were determined, and time kill kinetics revealed a strong action at very low concentrations. In particular, 0.25% extract was able to immediately inhibit the growth of *L. monocytogenes* ATCC 7644 and *S. aureus* ATCC 32760, while at the same concentration *Salmonella enterica* subsp. Derby S1 and *B. thermosphacta* ATCC 11059 were inhibited after 60 and 120 minutes, respectively. Moreover, growth dynamics were evaluated by means of automated turbidimetry. The analysis of growth parameters revealed a bacteriostatic action of olive wastewater extracts at concentrations lower than or equal to 0.5%, depending on the strain, and a bactericidal action at higher concentrations. Results obtained showed that olive mill wastewater extracts exerted a strong and fast *in vitro* antimicrobial activity against some spoiling and pathogenic microorganisms. Our data provide insight into a possible application of these extracts as antimicrobial compounds, although their application on foods still has to be optimized. Moreover, olive mill wastewaters have a strong polluting impact, and therefore their application as biopreserving agents could increase their value, converting a waste material into a powerful resource.

Key words: Olive mill wastewater extracts, *Listeria monocytogenes, Salmonella, Staphylococcus aureus, Brochothrix thermosphacta.*

INTRODUCTION

Food biopreservation is a promising strategy for food safety control and shelf-life extension, as it limits the use of chemical preservatives and strong technological treatments. At the same time, the emergence of environmental awareness moves research interest towards any method that may help reducing environmental pollution.

Polyphenols are widespread in nature and they are known to exert antioxidant action, while their antimicrobial activity is still under investigation. In particular, olives and olive oil are rich of tyrosol, hydroxytyrosol and verbascoside, which are associated with protection against oxidative stress. The same mixture of bioactive molecules could be also obtained by purification of olive mill wastewater (Servili and Montedoro, 1989), which represents an important polluting agent just because of polyphenols and organic material.

To investigate the antimicrobial activity of these compounds, polyphenol mixtures obtained from olive mill wastewater were tested against spoiling and pathogenic bacteria commonly isolated from meat products, such as *Brochothrix thermosphacta*, *Listeria monocytogenes, Salmonella enterica* subsp. Derby and *Staphylococcus aureus.*

MATERIALS AND METHODS

Type strains *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 32760, *Brochothrix thermosphacta* ATCC 11059, and strains *B. thermosphacta* B1, *Salmonella enterica* subsp. Derby S1 and *Salmonella enterica* subsp. Tiphymurium S4, isolated from meat products, were used in our experiments.

Olive mill wastewater extracts (OMWE) were obtained from Prof. Servili, University of Perugia; their concentration was adjusted in H_2O/EtOH 4:1, according to experimental needs, and then filtered before use.

Minimal Inhibitory Concentrations (MIC) of OMWE were determined by microdilution method. Time kill kinetics were determined in BHI plates, after 0-360 min of contact between polyphenolic extract and target strains (inoculum around 5.0 Log UFC/ml in BHI).

Growth dynamics were evaluated in BHI modified by addition of different OMWE concentrations, by means of automated turbidimetry (Bioscreen C, Oy Growth Curves ab Ltd, Finland). Strains were incubated at 30°C up to five days. Growth parameters were obtained by means of Gompertz equation modified by Zwietering *et al.* (1991).

RESULTS AND CONCLUSION

Data obtained for Minimal Inhibitory Concentrations (MIC) revealed antimicrobial activity at very low concentrations, e.g. around 0.25%, for all the tested strains.

Fig. 1 gathers the results regarding time kill kinetics, determined in BHI plates after putting in contact polyphenolic extract and target strains. A strong and rapid action was observed at very low concentrations. The extract (0.25%) immediately inhibited the growth of *L. monocytogenes* ATCC 7644 and *S. aureus* ATCC 32760, while at the same concentration *Salmonella enterica* subsp. Derby S1 and *B. thermosphacta* ATCC 11059 were inhibited after 60 and 120 minutes, respectively. No growth was observed in presence of higher concentrations, even after 360 minutes.

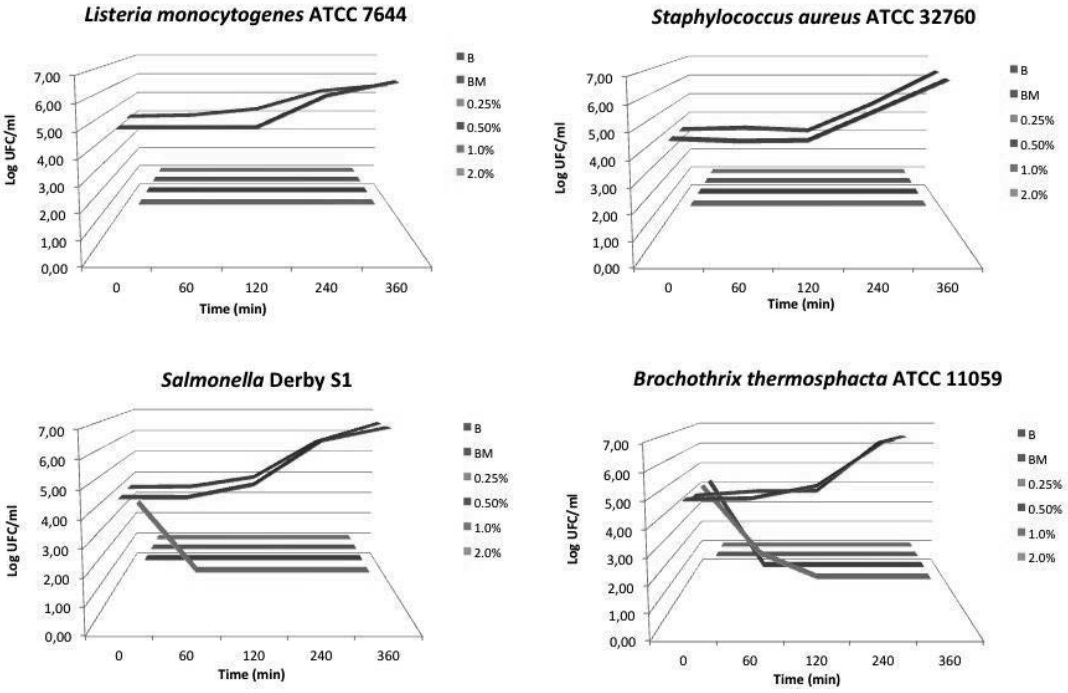

Fig. 1 - Time kill kinetics after 0-360 min of contact between OMWE and *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 32760, *Salmonella enterica* subsp. Derby S1 and *Brochothrix thermosphacta* ATCC 11059 (inoculum = 5.0 Log UFC/ml); (B = control; BM= control modified with H_2O/EtOH 4:1; 0.25-2.0% = OMWE in H_2O/EtOH 4:1).

Growth dynamics of survived cells during exposure to small OMWE concentrations were evaluated by automated turbidimetry (Fig. 2). Results confirmed what already observed by time kill kinetics. In particular, *L. monocytogenes* 7644 was unable to grow even in presence of the lowest OMWE concentration tested (0.25%). The analysis of growth parameters revealed a bacteriostatic action of extract concentrations lower than or equal to 0.5%, depending on the strain, and a bactericidal action at higher concentrations.

The antimicrobial action of OMWE has to be attributed to phenolic compounds, which are known to act on cytoplasmic membrane (Casas-Sanchez *et al.*, 2007), altering its structure and disturbing membrane potential (Paparella *et al.*, 2008; Serio *et al.*, 2010). Position and number of hydroxyl groups seem to be related to toxicity for microorganisms, with increasing activity at greater hydroxylation (Marjorie, 1999). Moreover, it was proved that mixtures of polyphenols exert greater activity with respect to single compounds (Obied *et al.*, 2007).

In conclusion, our findings demonstrate a strong and fast action of olive mill wastewater extracts on some spoiling and pathogenic microorganisms *in vitro*. Therefore, our data provide insight into a possible application of these extracts as antimicrobial compounds, although their application on foods still has to be optimized.

As olive mill wastewaters have a strong polluting impact, their application as biopreserving agents could increase their value, opening new perspectives for converting a waste material into a powerful resource.

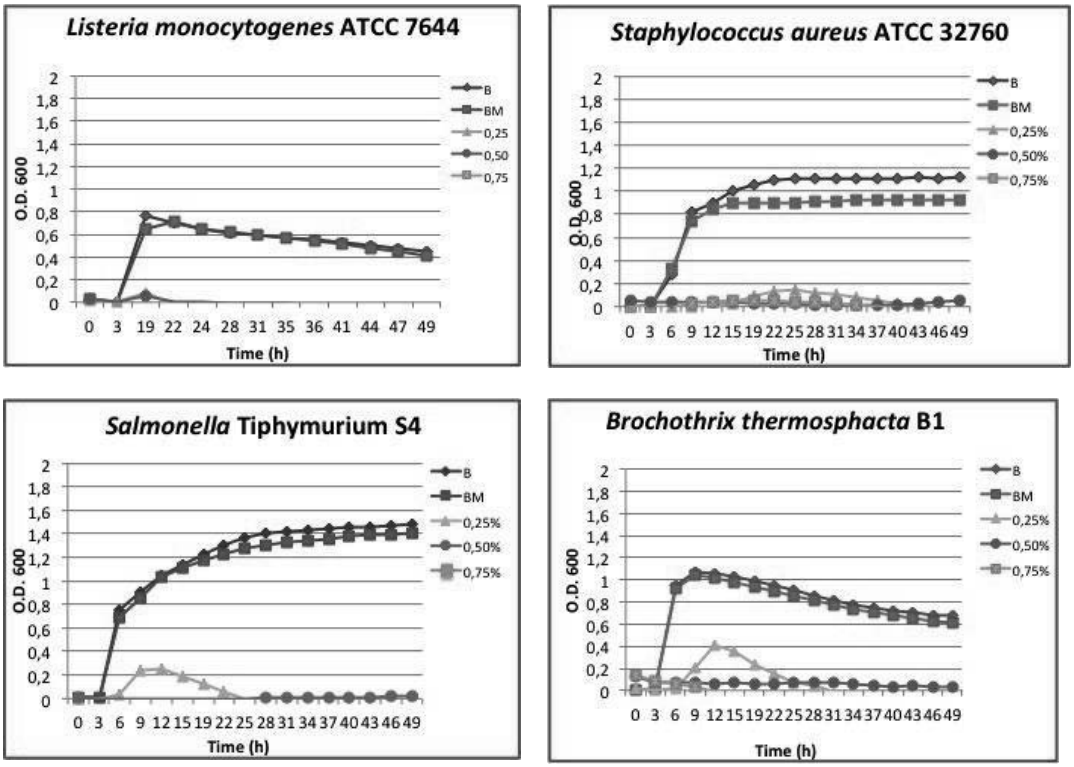

Fig. 2 - Growth dynamics of *Listeria monocytogenes* ATTC 7644, *Staphylococcus aureus* ATCC 32760, *Salmonella enterica* subsp. Tiphymurium S4 and *Brochothrix thermosphacta* B1 in BHI modified with small OMWE concentrations (B = control; BM= control modified with H_2O/EtOH 4:1; 0.25-2.0% = OMWE in H_2O/EtOH 4:1).

ACKNOWLEDGEMENTS

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EFFECTIVENESS OF SURFACE TREATMENTS WITH MIRENAT-N AGAINST *LISTERIA MONOCYTOGENES* IN TALEGGIO AND CACIOTTA CHEESES

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ABSTRACT

The anti-*Listeria* activity of a new compound, ethyl-N-lauroyl L-arginate hydrochloride (LAE) in Mirenat-N formulation, was evaluated in in Taleggio and Caciotta cheeses, which are considered able to support the growth of *L. monocytogenes*. Cheese samples were inoculated with *L. monocytogenes* ATCC 19114 or with a cocktail of three strains (ATCC 19114, strains 2 and 42 isolated from dairy products) and surface-treated with different Mirenat-N concentrations. During storage at 4°C for 28 days, Mirenat-N 4% was effective in reducing *L. monocytogenes* load immediately after treatment, and in containing its growth during time, with different dynamics between the cheeses. Mirenat-N was also effective against other microbial parameters such as mesophilic aerobic count and yeasts. LAE positive effects in extending milk shelf-life without sensory changes was already observed by other authors, but our results endorse LAE application on cheese surface. However, further studies are needed to extend knowledge on application, interactions and efficacy of these treatments..

Key words: Mirenat-N, *Listeria monocytogenes*, Taleggio cheese, Caciotta cheese.

INTRODUCTION

Control of unwanted microorganisms is one of the major concerns of food industry, because of great economic losses and increasing health risks for consumers. For this reason, new antimicrobial compounds are investigated to develop new effective strategies for shelf-life extension and food safety improvement.

Ethyl-N-lauroyl L-arginate hydrochloride (LAE; CAS number 60372-77-2) is a synthetic molecule, recently developed from lauric acid, L-arginine and ethanol. It is

considered safe for consumers, being naturally hydrolized in the human body, into common dietary compounds. In 2005, FDA recognized LAE as GRAS, and in 2007 EFSA released a positive opinion on this new compound (Heimbach, 2005; EFSA, 2012). While LAE is a powder, Mirenat-N is a formulation of LAE in propylene glycol.

The present study was aimed at the evaluation of this new compound, LAE, in Mirenat-N formulation, as antimicrobial agent, particularly against *L. monocytogenes* development in Taleggio and Caciotta cheeses. European Regulation on Microbiological Criteria for Foodstuffs includes these cheeses in the group of food products able to support the growth of *L. monocytogenes*. Special attention has to be paid to Taleggio, as this is a wash-rind and smear-ripened cheese, whose physical-chemical conditions could be particularly suitable for the pathogen growth.

MATERIALS AND METHODS

Two experimental data sets were prepared. First, cheese rinds were heat-treated by means of a Bunsen, then they were inoculated with *Listeria monocytogenes* ATCC 19114 at the end of logarithmic phase (5.0 log CFU/g), and subsequently surface-treated with Mirenat-N (Vedeqsa Inc, New York, US) at 4% concentration. In the second trial, after rind heat treatment, both cheeses were inoculated with *L. monocytogenes* ATCC 19114, strain 2 and strain 42, isolated from dairy products (7.0 log UFC/g), and surface-treated with Mirenat-N 4%. All samples were stored at 4°C for at least 21 days in ordinary atmosphere. Immediately after treatment and during storage, the following microbial parameters were determined: mesophilic aerobic count (in PCA, 30°C for 48 h), lactic acid bacteria (in MRS, 30°C for 48 h), yeasts (in YPD at 25°C for 48 h), and *L. monocytogenes* (in ALOA, Biolife Italiana, at 30°C for 48 h). All media were from Oxoid, except where differently specified. Also pH and a_w were determined during time, both on the surface and in the inner part, by using Mettler Toledo MP 220 pH-meter and Aqualab (Decagon Devices).

RESULTS AND CONCLUSION

During storage at 4°C for 28 days, Mirenat-N 4% was effective in reducing *L. monocytogenes* load immediately after treatment, and in containing its growth over time, with different dynamics in the two cheeses, as shown in Fig. 1. In particular,

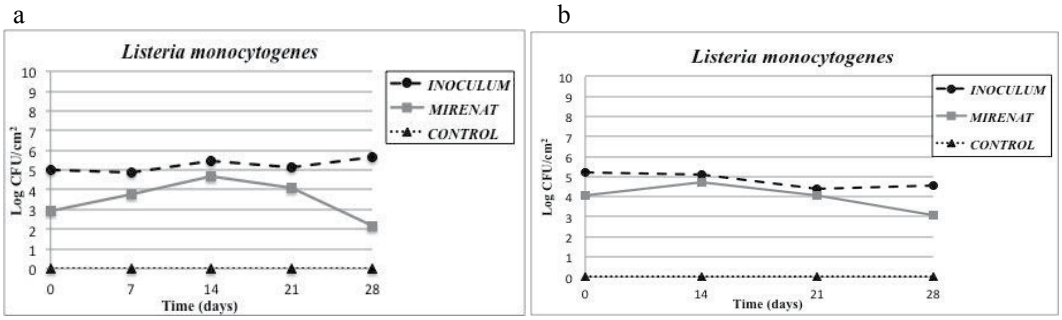

Fig. 1 - *Listeria monocytogenes* ATTC 19114 growth on Taleggio (a) and Caciotta (b) cheeses during storage at 4°C for 28 days, in trial 1 (inoculum 5.0 log CFU/g). (Inoculum: *L. monocytogenes* ATCC 19114; Mirenat: *L. monocytogenes* ATCC19114 + Mirenat-N 4%; Control: untreated sample).

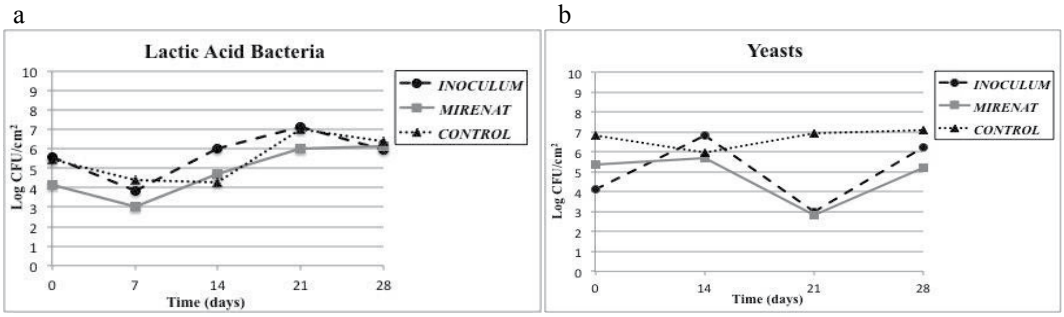

Fig. 2 - Lactic acid bacteria growth on Taleggio (a) and yeasts growth on Caciotta (b) cheeses during storage at 4°C for 28 days, in trial 1. (Inoculum: *L. monocytogenes* ATCC 19114; Mirenat: *L. monocytogenes* ATCC19114 + Mirenat-N 4%; Control: untreated sample).

at the end of storage *L. monocytogenes* load was significantly lower than in inoculated samples.

Mirenat-N was also effective against other microbial parameters, such as mesophilic aerobic count (data not shown), lactic acid bacteria and yeasts (Fig. 2). Physical-chemical parameters and sensory characteristics of the cheeses were not significantly affected by Mirenat-N application, both on the surface and in the inner part (data not shown).

In the second trial, where cheese samples were inoculated with a cocktail of three strains at higher counts, Mirenat-N immediately reduced *L. monocytogenes* count on Taleggio, while the best reduction was observed after three days on Caciotta (Fig. 3). For both cheeses, differences among treated and inoculated samples were progressively reduced during storage. The anti-listerial effect was less evident in comparison with trial 1, probably due to the higher loads inoculated. Moreover, in trial 2, the inoculum included two strains previously isolated from dairy products, possibly more adapted to cheese environment and more resistant.

Antimicrobial effects were also observed on mesophilic aerobic count, lactic acid bacteria and yeasts (data not shown).

LAE seems to act on cytoplasmic membrane, disturbing membrane potential and consequently altering metabolic processes (Rodriguez *et al.*, 2004). According

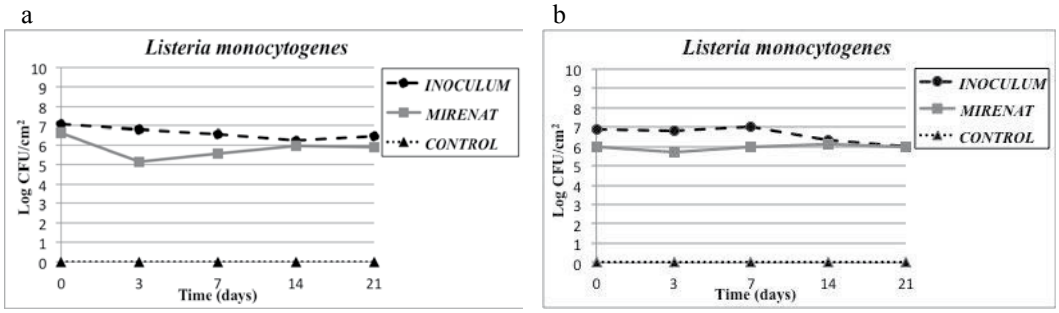

Fig. 3 - *Listeria monocytogenes* ATTC 19114, strains 2 and 42 growth on Taleggio (a) and Caciotta (b) cheeses during storage at 4°C for 28 days, in trial 2 (inoculum 7.0 Log CFU/g). (Inoculum: cocktail of *L. monocytogenes* ATCC 19114, strains 2 and 42; Mirenat: *L. monocytogenes* ATCC19114 + Mirenat-N 4%; Control: untreated sample).

to other authors, LAE breaks up the phospholipid structure, inhibiting cell growth (Bakal and Diaz, 2005). The antibacterial activity of LAE on *L. monocytogenes* was already documented on ham surface (Luchansky *et al.*, 2005).

In our study, we evaluated a very complex food matrix. In particular, on Taleggio surface, a stable microbial consortium made of yeasts, moulds, corynebacteria, coagulase-negative cocci and lactic acid bacteria insists. This particular condition may hamper Mirenat-N adsorption. For this reason, high Mirenat-N concentrations (4%) were used, with respect to researches of other authors (Woodcock *et al.*, 2009). Supporting our hypothesis, Mirenat-N producers recommend its use in beverages and ready-to-eat products, such as toppings and salads, which are simpler matrices than cheese.

In conclusion, our results support the effectiveness of LAE on cheese surface, in decreasing *Listeria monocytogenes* load and in reducing its growth over time, without sensory changes. Actually, our findings prove that LAE application on solid foods may have good perspectives, although further studies will be necessary to evaluate treatment methods, interactions and efficacy in working conditions.

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SESSION III

"Shelf Life Testing and Functional Food Properties"

PACKAGING REGULATION AND MIGRATION INVESTIGATION IN CHINA

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ABSTRACT

As an important part of food safety, the safety of food packaging has received much attention from both the ordinary people and the government. Therefore the government has enhanced its supervision of the impact of packaging on the food safety. This paper reviews the laws, regulations and standards on safety of food packaging, and the migration investigation in China. Special focus is on the issues like the additives in food packaging and paper packaging. The advances in migration testing, migration model and molecular dynamics simulation on migration in China are enhanced.

Key words: Regulation, migration, packaging materials, food packaging safety.

INTRODUCTION

Chinese packaging industry including packaging materials, packaging machinery and packaging printing has developed very fast. The value of the Chinese packaging industry's annual output soared from US$ 126 bn in 2008 to US$ 205 bn in 2011. Paper and plastic packaging materials, which represent over 60 percent of Chinese packaging industry's output, have become the two pillars. However, more and more food-packaging-related safety incidents like the addition of melamine in milk powder and the use of swill-cooked dirty oil for cooking have aroused concern

among people. As an important part of food safety, the safety of food packaging has received much attention from both the ordinary people and the government. Therefore the government has enhanced its supervision of the impact of packaging on the food safety. This paper reviews the laws, regulations and standards on safety of food packaging, and also the migration investigation in China.

REGULATIONS ON SAFETY OF FOOD PACKAGING

The most recent incident is the toxic capsules issue. China Central Television (CCTV) reported on April 15 that 13 commonly prescribed drugs had been packed into capsules made from industrial gelatin, which contains a much greater amount of chromium than edible gelatin. This issue caused great panic and fury among people. Although there are requirements set both by industrial standard and Chinese Pharmacopoeia, the crazy pursuit of profits made the enterprises ignore these. On April 19th China's State Food and Drug Administration (SFDA) issued an emergency notice to suspend the selling and consumption of the 13 medications produced by nine pharmaceutical companies. SFDA announced on April 27th excessive chromium was found in 74 batches of capsules produced by 15 enterprises. The Ministry of Public Security has arrested 9 people, detained 45 suspects, closed 10 illegal industrial gelatin and gel-capsule factories, and confiscated tonnes of industrial gelatin. The incident of toxic capsules reflects many problems of the supervision and administration mechanism, which is worthy to be noticed by the society.

In the aspect of law on safety of food and food packaging, "Food Safety Law of the People's Republic of China" has been implemented from 2009. This law contains safety standard of food packaging products, the legal duty and the punishment of violation of the law of the following people: the users and producers of food packaging products, the producers of new food packaging products and the users, producers and operators of imported food packaging products. Amendment 8 to the Criminal Law has been implemented last year, and criminals committing food safety related crimes face severer penalty.

The report of food safety information is included in the basic public health service items of China. "Schedule for Key Workings on Food Safety in 2011" mentioned that behaviour of producing food and food packaging materials failing to meet the safety standards would be investigated and treated. "Provisions on the Administration of Permit for New Varieties of Food Related Products" and "Declaration and Acceptance Provision of New Food-related Product Varieties" were issued last year. The food related products include food packaging materials, containers and additives. We would like to mention that for some new food-related product varieties, migration amount and/or residue amount, estimated dietary exposure and its estimation method should be provided.

Recent years the additives in food containers and packaging materials have caused great concern, especially Bisphenol A (BPA) in infant milk bottles. Now in supermarkets, these bottles were taken off from the shelves. "Circular for Clearance of Food Packaging Materials" was issued in 2009. All additives and resins that have not been listed on Chinese food packaging materials standards but used in China shall be reported to Ministry of Health (MOH). "Explanation of Clearance of Food Packaging Materials" and "Requirements on Application Material of Clearance of Food Packaging Materials" issued in 2010 gave the data requirement for clearance of food contact materials. "Letter on Seeking Public Opinions of Additives and

Resins to Be Approved for Food Packaging Materials" was issued last year. MOH has approved 196 additives and 116 resins. "Letter on Seeking Public Opinions of Forbidding Bisphenol A (BPA) Used in Containers for Baby Food" issued last year forbids the production and import of infant food containers such as milk bottles containing BPA. "Hygienic Standards for Uses of Additives in Food Containers and Packaging Materials" gives the principle of use of 959 varieties of the additives, allowable varieties of additives, scope of use, maximum amount, specific migration limits or maximum residue amount and other restricting requirements. A series of standards have been implemented concerning determination of toxic components in packaging materials and their migration to food simulants. In 2009, 22 national standards and 25 local standards containing specific migration limits were put into effects. At present, the migration limits are most concerned in the standards on packaging containers and materials.

Now paper packaging sector is the largest in packaging industry. Base paper and its products used for food packaging should meet the national hygienic standard. "Rules for Implementation of Production Permit of Paper Food Packaging and Containers etc." has been put into effect since 2007, which announced the first batch of paper food packaging and containers including 8 varieties for paper packaging, and 13 varieties for paper containers. The professional committees and censorships for market access of paper food packaging and containers have been set up. The enterprises can not produce the paper food packaging and containers that are not in the catalogue of the first batch. No units or person can sell or use the products without production permit.

MIGRATION INVESTIGATION IN CHINA

A. Research on migration

Both 10th and 11th Five National Science and Technology Support Projects funded the research of the migration and the control of toxic components in food contacting materials. A large number of universities and academic institutes in China have been researching on it, among which Packaging Engineering Institute of Jinan University has outstanding performance. In 2009, Packaging Engineering Institute of Jinan University obtained the project "research on control technology of harmful substances in food packaging materials" funded by the 11th Five National Science and Technology Support Project.

B. Migration testing

Table 1 is the summary of papers on migration testing published in recent years by Chinese researchers, mostly in international academic journals. It includes the contaminants, packaging materials, the destination (various food or simulant), sample pretreatment method, detection method and the specific migration limit (SML) of some contaminants. The contaminants include solvent residue like benzene, toluene and raw material like melamine. Primary aromatic amine (PAA) is the reaction product between the packaging materials and water-based food. Most contaminants investigated are additives in plastics, paper or can, and phthalates are most frequently studied. For example, the migration of DBP, DEHP, DOP and ATBC from paper packaging ink to milk powder was studied. Offset printing was used to simulate the real situation. Antimicrobials are added for active packaging. The release of these five antimicrobials (eugenol, isoeugenol, thymol, cinnamalde-

hyde and vanillin) from soy protein isolate films into olive oil followed the Arrhenius equation and their activation energies were calculated. Manufacturers of plastic food containers incorporate nanosilver to exploit its antimicrobial properties. But nanosilver is dangerous to organisms due to its dimension. The potential risks from its migration are preventing scientists and the public from fully supporting the advancement of nanosilver technology.

Table 1 - A summary of papers published by Chinese researchers.

Contaminant	Packaging Material	Food/Simulant	Pretreatment	Detection Method	SML(mg/kg)
VOC	Plastics	Apple etc	EA Extraction	GC-FID	1.2(toluene)
PAA	Plastics	Simulant	SPE	LC-UV	0.01
BPA	Can	Simulant	-	LC-FD	0.6
NOGE and BADGE-related	Can	Fish, meat etc	MAE+SPE	UPLC-ESI-MS/MS	1.0(BADGE)
		Fish, drink	SPE	LC-FD	
Plasticizer	Plastics	Drink, oil etc	SPE	LC-UV	30(BBP)
	FPVC	Simulant	-	LC-UV	0.3(DBP)
	Paper	Milk powder	Ethanol Extraction	GC-FID	1.5(DEHP) 5(DOP)
	Plastics	Ham sausage	SPE	GC-MS	9(DINP) 9(DIDP)
MEL	Plastics	Simulant	-	LC-DAD tITP-CE-UV	30
PIs	Plastics Carton	Milk	SPE	LC-ESI-MS/MS	0.6(BP)
	Paper	Simulant Milk	DCM or Ethanol Extraction	GC-FID	-
Antioxidant UV absorber	Plastics	Simulant	SPE	LC-DAD	30(BHA), 3(BHT), 1.5(Cyanox 2246), 2.4 (Irganox 1035), 6.0 (Irganox 1076)
BaP	Paper	Simulant	-	FD	-
PFCs	Carton	Milk, yoghurt etc	SPE	LC-ESI-MS/MS	-
Antimicrobial	SPI	Oliver oil	DCM Extraction	LC-UV	-
	Paper	Simulant	SPE	UPLC-ESI-MS/MS	
	Plastics	Simulant	Microwave Digestion	AAS, ICP-MS	
Metal element	Plastics	Simulant	Microwave Digestion	ICP-AES	-

After solvent extraction with or without SPE cartridges, the samples are commonly determined by methods like LC or GC coupled with various detectors depending on the properties of the contaminants. Chromatography coupled with tandem mass spectrometry can provide higher sensitivity and selectivity. Based on the indirect headspace gas chromatographic method (HS-GC), a volume ratio method was developed to measure the partition coefficient of organic contaminants between paper base packaging materials and air by Huang *et al.* (2008).

C. Migration model

Since the interaction between food contact materials and foodstuffs is foreseeable physical processes, one can build mathematical models to predict the migration processes instead of doing experiments during a period of time. Modelling is an attractive tool since fast computer is available.

The migration processes are mainly divided into three categories: Fick's diffusion, Fick's diffusion plus reaction and Non-Fickian processes. Most models are based on Fick's diffusion. Generally speaking, there are two methods to solve the Fick's diffusion equation to obtain the analytical solution. One is based on Crank method using method of separation of variables, and another on the error function using method of Laplace transform. It is difficult to obtain the analytical solution while studying the migration of contaminants in the multilayer composite materials. Therefore the numerical methods such as finite difference method and finite element method to get the numerical results of the migration are used. Based on the numerical method, we have developed corresponding software analyzing system "MigraSoft 2006". This system can analyze the migration process in up to 5 layer composite packaging materials.

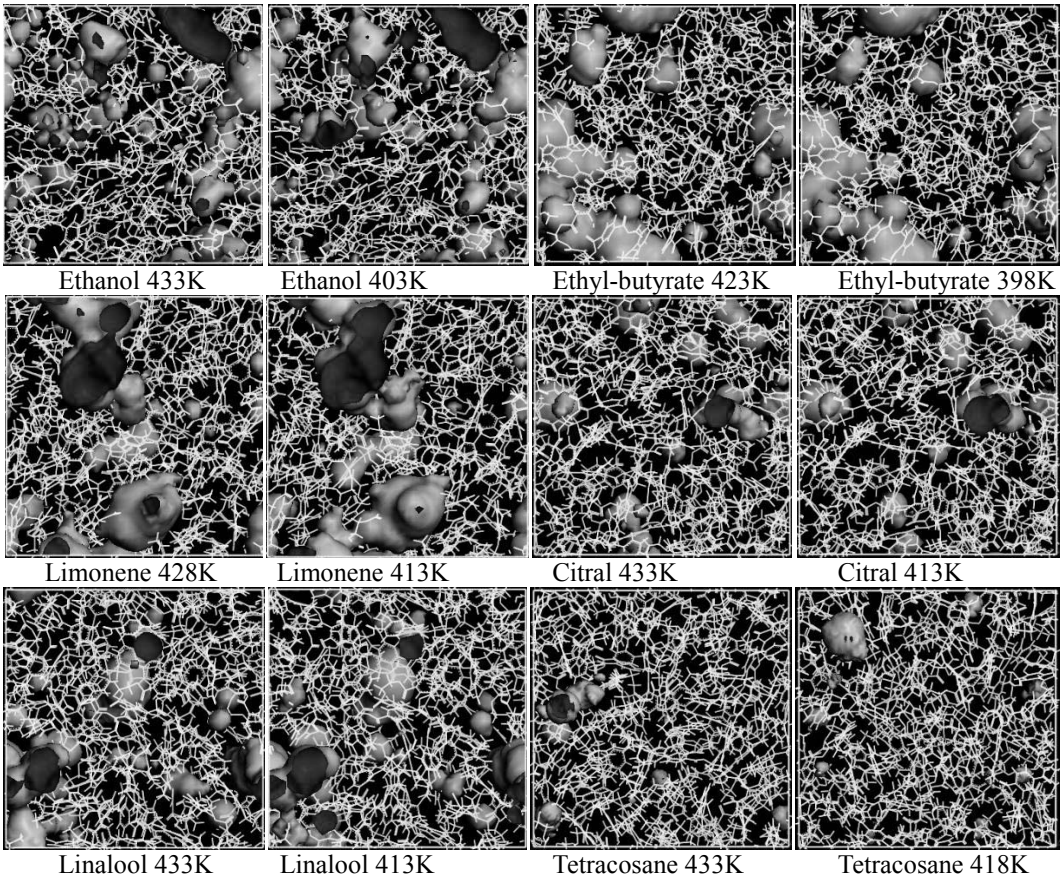

Fig. 1 - Simulated morphology of free volume voids in polymer models (Wang *et al.*, 2010).

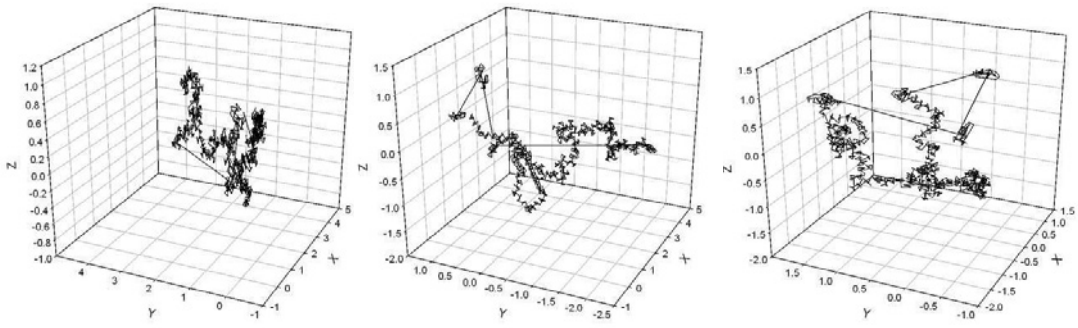

Fig. 2 - Movement trajectories of the limonene molecules in PP-H, PP-B and PP-R.

As we know, diffusion coefficient and partition coefficient are the two most important parameters influencing the migration process. The system can be used to investigate the changes of the migration process when the diffusion coefficient and partition coefficient vary.

D. Molecular dynamics simulation on migration

The molecular dynamics (MD) simulation technology has been used widely to investigate many engineering problems at the molecular level. It can also be used to investigate in detail the process, the mechanism and the influencing factors of the migration at the molecular level.

Understanding the diffusion of migrants in polyethylene terephthalate and calculation of diffusion coefficients are very important for the migration research. The migrations of 13 kinds of small molecules with molecular weights ranging from 32 to 339 $g.mol^{-1}$ from PET were investigated with using MD simulation. Fig. 1 shows the simulated morphology of free volume voids of 6 substances in polymer models at two temperatures by Wang *et al.* (2010).

The comparison among simulated, predicted and experimental diffusion coefficients as a function of molecular weight and as a function of temperature were obtained, and the simulated ones are closer to the experimental results than the predicted ones.

To evaluate the influence of types of polypropylene material on the migration behavior and to reveal the diffusion mechanism, the migration of limonene through three types of polypropylene materials (PP-H, PP-B, and PP-R) were studied by using MD simulation. The diffusion trajectories of the limonene molecules in PP-H, PP-B and PP-R were obtained by Wang *et al.* (2011), and shown in Fig. 2. The movement of limonene in PP-R is more active than that in PP-H and PP-B.

From the study of the migration of 13 substances from PET, and the migration of the same substance through PP with different structures, we are quite positive about the potential use of molecular dynamic simulation in the study of the migration.

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MOISTURE SORPTION CHARACTERISTIC OF SOY PROTEIN ISOLATE/ CARBOXYMETHYL CELLULOSE BLENDED FILM

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ABSTRACT

Soy protein isolate/carboxymethyl cellulose (SPI/CMC) blended films were prepared. The effect of ratios between SPI and CMC were used in a film forming solution on water vapor permeability (WVP) and a moisture sorption characteristic of SPI/CMC blended films was evaluated. When the CMC portion incresed, WVP of the blended films trended to increased. The sorption isotherm of each blended film provided the characteristic of sigmoid-shaped type II isotherm. The isotherms showed that the EMC of the films was concurrent with the water vapor permeability finding. The GAB, BET, Lewicki and Oswin models were tested to fit the experimental data. The Lewicki model was found to be the best fit model for SPI/CMC blended films with the lowest range of percent root mean square (12.2-66.1) at a_w as 0.11-0.75, at 25±2°C.

Key words: Biopolymer, carboxymethyl cellulose, soy protein isolate, sorption isotherm, water vapor transmission rate.

INTRODUCTION

The biopolymer films are non-polluting packaging materials which are biodegradable, edible, and nontoxic. They have been prepared from proteins, polysaccharides, and/or lipids. Protein films have better moisture, gas and oil barrier than some synthetic films. A wide range of proteins have been investigated (Rachtanapun and Suriyatem, 2012; Cho and Rhee, 2002). Soy protein has been widely used due to its low cost and decomposability. However, the purely polymeric films normally

conduce to be brittle. Thus, the improvement in the properties of protein films especially mechanical properties and permeability should be studied.

Carboxymethyl cellulose (CMC) is a cellulose ether that is water soluble, edible and nontoxic. CMC could improve the aggregation behavior of proteins between soy protein and whey protein (Yu *et al.*, 2004) and CMC could be well blended with cornflour and gelatin film to improve their permeability properties (Tongdeesoontorn *et al.*, 2009). To the date, there was no research which studied moisture sorption characteristic of SPI/CMC blended film. Thus, the objective of this work was to investigate the influence of CMC on the WVTR of SPI/CMC blended films. We also studied the moisture sorption isotherms of the films to see how well these films fit the standard.

MATERIALS AND METHODS

Film preparation Film forming solutions (5% w/v solid content) with different SPI and CMC ratios were prepared to yield different SPI/CMC blended films (Table 1). SPI was dispersed in distilled water and constantly stirred at 90°C for 30 minutes. CMC was dissolved in distilled water at 80°C for 10 min. The SPI and CMC solution were mixed and glycerol was added as a plasticizer. The mixture was degasified, cast on silicone plate, and dried at 25°C for 36 h.

Water vapor permeability (WVP) The WVP of the blended films was investigated according to ASTM E96 (ASTM, 1994) as previously described by Rachatanapun and Tongdeesoontorn (2009a, 2009b) with slight modofication. A saturated solution of NaNO3 was placed in a bottom of the desiccator to provide 65%RH. The test was carried out at 25°C for 7 days. All measurements were performed in triplicate.

Moisture sorption isotherm A film specimen (size 2 x 2 mm) was dried and preconditioned in a desiccator containing dried silica gel before testing. The film was placed in desiccators containing saturated salt solutions (LiCl, $MgCl_2$, $Mg(NO_3)_2$, $NaNO_3$ and NaCl) to create the specific RH (11, 34, 55, 65, and 75%) at 25°C. The film was weighed every 24 h until an equilibrium was achieved. The equilibrium moisture content

GAB (Guggenheim-Anderson-de Boer) model:

$$M = (M_0 C K a_w)/[(1-a_w)(1+(C-1)ka_w)] \quad (2)$$

Where, M; equilibrium moisture content on a dry basis, M_0; GAB monolayer moisture content, C; Guggenheim constant, k; factor correcting properties of the multiplayer molecules corresponding to the bulk liquid and a_w; water activity. The parameters (M_0, C, and k) of model were achieved from its second-order polynomial form ($y = \alpha x^2 + \beta x + \gamma$).

$$\alpha = k/[M_0(1/c-1)] \quad (3)$$

$$\beta = 1/[M_0(1-2/C)] \quad (4)$$

$$\gamma = 1/(M_0 kC) \quad (5)$$

This model was solved using linear regression analysis with the least sum of squares method to obtain α, β, and γ and subsequently the parameter values.

BET (Brunauer–Emmett–Teller) model:

$$M = [(M_0+T)Ca_w]/[(1-a_w)((1-a_w)+Ca_w)] \quad (6)$$

Where, M_0 and C are constants and obtained from slope and intercept of the linear plots of aw/ [(1-aw)*M] vs. aw.

$$M_0 = 1/ (\text{intercept} + \text{slope}) \quad (7)$$

$$C = 1/ (\text{intercept}*M_0) \quad (8)$$

Oswin model:

$$M = k[a_w/(1-a_w)]^C \quad (9)$$

Where, k and C are constants and achieved from slope and intercept of linear plots of log M vs. log [aw/(1-aw)], respectively.

Lewicki model:

$$M = [F/(1-a_w)^G] - [F/(1+a_w)^H] \quad (10)$$

Where, where F, G, and H = constants.

Fig. 1 - The model's equation from GAB, BET, Oswin, and Lewicki models for moisture sorption isotherm curve fitting.

(EMC) was calculated (Rachtanapun and Suriyatem, 2012) and sorption isotherm models were chosen to fit the experimental data of sorption isotherms. In literatures, the models found were shown as equations given in Fig. 1. (Rachtanapun and Suriyatem, 2012; Suppakul, 2006). All data was analyzed by one-way analysis of variance (ANOVA) and Tukey's multiple range test ($p \leq 0.05$) using statistical program SPSS version 11.

RESULTS AND CONCLUSION

The SPI/CMC-01 (pure SPI) film was transparent and light yellow while the SPI/CMC-07 (pure CMC) was also transparent and colorless (Fig. 2). The other films were translucent and showed the shade of very light yellow from the nature of soy protein (Fig. 2). The WVTR of SPI/CMC blends is related to the thickness of the film (Table 1). As the film thickness increased, there was a reduced resistance to water vapor transfer across it (McHugh, Avena-Bustillos and Krochta, 1993). The WVP value is the normalized transfer rate of the thickness and partial pressure gradient. It can then be compared even between films having different thicknesses according to the different ratios of SPI and CMC. The WVP of SPI/CMC blended films containing 0-50 g CMC/100g solid content was not significantly different. The WVP gradually increased when the CMC content was higher than 50 g/100g solid content. The related observations have been reported previously (Rachtanapun and Thondeesuoontorn, 2009a; Tongdeesoontorn et aThe isotherms of various SPI/CMC blended films showed the slow initial increase in moisture content with a_w increase of up to 0.55. And then the moisture content rapidly increased with

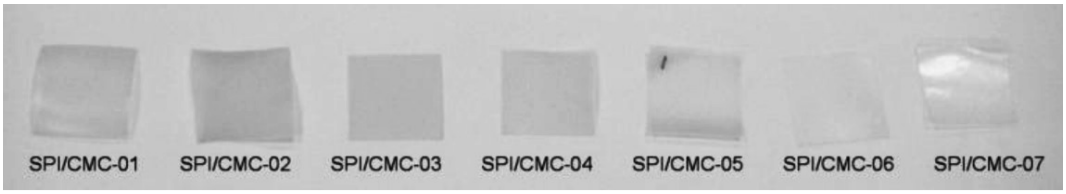

Fig. 2 - Characteristic of SPI/CMC blended films.

Table 1 - Water vapor transmission rate for SPI/CMC blended films at 65% RH, 25°C.

Type of films	SPI/CMC ratio	Thickness (mm)	WVTR ($g/day.m^2$)	WVP ($g.mm/m^2.mHg.day$)
SPI/CMC-01	100:0	0.135 ± 0.004	42.448 ± 0.000 a	3.702 ± 0.108 a
SPI/CMC-02	88:12	0.174 ± 0.009	31.482 ± 0.002 b	3.546 ± 0.192 a
SPI/CMC-03	67:33	0.171 ± 0.008	32.543 ± 0.005 c	3.702 ± 0.172 a
SPI/CMC-04	50:50	0.158 ± 0.009	37.849 ± 0.000 d	3.872 ± 0.219 a
SPI/CMC-05	33:67	0.153 ± 0.012	52.706 ± 0.003 e	5.244 ± 0.425 b
SPI/CMC-06	12:88	0.147 ± 0.004	67.209 ± 0.002 f	6.426 ± 0.193 c
SPI/CMC-07	0:100	0.157 ± 0.019	61.196 ± 0.000 g	6.232 ± 0.738 bc

Values followed with different letters in the same column are significant differences at the 5% level, according to Tukey ($P < 0.05$).

Table 2 - Sorption isotherm model constants of SPI/CMC blended films at 25°C.

Types of films	GAB					BET			
	M_0	C	K	r^2	%RMS	M_0	K	r^2	%RMS
SPI-CMC-01	0.1572	2.5694	0.2456	0.9985	18.0599	0.0716	1.3828	0.9985	17.9742
SPI-CMC-02	0.2249	3.2001	0.1422	0.9985	80.9947	0.0779	1.3186	0.9985	81.3768
SPI-CMC-03	0.1351	4.1573	0.2632	0.9983	14.9998	0.0807	1.8314	0.9983	14.9879
SPI-CMC-04	0.1526	3.8611	0.2563	0.9974	38.2462	0.0871	1.7331	0.9974	38.2163
SPI-CMC-05	0.1255	5.8144	0.3074	0.9991	10.9110	0.0905	2.4794	0.9991	10.9268
SPI-CMC-06	0.1307	7.5230	0.2712	0.9967	25.8064	0.0963	2.7690	0.9967	25.8056
SPI-CMC-07	0.1337	6.6607	0.3365	0.9980	8.2808	0.1031	2.9048	0.9980	8.2628
Types of films	Lewicki					Oswin			
	F	G	H	r^2	%RMS	k	C	r^2	%RMS
SPI-CMC-01	0.0541	1.1402	1.0501	0.9987	21.6976	0.0827	0.9349	0.9984	52.8715
SPI-CMC-02	0.0682	1.0438	1.5958	0.9987	66.1463	0.0883	0.9415	0.9985	86.1838
SPI-CMC-03	0.0720	1.0649	0.9277	0.9985	18.1698	0.1037	0.8816	0.9980	50.6284
SPI-CMC-04	0.0903	0.9668	1.3634	0.9976	27.7451	0.1103	0.8853	0.9974	58.3181
SPI-CMC-05	0.0968	0.9754	0.8553	0.9964	12.2163	0.1284	0.8261	0.9957	48.1298
SPI-CMC-06	0.1079	0.9545	0.8352	0.9964	25.6699	0.1408	0.8108	0.9957	50.1539
SPI-CMC-07	0.1091	0.9937	0.6724	0.9979	12.7725	0.1524	0.8060	0.9968	47.4586

the increase of a further a_w (Fig. 3). A higher amount of water than usual could be absorbed by the films when water activity was increased (Cho and Rhee, 2002; Rachtanapun and Suriyatem, 2012). The sorption isotherm of all films gave the characteristic sigmoid-shaped type II isotherm (Rachtanapun and Suriyatem, 2012; Tongdeesoontorn *et al.*, 2009; Cho and Rhee, 2002). The CMC film gave more EMC value than the SPI film which can be clearly seen at a_w as 0.75. The EMC of SPI/CMC blended film increased with the increase of CMC content. This confirmed that CMC is more hydrophilic than SPI in relation to the result of the WVP in this experiment. This result is similar to previous reports (Tongdeesoontorn *et al.*, 2009; Rachtanapun and Suriyatem, 2012).

Experimental sorption isotherm data were tested to fit the model equations. Corresponding coefficient r^2 and constants obtained are listed in Table 2. GAB and BET models were found to be the most accepted models for edible materials (Suppakul, 2006). Monolayer water content values (M_0) are used to determine the number of sorption sites. The GAB model provided a higher M_0 than the BET model for each SPI/CMC blended film (Table 2). Rachtanapun and Suriyatem (2012) and Rachtanapun and Tongdeesoontorn (2009a; 2009b) also found the relaled results. The C param-

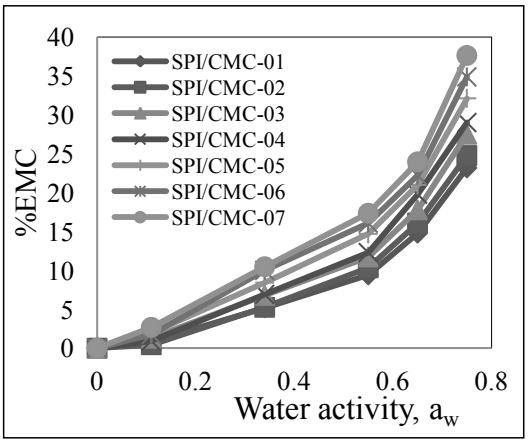

Fig. 3 - Water sorption isotherm of SPI/CMC blend films with different of SPI and CMC ratios.

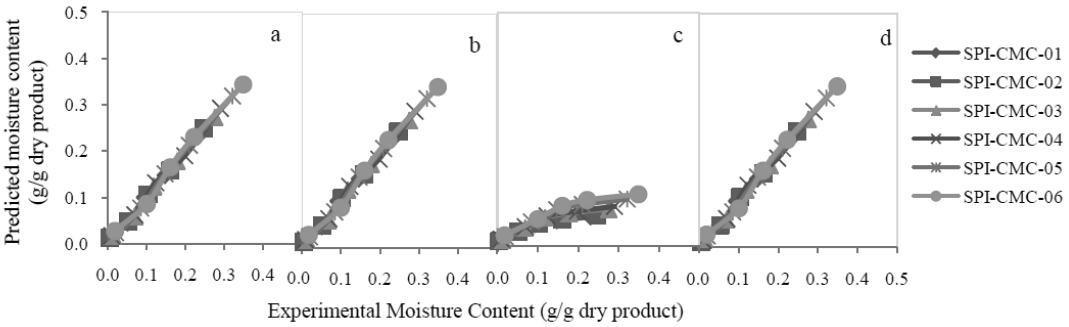

Fig. 4 - Comparison between the experimental moisture content predicted by (a) GAB model, (b) BET model, (c) Oswin and (d) Lewicki models of SPI/CMC blended films with various ratios between SPI and CMC.

eter in the GAB model is related to the difference of the magnitude in the upper layers and in the monolayer (Tongdeesoontorn *et al.*, 2009). For SPI/CMC blended films, the C parameter tends to increase when the CMC content is increased (Table 2) (Suppakul, 2006). The Oswin model commonly gave a good description of the moisture sorption isotherm throughout the entire range of water activity (Oswin, 1946). In this research, the %RMS value was obtained from 47.4 to 86.2%. The Lewicki model fits the moisture sorption data at a high humidity and then predicted the water content when the a_w reached 1.0 (Suppakul, 2006). In this work, the %RMS from this model ranged between 12.2-66.1%. In conclusion, the Lewicki model was found to be the better determiner for predicting the EMC of SPI/CMC blended films than the GAB, BET and Oswin models. Suppakul (2006) also reported that the GAB and Lewicki models gave the better prediction for cassava flour films. The experimental and predicted moisture contents by the GAB, BET, Oswin and Lewicki models for the SPI/CMC blended films with different CMC content are shown in Fig. 4. It shows that the GAB, BET, Oswin and Lewicki models could be employed to predict the moisture content of the SPI/CMC blended films at aw in range of 0.11 to 0.75.

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THE POST-PURCHASE CONSUMER BEHAVIOUR, SURVEY IN THE CONTEXT OF MATERIALS FOR FOOD PACKAGING

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ABSTRACT

In the food market packaging (primary packaging) can be considered one of the components of supply capable of generating utility (economic) and emotions (even if you consider the psychological aspect at the time of purchase) independent from any other attribute presented by the company. In particular, the investigation was focused on the main food packaging materials (glass, metal, plastics, paper and cardboard), with the aim of understanding the influence of packaging features on the consumer's choices and purchase preferences.

Key words: Glass, food-packaging, post-purchase, consumer behavior.

INTRODUCTION

Some products are considered undifferentiated with regard to intrinsic characteristics and brand (wine, oil, canned vegetables and canned fish), therefore consumers find on the packaging itself, rather than on the food product, the benefits sought from their habitual behavior. It was considered, therefore, interesting to point out the attractive effects and benefits arising from the presence of "strong" packaging, meaning with this term, the types of materials that have high market shares and enjoy the confidence of the consumer, and the "strength" is based on personal experience (loyalty) that the consumer has matured over time. This fidelity (loyalty) to the container becomes "a measure of attachment of the customer", and it is inversely correlated with the probability that a buyer can choose another different container, especially following a change of price or other material characteristic. If

customers show indifference to the packaging and, in fact, they buy considering only the food features, without taking into great consideration the characteristics of the material, then the box probably has a marginal value. Conversely, if customers continue to buy the container, even in the face of competitors who have higher food prices, then it means that they attribute a higher value to its function. Starting from the consideration that the sensory characteristics of food can only be verified after consumption (experience good), while the quality of the product "packaging" are considered observable and so assessable ex ante (search good), the survey was carried out at the retail self-service's exit, in particular, from the large retail chains with food department, located in major metropolitan cities of Sicily. The survey had a sample of 300 buyers-consumers, chosen in relation to certain factors that describe the individual's position in society (gender, age, economic situation, etc.), the availability of time and habitual behaviour at purchase. The data and information obtained highlight the competitive position of glass by recognizing some of the benefits sought - appearance, reliability, prestige, etc. - compared to other materials which show different degrees of involvement. To identify the importance of these benefits which involve, among other things, different purchasing patterns, a FCB matrix (Foote, Cone and Belding) was used: this tool does not consider, for the selected product (in our case, container), the differentiation between the various brands, but the benefit requested by consumers.

MATERIALS AND METHODS

The study was carried out during the second semester of 2011 at the exit of retail shops of big retail chains with stored food department of Eastern Sicily, collecting 326 questionnaires. Of these, 300 were considered valid for suitably grouping the consumers of foods and beverages, basing on their expressed preferences concerning primary packaging materials (glass, cellulose, metal and plastic) for such produces. In order to define the competitive positions of primary containers in the consumers' perceptions, the compensatory model method by Fishbein, suitably modified (Grandinetti, 2008) was used. The assessment (index) which derives is determined as the weighed mean of the benefits of the attributes (base services), corrected by a factor of importance, and presence perceived of the attribute (scale with minimum value = 1, maximum = 7, where 4 corresponds to indifference) for the primary packaging material, in particular:

$$Aj = \Sigma n\ vij = \Sigma n\ xij\ wi\ \Sigma n\ wi = 1$$

Therefore, by interchanging the dimensions, "importance of the attribute" (wi) and "perceived presence of the attribute" (xij) it is possible to identify the position (Aj) (behaviour of the consumer) of one container compared to another.

Once selected the material which had gained the highest number of preferences, the successive elaborations were aimed at assessing the connections and synergies between this packaging material and the type of food and/or beverage (Lee *et al.*, 2008), as a function of the personal profile of the consumer. The determinations done are useful for understanding the logics behind the consumers behaviour, i.e. the learning and involvement processes, which are tested on some kind of complementary services of the packaging material through a series of evaluation responses. The combination of the latter ones gives raise to four different paths

of a 2 by 2 matrix (*Foote, Cone & Belding* – FCB), characterized by a different sequence of macro-phases "Learn", "Feel" and "Do" adapted, in our case, as: *being informed* (Learn); *kind of perception, also physical* (Feel); *considering suitable, or sufficient* (Do). By crossing the responses (four options for each interviewed subject), the involvement model *Foote, Cone e Belding*, was set, which indicates the level of satisfaction (or dissatisfaction) of the consumer towards the selected primary container for food and/or beverages.

RESULTS AND CONCLUSIONS

Fig. 1 highlights a clear tendency (expressed by 300 subjects) towards glass (hollow glass). The same goes for Table 1 which shows the Fishbein index determined on the mean values relative to every question concerning the main basic services of the various packaging materials.

Table 2 reports the connections, as far as those subjects preferring hollow glass are concerned (200 in total), between such primary packaging material and some of the main food and beverage categories, numerically evaluated following a scale from 0 to 6, for verifying the importance of the benefits, also as a function of age and income.

For the complementary services (added value) of the hollow glass, the corresponding responses (4 for each of the 200 subjects) are shown in Table 3.

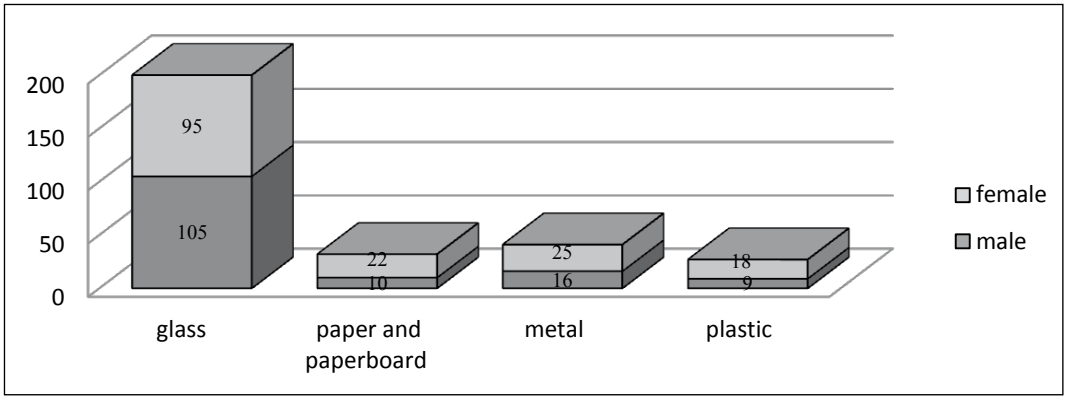

Fig. 1 - Number of interviewed, divided for sex, according to the preferred packaging material.

Table 1 - Distribution of the Fishbein index determined on the mean values relative to every question concerning the main basic services of the various packaging materials.

Attribute	Importance w_i	Glass x_{i1}	Paper x_{i2}	Metal x_{i3}	Plastic x_{i4}
Appearance	0.20	6	3	1	6
Resistance to transport	0.30	7	4	6	5
Lightness	0.10	2	5	4	5
Price	0.20	4	6	7	6
Ecology	0.20	6	7	1	1
Fishbein index		**5.5**	**4.9**	**4.0**	**4.6**

Table 2 - Distribution of evaluation responses of consumers who mainly use glass primary container for foods and/or beverages for main products, age and income classes (2011)(*).

Products	Fish preservers	Vegetable preserves	Oil	Fruit juices	Sauces	Wine	Total
Age and income ranges							
Up to 25 years old	12	30	36	12	24	30	144
From 26 to 45 years old	48	96	96	48	78	102	468
From 46 to 65 years old	48	90	90	42	66	108	444
Above 65 years old	12	24	30	18	24	36	144
Total	***120***	***240***	***252***	***120***	***192***	***276***	***1200***
Up to 10.000 €	12	42	48	18	30	48	198
From 10.001 to 20.000 €	24	60	66	24	42	72	288
From 20.001 to 40.000 €	48	90	96	48	72	102	456
Above 40.000 €	36	48	42	30	48	54	258
Total	***120***	***240***	***252***	***120***	***192***	***276***	***1200***

(*) Our elaborations on directly acquired data.

The survey on the consumers preference for the packaging material used for foods and/or beverages indicates that glass is preferred to other materials (more than two thirds of the interviewed). The use of hollow glass mainly concerns foods and beverages consumers (wine, oil and fruit juices) rather than preserves and sauces; the relative distinction into age, income and gender classes modifies this

Table 3 - Distribution of complementary attributed of hollow glass containers a function of entity of evaluation responses (2011)(*).

Complementary Services	**Interviewed**	**Responses**	**%**
Maintains the quality of product for a longer time?	18	72	
Guarantees the hygiene of the product?	21	84	
Does not transfer toxic or dangerous substances?	24	96	
Maintains the sensory characteristics of foods?	22	88	
A) Being informed			***42.5***
Makes the contained product more appealable?	15	60	
Is the material safe?	19	76	
Does the weight influence transport?	10	40	
Is it easy to handle?	9	36	
B) Kind of perception, also physical			***26.5***
Do you use it only for the products you desire?	19	76	
Is it easy to open and close?	17	68	
Do you re-use the container?	14	56	
Adequate location in cupboard	12	48	
C) Considering suitable, or sufficient			***31.0***
Total	***200***	***800***	
Total (A+B+C)			***100.0***

(*) Directly acquired data

ranking only slightly. Considerable are the results of the evaluation of choice of hollow glass as primary container through the FCB involvement model. The hollow glass primary containers aim at satisfying needs which are generated mainly by processes of experiential learning (II quadrant), but also by routine behaviours (III quadrant); less important are hedonistic processes (IV quadrant) and affectivity (I quadrant). To sum up, the evaluation determines actions which rely on rational behaviours rather than emotive ones. The involvement model for primary packages for foods and/or beverages is rather "*high*" and relies on the *intellectual learning.*

	Intellectual learning			***Emotive learning***	
	II			**I**	
	Learning			*Affectivity*	
High	Learn	96	76	Feel	
involvement	Feel	60	72	Learn	
	Do	56	48	Do	
Rationality ◄				►	**Emotivity**
	Routine			*Hedonism*	
	Do	76	68	Do	
Low	Learn	88	40	Feel	
involvement	Feel	36	84	Learn	
	III			**IV**	

Fig. 2 - FCB Matrix concerning hollow glass preferences. Our elaborations on directly acquired data.

The four groups reported in Fig. 2 were obtained from the values assigned to the evaluation responses following different sequences of the "*Learn–Feel-Do*" scheme in the construction of the FCB involvement model.

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SHELF LIFE TESTING OF KOREAN SPACE FOODS

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ABSTRACT

Seventeen Korean space foods sterilized by gamma irradiation were developed by Korea Atomic Energy Research Institute and certified as space foods usable in the International Space Station by Russian Institute of Bio-medical Problems (IBMP) by 2011. Shelf life of the foods was evaluated by the microbiological tests and organoleptic assessment under space simulating conditions for 51 days. Organoleptic tests such as taste, color, odor, and texture were assessed using a 5-grade scale. The results show that gamma irradiation did not change the organoleptic properties and successfully inactivated microorganisms to meet Russian regulation. All the Korean food products were certified by the IBMP for use in space flight conditions up to 30 days after their delivery on board.

Key words: Irradiation, shelf life test, space food.

INTRODUCTION

Irradiation is a physical method for food sterilization comparable to heat pasteurization. The process involves exposing foods to 1 of 3 types of ionizing energy: gamma rays, machine-generated electrons or X-rays. Irradiation is a promising technology that can be used to eliminate pathogens in foods (Farkas, 2006).

Korea Atomic Energy Research Institute (KAERI) has used irradiation technology to develop 17 Korean foods as space foods for astronauts: *Kimchi* (fermented vegetable), *Ramen* (ready-to-cook noodle), *Saengshik* (non-cooked nutrition bar), *Sujeonggwa* (persimmon and ginger beverage), *Bibimbap* (cooked rice mixed with red-pepper paste), *Bulgogi* (marinated beef with soy sauce), seaweed soup, mulberry beverage, mulberry jam, manila clam porridge, dried persimmon chocolate, dried blueberry, sweet pumpkin porridge, chicken curry-rice, chicken porridge, grilled chicken, and ox leg-bone–cabbage soup. The Korean space foods must be cetified by Russian Institue of Biomedical Problems (IBMP) before they can be provided to astonauts in the international Space Station (ISS). In this article, the procedures and results of shelf-life test for certification of Korean space foods by IBMP are described.

MATERIALS AND METHODS

The seventeen Korean space foods are prepared in a laboratory in KAERI. *Kimchi*, *Bulgogi*, and grilled chicken were made as ready-to-eat space foods packaged in metal can. *Ramen*, *Sujeonggwa*, *Bibimbap*, seaweed soup, mulberry beverage, manila clam porridge, sweet pumpkin porridge, chicken curry-rice, chicken porridge, and ox leg-bone-cabbage soup were freeze-dried for rehydration with the water supplied in the ISS. *Saengshik* bar, mulberry jam, dried persimmon chocolate, and dried blueberry were packaged in polymeric bags (Fig. 1A).

Thirty one samples of each food were irradiated in a cobalt-60 gamma irradiator (AECL, IR-79, Nordion, Ottawa, Canada) and provided to IBMP for certification tests. The source strength was approximately 320 kBq with a dose rate of 10 kGy/h and the actual doses were within 2% of the target dose. The absorbed dose was monitored with both free-radical and ceric/cerous dosimeters. *Kimchi*, *Bulgogi*, and grilled chicken were gamma irradiated at 25, 40, and 20 kGy, respectively, to prevent the cans from swelling because of the gas generated by microorganisms in the foods, whereas the other foods were gamma-irradiated at less than 10 kGy.

The shelf life of the foods was evaluated by the microbiological tests and organoleptic assessment of the foods under space-simulating conditions for 51 days. Briefly, the space foods were preserved in the climatic chamber for 21 days at approximately 20°C and 30 days under dynamic temperature condition at 25°C (27 days), 30°C (24 hours), and 35°C (48 hours). These temperature conditions are

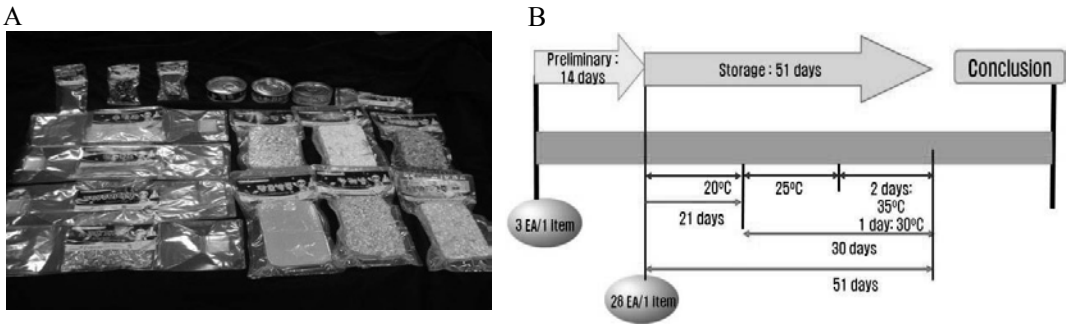

Fig. 1 - Korean space foods sterilized by gamma irradiation (A) and the procedure of certification test (B).

similar to those experienced in a space-flight environment. During the shelf-life test, the food products were also assessed for change in their organoleptic properties such as taste, color, odor, and texture using a 5-grade scale (Fig. 1B).

RESULTS AND CONCLUSION

Both safety and taste are important considerations in the development of space foods for astronauts in space. Because food-poisoning by harmful microorganisms can be fatal, space foods must meet rigid microbiological specifications (Bourland, 1993).

The microbiological test on the seventeen Korean space foods was performed in accordance with the Russian "rules concerning microbiological requirements to the quality and development of the products for cosmonauts". Microbial requirements of space foods vary with the type of food. The results of microbiological test after 51 days of storage in a chamber with fluctuating temperature are shown in Table 1.

Kimchi, Bulgogi, Dakgalbi packaged in metal can were confirmed as sterilized. *Kimchi* contains many fermentative microorganisms, especially lactic acid bacteria, and package-swelling from the CO_2 gas is an integral risk factor. Therefore, inactivation of all fermentative microorganisms is essential for the preparation of *Kimchi* as a space food (Song *et al.*, 2009). *Ramen* and *Saengshik* bar were failed to be sterilized, but the number of microorganisms in these foods was below the regulatary standards. Microorganisms were not detected in the other foods after storage for 51 days in the chamber with fluctuating temperature.

During the test period, we observed no changes in the organoleptic properties of any of the Korean space foods that were tested (data not shown).

In conclusion, for all the Korean space foods tested, no changes in the organoleptic properties and no increase in the number of microorganisms were observed, and all the tested foods met the regulatory standards of Russian rules. The 17 Korean food products were certified by the IBMP for use in space-flight conditions for up to 30 days after their delivery on board.

Table 1 - Microbiological test of Korean space foods after storage for 51 days in a chamber with fluctuating temperature.

Food products	Determined indicators	Test result	Legitimate value
Kimchi	Mesophilic aerobes	Absent in 2 g	Not allowed in 2 g
	Mesophilic anaerobes	Absent in 5 g	Not allowed in 5 g
Bulgogi	Mesophilic aerobes	Absent in 2 g	Not allowed in 2 g
	Mesophilic anaerobes	Absent in 5 g	Not allowed in 5 g
Dakgalbi	Mesophilic aerobes	Absent in 2 g	Not allowed in 2 g
	Mesophilic anaerobes	Absent in 5 g	Not allowed in 5 g
Ramen	Mesophilic aerobes	3 CFU in 1 g	< 5x103 CFU/g
	Coliform bacteria	Absent in 1 g	Not allowed in 1 g
	Mold fungi	< 10 CFU/g	< 50 CFU/g
	Bacillus cereus	< 3 CFU/g	< 10 CFU/g
Sujeonggwa	Mesophilic aerobes	< 3 CFU in 1 g	< 1x103 CFU/g
	Coliform bacteria	Absent in 1 g	Not allowed in 1 g
	Mold fungi	Absent in 1 g	Not allowed in 1 g

(continued)

Food products	Determined indicators	Test result	Legitimate value
	Escherichia coli	Absent in 10 g	Not allowed in 10 g
Bibimbap	Mesophilic aerobes	< 3 CFU in 1 g	< 5x103 CFU/g
	Coliform bacteria	Absent in 1 g	Not allowed in 1 g
	Mold fungi	< 10 CFU/g	< 50 CFU/g
	Bacillus cereus	< 3 CFU/g	< 10 CFU/g
Seaweed soup	Mesophilic aerobes	< 3 CFU in 1 g	< 5x103 CFU/g
	Coliform bacteria	Absent in 1 g	Not allowed in 1 g
	Mold fungi	< 10 CFU/g	< 50 CFU/g
	Bacillus cereus	< 3 CFU/g	< 10 CFU/g
Mulberry beverage	Mesophilic aerobes	< 3 CFU in 1 g	< 1x103 CFU/g
	Coliform bacteria	Absent in 1 g	Not allowed in 1 g
	Mold fungi	Absent in 1 g	Not allowed in 1 g
	Escherichia coli	Absent in 10 g	Not allowed in 10 g
Manila clam porridge	Mesophilic aerobes	< 3 CFU in 1 g	< 5x103 CFU/g
	Coliform bacteria	Absent in 1 g	Not allowed in 1 g
	Mold fungi	< 10 CFU/g	< 50 CFU/g
	Salmonella bacteria	Absent in 25 g	Not allowed in 25 g
	Bacillus cereus	< 3 CFU/g	< 10 CFU/g
Sweet pumpkin porridge	Mesophilic aerobes	< 3 CFU in 1 g	< 5x103 CFU/g
	Coliform bacteria	Absent in 1 g	Not allowed in 1 g
	Mold fungi	< 10 CFU/g	< 50 CFU/g
	Salmonella spp.	Absent in 25 g	Not allowed in 25 g
	Bacillus cereus	< 3 CFU/g	< 10 CFU/g
Chicken curry-rice	Mesophilic aerobes	< 3 CFU in 1 g	< 5x103 CFU/g
	Coliform bacteria	Absent in 1 g	Not allowed in 1 g
	Mold fungi	< 10 CFU/g	< 50 CFU/g
	Salmonella spp.	Absent in 25 g	Not allowed in 25 g
	Bacillus cereus	< 3 CFU/g	< 10 CFU/g
Chicken porridge	Mesophilic aerobes	< 3 CFU in 1 g	< 5x103 CFU/g
	Coliform bacteria	Absent in 1 g	Not allowed in 1 g
	Mold fungi	< 10 CFU/g	< 50 CFU/g
	Salmonella spp.	Absent in 25 g	Not allowed in 25 g
	Bacillus cereus	< 3 CFU/g	< 10 CFU/g
Ox leg-bone-cabbage soup	Mesophilic aerobes	< 3 CFU in 1 g	< 5x103 CFU/g
	Coliform bacteria	Absent in 1 g	Not allowed in 1 g
	Mold fungi	< 10 CFU/g	< 50 CFU/g
	Salmonella spp.	Absent in 25 g	Not allowed in 25 g
	Bacillus cereus	< 3 CFU/g	< 10 CFU/g
Saengshik bar	Mesophilic aerobes	4 CFU/g	< 1x102 CFU/g
	Coliform bacteria	Absent in 1 g	Not allowed in 1 g
	Mold fungi and yeast	Absent in 1 g	Not allowed in 1 g
Mulberry jam	Mold fungi and yeast	Absent in 2 g	Absent in 2 g
Dried persimmon chocolate	Mesophilic aerobes	< 3 CFU in 1 g	< 5x103 CFU/g
	Coliform bacteria	Absent in 1 g	Not allowed in 1 g
	Mold fungi	< 10 CFU/g	< 50 CFU/g
	Salmonella bacteria	Absent in 25 g	Not allowed in 25 g
Dried blueberry	Mesophilic aerobes	< 3 CFU in 1 g	< 5x103 CFU/g
	Coliform bacteria	Absent in 1 g	Not allowed in 1 g
	Mold fungi	< 10 CFU/g	< 50 CFU/g
	Salmonella spp.	Absent in 25 g	Not allowed in 25 g
	Bacillus cereus	< 3 CFU/g	< 10 CFU/g

ACKNOWLEDGEMENTS

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STRESS-CRACKING OF PET BOTTLE AS AN UNPREDICTABLE FACTOR LIMITING THE COMMERCIAL LIFE OF CARBONATED SOFT DRINKS

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ABSTRACT

The shelf life of carbonated beverages mainly depends on the carbon dioxide retention performances of the PET bottle, however the commercial life of such products can be compromised by ruptures and crackings of the material, which can occur in certain conditions. The so-called "stress cracking" of PET bottles occurs in correspondence with the injection point, which is more susceptible due to its amorphous nature, unlike the sides of the bottles, whose strength is a result of a certain degree of orientation. Stress cracking can be a consequence of various factors, which usually act in combination and are difficult to isolate: excessive internal pressure (excess carbonation), material distribution, altered intrinsic viscosity, exposure to UV rays and/or high temperatures, but most important is the contact with caustic substances (for example, the lubricant of the conveyors belts), etc. The present contribution focuses on a case study occurred at SIBEG s.r.l., concerning the non conformity of 1.5 L PET bottles of a popular carbonated soft drink, which caused the loss of an important amount of bottles, the disposal of defective products, and the careful inspection and selection of in-house stocks. Inspections revealed no failure in the blowing process, leading to hypothesize over-carbonation and transport conditions as the triggering events. Moreover, the analysis of intrinsic viscosity (IV) of bottles and preforms revealed the non-conformity with specification. Despite the product conformity concerning thermal stability, burst test and stress cracking test performed in alkaline conditions, PET bottles quality and performances may become altered if the IV of the material does not fulfill specifications. There-

fore, the IV determination plays a key role, complementary with other routine determinations, in the quality control of PET bottles addressed to carbonated soft drinks packaging. The use of such tool is fundamental for the prevention of severe problems which might occur along the distribution chain, such as claims, withdrawal and disposal of defective products.

Key words: Environmental stress-cracking (ESC), intrinsic viscosity (IV), over-carbonation, quality control, PET.

INTRODUCTION

The shelf life of carbonated beverages mainly depends on the CO_2 retention performances of the PET bottle. CO_2 starts decreasing just after bottling due to permeation through the packaging material, hence it is clear how the improvement of the PET bottle performance represents a competitive tool for the carbonated soft drinks industries and a means to guarantee the consumers with the original characteristics and quality (Coriolani *et al.*, 2007; Licciardello *et al.*, 2011). However, the quality of PET bottles is not only related with an adequate barrier to CO_2. Indeed, the commercial life of such products can be compromised by ruptures and crackings of the material occurring in certain conditions. The so-called "Environmental Stress Cracking" (ESC) of PET bottles is responsible for about 15% of the overall problems concerning packaging materials for carbonated beverages (Demirel and Daver, 2009). ESC occurs in correspondence with the injection point, which is more susceptible due to its amorphous nature, unlike the sides of the bottles, whose strength is a result of a certain degree of orientation. The problem of ESC appeared in the carbonated soft drink world with the introduction of "one piece" (or "stand-alone") bottles, typically with a petaloid base.

ESC can be a consequence of various factors, which usually act in combination and are difficult to isolate: excess carbonation, material distribution, altered intrinsic viscosity, exposure to UV rays and/or high temperatures, contact with caustic substances (for example, the lubricant of the conveyors belts), etc.

The present contribution focuses on a case study occurred at SIBEG s.r.l., concerning the non conformity of 1.5 L PET bottles of a popular carbonated soft drink, which caused the loss of an important amount of bottles, the disposal of defective products, and the careful inspection and selection of in-house stocks. The conventional determination performed on preforms and bottles could not be put in relation with the phenomenon, hence further investigations were carried out, with special regards for the intrinsic viscosity (IV) determination.

MATERIALS AND METHODS

Sibeg s.r.l routinely performs analyses on the preforms (*incoming inspection*), while other tests on bottles are performed at every change (design, volume etc.) of the package in order to verify the correspondence to standard specifications. The following tests were carried out on bottles in order to very their compliance with specifications given by The Coca-Cola Company: visual inspection; burst

test; thermal stability; stress cracking. All analyses were performed according to Guidelines by The Coca-Cola Company (Packaging Authorization for Non-Refillable Plastic Bottles).

In addition, the intrinsic viscosity (IV) of PET preforms was evaluated according to the standard method ASTM D 4603, with a phenol/tetrachloroethane solution (60/40 v/v) at 30°C.

RESULTS

The burst test performed on 12 bottles revealed the perfect conformity with standards, as the minimum and average values obtained were 11.59 and 12.52 bar, respectively, compared with 6.8 and 8.6 bar, which is the standard requirement. All of the 12 bottles sampled for visual inspection complied with requirements.

The stress cracking test carried out on twenty-four, 1.5-litre bottles coming from 2 different blowing lines, and on eight, 0.5-litre bottles, did not evidence content losses or bursts before 10 minutes, which is the minimum threshold. Smaller bottles recorded the lowest times, with minimum 11 min and average 12.5 min, while 1.5-litre bottles scored minimum 13 min (1 sample out of 24) and an average value of 16.8 min, which is far more than the specified standard.

Samples belonging to the batch where bottle crackings occurred, complied with requirements, as far as burst and stress cracking tests and visual inspection were concerned. Basing on the above tests, the occurring crackings to bottles could not be explained. For this reason further analyses were considered necessary. Intrinsic viscosity determination was carried out on preforms which produced defective and regular bottles, respectively. The IV test revealed the non-conformity for the first batch (Fig. 1), while the regular batch fell in the tolerance range for this parameter.

Further IV analyses were performed on three different preforms and on the corresponding bottles in order to select the most suitable products which could guarantee the company from future ESC episodes. Fig. 2 shows that the blowing process does not substantially alter the IV of preforms, but highlights significant differences in IV among different preform batches.

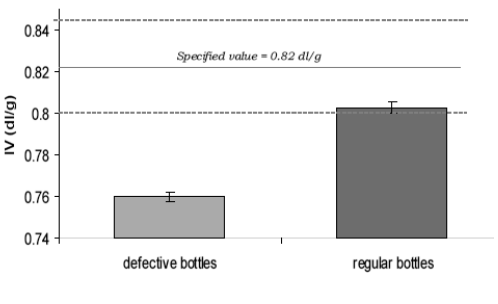

Fig. 1 - Intrinsic viscosity (IV) measured in bottles from a defective and regular batch.

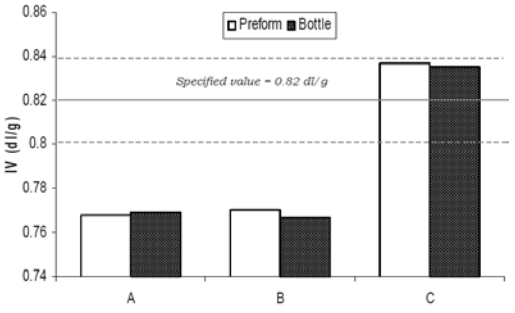

Fig. 2 - Intrinsic viscosity (IV) of preforms and bottles from different suppliers.

CONCLUSIONS

Stress cracking does not occur in normal conditions, but over-carbonation and extreme transport conditions might represent triggering events. Despite the product conformity concerning thermal stability, burst test and stress cracking test performed in alkaline conditions, PET bottles quality and performances may result altered if the intrinsic viscosity of the material does not fulfil specifications. The intrinsic viscosity determination plays a key role, complementary with other routine determinations, in the quality control of PET bottles addressed to carbonated soft drinks packaging. The use of such tool is fundamental for the prevention of severe problems which might occur along the distribution chain, such as claims, withdrawal and disposal of defective products.

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STRATEGIES FOR THE EXTENSION OF THE SHELF LIFE OF READY TO EAT PRICKLY PEAR FRUITS

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ABSTRACT

The prickly pear fruit (*Opuntia ficus indica* L. Miller) belongs to the Cactaceae family. The fruit is a berry, composed by an epicarp and the pulp, which represents the edible portion. At maturation, the epicarp turns yellow, red or white, depending on the cultivar. In Italy, the prickly pear is mainly cultivated in Sicily (90% of the national production). The fruit is very sensitive to low storage temperatures (< 5°C) which cause chilling injuries. The fruits can be successfully commercialized as a ready-to-eat product, peeled and suitably packaged. The main limit to its production is the formation of off-flavours due to different factors, such as the growth of microorganism and the action of endogenous enzymes (lipid oxidation). In fact, the oxidoreductases are directly responsible for the lipid oxidation, which has influence on the production of off-flavours, on the structure and on the shelf-life of the fruit. The lipoxygenase (LOX) is a dioxygenase which catalyzes the oxidation of polyunsaturated fatty acids to hydroperoxides. The aim of this work was to compare different packaging technologies to extend the shelf life of ready-to-eat prickly pear fruits. The LOX activity, microbial counts and gas composition were evaluated for non-treated samples packed in ordinary atmosphere and in two modified atmospheres having different O_2 and CO_2 composition (MA1 5% O_2, 2% CO_2, 93%N_2; MA2 2% O_2, 5% CO_2, 93% N_2) and for samples treated either with a blanching or with a blanching followed by a dipping in a citric acid solution. The pretreatment conditions are essential for the LOX activity, in particular the blanching reduced its activity by at least 30% especially in combination with fruit acidification and limited the microbial proliferation. As a result of suitable pretreatment and packaging operations the shelf life of ready-to-eat prickly pears can be successfully extended.

Key words: Blanching, LOX, packaging, prickly pear, shelf life.

INTRODUCTION

Appearance, aroma, consistance and nutritional value are the four main aspects considered by consummers in the choice of a food product. The packaging of minimally processed fruits and vegetables has two main goals: on one hand, to maintain the freshness of the produce without compromising its nutritional and sensory qualities, on the other hand, to obtain a shelf life sufficient to allow distribution and consumption in different geographical areas.

The issue of enzymatic degradations in minimally processed produces is of primary importance. The eventual rupture of cellular structures during processing causes the outflow of cellular components such as enzymes, substrates, metabolites and reserve substances, and the subsequent contact between enzymes and substrates, with an enhanced decay of the quality characteristics of the product. Prickly pear fruits are highly perishable at room temperature and are highly sensitive (chilling injuries) to refrigerated storage temperatures (0-4°C) (Di Cesare *et al.*, 1993). The main limits to storability of prickly pear fruits, indeed, are the loss of consistency and the formation of off-flavours which are due to different factors, such as microbial proliferation, oxidation processes and cellular degradation by endogenous enzymes. Oxidation is the main cause of the off-flavour production in foods containing fatty acids, even during storage at low temperatures.

A recent literature survey has highlighted a lack of researches concerning the study of the enzymatic activities of prickly pear fruits, while no study at all has been carried out on minimally processed prickly pears. The aim of the research was to evaluate suitable packaging solutions for minimally processed prickly pears and to study the shelf life of such product taking into account microbiological and enzymatic aspects.

MATERIALS AND METHODS

Prickly pear fruits were harvested in the area of Paternò, in the province of Catania, Italy. Fruits were manually peeled and packaged in ordinary atmosphere with a barrier film (***Control***) (PET 30 Melinex 850, permeability O_2 cc/m2/24h: 56, permeability H2O g/m2/24h: 13) and in two modified atmospheres having different O_2 and CO_2 composition (***MA1*** 5% O_2, 2% CO_2, 93% N_2; ***MA2*** 2% O_2, 5% CO_2, 93% N_2) (Cantwell *et al.*, 1995, Izumi *et al.*, 1996). Also, a part of the samples was treated with a blanching in water at 80°C for 10 min (***barrier+blanch***) or in water+ 2% citric acid (***barrier+blanch+citric ac***.) before packaging in ordinary atmosphere. Each tray contained four fruits. Microbial counts were determined by the official Oxoid methods and lipoxygenase activity was assessed by the colorimetric method by Gordon *et al.* (2001).

RESULTS AND DISCUSSION

Fig. 1 shows the gas composition in the package headspace. It can be inferred that O_2 in ***MA2*** packages decreases to not detectable levels after 3 days of refrigerated storage, while the same happens after 6 days for ***MA1***. A consequent increase of the CO_2 level was observed. Fig. 2 shows the gas trend inside packages containing fruits which had undergone blanching, in this case the O_2 level is close to zero

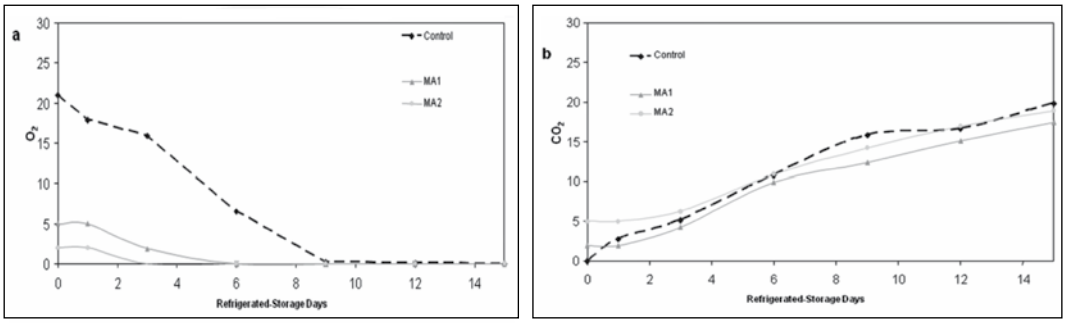

Fig. 1 - Headspace gas composition variations in packages with ordinary and modified atmosphere O2 (a) and CO2 (b).

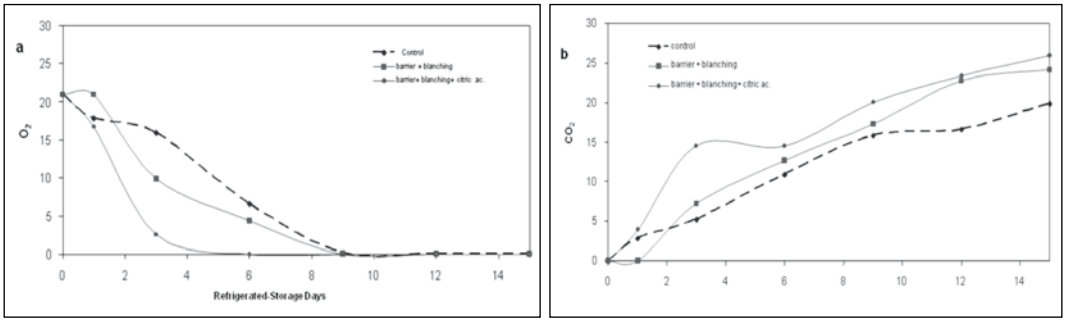

Fig. 2 - Headspace gas composition variations inside packages, as a function of pre-treatment: O_2 (a) and CO_2 (b).

after 9 days, while in fruits subject to blanching with ascorbic acid the same level is reached after 6 days. The CO_2 level increased up to about 26%.

Fig. 3 shows the microbiological counts for packed fruits during refrigerated storage. The modified atmospheres (***MA1*** and ***MA2***) determined only a slight decrease of the total bacterial counts (PCA), but a significative decrease of yeasts and molds (SAB).

Fig. 4 shows the time course for the LOX activity in the different packaging sys-

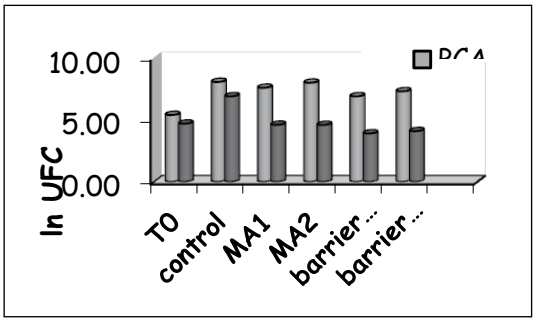

Fig. 3 - Total bacterial counts (PCA) and yeast and molds (SAB) in fresh and stored (12 days), yellow-flesh prickly pears.

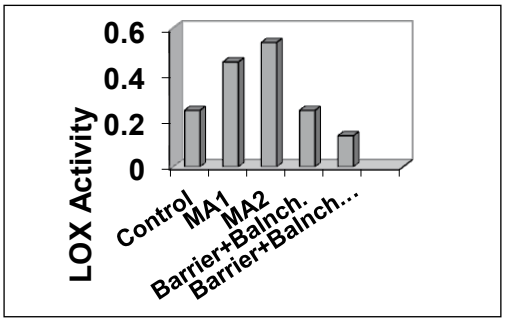

Fig. 4 - Total LOX activity during 12 days of storage in differently packed fruits, expressed as the curve integral.

tems. The enzyme was inhibited with the thermal treatment, while the citric acid treatment does not seem to determine significant variations.

The MA-packed samples showed a higher lipoxygenase activity, which is probably due to the higher presence of O_2, which slowly decreases with storage together with the increase of the CO_2 level.

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DEVELOPMENT OF COLOR INDICATOR TAG FOR MONITORING FRESHNESS OF INTERMEDIATE-MOISTURE DESSERT

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ABSTRACT

A color indicator tag based on dyes mix has been developed to monitor and indicate the shelf life of packaged traditional Thai dessert *"Thong-EK"*. At a mixing dyes ratio of Methyl red/Bromothymol blue 2:1 the maximum total color difference (TCD) in polyvinyl alcohol (PVOH) color indicator tag was observed. Visible color changes of tag correlated efficiently with carbon dioxide (CO_2) level which is a volatile spoilage metabolite from microbial growth. The in-package monitoring tests on tag at different packaging systems and storage conditions showed that the tags responsively tracked the increase in CO_2 level in the headspace of heat sealed multilayer aluminum pouch, heat sealed PP pouch and stapled PP pouch stored at 25 and 35°C. The response was also found to correlate to changing microbial counts (total viable count and yeast/mold count) which indicated the shelf life of packaged desserts were 4 days at 25°C and 3 days at 35°C, respectively.

Key words: Freshness, intelligent packaging, CO_2, intermediate-moisture dessert, shelf life.

INTRODUCTION

Control and monitor of food properties is critical to the shelf life and food safety especially for ready-to-eat packaged products. The increase in consumer demand on health awareness and confidence with regards to packaged food products and threat from contamination of pathogens has created a vast opportunity for application of advanced packaging technology. Freshness indicator, one of the emerging intelligent packaging technologies, that monitors the condition of packaged foods to give information regards to packaged food quality during transportation and storage.

The indicator is usually based on the detection of chemical changes related to the microbial growth in food products. In Thailand, the interest in the use of freshness indicator has increased in recent years but there is insufficient data to support the performance of freshness indicator in various packaged Thai food products. A traditional Thai dessert *"Thong-EK"* is wheat flour sweet with eggs yolks classified as the intermediate moisture foods (IMF) which are semi-moist with moisture content and water activity (Aw) in the region of 15-50% and 0.60-0.85 respectively. The sort of sweetmeat that is traditionally prepared for the propitious occasions is readily susceptible to microbial deterioration resulting in a short shelf life due to the enrichment of nutrients. The objective of this study is to develop a color indicator tag for detecting the freshness of Thai dessert and to investigate the effect of packaging systems and storage conditions on the performance of color indicator tag.

MATERIALS AND METHODS

Freshly made commercial *Thong-EK* dessert was obtained from a traditional Thai dessert shop just after production. A dessert was 10 g each individually wrapped in candy wrapping paper and packaged in heat sealed OPP pouch contained 250 g of product.

Characterization of Thai dessert *"Thong-EK"*

Thong-EK samples were packaged in heat sealed multilayer aluminum pouch and stored at 25 and 35°C. Then, pH, Aw, moisture, color change (ΔE), texture analysis (Stable micro system: TA-XT2i), brix, microbiological analysis (TVC, Yeast & Mold), CO_2 gas (portable gas analyzer OXYBABY®) were determined regularly until the end of shelf life.

Effect of mixing dyes ratio on the maximum total color difference (TCD)

Indicator solutions contained mixing dyes ratio of 0.1% Methyl red/ 0.1% Bromothymol blue 1:1, 1:2, 2:1, 2:3 and 3:2 were homogenized at 5,000 rpm with 6% (w/v) PVOH (BF-17) for 10 min and adjusted pH by 0.1% $NaHCO_3$. The coating solution was then coated on PVC sheet (100μm thick) using 50 g/m^2 coating weight and a coated sheet was air-dried at 40°C for 6 hr. Indicators tags were enclosed with CO_2 gas 1.0, 1.6, 2.1, 2.5, 3.0, 5.1 and 7.1% (v/v) in heat sealed multilayer aluminum pouch. Color change of indicator tag was analytically determined by spectrophotometer "Color Quest XT" and ΔE was then calculated.

Effect of packaging systems and storage conditions on color change of indicator tag

Indicator tags at ratio 2:1 (selected from previous part) were prepared and packaged with *Thong-EK* (100 g) in heat sealed multilayer aluminum pouch, heat sealed PP pouch and stapled PP pouch. All packaging systems were stored at 25 and 35°C. Then, CO_2 gas, ΔE and microbiological analysis (TVC, Yeast & Mold) were examined daily until the end of shelf life.

RESULTS AND CONCLUSION

Characterization of Thai dessert *"Thong-EK"*

The initial moisture content and Aw (Table 1) indicated that a product contained

very high moisture content which provided high active moisture. Besides, pH and °Brix offered decent conditions for promoting the microbial growth during storage. The microbial count was not detected at the beginning of storage. Although the moisture content and Aw were only slightly increased after storage, the shelf life of product packaged in high moisture barrier (aluminium pouch) was only 4 days at 25°C and 3 days at 35°C. The critical index of the end of shelf life was the microbial growth which was over the limit of microbiological standard (Nopwinyuwong *et al.*, 2010). Color changes were found to be pronounced in *Thong-EK*, the product gradually became darker. Total color change was significantly affected by storage conditions. It should be noted that the non-enzymatic browning might have influenced the variation of color changes. Hardness of texture significantly increased in higher storage temperature because the moisture in product changed to vapour phase in the headspace. Stickiness of texture significantly increased in lower temperature. The shelf life of *Thong-EK*, therefore, was substantially affected by intrinsic factors.

Effect of mixing dyes ratio on the maximum total color difference (TCD)

Total color change (TCD) or ΔE was calculated based on the L*a*b* measurements on indicator tags prepared from various mixing dyes ratios of Methyl red/ Bromothymol blue. Methy red shlfts from basic form (yellow, pH 6.2) to acidic form (red, pH 4.5) and Bromothymol blue changes from basic form (blue, pH 7.6) to acidic form (yellow, pH 5.8) (Nopwinyuwong *et al.*, 2010). Color change of indicator tags corresponded with change of CO_2 level. The 2:1 mixing dyes ratio exhibited the most response in ΔE when exposed to the CO_2 changes (Fig. 1). In addition, ΔE of the 2:1 ratio was linearly increased corresponding with the increase of CO_2 level (1-7.1%) as shown in Fig. 2. The indicator tag was initially blue and gradually turned to green-yellow, green and finally yellow.

Table 1 - Characterization of *Thong-EK* during storage conditions.

Properties	***Day 0***	***End of shelf life***	
		Day 4 25°C	**Day 3 35°C**
pH	6.95 ± 0.00	6.90 ± 0.01	6.90 ± 0.00
Aw	0.859 ± 0.008	0.870 ± 0.008	0.867 ± 0.009
Moisture (%)	21.85 ± 0.23	23.15 ± 0.17	23.27 ± 0.34
ΔE	-	10.11 ± 1.28	12.40 ± 2.59
Texture			
♦Hardness (g)	77.157 ± 1.31	82.397 ± 1.99	105.946 ± 2.15
♦Stickiness (g)	3.214 ± 0.28	20.694 ± 1.42	6.306 ± 5.05
°Brix	52 ± 0.00	50 ± 0.00	51 ± 0.00
Microbiological analysis			
♦TVC (log cfu/g)	ND	5.86 ± 0.08	5.94 ± 0.09
♦Yeast & Mold (log cfu/g)	ND	2.14 ± 0.08	2.08 ± 0.08
Note: microbiological standard: TVC 6.00 log cfu, yeast & mold 2.70 log cfu/g.			

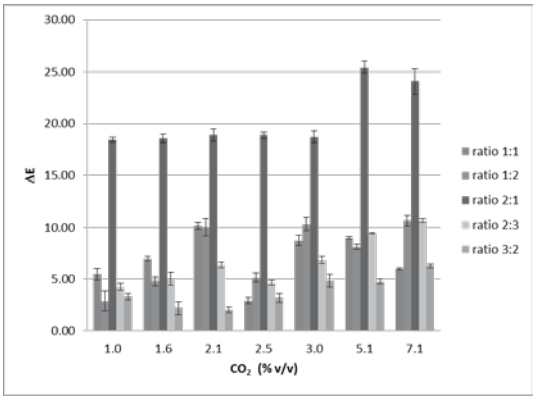

Fig. 1 - Response of TCD of indicator tags on CO_2.

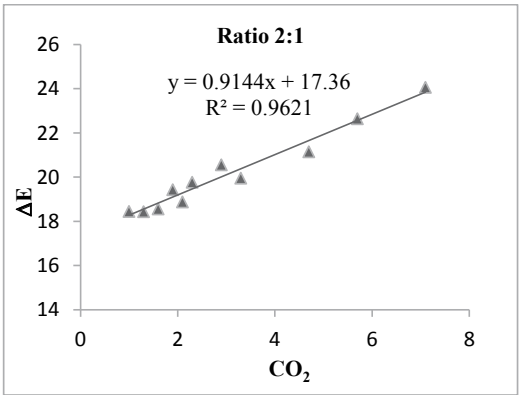

Fig. 2 - Response of TCD of indicator tag 2:1 ratio on CO_2.

Effect of packaging systems and storage conditions on color change of indicator tag

In order to determine the relationship between the microbial growth and the CO_2 concentration in the headspace of packages, the indicator tags prepared from 2:1 ratio were packaged with *Thong-EK* samples in heat sealed multilayer aluminium pouches. The microbial spoilage and CO_2 change were monitored at 25 and 35°C. The results that the total viable count, yeast & mold and CO_2 gradually increased over time suggested the correlation between the microbial growth and CO_2 level in headspace (Table 2). Furthermore, at higher temperature, the microbial growth tended to increase faster and produce higher CO_2 level. CO_2 is generally recognized as a volatile metabolite generated from microbial growth during food deterioration (Jung *et al.*, 2012; Puligundla *et al.*, 2012). Besides, CO_2 in the headspace rapidly dissolves in water and moisture vapour in the package, thereby lowering the pH by formed carbonic acid which reacts with the sensitive pH dyes mix embedded on the indicator tag. Figs. 3 and 4 showed CO_2 levels in the headspace of three sets of packaging systems during storage time. It was found that heat-sealed mul-

Table 2 - Microbial counts and CO_2 gas in headspace.

Temperature (°C)	Time (day)	Bacteria (LogCFU/g)	Yeast/mold (LogCFU/g)	CO_2 (%v/v)
25	0	ND	ND	0.00 ± 0.00
	1	2.42 ± 0.07	1.81 ± 0.07	0.10 ± 0.004
	2	4.64 ± 0.08	1.84 ± 0.08	0.12 ± 0.002
	3	5.60 ± 0.07	2.06 ± 0.07	0.26 ± 0.004
	4	5.86 ± 0.08	2.14 ± 0.08	0.34 ± 0.005
	5	6.37 ± 0.07	2.96 ± 0.07	0.92 ± 0.007
35	0	ND	ND	0.00 ± 0.000
	1	2.42 ± 0.07	1.81 ± 0.07	0.10 ± 0.004
	2	5.15 ± 0.07	2.04 ± 0.07	0.13 ± 0.003
	3	5.94 ± 0.09	2.08 ± 0.08	0.32 ± 0.005
	4	6.60 ± 0.07	2.22 ± 0.07	0.45 ± 0.006
	5	7.32 ± 0.08	3.79 ± 0.08	1.42 ± 0.007

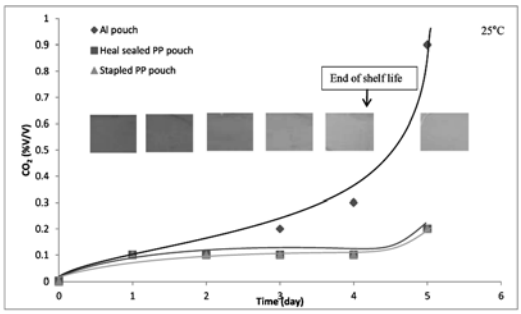

Fig. 3 - Change of CO_2 in *Thong-EK* enclosed with indicator tag at 25°C.

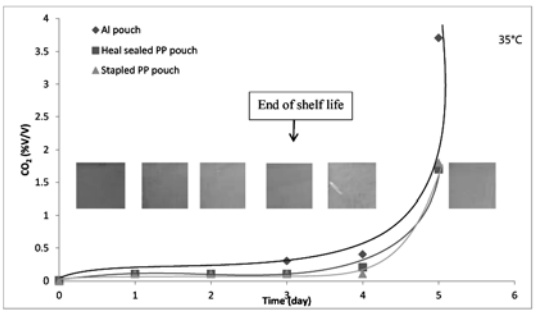

Fig. 4 - Change of CO_2 in *Thong-EK* enclosed with indicator tag at 35°C.

tilayer aluminium pouch which was considered the highest gas barrier compared to others, exhibited highest CO_2 level in headspace. In addition, CO_2 level at 35°C storage temperature was higher than that at 25°C as a result of shorter shelf life. Although packaging systems tested had different gas tight performance, no significant difference in visible response of TCD among sets of packaging systems was observed. Therefore, the color indicator tag prepared from dyes mix of Methyl red and Bromothymol blue is capable to offer real time visual monitoring freshness of intermediate dessert during in-package storage test. The increase in CO_2 is associated with the microbial growth and the most remarkable of TCD of indicator tag is observed at a mixing dyes ratio of 2:1. A comparison of the results from packaging systems and storage temperature suggested that the color indicator tag developed is able to track and provide a fast response on the shelf life of IMF packaged desserts.

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EXPLOITATION OF INDICATORS OF EGG PRODUCTS AND MILK PRODUCTS FOR THE ESTABLISHMENT OF SHELF-LIFE

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ABSTRACT

This study estimated the shelf–life of both egg and milk products. Egg powder product was subjected to an accelerated test, whereas a fermented milk product was subjected to an actual test. The shelf–life of egg powder was observed to be 13.67 months. Furthermore, the shelf–life of fermented milk was observed to be at 19 days at 10°C, 14 days at 15°C, and 12 days at 25°C. The shelf–life can be established by multiplying by a safety factor (< 1). Therefore, these methods offer the possibility to develop a guideline to determine the shelf–life of livestock products.

Key words: Acceleration test, egg products, indicator, milk products, shelf–life.

INTRODUCTION

The shelf–life is referred to as the "Sell-by Date" in Korea. According to surveys, Korean consumers stated that shelf–life is the most important piece of information and is typically checked before a purchase. Shelf–life is a food guide that determines the storage period in which food can be safely kept before its deterioration.

The shelf–life of general foods (i.e., agricultural products and processed foods) is enforced by several indicators and guidelines; however, the shelf–life of livestock

products is not yet available in Korea. Thus, the purpose of this study was to exploit indicators of both egg and milk products to establish their shelf–life.

MATERIALS AND METHODS

Shelf–life of regular egg powder is 1–2 years, determined by an accelerated test. Samples of egg powder were kept at 15, 25, 35, and 45°C. The accelerated test was assessed by pH, acid value, peroxide value, VBN (volatile basic nitrogen), and overall sensory evaluation. Accelerated test were performed at 6 day intervals over the course of 3 months. The indicator kinetics was calculated using the following equation (Eq. 1).

$$\begin{aligned} \text{Zero-order kinetic} \rightarrow -\frac{dA}{dt} = KA^{n} \rightarrow t = \frac{[A_0 - A_t}{K} \\ \text{First-order kinetic} \rightarrow -\frac{dA}{dt} = KA^{n} \rightarrow t = \frac{\ln[A_0] - \ln[A_t]}{K} \end{aligned} \quad (1)$$

where A is the quality indicator, t is the storage period, K is reaction rate constant, n is reaction order, dA/dt is the change of the A according to the storage period, A_0 is the initial A, and A_t is the A according to t.

The impact of temperature on the rate of physical-chemical, microbiological and sensory characteristics was determined using the Arrhenius equation (Eq. 2).

$$K = Ae^{-Ea/RT} \rightarrow \ln K = -\left(\frac{Ea}{R}\right) \times \left(\frac{1}{T}\right) + \ln A \rightarrow \ln K = \frac{S}{T} + I \rightarrow K = e^{(S/T)+1} \quad (2)$$

where A is the Arrhenius constant, Ea is the activation energy(cal/mol), R is the gas constant(1.987 cal/mol), T is the temperature (=°C+273), K is the reaction rate constant, S is the slope, and lnA and I are the intercept.

The shelf–life of egg powder was calculated using a linear regression equation and the Arrhenius equation (Eq. 3).

$$\begin{aligned} \text{Zero-order kinetic} \rightarrow t = \frac{A_0 - A_t}{K'} \rightarrow t = \frac{A_0 - A_t}{e^{\frac{S}{T}+I}} \\ \text{First-order kinetic} \rightarrow t = \frac{\ln [A_0] - \ln [A_t]}{K'} \end{aligned} \quad (3)$$

where K′ is annual reaction rate constant.

Fermented milk products were subjected to an actual test. Samples of fermented milk products were kept at 10, 15, and 25°C. Actual tests were started the day after samples arrived and were performed at 3 day intervals over the course of 25 days.

The quality limit of overall sensory evaluation was established at 5 points on a 9 point hedonic scale (Meilgard *et al.*, 1991). Legal quality limits were established based on the "Process Criteria and Ingredient Standards of Livestock Products" published by the Animal, Plant and Fisheries Quarantine and Inspection Agency in Korea. In contrast, non-legal quality limits were calculated by regression equations between the overall sensory evaluation and the non-legal quality criteria.

The limit day of each quality criterion was defined as the day when each of the samples reached the quality limit for each of the quality criteria. If R^2 of the regression equation was less than 0.9, non-legal quality criteria were excluded as quality criteria for establishment of the shelf–life.

RESULTS AND CONCLUSIONS

Linear regression equations of egg powder at 15, 25, 35, and 45°C is shown in Table 1. Changes followed an apparent zero-order kinetic. The shelf–life of egg powder was determined by the highest coefficient of determination (R^2) which was calculated from each indicator kinetic.

The estimated shelf–life of egg powder was found to be 13.67 months at 45°C according to our test results for pH (R^2=0.9921) (Table 2).

Table 1 - Linear regression equations was calculated at egg powder product according to storage temperatures.

Indicator	°C	Zero-order[1]		First-order[2]	
		Regression equation	R^2[3]	Regression equation	R^2
pH	15	y = -0.0026x + 9.1448	0.7731	y = -0.0003x + 2.2132	0.7738
	25	y = -0.0053x + 9.1247	0.9501	y = -0.0006x + 2.211	0.9507
	35	y = -0.0158x + 9.1502	0.9821	y = -0.0018x + 2.2145	0.9789
	45	y = -0.0315x + 9.1694	0.9921	y = -0.0038x + 2.2185	0.9894
Acid value	15	y = 0.0099x + 1.3101	0.9574	y = 0.0065x + 0.2738	0.9350
	25	y = 0.0092x + 1.3961	0.8729	y = 0.0058x + 0.3352	0.8384
	35	y = 0.0135x + 1.541	0.8599	y = 0.0074x + 0.4342	0.7977
	45	y = 0.0145x + 1.84	0.6597	y = 0.0072x + 0.5946	0.5574
Peroxide value	15	y = 0.0115x + 0.2175	0.9874	y = 0.0253x - 1.4352	0.9216
	25	y = 0.0125x + 0.2104	0.9911	y = 0.0261x - 1.4202	0.9481
	35	y = 0.0137x + 0.2226	0.9862	y = 0.0267x - 1.3671	0.9304
	45	y = 0.0139x + 0.2647	0.9769	y = 0.0252x - 1.2372	0.8963
VBN	15	y = 0.0296x + 9.8048	0.8135	y = 0.0029x + 2.2825	0.7941
	25	y = 0.0426x + 9.7292	0.8974	y = 0.004x + 2.277	0.8850
	35	y = 0.067x + 9.8719	0.9454	y = 0.0059x + 2.2931	0.9225
	45	y = 0.08x + 10.195	0.9321	y = 0.0066x + 2.3271	0.9040
Overall sensory evaluation	15	y = -0.0176x + 9.1911	0.6077	y = -0.0021x + 2.2197	0.6077
	25	y = -0.0383x + 9.3779	0.7281	y = -0.0048x + 2.2447	0.7246
	35	y = -0.0405x + 9.0156	0.9059	y = -0.0051x + 2.202	0.9075
	45	y = -0.0666x + 9.0871	0.9374	y = -0.0093x + 2.2203	0.9299

[1] Y=KX+B(X: storage period, Y: each quality criterion, K: reaction rate constant); [2] Y=KX+B(X: storage period, Y: lnA, B: lnA_0, K: reaction rate constant) (A: ln value according to storage period, A_0: ln value according to initial quality);
[3] Coefficient of determination

Table 2 - The shelf–life of egg powder product according to zero-order.

Indicator	Kinetic	R2 1)	A0–At2)	K'3)	Shelf–life (d)	Shelf–life (m)
pH	Zero-order	0.9921 (45°C)	1.70	1.49	416.44	13.67

1) Coefficient of determination; 2)A0: Initial quality, At: Quality standard; 3) Annual reaction rate constant

In the case of fermented milk product, the limit day of each quality criterion was established at 10, 15, and 25°C. The limit day of lactic acid bacterial counts was >25 days at 10, 15, and 25°C (Fig. 1a), whereas that of overall sensory evaluation was 16, 14, and 12 days at these temperatures, respectively (Fig. 1b). The limit days for titratable acidity(TA) were 19, 16, and 12 days (Fig. 1c), and those for pH value were 15, 14, and 12 days at 10, 15, and 25°C, respectively (Fig. 1d).

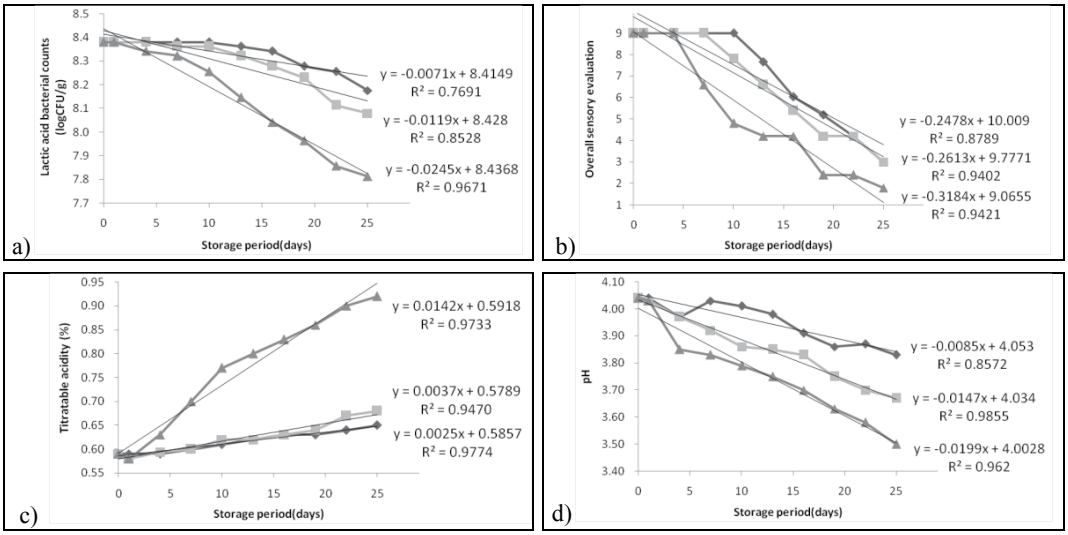

Fig. 1 - Regression equations between each quality criterion (y) and storage period (x) of fermented milk product at 10, 15, and 25°C. ◆: 10°C; ■: 15°C; ▲: 25°C.

Table 3 - Quality limit and limit day of fermented milk product at 10°C, 15°C, and 25°C.

	Quality criteria	Temperature	Quality limit	Limit day[4)]
Legal quality limit[1)] (Log CFU/mL)	Lactic acid bacterial counts	10°C	7	>25
		15°C	7	>25
		25°C	7	>25
	Overall sensory evaluation[3)]	10°C	5	16
		15°C	5	14
		25°C	5	12
Non-legal quality limit[2)]	Titratable acidity (TA) (%)	10°C	0.63	19
		15°C	0.65	16
		25°C	0.77	12
	pH	10°C	3.92	15
		15°C	3.62	14
		25°C	3.75	12

[1)]Legal quality limit: 'Process Criteria and Ingredient Standard of Livestock Products' by Animal, Plant and Fisheries Quarantine and Inspection Agency; [2)]Non-legal quality limit: estimated by regression equations between non-statutory quality criteria (y) and sensory evaluation (x); [3)]Panel number=10; 1= very much different from control, 5= different from control (quality limit of sensory evaluation), 9= same as control; [4)]Calculated period (d) at y= Quality limit.

Lactic acid bacterial counts at 10 and 15°C, overall sensory evaluation at 10°C, and pH at 10°C were excluded from the quality criteria for shelf–life ($R^2 \leq 0.9$). The shelf–life, was estimated as limited day of quality criteria that exceed quality limit among the quality criteria ($R^2 \geq 0.9$) at first. The shelf–life of fermented milk product was observed as 19 days at 10°C, 14 days at 15°C, and 12 days at 25°C, and coincidently, shelf–life of fermented milk is 10 days in the market (Table 3).

The shelf–life estimated in this study may lead to the establishment of shelf–life for livestock products.

ACKNOWLEDGEMENTS

This work was supported by the Animal, Plant and Fisheries Quarantine and Inspection Agency (Project # Z-FS03-2011-12-03).

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RADIOPROTECTIVE EFFECTS OF SILK PEPTIDE ON RAW264.7 MACROPHAGE CELLS

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ABSTRACT

Development of radioprotective agents is an important area of research because these agents have potential applications in several fields, such as space exploration, radiotherapy, and nuclear power plant accidents. In the present study, we verified the radioprotective effects of silk peptide acid hydrolysate (AH) and silk peptide enzyme hydrolysate (EH). Cytotoxicity tests on irradiated RAW264.7 macrophage cells revealed that the rate of cell proliferation was higher in the cells that were pretreated with AH and EH than in untreated control cells. However, there was no enhancement of cell survival in the post-treated group. Further, the levels of the cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-6 were significantly higher in the pre-treated cells than in the normal and irradiated control cells. These results suggest that silk peptide can elicit immune responses that inhibit radiation-induced cell death.

Key words: Radiation protection, silk peptide, RAW264.7 macrophage cell.

INTRODUCTION

Development of radioprotective agents is an important area of research because of the potential applications of these agents in both planned radiation exposure (e.g., radiotherapy) and unplanned radiation exposure (e.g., nuclear power plant accidents) (Arora *et al.*, 2005). Numerous synthetic and natural radioprotectors have been investigated, and in particular, many plants and herbal extracts have

shown potential biological activities against radiation-induced cell damage in mammalian systems.

The hydrolysate of silk peptide from silkworm cocoons consists of mainly 2 natural proteins, fibroin and sericin. Several biological activities of the silk peptide have been reported; these include anti-diabetic, hypocholesterolemic, anti-obesity, anti-tumor, and immunoregulatory activities (Byun *et al.*, 2010). In the present study, the radioprotective effects of silk peptide acid hydrolysate (AH) and silk peptide enzyme hydrolysate (EH) were assessed in RAW264.7 macrophage cell line by cell proliferation test and cytokine production assay.

MATERIALS AND METHODS

Sample preparation

The silk peptide acid hydrolysate (AH) and enzyme hydrolysate (EH) were supplied by World Way Co. Ltd. (Jeouneui, Republic of Korea) and used by storage at 4°C. Samples were dissolved in a sterilized Phosphate buffered saline (PBS) solution for followed studies.

Cell culture

RAW264.7 macrophage cell line was purchased from the Korean Cell Line Bank (KCLB, Seoul, Republic of Korea) and maintained in DMEM (Dulbecco's Modified Eagle's Medium) containing 10% fetal bovine serum, 100 unit/mL penicillin and 100 unit/mL streptomycin in complete medium under a humidified atmosphere of 5% CO_2 in air at 37°C.

Gamma irradiation

Cells were exposed to various irradiation doses (1.02 Gy/min) from a ^{137}Cs source (Gammacell 40 Exactor; MDS Nordion International Inc., Ottawa, Ontario, Canada) in the Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute (Jeoungeup, Republic of Korea). Control cells were submitted to the same conditions but not gamma irradiated.

Measurement of cell proliferation using MTT assay

RAW264.7 macrophage cells were divided into 2 batches (pre-treatment and post-treatment) and seeded at a density of 1×10^4 cell/well in 96-well plates. After incubation at 37°C for 3 h, the pre-treatment group cells were incubated with 500 μg/mL samples of AH and EH for 24 h and then irradiated. The cells in the post-treatment group were first irradiated and then incubated with AH and EH (500 μg/mL each) for 24 h. In each well, the medium was replaced by 50 μL of 5 mg/mL 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) solution and incubated for 15 min, and then, the cell monolayers were washed twice with DMEM containing 10% FBS. Intracellular formazan crystals were dissolved using 100 μL of dimethylsulfoxide (DMSO, Sigma, St. Louis., Mo). Product formation was measured at 595 nm in an automated micro-plate enzyme-linked immunosorbent assay (ELISA) reader (Zenyth 3100, Anthos Labtec Instruments, GmbH, Salzburg, Austria).

Cytokine productions assay

Supernatants cultured for 24 h were used for the cytokine production test.

ELISA kits (BD Biosciencs, San Jose, CA) were used by following the manufacturer's instructions and interleukin (IL)-6 and tumor necrosis factor-α (TNF-α) were evaluated at 450 nm ELISA reader (Zenyth 3100, Austria). The absorbance values were then converted to concentrations (pg/mL) of IL-6 and TNF-α using standard curves prepared with serial dilutions of recombinant IL-6 and TNF-α standards.

Statistical analysis

One-way analysis of variance was performed with SPSS software system (1997), and the differences among the mean values were compared using Duncan's multiple range tests. The statistical significance of the differences was evaluated for parallel experiments using Student's t-test. Mean values with standard errors of the mean (SEM) were reported, and the significance was defined at $p < 0.05$.

RESULTS AND CONCLUSION

The radioprotective effects of AH and EH were studied using MTT assay. RAW264.7 macrophage cells irradiated with 150 Gy gamma radiations were treated with AH and EH either before or after irradiation. Both the AH and EH pre-treated groups showed significant increase in cell proliferation compared to the irradiated PBS-treated control cells. However, the post-treated groups, compared to the irradiated control, did not show enhancement in cell proliferation. These results suggest that the silk peptide hydrolysates are potential candidates for functional foods with radioprotective activity; however, development of these hydrolysates as drugs would be difficult.

To determine how the silk peptide inhibits cell death caused by radiation, Raw264.7 macrophage cells were pre- and post-treated with AH and EH. The levels of 2 cytokines, tumor necrosis factor-α (TNF-α) and interleukin (IL)-6, were

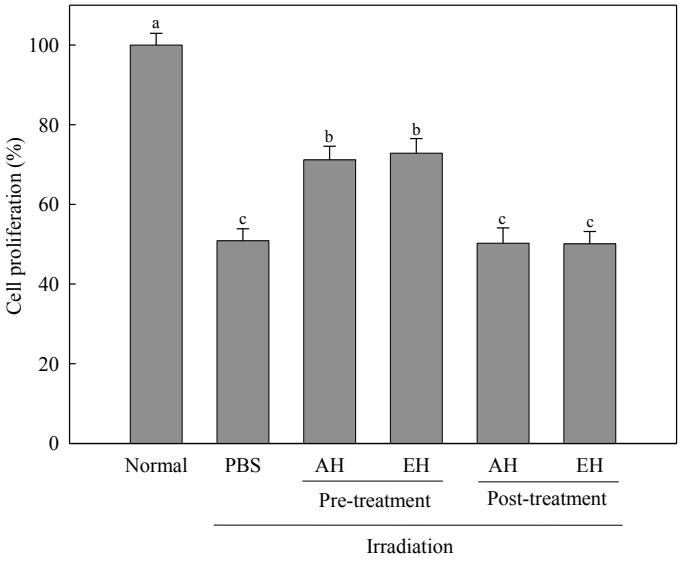

Fig. 1 - Radioprotective effects of silk peptide on cell proliferation in irradiated RAW 264.7 macrophage cells. RAW 264.7 macrophage cells irradiated with gamma radiation were pre- or post-treated for 24 h with acid hydrolysate (AH) and enzyme hydrolysate (EH) of silk peptide (500 μg/mL each).

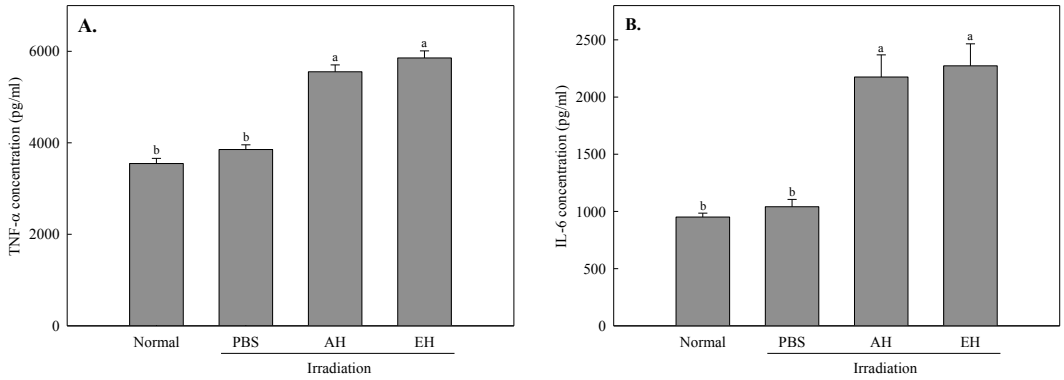

Fig. 2 - Cytokine (TNF-α, A; IL-6, B) production in irradiated RAW 264.7 macrophage cells pre-treated with AH and EH for 24 h (500 μg/mL each).

measured (Fig. 2).The levels of both the cytokines significantly increased after pre-treatment with AH and EH compared to the cytokine levels in normal and irradiated PBS-treated control cells. These findings suggest that the silk peptide can induce secretion of cytokines and inhibit radiation-induced cell death by eliciting immune responses.

ACKNOWLEDGEMENTS

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EFFECT OF GAMMA IRRADIATION ON MICROBIAL CONTAMINATION AND PHYSIOLOGICAL ACTIVITY OF ETHANOL EXTRACT FROM BOILED-WATER OF *ENTEROCTOPUS*

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ABSTRACT

In this study, microbial contamination and physiological activities were examined in a 70% ethanol extract of boiled-water in which *Enteroctopus* was processed. We evaluated the effects of gamma irradiation on the process water to determine whether the food processing by-product may have potential as a functional material for consumption or for cosmetic use. One log cycle reduction of cell counts was obtained by irradiating *Enteroctopus* extract at a dose of 1 kGy. Ferric-reducing antioxidant potential (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the *Enteroctopus* extract increased as a result of gamma irradiation. These results suggest that irradiation technology can be used to decontaminate and improve the usefulness of food by-products.

Key words: Gamma irradiation, *Enteroctopus* extract, decontamination, antioxidant.

INTRODUCTION

Enteroctopus is a popular seafood in Korean, Japan, and China. *Enteroctopus* is known to contain substantial amounts of taurine, which is effective in improving arteriosclerosis and hypertension. Taurine exhibits angiotension I converting enzyme (ACE) inhibitory activity, and inhibition of nitric oxide (NO) production. With the gradual increase in demand for ready-to-eat products, dried and canned forms of *Enteroctopus* are being sold in the market. During the manufacturing process, large volumes of boiled *Enteroctopus* extract are produced. The boiled extract contains

large quantities of proteins, carbohydrates, and phenolic compounds. However, these boiled extracts often are subject to microbial contamination because of open storage conditions and high nutrient content.

Gamma irradiation is known to be generally effective in reducing the microbial content of food products. In addition, recent studies have suggested that irradiation of natural food extracts can improve physiological activities, including electron donating ability and tyrosinase inhibition (Jo *et al.*, 2003). Therefore, in this study, we investigated the effect of gamma irradiation on boiled *Enteroctopus* extract, to determine whether the byproducts may have the potential for further use.

MATERIALS AND METHODS

Boiled-water of *Enteroctopus* was provided by WooYoung Ltd. (Pusan, South Korea). To remove debris, an extract was prepared with 70% ethanol solution at room temperature, which were filtered using Whatman filter paper No. 4 (Whatman International Ltd., Springfield Mill, Kent, England) and kept in a refrigerator (4°C) for further experiments.

Extracted samples were irradiated in a ^{60}Co irradiator (point source, ACEL, IR-79, MDS Nordion International Co. Ltd, Ottawa, Ontario, Canada) with doses of 0, 1, 3, 5, and 10 kGy. The source strength was approximately 11.1 PBq, and a dose rate of 10 kGy/h. The irradiation room temperature was 22°C. Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), calibrated against an international standard set by the International Atomic Energy Agency (Vienna, Austria).

For microbial content, samples (1 mL) were diluted with sterilized saline solution (9 g NaCl/L water, 9 mL) by mixing for 10 s at 3000 rpm (Maxi Mix II, Thermolyne, Dubuque, IA). Total aerobic bacteria were obtained from a plate count agar (Difco Laboratories, Detroit, MI).

The FRAP assay was performed as previously described by Benzie *et al.* (1996). The FRAP reagent contained 2.5 mL of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine, Sigma, St, Louis, MO) solution in 40 mM hydrochloric acid (HCl), 2.5 mL of 20 mM ferric sulfate ($FeSO_4$), and 25 mL of 0.3 M acetate buffer (pH 3.6). The FRAP reagent was prepared fresh and pre-warmed at 37°C. The extract sample (30 μL) was mixed with 90 μL of distilled water and 0.9 mL of FRAP reagent. An equal volume (0.9 mL) of methanol was added, instead of the FRAP reagent, as a control. After a 10-min incubation at 37°C, the absorbance was measured at 593 nm by UV/VIS spectrophotometer (UV-1601PC, Shimadzu, Tokyo, Japan). Standards of known Fe^{2+} concentrations ($FeSO_4 \bullet 7H_2O$) were run between 20 and 1000 μM concentration levels.

The free radical scavenging activity was estimated according to the method of Blois (1958). 10 times diluted extract samples (1 mL) were added to 1 mL of 0.2 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical solution (Sigma chemical Co.), and ethanol solution was used as the blank. The mixtures were shaken and left standing for 30 min at room temperature, being then measured at 517 nm with a spectrophotometer (UV-1601PC). The scavenging activity of the DPPH radicals was calculated as a percentage by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [1-(A_{sample} / A_{blank})] \times 100$$

where A_{sample} and A_{blank} are the sample and blank absorbances, respectively.

RESULTS AND CONCLUSION

Table 1 shows the total counts of aerobic bacteria in non-irradiated and gamma-irradiated samples of *Enteroctopus* extract. Irradiation at doses of 1 kGy or higher led to a minimum of one log cycle count reduction in analyzed microbiological populations, and doses > 3 kGy resulted in non-detectable (ND) counts (less than 100 CFU/mL). The same microbiological result has been reported for fruit juices (Song *et al.*, 2006). Microbial contamination of the *Enteroctopus* extract byproduct could be eliminated by heating during the manufacturing process. However, because of its rich nutrient content, *Enteroctopus* extract susceptible to microbial contamination under normal storage conditions (e.g., outside storage in open containers). The results in this study indicate that *Enteroctopus* extract is not suitable for application in the food and cosmetic industries without first undergoing decontamination. Gamma irradiation was shown to be efficient in reducing microbial contamination in *Enteroctopus* extract.

To investigate the effect of gamma irradiaiton on biological properties of *Enteroctopus* extract, antioxidant activities were measured using a variety of methods. The FRAP assay treats a sample's antioxidants as reductants in a redox-linked colorimetric reaction, and the resulting value reflects the reducing power of the antioxidants. Because this procedure is relatively simple and easy to standardize, it was used to analyze antioxidant activity of natural extracts following gamma irradiation. As shown in Table 1, the FRAP values of irradiated *Enteroctopus* extract increased as the dose of irradiation increased. The FRAP value of *Enteroctopus* extract without gamma irradiation was 1.87 mM, but this value increased to 1.99 and 2.28 mM at doses of 5 and 10 kGy, respectively ($p < 0.05$).

Table 1 - Effect of gamma irradiation on microbial populations and FRAP values of Enteroctopus extract.

Irradiation dose (kGy)	Viable cell counts (Log CFU/mL)	FRAP value (mM)
0	2.18±0.19[a]	1.87±0.09[c]
1	1.0±0.12[b]	-
3	ND-	
5	ND	1.99±0.11[b]
10	ND	2.38±0.1[a]

[a-c] in the same column indicate the values are significantly different ($p < 0.05$).

The DPPH assay has been widely used to evaluate the free radical scavenging ability of various natural extracts. Fig. 1 shows the DPPH radical scavenging activities of *Enteroctopus* extract at different doses of irradation. The DPPH radical scavenging activity of non-irradiated *Enteroctopus* extract was 68.7%, and the radical scavenging activities of the extract increased depending on the absorbed dose of irradiation. At a dose of 10 kGy, radical scavenging activity increased significantly ($p < 0.05$) to 73.6%.

Recently, Choi *et al.* (2010) reported that gamma irradiation of *Hizikia fusiformis* cooking drips increased the antioxidant activity of the byproduct through an increase in the total phenolic content. The results of the present study suggest that

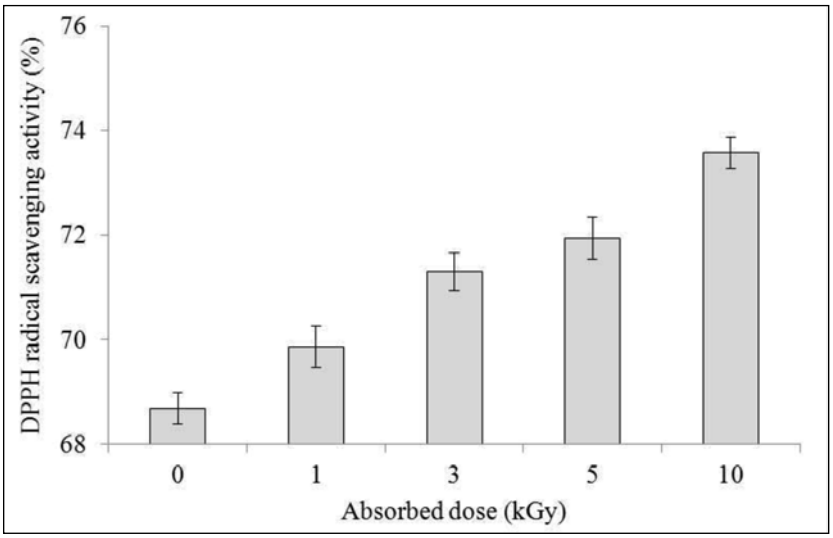

Fig. 1 - DPPH radical scavenging activity (%) of irradiated cooking drip extracts of *Enteroctopus* extract at the various absorbed doses.

Enteroctopus extract can be effectively decontaminated, and the antioxidant activities of the extract increased by by gamma irradiation. Furthermore, we suggest that the currently wasted byproduct of *Enteroctopus* processing may have potential for used in the food and cosmetic industries, if treated with gamma irradiation.

ACKNOWLEDGEMENTS

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IMMUNE-ENHANCING ACTIVITY OF SILK PEPTIDE IN A GAMMA-IRRADIATED MOUSE MODEL

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ABSTRACT

This study focuses on the immunomodulatory effects of silk peptide in gamma-irradiated mouse model. Six groups of mice (n = 60) were fed a standard diet, with some groups additionally receiving silk peptide administered at concentrations of 50 and 200 mg/kg body weight per day for 14 days. Mice were then gamma-irradiated (1.5 Gy). After irradiation, spleen weight, splenocyte counts, and cytokine production (IL-2 and IFN-γ) were significantly decreased in the mice that did not receive the silk-peptide treatment. In contrast, irradiated mice that received the silk-peptide treatment showed clear indications of enhanced immune system activity. Silk peptide may therefore have great potential value as a potent immunomodulatory agent.

Key words: Silk peptide, gamma irradiation, radiation protection, splenocyte counts, cytokine production.

INTRODUCTION

Bioactive peptides are specific protein fragments formed by the hydrolysis of various food proteins. They have a positive impact on body functions and may ultimately influence health (Korhonen and Pihlanto, 2003). Oral administration of bioactive peptides affects major body systems, including the cardiovascular, digestive, immune, and nervous systems (Horiguchi *et al.*, 2005). The activity of these functional peptides is based on their inherent amino acid composition and sequence.

Fibroin is mainly composed of the amino acids Gly, Ala, Ser, and Tyr (Nahm and Oh, 1995). Recent studies have shown that fibroin-derived peptides have specific

biological effects (Park *et al.*, 2005). However, no study has described their protective effect against gamma-irradiation-induced immune suppression in *in vitro* and *in vivo* models.

The present study was therefore conducted to investigate the protective effects of silk peptide (SP) using gamma-irradiated immune suppressed BALB/c mice as a model. We evaluated the use of SP as an effective and safe functional food.

MATERIALS AND METHODS

Sample preparation

SP was supplied by Worldway Co. Ltd. (Jeouneui-Myun, Korea) and stored at 4°C. For experimental use, SP was dissolved in a sterile phosphate buffered saline solution.

Animals and diet

All animal studies were conducted according to animal experimental protocol approved by The Institutional Animal Care and Use Committee (IACUC) at Korea Atomic Energy Research Institute. 7-week-old female BALB/c mice (body weight (B.W.), 19–21 g) were obtained from Orient Inc. (Charles River Technology, Seoul, Korea). For adaptation, the mice were housed in a polycarbonate cage and fed a standard animal diet and water *ad libitum* at a controlled temperature (22 ± 2°C) and humidity level (55 ± 10%) with 12-h light/dark cycles for 1 week. After acclimation, the mice were randomly divided into 6 groups (10 mice per group).

Sample administration and gamma-irradiation

After adaptation, the mice were given an oral administration of SP for 2 weeks (50 and 200 mg/kg B.W. per day) and control mice were even given phosphate buffered saline solution. Mice were then gamma-irradiated at a dose of 1.5 Gy (1.02 Gy/min) from a ^{137}Cs source (Gammacell 40 Exactor; MDS Nordion International Inc., Ottawa, Ontario, Canada) at Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute (Jeoung-Eup, Korea). Non-irradiated SP treated group was compared with gamma-irradiated group.

Splenocyte count

Four days after irradiation, all animals were sacrificed to obtain spleen tissue. To prepare splenocytes, the spleen tissue samples were homogenized in a glass homogenizer (Corning Inc., Lowell, MA, USA). Homogenates were transferred to a sterile universal tube and centrifuged at 600 × *g* for 5 min. The supernatant was discarded, and the cell pellet was gently tapped to resuspend it. Two microliters of sterile red blood cell lysis buffer (eBioscience Co., San Diego, CA, USA) was added to the cell suspension and incubated at room temperature for 1 min. For washing cells, 18 mL of RPMI 1640 medium was then added to the cell suspension and centrifuged at 600 × *g* for 5 min, after which the supernatant removed. This step was repeated twice to wash out residual red blood cell lysis buffer and red blood cells, leaving a pellet of splenocytes. Splenocytes were then resuspended in 10 mL of RPMI 1640 medium containing 10% fetal bovine serum, 100 units/mL penicillin, and 100 units/mL streptomycin (complete medium). Splenocyte count was then determined using a trypan blue assay (Cederbrant *et al.*, 2003). For measurement of cytokine production, 2 × 10^6 cells from each splenocyte

preparation suspended in the complete medium were transferred to wells of a 48-well tissue culture plate. Splenocyte suspensions were then incubated at 37°C for 24 h under 5% CO_2. After 24 h, the supernatants were collected and stored in –80°C until further use.

Cytokine production

ELISA kits (BD Biosciences, San Jose, CA, USA) were used according to the manufacturer's instructions to estimate cytokine production. Absorbance values were converted to concentrations (pg/mL) of IL-2 and IFN-γ by using standard curves prepared with serial dilutions of recombinant IL-6, IL-2, IFN-γ, and TNF-α standards contained in the kit.

Statistical analysis

One-way analysis of variance was performed using the SPSS software system (1997), and Duncan's multiple range test was used to compare the differences between the mean values. Mean values with standard errors of the mean (SEM) were reported, and statistical significance was defined at $p < 0.05$.

RESULTS AND CONCLUSION

Change of body and tissue weight

Fig. 1 shows the change of spleen weight. Gamma irradiation has been shown to induce a reduction in body and tissue weight (Chintalwar *et al.*, 1999). In our study, body and liver weight were reduced in gamma-irradiated mice that did not receive SP treatment, but no significant difference was observed between the control group and the SP-treated groups (data not shown). Furthermore, significant reductions in the spleen weight were observed in gamma-irradiated mice except for those treated with SP at 200 mg/kg B.W. per day, where the spleen weight had increased. Gamma irradiation causes severe damage to splenocytes, leading to apoptotic cell death (Chintalwar *et al.*, 1999). A previous report by Jo *et al.* (2006) suggested that administration of SP and chitosan oligosaccharides increased splenocyte proliferation compared to an untreated group. In addition,

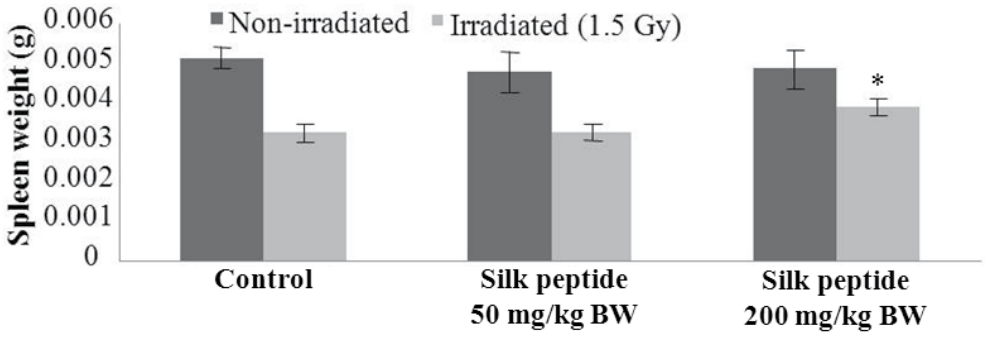

Fig. 1 - Effect of silk peptide treatment on change of spleen weight in gamma-irradiated (1.5 Gy) and non-irradiated mice. Silk peptide was orally administrated at the concentration of 50, 200 mg/kg body weight (BW) for 3 weeks. Four days after γ-irradiation, spleen separated from mice was weighed.

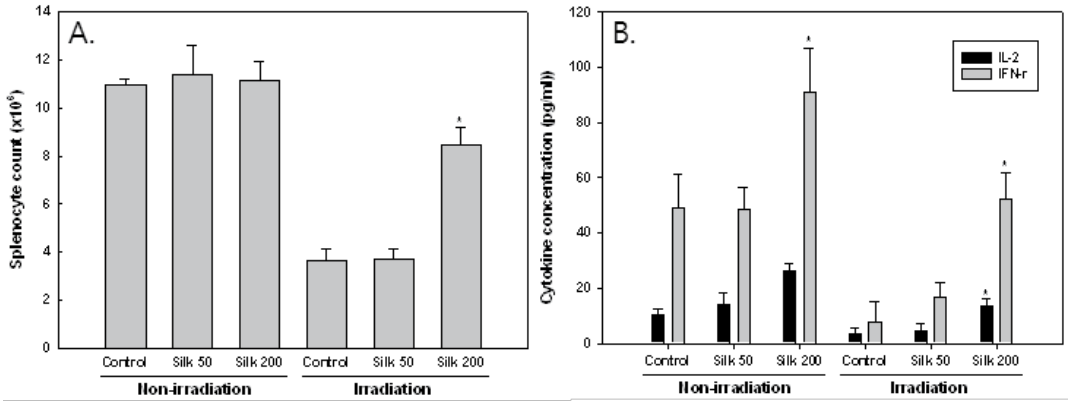

Fig. 2 - Effect of silk peptide treatment on change of splenocyte count (A) and cytokine (IL-2 and IFN-γ) production in gamma-irradiated (1.5 Gy) and non-irradiated mice. Silk peptide was orally administrated at the concentration of 50, 200 mg/kg body weight (BW) for 3 weeks. Four days after gamma irradiation, spleen separated from mice, and splenocyte count and cytokine production were then measured.

Byun *et al.* (2010) reported that tumor size in mice was significantly reduced in the SP-treated group, and this effect explained the observed increase in the immunomodulatory activity. Taken together, therefore, these results suggest that treatment with SP may enhance the immune system of mice in response to exposure to gamma irradiation.

Splenocyte count and cytokine production

As shown in Fig. 2, splenocyte count and cytokine (IL-2 and IFN-γ) production significantly decreased in gamma-irradiated mice except for the SP-treated mice, which showed an increased splenocyte count and cytokine production. Splenic lymphocytes primarily participate in innate (monocytes and NK cells) and acquired (T and B cells) immune defense, and previous studies have shown that beneficial effects on the immune response to microbial pathogens and tumors were directly correlated with the ability of natural food and herbal products to stimulate lymphocyte proliferation (Dalloul *et al.*, 2006). IL-2 and IFN-γ are important cytokines that are mainly secreted by splenic lymphocytes. They contribute a protective effect against various microbial pathogens by enhancing the host's immune system (Lillehoj *et al.*, 2004; Ding *et al.*, 2004). In this study, SP treatment prevented gamma-irradiation-induced immune suppression, and it was thought to stimulate immune cell activity. This finding suggests that SP can be used a natural substance to combat gamma-irradiation-induced immune suppression.

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EFFECTS OF IRRADIATION ON BACTERIAL GROWTH AND PHYSIOCHEMICAL AND SENSORY PROPERTIES OF *DAKGALBI*, A KOREAN CHICKEN-BASED DISH, COOKED USING ELECTIC PAN FRYING OR CHARCOAL BROILING

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ABSTRACT

The objective of this study was to compare the total bacterial population and sensory quality of irradiated *Dakgalbi* prepared using either electric pan frying or charcoal broiling. Fresh deboned chicken meat was divided into 2 portions, and each was cooked using either electric pan frying or charcoal broiling. The samples were then γ irradiated at 0, 5, 10, 15, 20, and 25 kGy and stored under accelerative conditions at 35°C for 7 days. Total bacterial populations in *Dakgalbi* prepared using either cooking method significantly decreased in an irradiation dose-dependent manner and were below the detection limit (1 log CFU/g) at doses >20 kGy. The levels of thiobarbituric acid reactive substances increased with an increase in the storage period. Although use of electric pan frying as well as charcoal broiling led to decreased sensory quality in *Dakgalbi*, charcoal-broiled *Dakgalbi* had better sensory quality. These results indicate that charcoal broiling is a better cooking method than electric pan frying for minimizing bacterial growth and improving the sensory quality of irradiated *Dakgalbi*.

Key words: Bacterial growth, charcoal broiling, *Dakgalbi*, electric pan frying, irradiation, physiochemical and sensory quality.

INTRODUCTION

Dakgalbi is a popular food in Korea. It is a chicken-based dish prepared by stir-frying marinated diced chicken in red pepper paste sauce along with rice cakes and vegetables such as cabbage, scallion, onion, and sweet potato. *Dakgalbi* is considered as a healthy food because it is low in calories, fat, and cholesterol; moreover, it is rich in proteins and dietary fiber due to the chicken meat and vegetables, respectively. However, *Dakgalbi* has a short shelf life due to the presence of thermo-resistant bacteria in red pepper paste sauce (Yoon *et al.*, 2009). This hinders commercial development of ready-to eat *Dakgalbi* products in the food industry.

γ-irradiation is a well known decontamination technique for ensuring the microbial safety of food products. It decreases the incidence of food-borne illnesses by reducing or eliminating disease-causing pathogens and extends the shelf-life of foods by reducing spoilage. Compared to heat- or freezing-treatment, this technique has economical and nutritional advantages.

In this study, *Dakgalbi* was prepared either by electric pan frying or charcoal broiling, and the effects of γ-irradiation on the bacterial populations and sensory properties of *Dakgalbi* prepared by the 2 methods were compared.

MATERIALS AND METHODS

Fresh refrigerated chicken meat was cut into small pieces using a sterile knife at ambient room temperature. The meat was then marinated with *Dakgalbi* sauce, containing water (22.5%), red pepper paste (45%), corn syrup (10%), garlic (5%), soy sauce (5%), sugar (5%), ginger (4%), turmeric powder (2.5%), and curry powder (1%) for 2 h at 4°C. The ratio of meat to sauce was 4:1 (w/w). The marinated sample was divided into 2 portions; 1 aliquot was subject to electric pan frying according to our previously published method (Yoon *et al.*, 2012), while the other was subject to charcoal broiling for 12 min, followed by cooling at room temperature. The samples were then packed in sterile vacuum bags (aluminum-laminated, low-density polyethylene; density, 0.92 g/cm^3) and subsequently γ-irradiated at doses of 0, 5, 10, 15, 20, and 25 kGy at room temperature using a cobalt-60 irradiator (IR-221; MDS Nordion International Co. Ltd., Ottawa, Canada) at the Advanced Radiation Technology Institute (Jeongeup, South Korea) of the Korea Atomic Energy Research Institute. The source strength was approximately 300 kCi with a dose rate of 10 kGy/h. Dosimetry was carried out using a 5 mm diameter alanine dosimeter (Bruker Instruments, Rheinstetten, Germany), which was calibrated with an international standard set provided by the International Atomic Energy Agency (Vienna, Austria). All *Dakgalbi* samples were stored under accelerative conditions at 35°C for 7 days.

Measurements of total bacterial populations of *Dakgalbi* samples were performed according to our previously published method (Yoon *et al.*, 2012). Briefly, 10 g samples were transferred into sterile bags (Sunkyung Co., Seoul, South Korea) containing 90 mL of 0.1% peptone water, which were then homogenized by pummeling in a stomacher (Model 400; Tekmar Co., Los Angeles, USA) for 2 min. The supernatant was serially diluted and 1 mL of each diluent was mixed with 20 mL of plate count agar on a petri culture plate. The plates were incubated at 35°C for 48 h, and bacterial colonies were manually counted. Additionally, the levels of thiobarbituric acid reactive substances (TBARS) were measured according to our previously published method (Park *et al.*, 2010). Samples (5 g) were homogenized

with 15 mL distilled water containing 0.024% butylated hydroxyanisole in 0.33% ethanol. One milliliter of the homogenate was then mixed with 3 mL of 20 mM 2-thiobarbituric acid (15% in trichloroacetic acid solution), heated in boiling water for 15 min, and centrifuged for 10 min at 2500 × *g*. The absorbance of the supernatant was measured at 532 nm, which provides a measure (in μg) of malondialdehyde per gram of sample. Sensory evaluation of *Dakgalbi* was conducted by 10 panelists who were trained as previously described by Civille and Szczesniak (1973). Appearance, taste, flavor, texture, off-flavor, and overall acceptance of the samples were tested using a 7-point descriptive scale from 1 (extremely dislike or extremely weak) to 7 (extremely like or extremely strong).

All data were analyzed using the generalized linear model procedures of SAS version 9.2 (SAS Institute, Cary, USA). Tukey's multiple range tests were used to compare least-squared means, and a *p*-value of 0.05 was considered statistically significant.

RESULTS AND CONCLUSION

The results of the measures of total bacterial populations in irradiated *Dakgalbi*, cooked using electric pan frying or charcoal broiling, are presented in Table 1. Without γ-irradiation, the bacterial populations of *Dakgalbi* cooked using electric pan frying or charcoal broiling were 3.4 and 4.2 log CFU/g, respectively, on day 0. Irradiation significantly reduced the initial bacterial populations in a dose-dependent manner, and the populations were below the detection limit (<1 CFU/g) at doses ≥15 kGy. However, after accelerative storage for 1 day, bacteria was detected in the 15 kGy-irradiated *Dakgalbi* cooked using both methods, suggesting that the dose was insufficient to kill all bacteria present. No bacteria were detected (<1 CFU/g)

Table 1 - Total bacterial populations (log CFU/g) of irradiated *Dakgalbi* cooked using electric pan frying or charcoal broiling during accelerative storage for 7 days at 35°C.

Cooking method	Dose (kGy)	Storage period (days)							
		0	1	2	3	4	5	6	7
Electronic pan frying	0	$3.4\pm0.2^{C,b}$	$5.8\pm0.1^{B,a}$	$6.9\pm0.3^{A,a}$	-[1)]	-	-	-	-
	5	$2.3\pm0.4^{E,cd}$	$3.9\pm0.6^{D,bc}$	$5.2\pm0.3^{C,bc}$	$6.5\pm0.4^{B,ab}$	$7.6\pm0.3^{A,a}$	-	-	-
	10	$1.2\pm0.3^{E,e}$	$2.7\pm0.4^{D,de}$	$4.0\pm0.2^{C,d}$	$5.9\pm0.5^{B,bc}$	$7.0\pm0.2^{A,b}$	-	-	-
	15	ND[2)]	ND	$3.1\pm0.3^{D,e}$	$4.9\pm0.4^{C,d}$	$6.3\pm0.1^{B,c}$	$7.5\pm0.2^{A,a}$	-	-
	20	ND	ND	ND	ND	ND	ND	ND	ND
	25	ND	ND	ND	ND	ND	ND	ND	ND
Charcoal broiling	0	$4.2\pm0.4^{B,a}$	$6.1\pm0.2^{A,a}$	$7.4\pm0.8^{A,a}$	-	-	-	-	-
	5	$2.7\pm0.5^{E,c}$	$4.4\pm0.3^{D,b}$	$5.9\pm0.4^{C,b}$	$6.9\pm0.3^{B,a}$	$7.9\pm0.2^{A,a}$	-	-	-
	10	$1.9\pm0.2^{E,d}$	$3.2\pm0.5^{D,cd}$	$4.8\pm0.3^{C,c}$	$5.8\pm0.6^{B,bcd}$	$7.7\pm0.3^{A,a}$	-	-	-
	15	ND	$2.1\pm0.3^{E,e}$	$3.4\pm0.1^{D,de}$	$5.5\pm0.4^{C,cd}$	$6.8\pm0.2^{B,b}$	$8.1\pm0.3^{A,a}$	-	-
	20	ND	ND	ND	ND	ND	ND	ND	ND
	25	ND	ND	ND	ND	ND	ND	ND	ND

[1)] Dash indicates no bacterial populations found because of spoilage; [2)]ND, not detected within detection limit (< 1 log CFU/g);
[A-E]Mean±standard deviation values within a row with different superscripts are significantly different ($p<0.05$);
[a-e]Mean±standard deviation values within a column with different superscripts are significantly different ($p<0.05$).

Table 2 - TBARS value of irradiated *Dakgalbi* (μg of malondialdehyde/g sample) cooked using electric pan frying or charcoal broiling during accelerative storage for 7 days at 35°C.

Cooking method	Dose (kGy)	Storage period (days)							
		0	1	2	3	4	5	6	7
Electronic pan frying	0	1.65±0.03^{A,f}	1.62±0.10^{A,f}	1.75±0.06^{A,f}	-[1)]	-	-	-	-
	5	1.71±0.05^{E,ef}	1.81±0.09^{DF,de}	1.91±0.07^{D,d}	2.55±0.14^{C,a}	1.94±0.04^{D,c}	2.61±0.03^{C,c}	2.88±0.08^{B,c}	3.38±0.09^{A,d}
	10	1.79±0.11^{F,def}	1.77±0.12^{F,def}	1.88±0.11^{F,ce}	2.22±0.13^{E,bcd}	2.41±0.03^{D,b}	2.63±0.06^{C,c}	2.94±0.05^{B,c}	3.44±0.10^{A,d}
	15	1.82±0.07^{F,cde}	1.90±0.04^{F,cd}	2.15±0.08^{F,b}	2.29±0.07^{D,bc}	1.97±0.12^{D,c}	2.78±0.05^{C,b}	3.08±0.10^{B,b}	3.64±0.09^{A,c}
	20	1.89±0.01^{G,abc}	1.99±0.04^{F,bc}	2.21±0.10^{E,b}	2.18±0.02^{E,cd}	2.45±0.07^{D,b}	3.02±0.10^{C,a}	3.28±0.02^{B,a}	3.77±0.03^{A,abc}
	25	1.99±0.09^{G,a}	2.21±0.04^{F,a}	2.33±0.02^{E,a}	2.41±0.03^{E,ab}	2.57±0.06^{D,a}	3.01±0.04^{C,a}	3.29±0.02^{B,a}	3.86±0.05^{A,ab}
Charcoal broiling	0	1.62±0.07^{A,f}	1.64±0.05^{A,fg}	1.71±0.09^{A,e}	-	-	-	-	-
	5	1.73±0.04^{G,ef}	1.69±0.08^{G,efg}	1.87±0.05^{F,de}	2.08±0.06^{E,d}	2.38±0.11^{D,b}	2.55±0.06^{C,cd}	2.71±0.09^{B,d}	3.12±0.05^{A,e}
	10	1.75±0.06^{F,def}	1.80±0.08^{F,de}	1.99±0.10^{E,cd}	2.16±0.07^{D,cd}	2.29±0.12^{D,b}	2.47±0.04^{C,d}	2.66±0.09^{B,d}	3.08±0.08^{A,e}
	15	1.80±0.11^{F,cde}	1.86±0.06^{F,cd}	2.08±0.02^{E,bc}	2.17±0.08^{DE,cd}	2.33±0.06^{C,b}	2.27±0.05^{D,ce}	2.87±0.03^{B,c}	3.44±0.07^{A,d}
	20	1.86±0.02^{F,bcd}	1.96±0.07^{FG,bc}	2.16±0.08^{E,b}	2.09±0.19^{EF,d}	2.37±0.02^{D,b}	2.78±0.11^{C,b}	3.16±0.09^{B,ab}	3.74±0.06^{A,bc}
	25	1.95±0.02^{H,ab}	2.09±0.08^{G,ab}	2.21±0.12^{F,ab}	2.37±0.05^{E,ab}	2.56±0.01^{D,a}	2.91±0.04^{C,a}	3.24±0.05^{B,a}	3.88±0.04^{A,a}

[1)]Dash indicates no determination of TBARS value because of spoilage; $^{A-F}$Mean±standard deviation values within a row with different superscripts are significantly different ($p<0.05$); $^{a-e}$Mean±standard deviation values within a column with different superscripts are significantly different ($p<0.05$).

during the storage period in *Dakgalbi* irradiated at doses >20 kGyTBARS values of irradiated *Dakgalbi* cooked using electric pan frying (1.65–1.99 μg/g) or charcoal broiling (1.62–1.95 μg/g) were not significantly different up to 10 kGy, but the values for samples irradiated at doses >15 kGy significantly increased (Table 2). Further, the values significantly increased with increasing storage period, regardless of the irradiation dose. Meanwhile, TBARS values of irradiated *Dakgalbi* during storage

Table 3 - Sensory quality of *Dakgalbi* cooked using electric pan frying or charcoal broiling on day 0.

Cooking method	Dose (kGy)	Attributes					
		Appearance	Taste	Flavor	Texture	Off-flavor	Overall acceptability
Electronic pan frying	0	5.5±0.2^{bc}	6.2±0.3^{bc}	6.1±0.1^{abc}	6.4±0.2^{ab}	1.0±0.1^{g}	6.1±0.3^{bc}
	5	5.7±0.1^{a}	5.8±0.2^{cd}	5.8±0.3^{cde}	6.1±0.1^{bc}	1.3±0.1^{fg}	5.9±0.2^{cde}
	10	5.5±0.2^{bc}	5.1±0.1^{f}	5.0±0.2^{f}	5.5±0.3^{e}	1.5±0.2^{df}	5.6±0.1^{de}
	15	5.4±0.1^{bc}	4.5±0.1^{g}	4.4±0.1^{g}	4.8±0.2^{f}	2.6±0.2^{c}	4.1±0.3^{fg}
	20	5.5±0.1^{bc}	4.1±0.2^{gh}	4.0±0.3^{h}	4.4±0.1^{f}	3.5±0.2^{b}	3.7±0.3^{g}
	25	5.3±0.3^{c}	3.9±0.4^{h}	3.1±0.4^{i}	3.7±0.3^{g}	4.1±0.1^{a}	2.6±0.1^{h}
Charcoal broiling	0	6.6±0.1^{a}	6.7±0.2^{a}	6.5±0.2^{a}	6.6±0.3^{a}	1.0±0.1^{g}	6.7±0.2^{a}
	5	6.6±0.2^{a}	6.5±0.1^{ab}	6.4±0.1^{ab}	6.4±0.2^{ab}	1.0±0.1^{g}	6.5±0.2^{ab}
	10	6.4±0.1^{a}	6.0±0.3^{cd}	6.0±0.2^{bcd}	5.8±0.4^{cde}	1.3±0.2^{fg}	6.0±0.1^{cd}
	15	6.5±0.2^{a}	5.9±0.2^{cd}	6.0±0.3^{bcd}	6.1±0.2^{bc}	1.8±0.3^{de}	5.8±0.2^{cde}
	20	6.5±0.3^{a}	5.7±0.3^{de}	5.6±0.1^{de}	6.0±0.1^{bcd}	2.0±0.3^{d}	5.5±0.3^{e}
	25	6.6±0.1^{a}	5.3±0.2^{df}	5.5±0.1^{e}	5.6±0.2^{de}	2.5±0.2^{c}	4.5±0.4^{f}

$^{a-g}$Mean±standard deviation values within a column with different superscripts are significantly different ($p<0.05$).

were not significantly different in the samples prepared by electric pan frying and charcoal broiling.

The effect of irradiation on the sensory quality of *Dakgalbi* was evaluated on day 0. γ-irradiation decreased the sensory quality of *Dakgalbi* cooked using both methods, with off-flavor increasing as irradiation dose increased (Table 3). However, after considering all tested categories including overall acceptability, the results showed that irradiated *Dakgalbi* cooked using charcoal broiling was much more acceptable than irradiated *Dakgalbi* cooked using electric pan frying. The results of this study suggest that charcoal broiling might be a better cooking method than electric pan frying for minimizing bacterial growth and maintaining the sensory quality of irradiated *Dakgalbi*. However, further studies are needed on the use of factors such as modified atmosphere packing, low-temperature irradiation, and the addition of antioxidants for improving the quality of irradiated *Dakgalbi*.

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EFFECT OF IONIZING IRRADIATION ON *IN VITRO* CYTOTOXIC AND NITRIC OXIDE PRODUCING ABILITIES OF MISTLETOE (*VISCUM ALBUM*) EXTRACT

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ABSTRACT

The aim of this study was to evaluate the effect of ionizing radiation on the cytotoxicity and nitric oxide (NO) inducting activities of mistletoe water extract (MWE). MWEs were individually irradiated with gamma rays and electron beams at doses of 0, 10, 30, 50, 70, and 100 kGy. Heated-treated MWE was used as the control. A cell proliferation assay showed that gamma- or electron beam-irradiated MWE was less cytotoxic to RAW 264.7 macrophage cells than non-irradiated MWE, and the cytotoxicity decreased as the irradiation dose increased. A similar cytotoxicity reducing effect was observed in different tumor cell lines such as THP-1, Colon26, B16BL6, and AGS. Heat treatment also reduced the cytotoxicity of MWE in all the tested cells and was comparable to that of irradiated MWE. In contrast, gamma- and electron beam-irradiated MWE induced significant levels of NO in macrophage cells. However, unlike irradiated MWE, heat-treated MWE had no effect on NO production. These results suggest that ionizing irradiation could be a useful tool for obtaining detoxified MWE with sustained immunomodulatory activity.

Key words: Gamma irradiation, cytotoxicity, electron beam irradiation, immunomodulatory activity, mistletoe, nitric oxide.

INTRODUCTION

Mistletoe (*Viscum album*), a common semiparasitic evergreen plant that grows in trees, has been used as a folk medicine for therapeutic purposes against human

diseases, such as cancer, diabetes, hypertension, and others, since ancient times. In Europe, mistletoe is mostly prepared as an alcohol-water extract, fermented water extract using lactic acid bacteria, or as a mixture with other plant extracts in the form of functional or medical foods. In eastern Asia, the plant is also made as a tea by hot water infusion or a decoction with heat treatment for long periods of time. However, because of its toxicity due to toxic components such as lectins, viscotoxin, and alkaloids, the use of mistletoe in humans has been limited. Therefore, to increase the application of mistletoe preparations, the development of methods that reduce its toxicity are required.

Ionizing irradiation is a well-known sterilizing technology that improves food safety by inactivating food pathogens and reducing food borne illnesses. This technology also extends the shelf life of foods by killing pathogenic and spoilage microorganisms and reducing enzymatic activity in the final products without changing food quality. Recently, this technology has been used to improve the biological properties of natural materials, by inhibiting the allergy induction of egg ovalbumin (Seo *et al.*, 2007), increasing the immunomodulatory activity of β-glucan (Sung *et al.*, 2009), and inhibiting the side effects of doxorubicin (Lee *et al.*, 2008).

In this study, the effect of ionizing radiation on the cytotoxicity and nitric oxide inducing activities of mistletoe water extract (MWE) was investigated as a possible method for reducing its cytotoxicty and increasing its utilization as a material in functional foods.

MATERIALS AND METHODS

Mistletoe preparation and ionizing radiation

Fresh mistletoe growing on oak trees was collected in January 2007 from the Naejang Mountains, Jeollabuk-Do, Republic of Korea, and then stored at -70°C until used. MWE was prepared by homogenizing 100 g of mistletoe in 1 L of cold distilled water (DW). The extract was then centrifuged at 20,000 × *g* for 20 min. The supernatant was sterile-filtered and lyophilized. Heat -treated mistletoe extract was also prepared by heating the filtered supernatant in boiling water for 30 min, which was then also lyophilized. The obtained powders were stored at -70°C until use. Irradiation of mistletoe extract with gamma rays or an electron beam was performed as follows; first, MWEs were prepared by solubilizing lyophilized mistletoe powder in DW at a concentration of 10 mg/mL. The samples were then gamma or electron beam irradiated at doses of 0, 10, 30, 50, 70, and 100 kGy. For gamma irradiation, a cobalt-60 irradiator (IR-79; Nordion International Ltd., Ottawa, Canada) with an 11.1 PBq source strength was used, and the dose rate was 10 kGy/h. For electron beam irradiation, an ELV4-electron accelerator (energy 10 MeV, beam power 570 kW) was used and the beam current was 1 mA. Dosimetry was performed using cellulose triacetate film. Both the gamma- and electron beam-irradiated MWE samples were immediately stored in a refrigerator at 4°C to test cytotoxicity and nitric oxide (NO) induction.

In vitro cytotoxicity and NO inducing ability of mistletoe extract

Murine RAW 264.7 macrophage cells, human monocytic tumor (THP-1) cells, murine colon adenocarcinoma (Colon26) cells, murine melanoma (B16BL6) cells, and human stomach tumor (AGS) cells were obtained from the Korean Cell Line Bank (KCLB, Seoul, Republic of Korea). Each cell line was cultivated as follows:

RAW 264.7 in Dulbecco's modified Eagle's medium (DMEM), B16BL6 in minimum essential medium (MEM), and Colon26, THP-1, and AGS in Roswell Park Memorial Institute medium (RPMI-1640) containing 10% fetal bovine serum, 100 units/mL penicillin (Invitrogen, CA, USA) and 100 units/mL streptomycin (Invitrogen) at 37°C in 5% CO_2. To measure the cytotoxic effects of irradiated MWEs, the MTT method was employed (Goodwin *et al.*, 1995). Briefly, cells (2×10^4 cells/well) were seeded in a 96-well culture plate and incubated at 37°C in an atmosphere containing 5% CO_2 for 24 h. Different concentrations of irradiated MWEs were added to each well of the plate and incubated for an additional 24 h. After incubation, dimethylthiazol tetrazolium bromide (MTT) solution was added, and the plate was further incubated at 37°C for 2 h. The absorbance of the samples was then measured at 595 nm using a microplate reader (Zenyth 3100; Anthos Labtec Instruments GmbH, Salzburg, Austria). The ratio of the optical density (OD) of the sample with no MWE added to the OD of samples with added MWE was calculated to determine the percentage of proliferation. Heat-treated MWE was used as a control. NO production was also measured in RAW 264.7 macrophage cells. The cells were seeded (1×10^5 cells/well) in 48-well plates and incubated for 4 h. Irradiated MWE was added at a concentration of 125 μg/mL, and the cells were incubated for 24 h. After incubation, the culture supernatant was separated by centrifugation. Equal volumes (100 μL) of culture supernatant and Griess reagent (Sigma-Aldrich Co., St. Louis, MO, USA) were mixed and incubated for 15 min. The absorbance was measured at 595 nm using a microplate reader. The absorbance values were then converted to the corresponding NO concentration (μM) using standard curves prepared with serial dilutions of $NaNO_2$ standards.

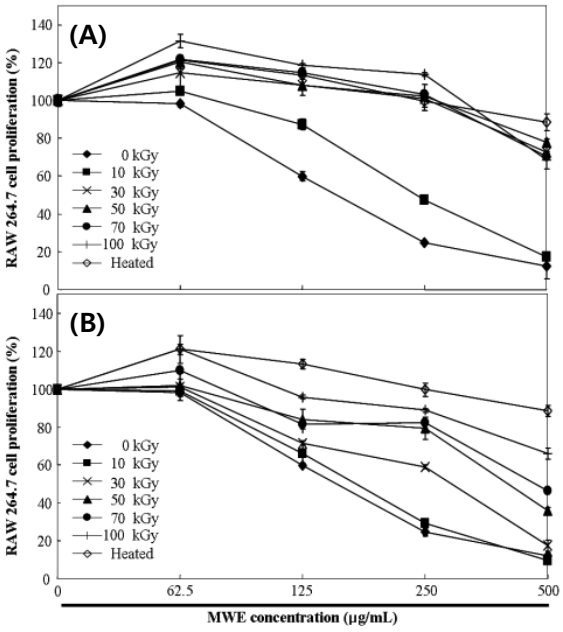

Fig. 1 - Effects of detoxified mistletoe water extract by gamma (A) and electron beam (B) irradiation on the proliferation of RAW 264.7 macrophage cells.

All data were analyzed using SPSS software (Statistical Package for Social Sciences, 10.0, 2000) to obtain the mean and standard deviation. For all experiments, the SPSS ANOVA, LSD-test was used. A P value less than 0.05 was considered statistically significant.

RESULTS AND CONCLUSION

Reduction of MWE cytotoxicity by gamma or electron beam irradiation

The effects of gamma or electron beam irradiation on the cytotoxicity of MWE against RAW264.7 macrophage cells are shown in Fig. 1. Non-irradiated MWE had a strong cytotoxic effect on the macrophage cells, and this effect was concentration dependent. Irradiation of MWE reduced its cytotoxicity, and the cytotoxic effect

decreased as the irradiation dose increased. The reduction in the cytotoxicity of MWE did not differ between extracts treated with gamma irradiation and those treated with electron beam irradiation. When MWE was heat treated, no cytotoxicity against the macrophage cells was detected.

Next, to confirm that the reduction in the cytotoxicity of MWE due to irradiation was reproducible in other cell lines, different types of tumor cells, such as THP-1 cells, B16BL6 cells, AGS cells, and Colon26 cells, were treated with gamma-irradiated MWE (Fig. 2). Expectedly, non-irradiated MWE exhibited strong cytotoxicity in all tumor cells tested, and this effect was concentration dependent. Among the tested cells, murine melanoma B16BL6 cells were the least affected by the cytotoxicity of non-irradiated MWE treatment. Gamma-irradiated MWE was less cytotoxic than non-irradiated MWE, but it still had fairly high cytotoxicity. However, the cytotoxic effect gradually decreased as the irradiating dose increased. In contrast, heat-treated MWE was still cytotoxic; however, the cytotoxic effect was very small. Only high concentrations of heat-treated MWE (greater than 60 μg/mL) were found to be somewhat cytotoxic. The overall cytotoxicity of heat-treated MWE in the tested tumor cells was much weaker than that of gamma-irradiated MWE.

NO inducing ability of irradiated MWE

To measure the immunomodulatory ability of detoxified MWE, NO production was measured in RAW264.7 macrophage cells (Fig. 2). The concentration of NO

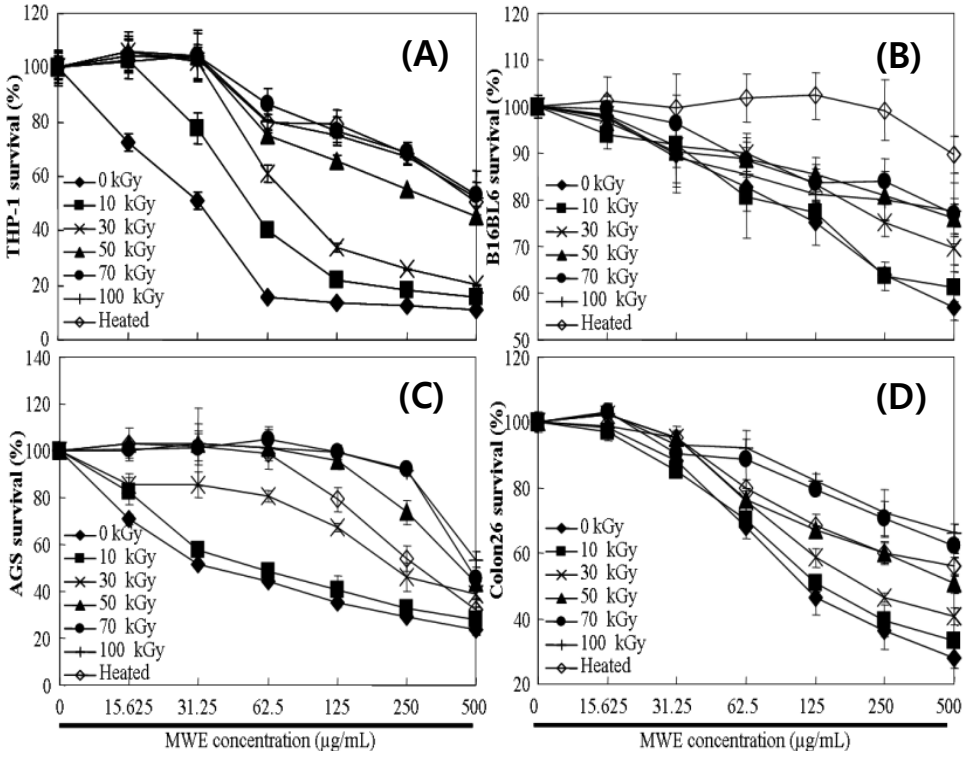

Fig. 2 Effects of detoxified mistletoe water extract by gamma irradiation on various tumor cell lines. (A) THP-1, (B) B16BL6, (C) AGS, (D) Colon26.

produced by non-treated (PBS) cells was 6.2 ± 0.08 μM. Treatment of macrophage cells with non-irradiated (0 kGy) MWE significantly enhanced NO production to greater than 13 μM, which is more than twice that produced by non-treated cells. Cells treated with gamma-irradiated MWE also produced more NO than non-treated cells; however, the concentration of NO decreased as the irradiation dose to MWE increased. Nevertheless, cells treated with MWE that was irradiated with more than 50 kGy showed fairly high NO production, almost 9 μM compared to non-treated cells. A similar tendency on NO production was observed in electron beam-irradiated MWE-treated cells.

In contrast to irradiated MWE-treated cells, which did produce NO, heat-treated MWE-treated cells did not produce NO. The NO concentration generated by these cells was 6.4 ± 0.26 μM, which was the same as that produced by non-treated (PBS) cells, indicating that heat treatment does not preserve the immunomodulatory activity of MWE. However, these data suggest that the immunomodulatory activity of MWE is maintained to some extent after irradiation with gamma rays or electron beams. It should be noted that there was no difference in NO production between gamma-irradiated and electron beam-irradiated MWE-treated cells.

NO is an important biomarker in the body that is associated with various biological responses. These biological activities play significant roles in antibacterial, antiparasitic, and tumoricidal activities in mice (Nathan and Hibbs, 1991). In addition, the NO produced in innate immune cells is essential for the treatment of tuberculosis in mice and humans (MacMicking *et al.*, 1997). In this study, NO production was observed in gamma- or electron beam-irradiated MWE-treated cells, indicating that the immunological activities of MWE were retained even after irradiation. The results of this study indicate that ionizing irradiation could be an effective method for reducing the cytotoxicity of the mistletoe extract while maintaining its immunomodulatory activity. However, further studies, including structural analyses, are required to clarify the detoxification mechanisms of MWE.

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SPME-GC METHOD FOR THE DETERMINATION OF VOLATILE AMINES AS INDICES OF FRESHNESS IN FISH SAMPLES

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ABSTRACT

Volatile amines including, methylamine, dimethylamine and trimethylamine have been widely used for measuring freshness of seafood. In the present work an SPME-GC-FID method to evaluate the volatile amine contents in fish samples stored under controlled conditions was developed. The optimum conditions for the extraction of amines were as follows: 50/30 μm DVB/Carboxen/PDMS fibre, extraction time and temperature of 15 min and 21°C. In addition, total volatile basic nitrogen (TVB-N) was determined by a titration method. Results showed good agreement between two determinations.

Key words: Freshness, fish quality parameters, SPME-GC, TVB-N, volatile amines.

INTRODUCTION

Fish quality has been commonly evaluated by sensory and chemical tests. Among chemical indices, volatile amines including, methylamine (MA), dimethylamine

(DMA) and trimethylamine (TMA) have been widely used as indices of freshness and spoilage of seafood.

Different techniques including colorimetric assays, gas chromatography and capillary electrophoresis among others have been reported in the literature for their determination. In recent years solid-phase microextraction coupled with gas chromatography (SPME-GC) appears as a promising methodology for the analysis of volatile and semi-volatile compounds. This technique combines simultaneously the extraction and pre-concentration steps. Besides, it is a fast technique and no solvents are required. Simple and rapid methods for the estimation of freshness in seafood for quality control laboratories are required (Pena-Pereira *et al.*, 2010; Chan *et al.*, 2006).

The aim of this work is to develop an SPME-GC-FID method to determine volatile amines in fish samples. Additionally, total volatile basic nitrogen (TVB-N) was determined according Commission Regulation (EC) No 2074/2005.

MATERIALS AND METHODS

Chemicals and analytical standards

Hydrochloric acid and sodium hydroxide were supplied by Merck and Panreac, respectively. The analytical standards: Trimethylamine hydrochloride (CAS 593-81-7), dimethylamine hydrochloride (CAS 506-59-2) and propylamine hydrochloride (CAS 556-53-6) used as internal standard were obtained from Aldrich and methylamine hydrochloride (CAS 593-51-1) was from Fluka, Analytical. Water used for all solutions was obtained from Milli-Q water purification system (Millipore) (Bedford, MA, USA). Stock standard solutions of amines at a concentration of 1000 mg/L were prepared in 0.5 N HCl and stored at 4 °C: Working standard solutions were prepared by dilution.

Samples

Fish samples of roundnose grenadier (*Coryphaenoides rupestris*) were stored in ice and inside a refrigerator at 5 °C.

SPME procedure

Amines were extracted from fish samples as follows: 20 mL of 0.5 N HCl were added to 30 g of sample, and mixed in a vortex (Minishaker, IKA) for five min. Then, the mixture was centrifuged (Hettich zentrifugen) at 5000 rpm for 10 min at 5 °C. The supernatant was removed and made up to 10 mL in a volumetric flask containing the internal standard. 500 µL of the resulting solution and 1 mL of 15 N NaOH was transferred into 20 mL headspace vial and a small magnetic stirring bar was also added to the vial before was capped. Then, the SPME fibre was exposed to the headspace of the sample for 15 min. The fibre was conditioned into the GC injector at 270 °C during 1 h following the supplier's instructions prior the first use.

A SPME fibre coated with a 50/30 µm layer of divinylbenzene–carboxen–polydimethylsiloxane (DVD–CAR–PDMS) (Supelco, Bellefonte, PA, USA) was used for extraction of amines.

GC-FID analysis

Analyses were carried out on a GC 8000 TOP CE Instruments gas chromatograph equipped with a flame ionization detector (FID). Amines were separated on an Rtx®-VolatileAmine (30 m x 0.32 mm I.D.) column.

The GC conditions were as follows: the injector temperature was 200 °C, and the split ratio 1:25. Helium was used as carrier gas and the flow rate was 1.3 mL/min. Detector temperature was kept at 250 °C. The ramp temperature was initially set at 40 °C (5 min) then increased at the rate of 10 °C/min until 120 °C and held for 2 min. The software Chrom-Card for Windows was used for data processing.

Total volatile basic nitrogen (TVB-N)

TVB-N was determined according Commission Regulation (EC) No 2074/2005. The method involves distilling an extract deproteinised by perchloric acid. Results were expressed as mg /100 g.

RESULTS AND CONCLUSIONS

Solid-Phase-MicroExtraction (SPME) has been successfully applied to extract the volatile amines from fish samples. It is a simple, fast and solvent-free technique that combines the extraction and pre-concentration into one step.

A triple-coated fibre 50/30 μm DVB/Car/PDMS has demonstrated to be suitable for the analytes studied. A complete extraction was achieved after 15 min of exposure.

Fig. 1 shows a chromatogram of volatile amines of a fish sample. The compounds were identified by comparison of their retention times with those of pure standards.

For quantitative analysis a series of volatile amines standards of known concentrations were used. The calibration curves were constructed based on mg/L concentration levels ranging between 0.5–10 mg/L for MA, 0.25–10 mg/L for DMA and 0.1–10 mg/L for TMA. A constant amount of the internal standard was added. Each point of the calibration curve is the average of three peak-area measurements.

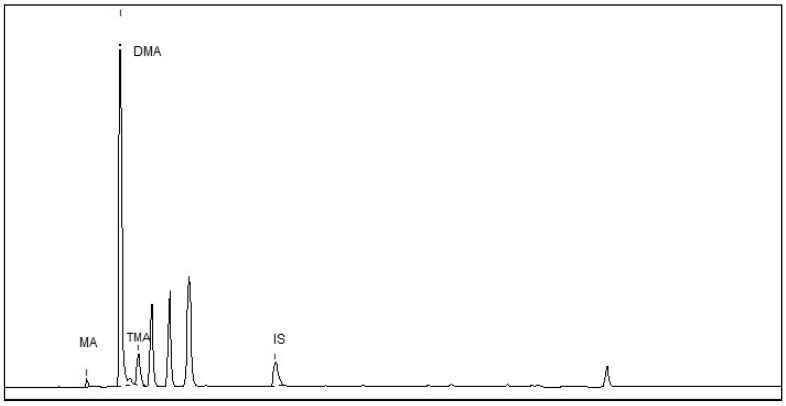

Fig. 1 - GC-chromatogram of volatile amines of a fish sample.

The equations and determination coefficients obtained were the follow: $y=0.0305x+0.0106$ ($R^2=0.9991$) for MA; $y=0.1387x+0.0183$ ($R^2=0.9961$) for DMA and $y= 0.1796x-0.0163$ ($R^2=0.9847$) for TMA.

Detection limits, calculated in accordance with ACS Guidelines (defined as a

signal three times the height of the noise level) were 0.25 mg/L for MA, 0.1 mg/L for DMA and 0.05 mg/L for TMA.

Once the analytical conditions were established the method was applied to determine volatile amines in fish samples stored in ice and inside a refrigerator at 5 ºC in three consecutive days. The TVB-N was also determined. The samples were analyzed in triplicate and the values were expressed as mg N/100g sample.

The results obtained were the following: Day 1: (MA) 0.5691±0.3242; (DMA) 0.3636±0.0028; (TMA) 0.0310±0.0219; (TVB-N) not detected; Day 2: (MA) not detected; (DMA) 2.3436±0.0600; (TMA) 0.0060±0.0009; (TVB-N) 1.33±0.05 and Day 3: (MA) no detected; (DMA) 1.6055±0.0045; (TMA) 0.0060±0.0014; (TVB-N) 1.49±0.73. The MA content decreases along the time whereas the DMA and the TVB-N levels increase progressively during storage. Results showed good agreement between two determinations.

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IN VIVO TOXICOLOGICAL SAFETY OF HIGH DOSE IRRADIATED *TARAKJUK* (MILK PORRIDGE) AS A PATIENT FOOD

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ABSTRACT

The purpose of this study was to evaluate the safety of *Tarakjuk* irradiated with a dose of 30 kGy over a course of a 3-month period in order to determine its possible use as a patient food. Assessment of subacute toxicity was performed by observing effects on appearance, behavior, mortality, body weight, organ weight, and hematological parameters in mice following *Tarakjuk* consumption. Male and female ICR mice were fed AIN39G diet comprising 30 kGy-irradiated or non-irradiated freeze-dried *Tarakjuk*; mice fed AIN39G diet without *Tarakjuk* were termed as controls. During the experimental period, no significant differences in appearance, behavior, mortality, body weight, organ weight, or food consumption were observed between the test and control mice. In histopathological examination including hematological analyses, no significant difference was found between the test mice and controls. These results suggest that irradiation of *Tarakjuk* with doses up to 30 kGy does not cause any toxic histopathological effects and that irradiated *Tarakjuk* could be used in the development of safe patient food.

Key words: *Tarakjuk*, milk porridge, high-dose irradiation, subacute toxicity, ICR mice.

INTRODUCTION

Energy-efficient non-thermal sterilization is an advanced application of radiation technology (Byun, 1997). In pasteurization or sterilization of foods, radiation has been shown to effectively control biological hazards associated with foods without compromising their nutritional properties (Skala *et al.*, 1987; Thayer *et al.*, 1986). It has been reported that when gamma irradiation with doses below 10 kGy was applied to *Tarakjuk* (a traditional Korean milk porridge), its hygienic quality and shelf-life were improved (Han *et al.*, 2011). *Tarakjuk*, made with milk and waxy rice, is used to improve nutrition for senior and patients. Therefore, irradiated *Tarakjuk* have to be considered in consumer acceptance and industrial application. The wholesomeness of irradiated foods has been evaluated in many *in vitro* and *in vivo* systems, and the results have shown enhanced food preservation (WHO, 1999). Nevertheless, many consumers and managers working in the food industry require safety assurances for irradiated items (ICGFI, 1994). For this reason, evaluating the wholesomeness of high-dose irradiated *Tarakjuk* (with doses more than 10 kGy) is necessary to address concerns regarding its human consumption. This study evaluated the wholesomeness of *Tarakjuk* irradiated with a dose of 30 kGy for patient consumption by using indicators of subacute toxicity, such as body weight, food consumption, organ weight, and hematology assays in ICR mice fed high-dose irradiated *Tarakjuk* for 3 months.

MATERIALS AND METHODS

Tarakjuk preparation and ionizing radiation

Tarakjuk was prepared using 1000 mL of milk (Seoul milk, Yangju, Korea) and 200 g of worked waxy rice (Jeongup, Korea). Waxy rice was prepared by soaking in water at 20°C for 6 h and dried for 16 h at the same temperature. Dried waxy rice was powdered using a mixer (HMF-1000A, Hanil electric, Incheon, Korea). Milk and waxy rice were purchased from local supermarkets. Powdered waxy rice was roasted at 150°C for 10 min, and 400 mL of water was added to the roasted waxy rice powder and boiled at 100°C for 2 min. The rice was then boiled with milk 3 times at 80°C for 15 min. The *Tarakjuk* was irradiated at a dose of 30 kGy by gamma ray. Gamma irradiation was performed using a cobalt-60 irradiator (IR-79; Nordion International Ltd., Ottawa, Canada) with 11.1 PBq source strength, and the dose rate was 10 kGy/h. Dosimetry was performed using cellulose triacetate film.

Diet preparation, animals, and dietary administration

The samples irradiated with 0 and 30 kGy were lyophilized and pulverized. The laboratory animals were fed AIN93G standard feed. The subacute toxicity study of *Tarakjuk* irradiated with 30 kGy was conducted on the basis of Kang's method (Kang *et al.*, 2001). Male and female Crj:CD-1 (ICR) mice aged 5 weeks were used after a 1 week acclimatization period. On the day before study initiation, mice were divided into 4 groups of each sex, with a total of 8 groups (10 mice/group of each sex). During this period, and throughout the experiment, the mice were housed individually in plastic cases with cedar chip bedding, and were kept under controlled temperature (25 ± 1°C) and relative humidity (55 ± 5%) conditions with a 12-h light/dark cycle. All *in vivo* experiments were carried out with the approval of The

Institutional Animal Care and Use Committee (IACUC) at Korea Atomic Energy Research Institute.

Clinical observation, body weight, and food consumption

During the experimental period, the mice were observed daily, and clinical signs and mortality were recorded. Body weight and food intake were measured weekly throughout the study period. At the end of the administration period, the mice were killed by decapitation.

Tissue preparation and organ weight

Gross observations were made at autopsy, and recorded. The organs from each mouse were weighed.

Histopathological examination

Fresh organs were fixed in 10% phosphate-buffered formalin and embedded in paraffin wax. The wax-embedded organs were cut into 7 μm sections, stained with hematoxylin and eosin, and then examined by light microscopy (Opticaphot-2, Nikon, Tokyo, Japan).

Data were examined for equal variance and normal distribution prior to statistical analysis. Mean values were compared using the general linear model procedure by using SAS 9.2 (SAS Institute, Cary, NC, USA). Tukey's multiple range tests were used. A P value of less than 0.05 was considered statistically significant.

RESULTS AND CONCLUSION

Body weight, food consumption and organ weight

No deaths were observed in the control, 0 kGy, and 30 kGy groups during the 12 week experimental period (Table 1 & 2). The measurements of initial body weight (males, 28.7 – 29.0 g; females, 22.0 – 23.1 g), final body weight (males, 38.4 – 39.2 g; female, 32.4 – 32.6 g), body weight gain (males, 9.6 – 10.4 g; females, 9.3 – 10.5 g), and food intake (males, 4.3 – 4.7 g/day; females, 4.1 – 4.2 g/day) indicated no significant differences between the control, 0 kGy, and 30 kGy groups.

Table 1 - Effect of 30 kGy-irradiated *Tarakjuk* on body weight and food consumption of IRC mice

Sex	Group	Initial body weights (g)	Final body weights (g)	Body weight gain (g)	Food Intake (g/day)
Male	Control	28.78±1.10[a]	38.43±1.65[a]	9.61±1.81[a]	4.58±0.51[a]
	0 kGy	29.00±1.78[a]	38.67±1.37[a]	9.67±0.70[a]	4.39±0.71[a]
	50 kGy	28.80±0.44[a]	39.20±1.31[a]	10.40±0.36[a]	4.76±0.55[a]
Female	Control	22.47±1.00[a]	32.67±1.21[a]	10.20±0.56[a]	4.21±0.45[a]
	0 kGy	23.10±0.70[a]	32.47±2.61[a]	9.37±0.90[a]	4.16±0.55[a]
	50 kGy	22.07±0.81[a]	32.60±1.18[a]	10.50±1.22[a]	4.10±0.62[a]

Intergroup differences in organ weights of the liver (males, 5.1 – 5.2 g/100g BW; females, 4.4 – 4.7 g/100g BW), spleen (males, 0.2 – 0.3 g/100g BW; females, 0.3 g/100g BW), testis/ovary (males, 0.7 g/100g BW; females, 0.3 g/100g BW), lung (all, 0.5 – 0.6 g/100g BW), and heart (males, 0.5 g/100g BW; females, 0.4 g/100g BW) were not significant. Body weight, food consumption, and organ weight showed no statistically significant effects of irradiation.

Table 2 - Effect of 30 kGy-irradiated *Tarakjuk* on organ weight of IRC mice.

Sex	Group	Liver (g/100g BW)	Spleen (g/100g BW)	Kidney (Left) (g/100g BW)	Kidney (Right) (g/100g BW)	Testis/Ovary (g/100g BW)	Lung (g/100g BW)	Heart (g/100g BW)
Male	Control	5.11±0.56^a	0.31±0.04^a	0.69±0.07^a	0.66±0.08^a	0.74±0.01^a	0.54±0.07^a	0.52±0.08^a
	0 kGy	5.20±0.08^a	0.25±0.06^a	0.69±0.07^a	0.70±0.03^a	0.71±0.13^a	0.61±0.03^a	0.51±0.06^a
	50 kGy	5.22±0.29^a	0.26±0.03^a	0.71±0.02^a	0.67±005^a	0.74±0.03^a	0.54±0.06^a	0.52±0.05^a
Female	Control	4.42±0.22^a	0.31±0.04^a	0.44±0.08^b	0.48±0.03^b	0.35±0.05^a	0.56±0.03^a	0.40±0.06^a
	0 kGy	4.76±0.41^a	0.34±0.04^a	0.56±0.04^a	0.56±0.02^a	0.37±0.04^a	0.62±0.07^a	0.43±0.07^a
	50 kGy	4.48±0.30^a	0.32±0.05^a	0.53±0.03^{ab}	0.50±0.03^{ab}	0.35±0.09^a	0.58±0.05^a	0.42±0.09^a

Histopathological examination

Gross pathological examination of the liver and kidney indicated no abnormalities (Fig. 1). Moreover, no alterations were seen in the microscopic examination of the internal organs. Specifically, cellular degeneration (tissue atrophy, necrosis, exfoliation, and inflammation) was unremarkable in all groups and sexes.

Many studies of subchronic toxicity have been performed with irradiated foods.

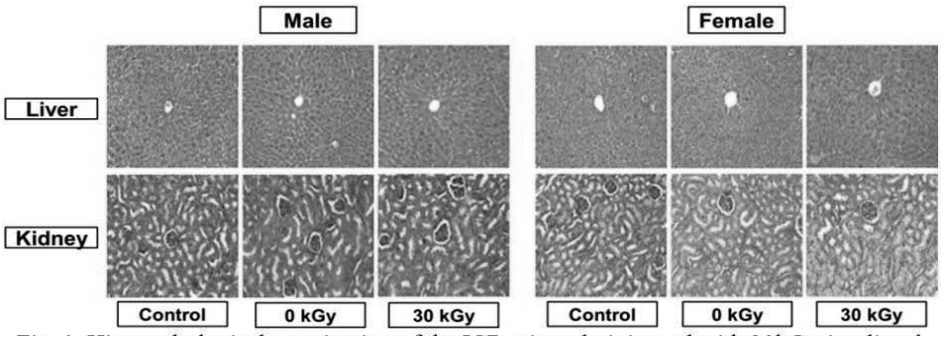

Fig. 1 - Histopathological examination of the ICR mice administered with 30kGy-irradiated *Tarakjuk* for 3 months (× 250).

Previous studies that involved the use of high-dose-irradiated diets in animals such as rats and mice, showed no toxicological hazards (Kang *et al.*, 1998; Monsen, 1960; Thompson *et al.*, 1965). The results of our present study are similar to previously reported results, and indicate that irradiation of *Tarakjuk* with doses up to 30 kGy does not produce any toxic effects. Thus, high-dose-irradiated *Tarakjuk* could be used in the development of safe patient food.

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