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G.G. Buonocore & E. Torrieri

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This Special Issue of the Italian Journal of Food Science collects the presentations given at the "SLIM 2019, Shelf-Life International Meeting" organized by the Italian Scientific Group of Food Packaging (GSICA), the Department Of Agricultural Science, University Of Naples Federico II and the Institute Of Polymers, Composites And Biomaterials, National Research Council, held in Naples, Italy, on June 17-20, 2019.
These papers were reviewed by the Scientific Committee of SLIM 2019 before their presentation but they did not undergo the conventional reviewing system of the Italian Journal of Food Science.

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The Italian Journal of Food Science is an international journal publishing original, basic and applied papers, reviews, short communications, surveys and opinions on food science and technology with specific reference to the Mediterranean Region. Its expanded scope includes food production, food engineering, food management, food quality, shelf-life, consumer acceptance of foodstuffs, food safety and nutrition, energy and environmental aspects of food processing on the whole life cycle.

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

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INTRODUCTION

The current volume includes the selected manuscripts peer-reviewed by the Scientific Committee of the 9th Shelf Life International Meeting (SLIM 2019), which was held in Naples, ITALY on 17-20 June 2019. The conference was organized by the Department of Agricultural Science, University of Naples Federico II and the Institute of Polymers, Composites and Biomaterials, National Research Council, in collaboration with the Italian Scientific Group of Food Packaging (GSICA).

The focus of the 9th SLIM was on *Shelf life assessment, prediction and extension by applying new, safe and sustainable materials and technologies*. The conference purposed to offer a knowledge sharing platform for experts around the world with the aim to disseminate the fundamental aspects, research findings as well as industry applications and innovation on food shelf life and sustainability. The diversity of the attendees and the careful selection of the contributes from both industries and academic institutions significantly contributed to the advancement of knowledge and to promote scientific discussions in the fields of food science, packaging and shelf life.

The Conference Proceedings, published once again with the support of Chiriotti Publisher as a special issue of the Italian Journal of Food Science, contain research reports presented as oral and poster papers during SLIM 2019. The conference was organized into five sessions: Shelf Life Assessment and Prediction, New Technologies for Shelf Life Extension, Safety Issues of Packaging Materials, New Materials for Shelf Life Extension, Sustainability and Shelf Life. The conference features multidisciplinary lectures from five distinguished keynote speakers, 37 oral presentations, and 55 poster presentations; and the participants who contribute to the forum are from fourteen countries, which are Belgium, Brazil, Colombia, Germany, Greece, Italy, Mexico, Philippine, Portugal, Korea, Spain, UK, USA and Thailand.

As it was officially announced within the final remarks of the conference, the 10th Shelf Life International Meeting (SLIM 2022) will be held in Bogotà, Colombia. The Instituto de Ciencia y Tecnología de Alimentos (ICTA) of the National University of Colombia confirmed its commitment to organize the next conference. The local organizing committee is chaired by Prof. Carlos Alberto Fuenmayor. Further useful information will be disclosed soon. Hope to see you at our next SLIM 2022 meeting!

G.G. Buonocore & E. Torrieri

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SESSION I

Shelf Life Assessment and Prediction

APPLICATION OF MORINGA SEED AS SWEETNESS-INDUCING SUBSTANCE

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ABSTRACT

The study aimed to establish sweetness-inducing property of moringa seed and its application in beverage. The flavor profile was evaluated by descriptive analysis. The sweetness of drinking water was significantly increased after tasting moringa seed. This property was further investigated at pH 2, 4, 6, and 8 and the stability was examined at 40, 60, 80, and 100°C. The results revealed that increasing pH of moringa infusion significantly increased sweetness of drinking water, while sweetness intensity decreased with increase in temperature. The application in green tea revealed that 5% of moringa seed was sweeter than 7% of fructose.

Keywords: flavor, taste, sweetness-inducing substance, moringa (*Moringa oleifera* Lam.) seed, sugar-sweetened beverage

1. INTRODUCTION

High consumption of sugar-sweetened beverages (SSBs) is the one of reason increases the risk of obesity (PAPIER *et al.*, 2017). SSBs taxation is currently extensively discussed and researched to decrease the problems of overweight and obesity. In diverse countries such as France, Hungary, Mexico, Thailand and various states of the USA have already consummate impel taxes on SSBs (HEISE *et al.*, 2016). Therefore, reducing sugar consumption by using sugar substitute is becoming more popular. However, some artificial sugar substitutes, such as saccharin and aspartame, may affect health (ZHAO *et al.*, 2018; OKASHA, 2016). Sugar substitutes from natural sources, such as stevioside and monk fruit extract, become more popular (CARDELLO *et al.*, 1999; PARKER *et al.*, 2018). Moreover, identification and application of natural sweetness-inducing or taste-modifying compounds have been studied. For example, BARTOSHUK *et al.* (1972) identified the chlorogenic acid, the phenolic acid in artichoke, which induced sweet aftertaste and FRY (2012) study the sweetness-inducing characteristic of protein neoculin in lemba fruit. MISAKA (2013) also reported the protein miraculin in miracle fruit, which modified the sour taste to sweetness. Besides the above mentioned, the sweetness of drinking water can be induced after consumption of mature moringa (*Moringa oleifera* Lam.) seed. This is the phenomenon of Thai traditional medicine but there is a lack of scientific explanation. Therefore, this research aimed to evaluate flavor profile and taste property of moringa seed. Influencing factors of induced sweetness and application in green tea had been studied as well.

2. MATERIAL AND METHODS

2.1. Materials

Dried moringa seeds were supplied from Lop Buri province, Thailand and the references for descriptive sensory analysis were purchased from the local supermarket. Citric acid (food grade), sodium bicarbonate (food grade), fructose powder (food grade) and unsweetened green tea were obtained from R&B Food Supply Co., Ltd. (Ayutthaya, Thailand), Continental Food Co., Ltd. (Bangkok, Thailand), Ma-jusmin Co., Ltd. (Nonthaburi, Thailand) and Ichitan Group Co., Ltd. (Ayutthaya, Thailand), respectively.

2.2. Flavor profile and taste property of moringa seed

The flavor profile and taste property of three samples including moringa seed, drinking water after consuming seed and moringa seed infusion were investigated by using descriptive sensory analysis. Moringa seed powder was prepared in drinking water (10% w/w), stirred at 250 rpm for 30 min and filtered through cotton cloth to obtain the infused water. The 11 panelists (3 male and 8 female, 25-30 years old) were selected and trained to describe flavor characteristics of samples (Table 1). The attribute intensities were scored on 15-cm line scale.

2.3. Influencing factors of induced sweetness

The effects of pH and temperature on sweetness-inducing property of moringa seed were studied by sensory approach. Moringa seed infusion was prepared as previous described and the pH was adjusted to 2, 4, 6 or 8 by 1 M citric acid or 1 M sodium bicarbonate. The pH-adjusted samples were randomly served at room temperature. During serving samples, cracker and drinking water were used as a palate cleanser. Eighteen panelists were scored a sweetness intensity of moringa seed infusion and drinking water after consuming infused water by using a category scale. For the effect of temperature, moringa seed infusion was stirred and heated to reach the set temperature at 40, 60, 80 and 100 °C and held for 15 min. The samples were immediately cooled and randomly served to score the sweetness intensity.

Table 1. Flavor lexicon of moringa seed.

Attribute	Definition	Reference	Intensity
green	aromatic associated with green herb	2 g of green tea (Ban-Cha, Japan) in 100 mL of boiled water	5
woody	aromatic associated with bark	2 g of *Ganoderma lucidum* tea (Dr. green®, Thailand) in 100 mL of boiled water	7
bitter	bitter taste stimulated by bitter herbs	0.02 g of *Andrographis paniculata* (Burm. f.) Wall. Nees powder in 100 mL of water (Abhaibhubejhr®, Thailand)	10
sweet	sweet taste stimulated by sweetener	4 g of stevia in 100 mL of water (Equal®, USA)	10
astringent	dry and puckering feeling in the mouth	2 g of *Ganoderma lucidum* tea (Dr. green®, Thailand) in 100 mL boiled of water	6
nutty	sensation in the mouth associated with legumes	sunflower seed (Flowerfood®, Thailand)	5

2.4. Application in green tea

Unsweetened green tea was used to prepare 7% (w/v) fructose- and 5% (w/v) moringa seed-sweetened green tea. Those models were filtrated to obtain clear beverages before serving at room temperature. Thirty panelists were scored the sweetness intensity of green tea samples using a category scale.

3. RESULTS AND DISCUSSION

3.1. Flavor profile and taste property of moringa seed

Flavor of moringa seed were described by two retronasal (green and woody), two taste (bitter and sweet), and other two sensation (astringent and nutty) attributes (Fig. 1). According to the odor quality of the volatile, the woody note of moringa seed might be contributed by β-caryophyllene and humulene. Woody note of herb is normally derived from the terpenoid pathway. β-Caryophyllene was the most abundant terpene in moringa seed oil and humulene presented in lower amount (ABBASSY *et al.*, 2018). In addition, green note of moringa seed might be from aliphatic C6 volatiles which derived from C18 unsaturated fatty acids. Moringa seed contained a significant amount of oil (36.7%) and oleic was the major fatty acid (73.57% of total fatty acids) followed by linoleic and linolenic (LEONE *et al.*, 2016). These unsaturated fatty acids involved in lipoxygenase pathway to yield aliphatic C6 volatiles such as hexanal and *trans*-2-hexenal which delivered the scent of green note (NEGRE-ZAKHAROV *et al.*, 2009). High content of oleic acid might associate with the nutty sensation of moringa seed as well. The receptors of fatty acid at human gustation and olfactory epithelium were named as GPR120 (GALINDO *et al.*, 2012) and CD36 (KINDLEYSIDES *et al.*, 2017), respectively. In addition, the phenolic compounds could cause astringent and bitterness of moringa seed. Moringa seed contained high content of phenolic compounds such as gallic acid, caffeic acid, catechin and quercetin (SINGH *et al.*, 2013) and the later three polyphenols had astringent and bitter characteristics. Moreover, alkaloids, glucosinolates and isothiocyanates, the bioactive compounds in moringa seed (CHODUR *et al.*, 2018), could provide bitter taste (PIZIO *et al.*, 2018) as well.

The bitterness was the highest attribute in moringa seed and the intensity decreased after drinking water (Fig. 1). On the other hand, the sweetness intensity of drinking water after seed consumption was four-fold increase (Fig. 1). This sweetness-inducing property could also be observed in moringa seed infusion. The results indicated that moringa seed contained sweetness-inducing water-soluble substance and had high potential to use in beverage.

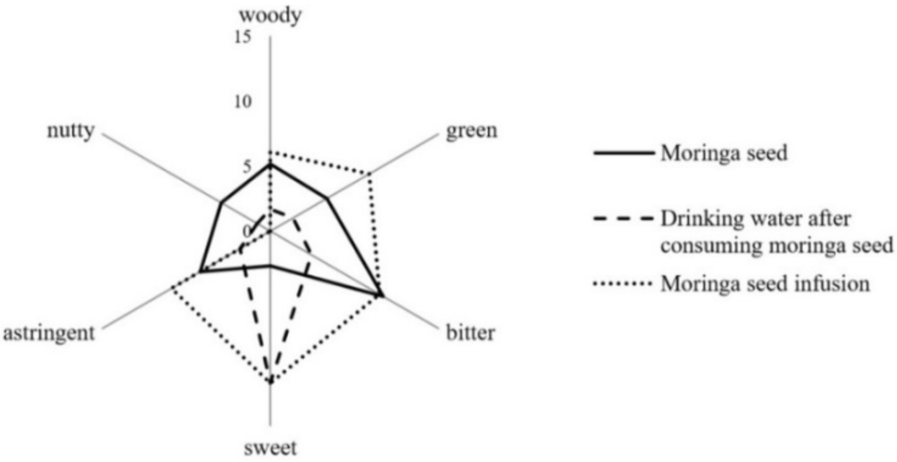

Figure 1. Flavor profiles of moringa seed, drinking water after consuming moringa seed and its infusion (n=11).

3.2. Influencing factors of induced sweetness

There are many factors such as taste quality, calorie, cost, stability and solubility that should be considered for sweetener selection, especially in different kinds of beverages. The pH is one of the most important processing parameters for quality assurance of sweetener application in beverages. The whole pH range of non-alcoholic beverages was approximately 2-7 (REDDY *et al.*, 2007). The sensory results indicated that moringa seed cloud induce sweetness of beverage models at pH 2, 4, 6 and 8 (Fig. 2). The moringa seed showed better sweetness-inducing ability at neutral to alkaline pHs than those at acidic pH. The less sweetness-inducing potency of moringa seed at low pH might be due to the degradation of sweetness-inducing substance. Furthermore, sweetness intensity of drinking water was higher than those of infusion at acidic pHs, especially at pH 2. The result implied that strong sour taste from acidulant could suppress the sweet perception. This result was supported by the experiment of STAMPANONI (1993), which reported the sweetness reduction of 17% sucrose solution after increasing of acid content. The effect of pH on sweetness-inducing property has also been established in miraculin. The sweetness of lemonade juice was induced by micraculin but there was no significant sweetness intensity when applying in green tea (ANDRADE *et al.*, 2019).

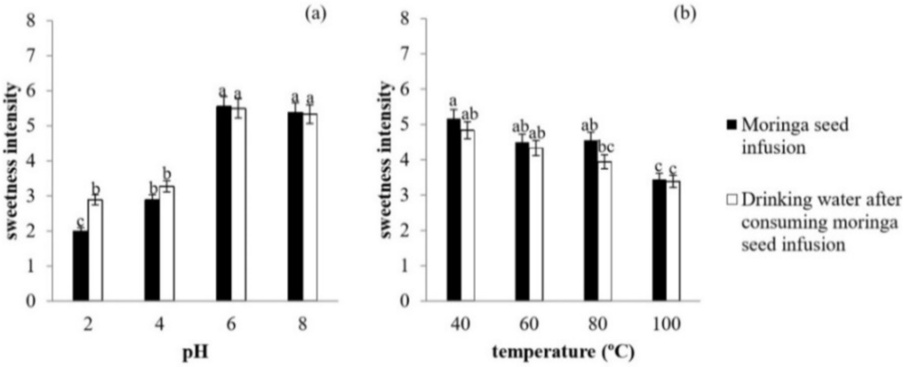

Figure 2. Effect of pH and temperature on sweetness-inducing intensity of moringa seed infusion and drinking water after consuming the infusion (n=18).

The temperature is another consideration factor for sweetener choice in beverage production. The tested temperature in this experiment was set according to the process of beverage such as blanching, pre-treatment and pasteurization. Moringa seed infusions heated at 40, 60, 80 and 100ºC were still able to induce sweetness (Fig. 2). However, the inducing potency was decreased when increasing the temperature and the significantly reduction was observed at 100ºC. The panelists also perceived the sweetness of drinking water after consuming all heated moringa seed infusion. This result was similar to the study of neoculin by FRY (2012). The author reported that the sweetness-inducing potency of neoculin was reduced when the temperature was above 50ºC and the property was completely loss at 75°C.

3.3. Application in green tea

Green tea was chosen as beverage model in this experiment because its pH, approximately 6-7, was optimum for sweetness-inducing property of moringa seed. Green tea is also one of the most famous beverages and worldwide consumed. The sensory results indicated that moringa seed had ability to induce sweetness of green tea and drinking water after consumption. In addition, 5% moringa seed-sweetened green tea was sweeter than 7% fructose-sweetened sample (Table 2). This result showed superior application of moringa seed in commercial beverage compared with the miraculin. Unable to use miraculin as sweetness-inducing substance in green tea was stated by ANDRADE *et al.* (2019). They found that miraculin-sweetened green tea was as sweet as unsweetened green tea. Thus, the limitations of miraculin in neutral-basic beverages were resolved by moringa seed.

Table 2. Sweetness intensities of green tea samples.

Sample	Sweetness intensity[a]	
	Green tea	Water after consuming sample
unsweetened (control)	0.77±0.72d	0.13±0.34d
7% (w/v) fructose-sweetened	5.57±1.02b	0.27±0.44d
5% (w/v) moringa seed-sweetened	5.90±1.47a	2.37±0.87c

[a] Sweetness intensity scored using a category scale (8 = extremely sweet to 0 = non-sweet). Means ± standard deviations (n=30) followed by the same letter indicated no significantly difference ($p > 0.05$).

4. CONCLUSIONS

Moringa seed has the ability to induce sweet taste of water and infusion, especially at the optimum pH of 6-8 and the temperature below 100ºC. Although the sweetness-inducing efficiency of moringa seed is pH-dependent, its property can be apply to the whole pH range of various non-alcoholic beverages. Therefore, moringa seed has high potential implications for alternative sugar substitute. Some evidence of this research can help to decrease the risk of health problems from high sugar consumption.

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FOOD LIFE EXTENSION BY CELLULOSE NANOCRYSTALS COATINGS

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ABSTRACT

The scope of this paper was to use the cellulose nanocrystals (CNCs) coatings with outstanding gas barrier properties to implement fully compostable laminates (LAM_{CNC}) structured of "Cellophane (19μm)/Aluminum metallization (<1 μm)/Tie (2μm)/CNCs (1μm)/Tie (3μm)/PLA (55μm)" in replacement of synthetic laminates (LAM_{EVOH}) "PET (28μm)/Tie (1.2μm)/EVOH (3.3μm)/Tie (1.2μm) PET (25μm)/Tie (1.2μm)/PE (12.25μm)" currently used for oxidation-sensitive food products. Subsequently, a comparative two-month food shelf-life assessment was designed by using the two types of laminates to fabricate identical-sized pouches and filled with ground coffee and grated cheese then sealed under 100% N_2 to be finally stored at 23°C/30°C/40°C and 5°C/23°C respectively.

Keywords: cellulose nanocrystals (CNCs), food oxidation, food shelf-life, fully compostable and synthetic laminates

1. INTRODUCTION

In recent years, oxidation has been one of the major challenges for the scientific and manufacturing community in terms of food quality decay, economic losses and environmental concerns. All foods containing lipids can undergo oxidation compromising their quality with adverse physicochemical changes (LABUZA and DUGAN, 1971). Nowadays, to prevent food spoilage from oxidation, vacuum, oxygen-scavengers, modified atmosphere packaging (MAP) and oxygen-barrier synthetic laminates based on EVOH copolymer (Ethylene vinyl alcohol) are employed. However, with the growing awareness of the environmental impact of these non-renewable oil-based materials, the search for more sustainable alternatives has recently been scientists' focus (Li et al., 2015). Bio-polymers such as PLA (Polylactic acid) and PHAs (Polyhydroxyalkanoates) might be valid alternatives; however, they lack gas barrier properties. Recently, nanotechnology has become a very captivating field because it allows materials to be manipulated on the nanometric scale for providing new properties useful for the development of more advanced materials (RASHIDI et al., 2011). Cellulose is the most abundant natural polymer that consists of millions of glucoses linked by glicosidic bonds forming a hierarchical organization containing amorphous and crystalline regions. By obtaining an oxygen permeability of 0.06 $cm^3*24h^{-1}*m^{-2}*bar^{-1}$ at 0% RH of PET (Polyethylene terephthalate) films coated with less than 1 μm thick CNCs, Li et al., 2015 confirmed the better improvement in gas barrier properties of the materials coated with CNCs in comparison with 3-4μm thick EVOH (MOKWENA and TANG, 2012). Generally, gas diffusion through any materials broadly depends on direct factors such as crystallinity index, permeant solubility and concentration gradient. Other extrinsic factors such as temperature and relative humidity may also have a significant influence on the permeation of the gas (PIRINGER et al., 2008). Henceforth, CNCs being very hydrophilic by nature due to the presence of many hydrogen bonds and polar groups on their surface tend to swell in high relative humidity (from 40% to 100%), which then causes a gradual disentanglement of the crystalline lattice and causes a dramatic permeation of gases through the coated materials (FOTIE et al., 2017). In this paper, it was demonstrated that one of the best solutions to alleviate the water sensitivity of the CNCs, was to concur to the insulation of CNCs coatings through their lamination between two layers such as a water-repellent structure like the metalized cellophane and a sealable polymer like PLA to set up fully compostable laminates.

2. MATERIAL AND METHODS

2.1. Materials

Fully compostable laminates implementation, 55μm PLA and 19 μm metalized cellophane films and 72 μm synthetic laminates (LAM$_{EVOH}$) constituted of PET (28μm)/Tie (1.2μm)/EVOH (3.3μm)/Tie (1.2μm) PET (25μm)/Tie (1.2μm)/PE (12.25μm were provided by Goglio spa, Italy. Solvent-based adhesive was procured from Sapici spa, Italy and the cellulose nanocrystals isolated by acid hydrolysis (sulfuric acid) from wood pulp were bought from CelluForce, Canada. For food shelf-life assessment, grated cheese with 28% fat content of which 68% saturated and ground coffee with 46% of linolenic acid content were provided by food companies. All chemicals were bought from SIGMA-Aldrich, Italy.

2.2. Methods

2.2.1 CNCs morphology assessment

apparent dynamic diameter of water-dispersed CNCs 1wt% at pH 5.5 was measured by using the DLS (dynamic light scattering) technology (mod. Litesizer 500, Anton Paar, Graz, Austria). Measures were read at 90° detection angle by (90° and 25.0 ±0.1 °C, by means of a 35 mW diode laser (λ = 658 nm) were replicated 5 times. The actual dimensions of the CNCs were evaluated via Transmission Electron Microscopy (TEM).

2.2.2 Evaluation of CNCs charges density

Z Potential of water-dispersed CNCs 1wt% at pH 6 was measured at 15° detection angle by electrophoretic light (ELS) by using the PALS technology (mod. Litesizer 500, Anton Paar, Graz, Austria) replicated 5 times, at 25.0 ± 0.1 °C, by means of a 35 mW diode laser (λ = 658 nm). Strong acid ($-OSO_3H$) and weak acid (-OH) concentrations were calculated through the conductometric titration (mod.Titronic 300, SI Analysis and Multi 3620 IDS, YSI, USA).

2.2.3 PLA coating with CNCs and fully composites lamination

PLA films were coated with water-dispersed CNCs 4wt% through rotating roll then dried in tunnel at 70°C. Subsequently, coated PLA films (1st web unwind) were coupled with metalized cellophane (2nd web unwind) through the laminating process by rotating roll filled of solvent-based polyurethanic liquid adhesives. The lamination was set up at room temperature and the fully compostable laminated (LAM_{CNC}) were stored at the drying oven to allow the solvent to evaporate.

2.2.4 Thickness assessment of CNCs and adhesive layers

For the evaluation of the CNCs layer's thickness, the gravimetric method was adopted. Four samples of coated PLA (10 × 10 cm^2) were weighed (m_1, g), then the coating was removed by washing out the coating hot water (~70 °C) and the uncoated film obtained was dried and weighed (m_2, g). The coating thickness (L, cm) was estimated by Equation: $L = (m_1 - m_2)/100\varrho$, where ϱ = 1.58 g cm^{-3} is assumed as the density of the CNCs. The thickness of the adhesive in LAM_{CNC} was calculated by the difference between the total thickness of the laminated and the thickness of other constituents.

2.2.5 Gas permeability measurements of LAM_{CNC} and LAM_{EVOH}

All the carbon dioxide, oxygen and water vapor permeability measures were performed by an isostatic permeabilimeter (mod. Multiperm, PERMTECH S.r.l., Pieve Fosciana, Italy) according to ASTM standard methods (D-3985 and F-1249 respectively). Oxygen (PO_2) and carbon dioxide (PCO_2) permeability of both laminates measured at 23 °C under 35% RH as for the water vapor transmission rate (WVTR) which was measured at 38°C and 80% RH.

2.2.6 Pouches fabrication, storage and sampling

Pouches (20.5 x 12 cm) were fabricated with the two different laminates, filled with 80 g of ground coffee and 60 g of grated cheese and sealed under 100% of N_2. Subsequently, both types of pouches were stored in dark conditions, at 23, 30 and 40 °C for ground coffee and at 5 and 23 °C for grated cheese at 35% RH. The pouches withdrawal in triplicate was set at time 0 and then 14, 39, 53 and 67 days to perform the headspace gas composition, chemical changes, color by CIE-Lab, microbiological changes (mesophilic total bacterial count) and sensory changes (assessed by 20 untrained tasters).

3. RESULTS AND DISCUSSION

3.1. CNCs dimensions and charges density

Apparent hydrodynamic diameter of CNCs was confirmed by TEM and the CNCs length was found to be 115±4 nm moreover, the Z potential measured was -40±1 mV and the concentration of strong and weak acids were 120 mmol/kg and 350 mmol/kg respectively. The size obtained confirms the nanometric scale of the CNCs and the high Z potential demonstrates the stability of the water-dispersed CNCs and strong charges residues derive from the sulfuric acid reaction with the cellulose.

3.2. Oxygen, carbon dioxide and water permeability

The CNCs coatings thickness was found to be 1 μm and the adhesive thickness was less than 1μm. Gas permeability measures of laminates based on CNCs and EVOH are indicated in Table 1.

Table 1. Permeability values measured on the two laminates used in the Shelf Life tests.

Laminates	T (°C)	OTR at 35% RH ($cm^3d^{-1}bar^{-1}m^{-2}$)		CO_2TR/OTR Gas Selectivity	WVTR at 80% ΔRH ($g\ m^{-2}\ d^{-1}$)
		OTR	CO_2TR		
LAM_{CNC} (compostable)	23	0.47	1.33	2.83	-
	30	0.60	1.92	3.20	-
	38	-	-	-	6.31
	40	0.77	2.21	2.90	-
LAM_{EVOH} (synthetic)	23	0.67	1.38	2.00	-
	30	1.50	3.15	2.16	-
	38	-	-	-	3.84
	40	3.70	9.55	2.58	-

It is clearly evident that the oxygen and carbon dioxide permeability of the two materials are quite similar at 23°C but very different at 30 and 40°C, therefore it was reasonable to evaluate the activation energy of gas diffusion (Ea).The Ea (kJ/mol) estimated were 21.6 and 24.24 respectively, for O_2 and CO_2 for the fully compostable laminate, while they were 77.0 and 87.5 respectively for O_2 and CO_2, in the standard laminates, which confirmed that compostable laminates are less sensitive to the temperature. In addition, the water vapor transmission rate (WVTR) was slightly high in the compostable laminates, which can be explained by their sensitivity in wet environment.

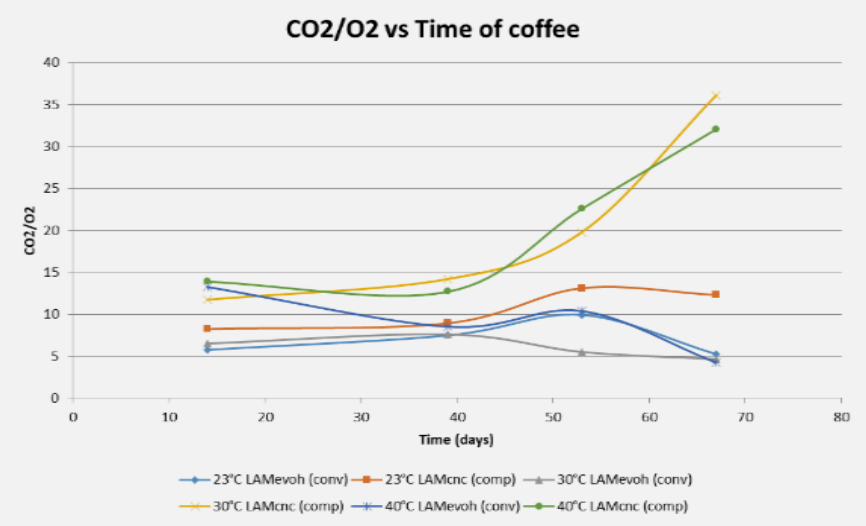

Figure 1. Gas evolution in the pouches of ground coffee.

3.3 Headspace gas composition

Gas composition was evaluated by GC-TCD and the selectivity (CO_2/O_2) was then calculated in the pouches containing the ground coffee (Fig. 1), moreover, the two laminates tested did not show relevant discrepancies in gas permeability, at least at 23°C and partially at 30°C; a bigger difference however, was observed at 40 °C, with a better barrier offered by the compostable laminate. Less than 1% O_2 was found in the pouches with grated cheese during the entire shelf-life.

3.4. Food Colour Changes

Colour difference was estimated according to CIE-Lab 1976 by the equation $\Delta E = [(L_2-L_1)^2 + (a_2-a_1)^2 + (b_2-b_1)^2]^{1/2}$, where 'L' is the Lightness, 'a' the Redness and 'b' the Yellowness of the product colour. A colour difference detectable by human eye is generally considered when DE is higher than 1.8 but this value was never reached in our experiments as the Fig. 2 (left and right) clearly indicates.

3.5. Microbiological analysis

The microbiological control was carried out only on cheese. From the Fig. 3, total bacterial count increases with the increase of the temperature although slight discrepancies were found between both synthetic and compostable laminates.

3.6. Sensory analysis

Five different attributes of sensory quality of the ground coffee were evaluated by a panel of 20 tasters: overall odour intensity, overall acceptability, greasiness, rancid odour, odour persistence. 11 different sensory attributes were evaluated by the same panel for the grated cheese in the two types of laminates and at two different temperatures, as shown in Fig. 4. In any case, no significant difference was found between the two different pouches.

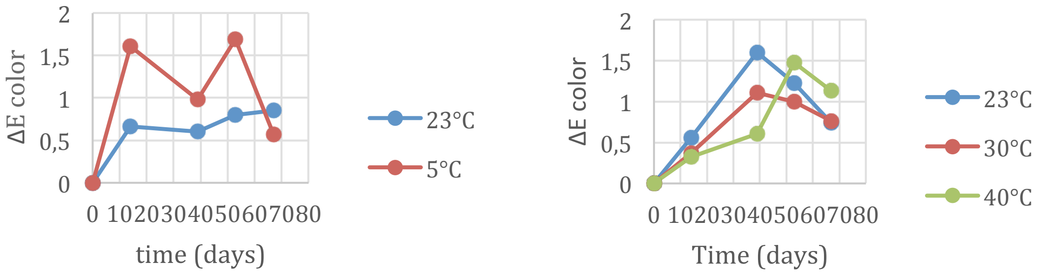

Figure 2 (left). Colour difference of grated cheese and figure 2-right ground coffee over time.

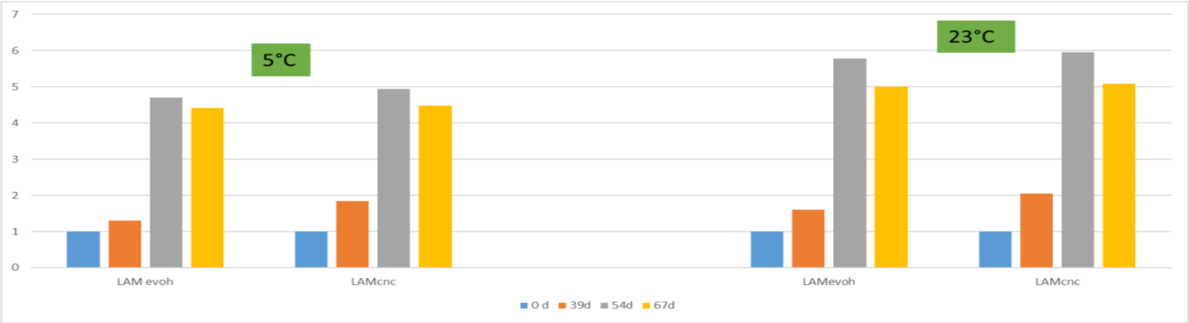

Figure 3. Mesophilic total bacterial count of grated cheese at the two temperatures in both laminates.

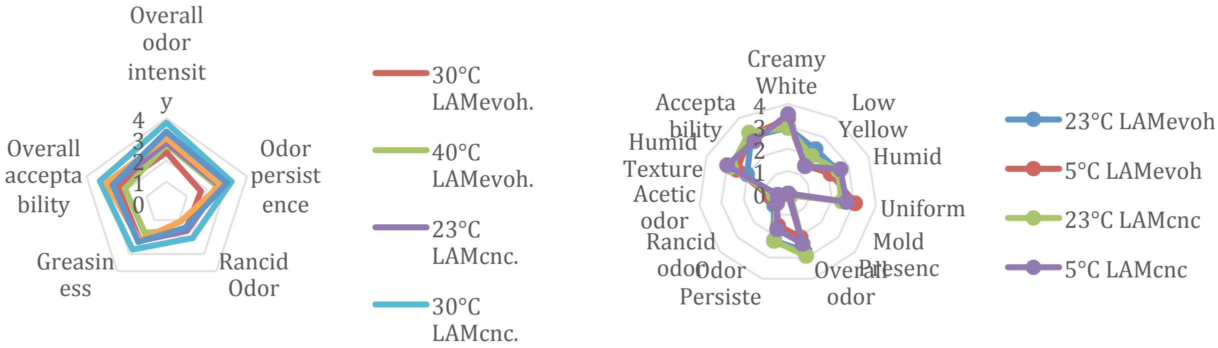

Figure 4. Sensory analysis of ground coffee at three different temperatures (left) and grated cheese at two different temperatures (right) in the two different pouches.

4. CONCLUSIONS

It can be firmly assumed that no significant difference was found between samples packed in different pouches after more than two months at different temperatures even though, it was noted that LAM$_{CNC}$ pouches of ground coffee stored at 30°C and 40°C were the most appreciated by the tasters probably due to higher CO2 barrier of the fully compostable pouches which allowed the accumulation of higher amount of CO$_2$ released by the coffee. This positively affects the sensory attributes of the coffee and the aroma perception by the tasters. In addition, the high WVTR found in compostable laminates does not seem to influence the shelf-life of products. Therefore, in light of the results, it can be concluded that the replacement of synthetic gas barrier with the CNCs-based gas barrier is a certainty, and this will help to implement more advanced and sustainable food packaging materials.

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EFFECT OF *L*-GLUTAMINE ADDITION ON AROMA OF COCONUT SUGAR

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ABSTRACT

This research aimed to determine the impact of *L*-glutamine addition on aroma of coconut sugar. *L*-Glutamine was dissolved in sap at 0, 250, 750, and 1,250 mg/L before preparing sugar. The headspace volatiles were analyzed and the major compounds were acetic acid, 2-methyl pyrazine, furfural, 2,6-dimethyl pyrazine and methional. The results also indicated that addition of *L*-glutamine increased Maillard reaction products, especially 2-methyl pyrazine, vinyl pyrazine, furfural, 2-acethylfuran, 5-methyl-2-furanmethanol, and 2-formylpyrrole. The instrumental analysis was in agreement with sensory profiles. Increase of 2-methyl pyrazine and 2-acethylfuran resulted in higher nutty note, while decrease of 2-methylbutanal caused the reduction of malty.

Keywords: flavor, aroma, coconut sugar, *L*-glutamine, Maillard reaction

1. INTRODUCTION

Coconut (*Cocos nucifera* L.) is a member of the palm tree family (Arecaceae) and it is an important economic crop in Asia. The famous coconut products are juice, milk, oil, sap, and sugar. Coconut sap is a white opaque liquid harvested by cutting the end of inflorescence and collected in a container. Sap has a sweet taste and neutral pH (HEBBAR *et al.*, 2015). It also contains various nutrients such as carbohydrates, amino acids, vitamins and minerals. The majority of sugar in coconut sap is sucrose, while glucose and fructose are small amounts in fresh sap (NAKAMURA *et al.*, 2004). There are many amino acids in coconut sap, for example, glutamic acid, serine, asparagine, aspartic acid, arginine, lysine, alanine, and glycine and glutamine has been reported as a predominant amino acid (0.8 g/L) in coconut sap (HO *et al*, 2007; NAKAMURA *et al.*, 2004). Coconut sap consists of the vitamin B complex, vitamin C, Cu, Zn, Fe, Mg, Na, K and Mn (ALBERSBERG *et al.*, 1997; NAKUMURA *et al.*, 2004). It also contains phenolic compounds, such as, gallic acid, protocatechuic acid, caffeic acid, and *p*-coumaric acid (XIA *et al.*, 2011). Sap can be consumed fresh or processed to coconut sugar.

Coconut sugar is made by heating of inflorescence sap until it turns to brown and granulate. The sugar is an Asian traditional sweetener and it has been a growing interest in Europe and North America as a natural sugar alternative because it has low glycemic index around 35 (TRINIDAD *et al.*, 2010). Coconut sugar usually uses as an ingredient in many foods and drinks to provide a pleasant flavor. KABIR and LORJAROENPHON (2014) reported that the major aromas of coconut sugar consisted of heterocyclic compounds that occurred via Maillard reactions. Maillard reaction is a non-enzymatic browning started from the condensation between a carbonyl group of reducing sugar and an amine group of amino acid, peptide or protein. The intermediates rearrange structure to Amadori or Heyns products and these products undergo dehydration, fragmentation, cyclization, polymerization or Strecker degradation. The later reaction provides various flavor compounds such as aldehydes and pyrazines (LU *et al.*, 1997). Maillard is an important reaction to produce process flavor and the sap components, especially sugars and amino acids, can act as the precursors. These aspects affect both flavor quality and quantity in coconut sugar. Therefore, the aim of this research was to determine the impact of *L*-glutamine addition on the aroma of coconut sugar.

2. MATERIAL AND METHODS

2.1. Materials

Coconut sap was purchased from Amphawa Chaipattananurak Conservation Project (Samut Songkhram province, Thailand). *L*-Glutamine was obtained from Himedia Laboratories Pvt., Ltd. (Mumbai, India). 2-Methyl-3-heptanone (98.0%) and *n*-alkanes (C_6-C_{26}, C_{28}, C_{30}) (≥ 99.0%) were purchased from Sigma-Aldrich (MO, US). The authentic standards for identification were obtained from Sigma-Aldrich (MO, US) and Fluka (Steinheim, Switzerland).

2.2. Sample preparation

L-Glutamine (0 (as control), 100, 300 or 500 mg) were dissolved in 400 mL of coconut sap. The mixtures (0, 250, 750 and 1,250 mg/L concentrations) were heated and stirred to obtain light brown crystalline sugar. All sample treatments were prepared in triplicate.

2.3. Analysis of volatile compounds

Coconut sugar (4.5 g), 1 g of NaCl, 0.5 mL of odorless deionized water and 2 μL of 2-methyl-3-heptanone (0.68 mg/10 mL in methanol) as internal standard (IS) were prepared in the headspace (HS) vial. The sample was stirred at 200 rpm and equilibrated at 55 °C for 30 min. The solid-phase microextraction (SPME) fiber (50/30 μm divinylbenzene/carboxen /polydimethylsiloxane; DVB/CAR/PDMS) (Supelco; PA, US) was exposed to the HS for 40 min at the same condition.

The analysis was performed on a GC 7890A (Agilent Technology; CA, US) coupled with ToFMS Pegasus 4D (LECO®; MI, US) as described by KABIR and LORJAROENPHON (2014). The separation was achieved on a Stabilwax®-MS column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) (Restek; PA, US). Splitless injection temperature was 250 °C and helium was used as a carrier gas at a constant flow rate of 1 mL/min. The temperature program was started from 35 °C and held for 5 min. The oven temperature was raised at 4°C/min to 225 °C and held for 20 min. The retention indices (RIs) of volatiles were calculated with a series of *n*-alkane before comparison with the literature and mass spectra were compared with the database of National Institute of Standards and Technology (NIST) Mass Spectral Library version 2.0 g, 2011. The positive identification was further conducted using the authentic standards. The relative concentration was calculated from the ratio of compound and IS area.

2.4. Aroma descriptive sensory analysis

The procedure was followed the method of Meilgaard *et al.* (2007). Selected ten panelists were trained to describe aroma attributes of coconut sugar and to use the 15-cm line scale. Nine aroma attributes, definitions, and intensities were generated (Table 1). Twenty grams of coconut sugar samples were dissolved in 10 mL of odorless deionized water. Samples were prepared in Teflon™ sniff bottle (Thermo Scientific; NJ, US) covered with aluminum foil and labeled with a random 3-digit code. There were randomly monadic served to each panelist to avoid any bias. Panelists evaluated the intensity of aroma attribute in coconut sugar and illustrated the results as radar chart (Fig. 1).

Table 1. References used to describe the aroma attributes of coconut sugar.

Attribute	Definition (reference - intensity)
toast	aromatic associated with toasted (1 g of toasted bread crust - 12)
caramelized	aromatic associated with caramel, burnt sugar, sweet and sharp note (10% w/v of caramel - 4) (20 mL of burnt sugar (braised 50 g of white sugar with 25 mL of water) - 7)
nutty	aromatic associated with boiled peanut (0.5 g of boiled peanut shell - 9)
malt	aromatic associated with malt (2.5% w/v of Ovaltine - 10)
vanilla	aromatic associated with vanilla (0.01% w/v of vanilla flavor - 7)
sweet potato	aromatic associated with steamed sweet potato (2 g of boiled sweet potato - 7)
fermented	aromatic associated with sour fermentation of LAB (0.5% w/v of miso paste - 5)
briny	aromatic associated with brine (1% w/v of sweet soy sauce - 3)
coconut	aromatic associated with coconut milk (5% w/v of coconut milk - 5)

3. RESULTS AND DISCUSSION

A total of 31 volatile aroma compounds including aldehydes, ketones, carboxylic acid, alcohols, phenolic compound, pyrazines, furans, pyrroles, thiazoles, and lactone were identified in coconut sugar (Table 2). All of these compounds, except phenol, were Maillard reaction products. Phenol in coconut sugar might be generated from the thermal degradation of lignin or *p*-coumaric acid. Kiam (*Cotylelobium lanceolatum*) woodchips, which added to prevent fermentation during the collection of coconut sap could provide lignin residue and this wood polymer was broken-down to form phenol during heating. The result was similar to the study of MANCILLA-MARGALLI and LÓPEZ (2002), which reported the formation of phenol in agave (*Agave tequilana* Weber var. azul.) exudate via thermal degradation of lignin. Coconut sap also contained *p*-coumaric acid (XIA *et al.*, 2011) which undergone thermal decarboxylation to form phenol volatile. This result was supported by the formation of phenol from *p*-coumaric acid in the steam distillate of rice bran (FUJIMAKI *et al.*, 1977).

Among all volatiles, the major aroma compound in coconut sugar was acetic acid followed by methional, 2,6-dimethyl pyrazine, 2-methyl pyrazine and furfural (Table 2) and the concentration of these abundant volatiles was up to 80% of total aroma content. Although coconut sap contained a higher amount of fructose compared with glucose (NAKAMURA *et al.*, 2004), the hydrolytic *β*-dicarbonyl cleavage to form acetic acid during Amadori degradation of glucose was faster than those of fructose (DAVÍDEK *et al.*, 2006; HUANG *et al.*, 2011). This finding is not in agreement with the general characteristic of Maillard reaction which shown that pentose generally tends to react with amino acid greater than hexose. Besides Maillard reactions, some molecules of acetic acid might be formed via fermentation during sap collection. Coconut sap is a good source for microbial fermentation because it contains various nutrients such as sugars, amino acids, vitamins, and minerals. In addition, coconut sap is usually collected under sunlight at ambient temperature for a long period of time. Improper sanitation during traditional sap collection also causes fermentation (HEBBAR *et al.*, 2015). Thus, sap composition affected aroma formation of coconut sugar.

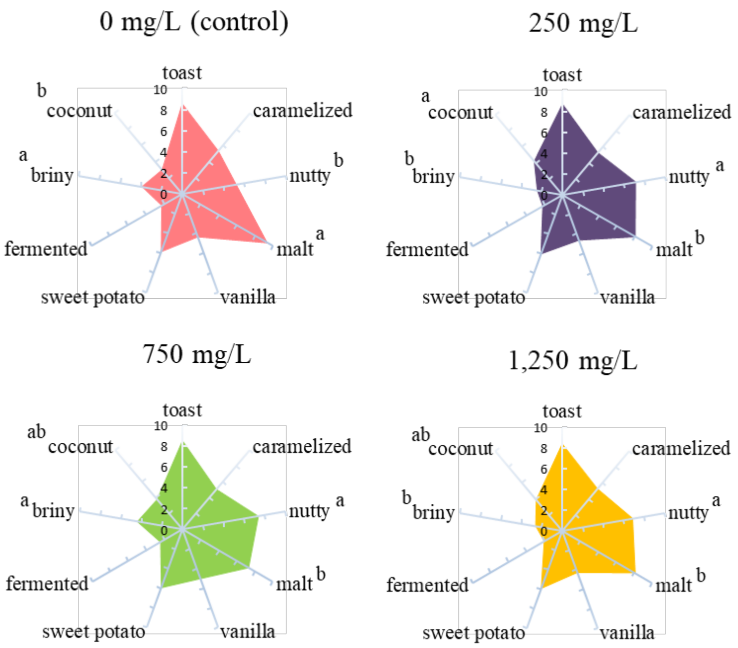
Addition of *L*-glutamine in coconut sap prior sugar preparation significantly increased the concentrations of 2-methyl pyrazine, vinyl pyrazine, furfural, 2-acetylfuran, benzaldehyde, 5-methyl-2-furanmethanol and 2-formylpyrrole (Table 2). *L*-Glutamine has been reported as the precursor of 2-methyl pyrazine, vinyl pyrazine, furfural, and 2-formylpyrrole in the model systems (CHEN and HO, 1999). Interestingly, the generation of 2-acetylfuran, benzaldehyde and 5-methyl-2-furanmethanol from *L*-glutamine in coconut sugar was stated for the first time in the real food system.

L-Glutamine donated the nitrogen atom to the aromatic ring structure of those two pyrazines and HWANG *et al.* (1993) reported that the involvement of nitrogen atom from amide chain had higher impact compared with those from *α*-amino group. In addition, the concentration of 2-methyl pyrazine was more than 50-fold of vinyl pyrazine content (Table 2). This result indicated that the contribution of *L*-glutamine to form a nitrogen-containing aromatic ring of 2-methyl pyrazine was greater than those of vinyl pyrazine. It should be noted that not only the amino acid structure but also the types of reducing sugars affected the volatile aroma profile. CHEN and HO (1999) reported that only fructose participated to 2-formylpyrrole production, while 2-methyl pyrazine, vinyl pyrazine, as well as furfural were generated from either glucose or fructose. Furthermore, furfural was generated greater from fructose than glucose.

Table 2. Relative concentrations of volatile aroma compounds in coconut sugar added with *L*-glutamine at difference concentrations.

RI[a]	Compound	Odor description[b]	Relative concentration (μg/kg)[c]			
			control	**250 mg/L**	**750 mg/L**	**1,250 mg/L**
<1211	2-methylbutanal	cocoa, malt	14.86a	7.12b	9.18ab	4.91b
<1211	2,3-butanedione	buttery	10.12	12.19	14.63	13.62
<1211	2,3-pentanedione	buttery	3.37	6.69	6.46	4.24
<1211	pyrazine	roasted hazelnuts	0.95	1.59	1.30	1.10
1211	2-methyl pyrazine	nutty, cocoa	39.98b	72.02ab	85.97ab	90.67a
1224	3-hydroxy-2-butanone	creamy, buttery	2.28	2.90	2.88	3.28
1237	1-hydroxy-2-propanone	green, sweet	2.02b	5.27a	2.70b	1.60b
1265	2,5-dimethyl pyrazine	roasted nut	3.17	7.86	7.30	4.97
1270	2,6-dimethyl pyrazine	roasted nut	40.88	62.03	57.15	60.73
1285	2,3-dimethyl pyrazine	nutty, cocoa	0.38	0.80	0.76	0.75
1326	2-ethyl-6-methyl-pyrazine	roasted, nutty	0.33	0.64	0.47	0.62
1343	trimethyl pyrazine	roasted peanut	0.63	0.88	0.84	1.30
1365	vinyl pyrazine	green, burnt	0.54b	1.21ab	1.59a	1.50a
1376	acetic acid	pungent, vinegar	95.20	127.40	152.23	148.34
1379	methional	potato	53.37	55.54	89.09	64.18
1386	furfural	almond, caramel	38.52b	68.79ab	104.70a	72.49ab
1415	2-ethenyl-6-methyl-pyrazine	roasted potato	0.85	1.93	1.58	1.93
1422	2-acetylfuran	sweet, nutty	1.86b	7.62a	6.68a	5.76a
1430	pyrrole	sweet, burnt	0.21	0.21	0.18	0.11
1439	benzaldehyde	burnt sugar	0.45b	0.63b	1.17ab	2.30a
1482	5-methylfurfural	bread, caramel	0.87	2.19	2.36	1.84
1523	γ-butyrolactone	creamy	0.60	1.05	0.75	2.40
1545	phenylacetaldehyde	floral, green	1.75	2.27	3.89	3.19
1546	2-acetylthiazole	nutty, toasted	1.06	1.43	1.24	0.94
1560	2-furanmethanol	caramel	9.24	15.78	12.98	7.48
1617	5-methyl-2-furanmethanol	sweet caramel	3.89b	7.84ab	9.08a	7.32ab
>1617	phenylethyl alcohol	floral, rose-like	2.13	2.27	1.77	1.67
>1617	2-acetylpyrrole	floral, cherry	1.00	2.10	2.24	1.69
>1617	phenol	medicinal	0.25	0.26	0.30	0.39
>1617	2-formylpyrrole	burnt	0.63b	1.40ab	1.65ab	1.91a
>1617	2,5-dimethyl-4-hydroxy-3(*2H*)-furanone	fruity, caramel	0.28	0.75	0.59	0.22

[a] Retention index from Stabilwax® column. [b] Odor description obtained from KABIR and LORJAROENPHON (2014) and LEFFINGWELL (2004). [c] The different lowercase letters represent significantly difference at $p \leq 0.05$.


0 mg/L (control)

250 mg/L

750 mg/L

1,250 mg/L

Figure 1. Aroma profiles of coconut sugar added with *L*-glutamine at different concentrations (n=10). The different superscripts represent significantly difference at $p \leq 0.05$.

According to the odor quality from the literature, the GC results of 2-methyl pyrazine, furfural, 2-acetylfuran, and benzaldehyde correlated well with significant increase of nutty note from descriptive sensory analysis (Fig. 1). In addition, lower intense malt note in *L*-glutamine-added sugar might be due to the significant decrease of 2-methylbutanal (Table 2). 2-Methylbutanal was the intermediate of pyrazine formation. HWANG *et al.* (1995) reported the production of various pyrazines from 2-methylbutanal via two pathways. This aldehyde was condensed with carbonyl compound via aldol condensation to form α-diketone or α-hydroxyketone and further converted to pyrazines by Strecker degradation. 2-Methylbutanal could interact with dihydropyrazine via dehydration reaction to yield pyrazines as well. In addition, only isoleucine and leucine were reported as the precursors of 2-methylbutanal (HWANG *et al.*, 1995). Thus, *L*-glutamine might not be the precursor of this malt-note aldehyde in the coconut sugar.

4. CONCLUSIONS

All volatile aroma compounds, except phenol, in coconut sugar are Maillard reaction products. The accumulation of 2-methyl pyrazine, furfural, 2-acetylfuran, and benzaldehyde in coconut sugar after addition of *L*-glutamine results in more intense nutty note. However, the intensity of malty note is decreased from the degradation of 2-methylbutanal. Therefore, the information from this study can be used as a quality assurance to produce premium quality coconut sugar and the great profit margin can accomplish accordingly.

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OXIDATION KINETICS OF READY TO USE THERAPEUTIC FOOD FORMULATIONS

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ABSTRACT

Ready-to-Use Therapeutic Foods (RUTFs) have to recover health status of malnourished children without clinical complications. If produced in loco, RUTFs should be stable when stored at room temperature and unprotected atmosphere. The aim of this work was to verify the oxidative stability of 4 RUTFs, by means of conjugated dienes and malondialdehyde (MDA) content at 3 temperatures for 4 weeks. RUTFs initially presented a different content of peroxydiene and their increase was well described by pseudo zero order models. Kinetic constants at 60°C were higher than those estimated at 30° and 40°C. MDA values were very low (≈0.48 μg/ml after 4 weeks). Those products were quite stable over 2 weeks even if stored at 40°C in an unprotected atmosphere.

Keywords: conjugates dienes, malondialdehyde, RUTF

1. INTRODUCTION

Ready to use Therapeutic Food (RUTF) is an energy-dense, micronutrient-enriched paste, used for the Community based Management of Acute Malnutrition (CMAM) in under-five children of low-income countries, avoiding hospitalization. Actual RUTF mainstream formulation is affected by some issues, as high cost of skimmed milk powder, nutritional properties of palm oil, allergenic power of peanuts. The commercial RUTF, called Plumpy'Nut ®, is affected by some drawbacks like high production costs and low acceptability in some regions (SANTINI et al., 2013). Alternative RUTF recipes should be defined both to reduce production costs and to permit the in loco production, by using inexpensive production technologies and locally available ingredients. Although the benefits of localizing RUTF production close to malnourished patients (CLARK & HOBBS, 2015; KOMRSKA, 2012; UNICEF, 2014) are known, a limited research on adjusting RUTF ingredients to the local crop selection and environmental constraints is nowadays available. Some recent reviews (WEBER et al., 2017; BRIXI, 2018) reported that some RUTF formulations have been proposed in Ethiopia, Ghana, Pakistan, India, based on different legumes (almond, lentil, soybean), cereals (maize, oat), other oils (Canola, rapeseed, Coconut, Sunflower) and different forms of whey (acid whey milk, whey protein concentrate). The main problem of those RUTF formulations is the inclusion of an expensive vitamin and mineral premix, to ensure a balanced micronutrient intake of the final product. Starting from those consideration, during a previous work (ARMINI et al., 2018), the ingredient composition of an alternative RUTF, based on Spirulina microalgae, soy, sorghum, icing sugar and sunflower oil, was optimized and four optimal formulations (1-2-3-4) came out. In order to set up their production and distribution in a low-income country at environmental temperature, RUTFs were prepared again and their oxidation stability was determined by means of primary and secondary oxidation indexes.

2. MATERIAL AND METHODS

2.1. Materials

For RUTF formula optimization, the following ingredients were used: sunflower oil (Grazia, Lucca, Italy) and soy lecithin (ACEF, Piacenza, Italy), as lipidic phase, dehulled and roasted soy flour (Consorzio Agrario dell’Emilia Romagna, Bologna, Italy) and dehulled and roasted sorghum flour (Consorzio Agrario dell’Emilia Romagna, Bologna, Italy), as flour mix, and icing sugar (Eridania, Bologna, Italy). Microalgae Spirulina dried powder was also used (produced in CAISIAL-University of Naples, Portici, Italy).

2.2. RUTF preparation

Four different RUTF samples were prepared (Table 1). A stirred ball mill was used (model PM 200, Retsch, Haan, Germany), equipped with two jars (250 mL) and 9 spheres (5 of 2 cm diameter and 4 of 1 cm diameter, respectively) were used for cream production. Refining was carried out at 500 rpm for 120 min, at 40°C.

2.3. Experimental design

To study lipid oxidation, conjugated dienes and malondialdehyde (MDA) were determined during RUTF samples storage at different temperature (30, 40 and 60 °C), for different times (from 0 to 4 weeks) and in unprotected atmosphere. Three replications for each sample were prepared.

2.3.1 Conjugated Dienes

In order to evaluate the conjugated dienes formation, a modified lipid cold extraction method proposed by Bligh and Dyer (1959) was used. 0.1 g of the lipid fraction were dissolved in hexane forming a solution at 0.1% (w/v); the absorbance of the solution was measured at 232 nm (UV-VIS Spectrophotometer Jasco V-550).

2.3.2 MDA

Thiobarbituric Acid Reactive Substances (TBARS) analysis was carried out according to Maraschiello et al. (1999), which included the preparation of MDA standard, the construction of the calibration line and finally the analysis of the samples.

Table 1. Ingredients (g/100g) of each optimal RUTF formulation.

Formulation	Soy flour %	Sunflower oil %	Icing sugar %	Sorghum flour %	Soy lecithin %	*Spirulina* %
1	31.80	32.95	24.25	7.00	1	3.00
2	23.47	32.95	32.58	7.00	1	3.00
3	22.10	32.95	33.95	7.00	1	3.00
4	31.80	30.16	27.04	7.00	1	3.00

2.4. Data analysis

Conjugated dienes and malondialdehyde data were fitted with a pseudo zero order model to determinate the kinetic constants (k) (day^{-1}). A two-way Anova (formulation, storage time and their interaction effects) and Duncan's multiple comparison test were performed on kinetic constants ($p<0.05$).

3. RESULTS AND DISCUSSION

In Fig. 1 the conjugated dienes for each RUTF sample, during 4 weeks storage, were reported.

RUTF samples initially presented a different content of peroxydiene, due to their different formulation (Fig. 1), in particular, samples 1 and 4 presented similar CD content and higher than those of samples 2 and 3. At 30°C and 40°C peroxydiene content grew up slowly in all the four optimal formulations, showing a similar behavior. The peroxydiene formation, due to primary lipid oxidation, has been well described by a pseudo zero order kinetic model and the estimated k values were reported in Table 2.

Formulation ($p<<0.001$), temperature ($p<<0.001$), and their interaction ($p=0.008$) significantly affected estimated k values. In particular, sample 1 presented the slowest primary oxidation kinetic constant. With the exception of sample 2, k at 60°C were higher than those estimated at 30° and 40°C, for which no differences were observed. Meanwhile, sample 3 resulted the more stable RUTF considering both the initial CD content (Fig. 1) and its kinetic constant (Table 1).

In Fig. 2 the malondialdehyde for each RUTF sample, during 4 weeks storage, were reported.

Regarding secondary oxidation, there were small differences among the samples, at all the temperatures considered (Fig. 2). In general, MDA content was very low, showing a constant value during the first two weeks of storage and a slight increment after the third week. Malondialdehyde formation, was not well described by any kinetics models.

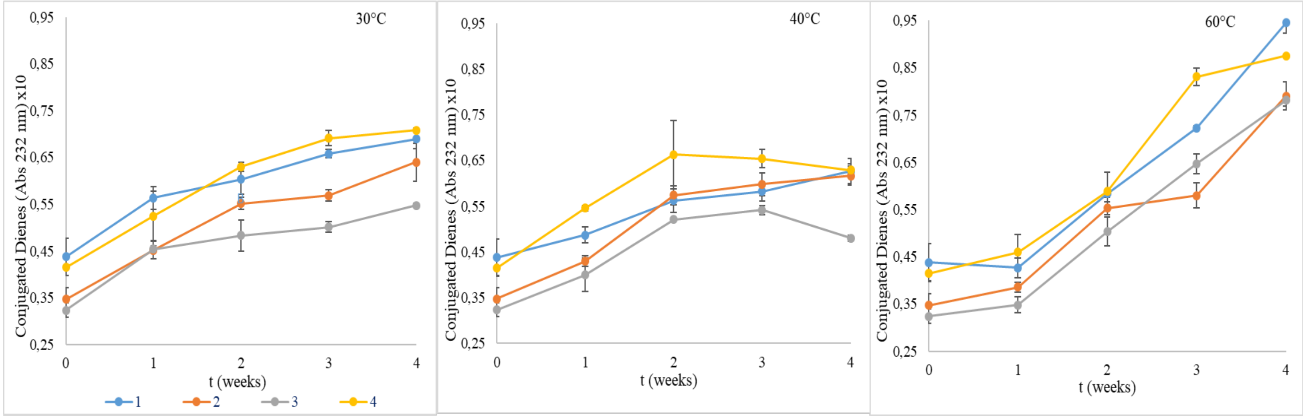

Figure 1. Conjugates dienes (CD) over time.

Table 2. Kinetic constants (k) of primary lipid oxidation at different temperatures.

Sample	k (day^{-1})		
	30°C	40°C	60°C
1	0.027^{aA}	0.02^{aA}	0.035^{aB}
2	0.035^{b}	0.035^{c}	0.041^{b}
3	0.027^{aA}	0.026^{bA}	0.056^{bcB}
4	0.025^{aA}	0.026^{bA}	0.04^{abB}

For each line (upper case) and for each column (lower case) at different letters correspond significantly different values ($p<0.05$) from Duncan's test.

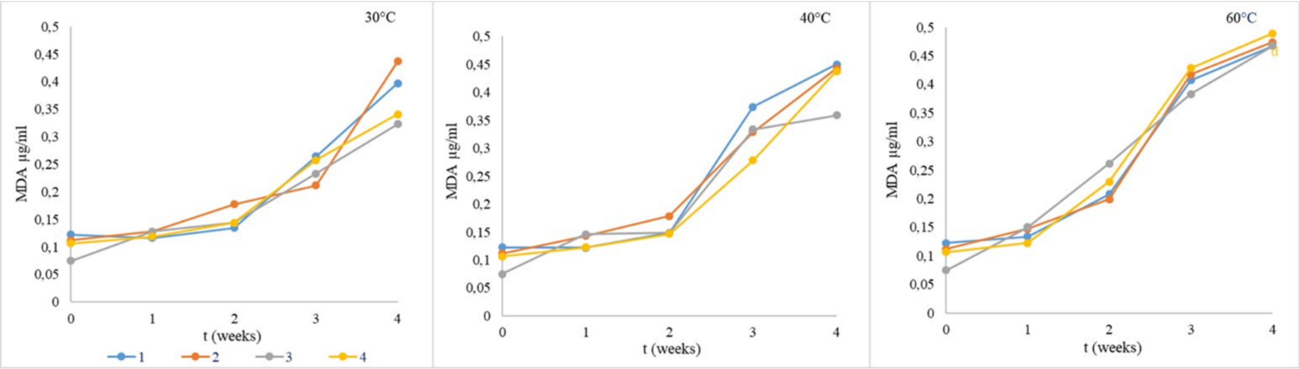

Figure 2. Malondialdehyde (MDA) over time.

4. CONCLUSIONS

This analytical framework suggests that the storage of the alternative RUTFs in an unprotected atmosphere for four weeks at temperature under 40°C should not determine a drastic loss of quality due to lipid oxidation. Sample 3 seems to be the most stable one.

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QUALITY INDICES OF CHEESE OXIDATION DURING STORAGE

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ABSTRACT

The objective of the work was to identify the quality indices of lipid oxidation of Grana Padano cheese and to optimize the methods for their determination. Cheese was stored at 4°C and at 20°C for 60 days. Free fatty acids (FFA) content, peroxide value (PV) and volatile organic compounds (VOC) were studied during storage. No difference for FFA values in cheese during the storage was found, while PV increased after 60 days at 20°C. The concentration of VOC increased during the storage. Results showed that VOC were the best quality indices to discriminate samples during storage.

Keywords: cheese, lipid oxidation, quality indices, shelf-life, storage, volatile compounds

1. INTRODUCTION

Lipid oxidation is one of the processes responsible for the reduction in food shelf-life since it leads to off-flavour, off-odour and has been linked to oxidation reactions that cause product discoloration and loss of nutrients (DALSGAARD *et al.*, 2010).

Non-enzymatic browning and lipid oxidation are the main alteration process of grana Padano cheese. The main effects of alteration process are: change of organoleptic value due to rancid flavors production as well as production of reactive oxygen species implicated in inflammation and cardiovascular diseases (KRISTENSEN *et al.*, 2001; FEDELE and BERGAMO, 2001). Storage conditions affect the oxidative stability of cheese, and both modified atmosphere and light exposure have an impact on the formation of oxidation products.

So, the objective of this work was to identify both the critical quality indices of lipid oxidation and lipolysis able to describe the kinetics of alteration and the analytical methods for their determination during the storage of cheese at 4°C and 20°C.

2. MATERIAL AND METHODS

2.1. Sampling cheese

Grana Padano cheese (10 months-aged) has been used as a case study. The cheese was packed in air with a barrier film and stored at 4°C and at 20°C for 60 days. After 20, 40, and 60 days the following quality indices of the lipid oxidation and lipolysis were studied: free fatty acid content, peroxides value and volatile organic compounds.

2.2. Extraction of fat by cheese

The fat content was gravimetrically determined by using with some modifications the method described in D.M. 1986 based on the Schimith-Bondzynski-Ratzla traditional method of extraction of lipids. Ten grams of grated cheese were hydrolyzed using 10 mL of 37% hydrochloric acid and 7 mL of 95% (v/v) ethanol. The cheese suspension was homogenized with an ultrasound for 10 min and it was incubated for 30 min at 50°C in thermic bath. After cooling, the fatty matter was extracted using 10 mL of ethyl ether-petroleum ether (1:1) solution and the suspension was centrifugated for 10 min at 6000 rpm. The organic phase was collected and the extraction protocol was repeated 3 times.

Three organic extracts were pooled, dried over anhydrous sodium sulfate, filtered with a paper filter, evaporated under reduced pressure in a rotary evaporator and weighed.

2.3. Peroxide value

To evaluate the number of peroxide value (PV), a titration method was used, followed by the procedure of SENGUL *et al.*, (2014) with some modification. Briefly, 500 mg of fat was weighed after dissolving in acetic acid/chloroform (3:2 v/v) solution, then stored in the dark for 5 min after the addition of saturated potassium iodide. Finally, 7.5 mL deionized water and 1% starch solution was added to mixture and titrated with 0.001 N $Na_2S_2O_3$ solution. PV was expressed as meqO_2/kg fat.

2.4. Free fatty acids

To quantify free fatty acids (FFA) a titration method was used, followed by the procedure of KONIECKO (1979) with some modifications. Briefly, 500 mg of fat was weighted and 5 ml of ethanol/ethyl-ether (1:2) were added. NaOH 0.01N was used to neutralize sample acidity and phenolphthalein was used as indicator. Results are expressed as % of oleic acid.

2.5. Volatile organic compounds analysis

The extraction and analysis of volatile organic compounds (VOC) was performed using SPME-GC/MS, according to LEE *et al.* (2013) and MANZO *et al.* (2019) with modifications. The solid-phase microextraction (SPME) device equipped with a 50/30-μm thickness divinyl-benzene/carboxen/polydimethylsiloxane fiber coated with 2-cm length stationary phase was used. Then, 1.5 g of frozen grated cheese was transferred into a 10 mL vial added with 3 mL of deionized water and 15 *μ*L of 2-methyl-3-heptanone as internal standard (408 mg/L). Samples were homogenized and heated on a heating magnetic stirrer. Then the SPME device was hermetically put in the vial containing the samples and left for 1 hour at 50°C. The SPME was introduced directly into the GC injector where the thermal desorption of the analytes was performed at 250 °C for 10 min. A GC system 6890N equipped with a mass detector 5973 was used. The VOC were separated on a 30 m × 0.250 mm capillary column coated with a 0.25 μm film of 5% diphenyl 195% dimethylpolysiloxane. Splitless injection was used for the samples. The column oven temperature was held to 40°C for 2 min and increased from 40°C to 160°C at 6°C/min and from 160 to 210°C at 10°C/min, which was held for 10 min. The injection and ion source temperatures were 250 and 230 °C, respectively. Helium was used as carrier gas at a flow rate of 1 mL/min. The ionizing electron energy was 70 eV and the mass range scanned was 40-450 amu in full-scan acquisition mode. The compounds were identified using the NIST Atomic Spectra Database version 1.6 and verified by the retention indices. The VOC were calculated by the internal standard method and were expressed as mg/kg of cheese.

2.6. Statistical analysis

Results were expressed as means ± standard deviation (SD). Statistical analysis was performed with the statistical package SPSS for Windows (version 20, SPSS Inc. Chicago, IL, USA). The difference between means was tested using Student's t-test. Statistical significance was set at level of $p<0.05$.

3. RESULTS AND DISCUSSION

Table 1 shows the results about the PV and FFA during the storage at different temperatures.

Results showed that no significant difference for FFA values in cheese stored at different time was found. The PV was increased after 60 days at 20°C, while no difference was found with the storage at 4°C.

Fig. 1 shows the results about the VOC concentration during the storage at different temperatures.

In terms of the VOC, an increase of ketones and alcohols during the storage at both the temperatures was found, while the concentration of aldehydes increased significantly only after 60 days at 20°C (Fig. 1).

Table 1. PV and FFA during the storage at 20°C and 4°C. Different letters indicate significant difference ($p<0.05$) during the time at same temperature, while the asterisk indicates significant difference ($p<0.05$) between the different storage temperatures.

T (°C)		Time (days)			
		0	20	40	60
4	PV (meq O2/Kg)	8.5±0.4a	9.1±0.2a*	9.1±0.01a*	8.6±0.01a
20	PV (meq O_2/Kg)	8.5±0.4b	7.5±0.3b*	7.6±0.2b*	9.1±0.4a
4	FFA (% oleic acid)	2.6±0.1a	3.1±0.02a	2.9±0.01a	2.8±0.01a
20	FFA (% oleic acid)	2.6±0.1a	2.9±0.01a	3.1±0.01a	2.9±0.01a

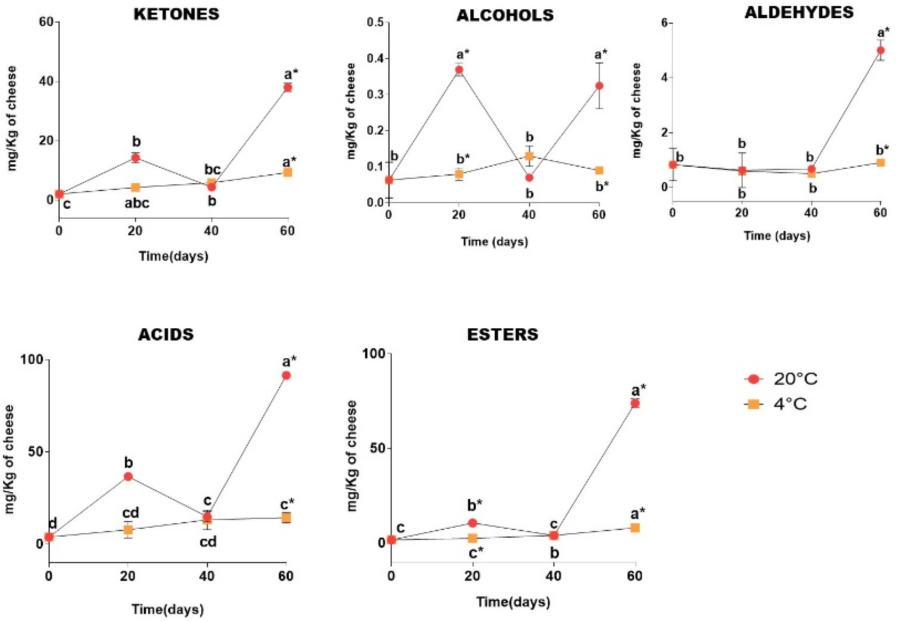

Figure 1. VOC concentration during the storage. Different letters on the lines indicate significant difference ($p<0.05$) during the time at same temperature, while the asterisk indicates significant difference ($p<0.05$) between the different storage temperatures.

Furthermore, the concentration of acids increased during the storage at both temperatures, but the highest concentration of acids was found after 60 days at 20°C (Fig. 1). Finally, the concentration of esters increased after storage at 20°C with the highest concentration after 60 days, while at 4°C, an increase of esters after 60 days was found (Fig. 1).

The results showed that the VOC were the best quality indices to discriminate samples during storage.

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SHELF LIFE STUDY OF EDIBLE WILD FLOWERS

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ABSTRACT

The interest in edible flowers is increasing, chef and consumers purchase packaged flowers for use in meals. Our aim was to evaluate the shelf life of the *Glebionis segetum*, *Malva sylvestris*, *Papaver rhoeas* and *Tropaeolum majus* to protect them from desiccation. The flowers have been packaged in different polypropylene films, then stored in a refrigerator at +4±1°C in the dark and in the presence of light, and their shelf life have been analyzed for 12 days.
The light has always a negative influence. The MICRO film gives the best performance, limiting the weight loss, preserving the color and guaranteeing good enzymatic results in

Keywords: visual quality, lightening, color

1. INTRODUCTION

The interest in edible flowers is increasing as also demonstrated by high numbers of scientific papers and dissemination activities (LU, et al., 2016); they are also mentioned in connection with biologically active substances and their presence is always more frequent in discount. Always more chef and consumers purchase packaged flowers for use in meals as a garnish or ingredient in salads, entrees, desserts, and drinks.

The main factor that determines the purchase of edible flowers by consumers is their appearance, so particular attention must be paid to their shelf life. The potential number of suitable species is very high (over 1,000) and some species are also present in the spontaneous flora of Mediterranean environment. Flowers must be protected from desiccation and their quality preserved, so the aim of this study was to evaluate the shelf life of some wild edible flowers of Mediterranean region: *Glebionis segetum* (G)*, Malva sylvestris* (M)*, Papaver rhoeas* (P) *and Tropaeolum majus* (T). The tests have been conducted in the dark and in the presence of light to simulate the sales conditions and understand how the lightening could affect the shelf life and bioactive components of the flowers.

2. MATERIAL AND METHODS

Wildflowers were picked up in the rural area's surroundings the urban area of Catania. After selection, they were placed in PET trays (Fig. 1). The number of flowers, according to their sizes, was 12 for T, 20 for G, 14 for P and 42 for M. The flowers are packed in ordinary atmosphere using bags from three different polypropylene (PP) films: MACRO perforated PP with a piercing density of 7 holes/cm^2 (Bemis Le Trait sas, France), was used as *control*; CAST (OTR 3000 cc/m^2/24h, 23°C 0% RH); MICRO perforated coextruded PP with a row of holes spaced each 3 cm (OTR 38800 cc/m^2/24h) kindly supplied by Coralife Swaf C (Corapack s.r.l., Italy).

Figure 1. Arrangement of wild flowers in PET trays.

Successively samples were stored in two refrigerator at +4±1°C, one kept in the dark, while the second was with an internal light made by a "COLD LIGHT 6500 K" LED lamp, to simulate the sale conditions. Samples have been analyzed every 3 days for 12 days. The weight losses and the color degradation were determined as reported by RIZZO *et al.* (2017); the headspace gas composition was tested using a CheckPoint portable gas analyser (PBI Dansensor, Denmark), while enzymatic analysis were performed as described in RIZZO *et al*. (2019). Analyses were carried out on three replicate at each sampling time.

3. RESULTS AND CONCLUSIONS

The key to extending flower life after harvest is understanding the biological processes governing postharvest physiology, as respiration, water loss (transpiration), hormonal imbalance and the activation of enzymes associated with flower discoloration and leaf yellowing (chlorophyll loss) (FERRANTE and REID, 2006).
As expected, the light has always a negative influence, reducing shelf life. The weight loss of control samples were under the 6% in all flowers stored in the dark, with the exception of G which reached the 19% during the first 3 days, in MACRO. Differently, samples packaged in MICRO and stored in the dark, had a weight loss of 1-2%. The CAST packages had always the worse performance in the dark, reaching the 25% in G after 3 days, and values similar to MICRO under light exposition (Fig. 2).

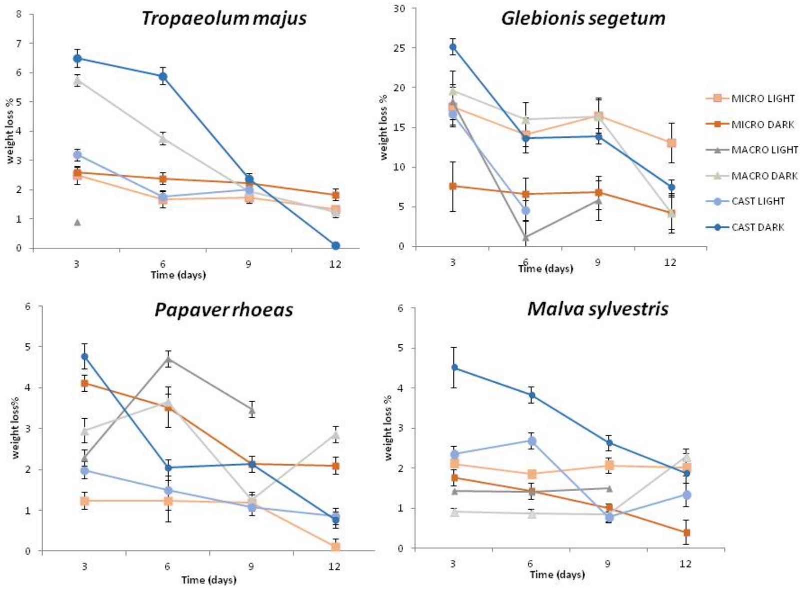

Figure 2. Weight loss % registered in PP plastic film tested in *Tropaeolum majus (A), Glebionis segetum (B), Papaver rhoeas (C) and Malva sylvestris (D).*

Respiration is the fundamental metabolic process responsible for providing energy in all living cells (KELLEY *et al*., 2003). In our study, the gas composition did not change too much during shelf life, with a variation by 0.2%. External quality (appearance) is the first sensorial sensation and the most important to the consumer (RIVA *et al*., 2005) (Fig. 3). Color results, obtained from the software of image analysis, demonstrated not statistical difference between dark and light exposure, while the highest variation of RGB were in MACRO, followed by CAST then in MICRO where parameters were nearest starting values.

Figure 3. Visual appearance of damaged wild flowers studied.

When plants are exposed to stress condition, reactive species of oxygen (ROS) increase inside plant tissues, enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione perossidase (GPX, data not shown), which are existing in the cells; particularly in chloroplast and mitochondria are used to scavenge ROS (BANIASADI *et al.*, 2018). Edible flowers showing a high antioxidant activities and having a marked inhibitive effect on free radicals (MLCEK and ROP, 2011). Enzymatic results pointed out the MICRO as best packaging film if considered in the dark (Fig. 4).

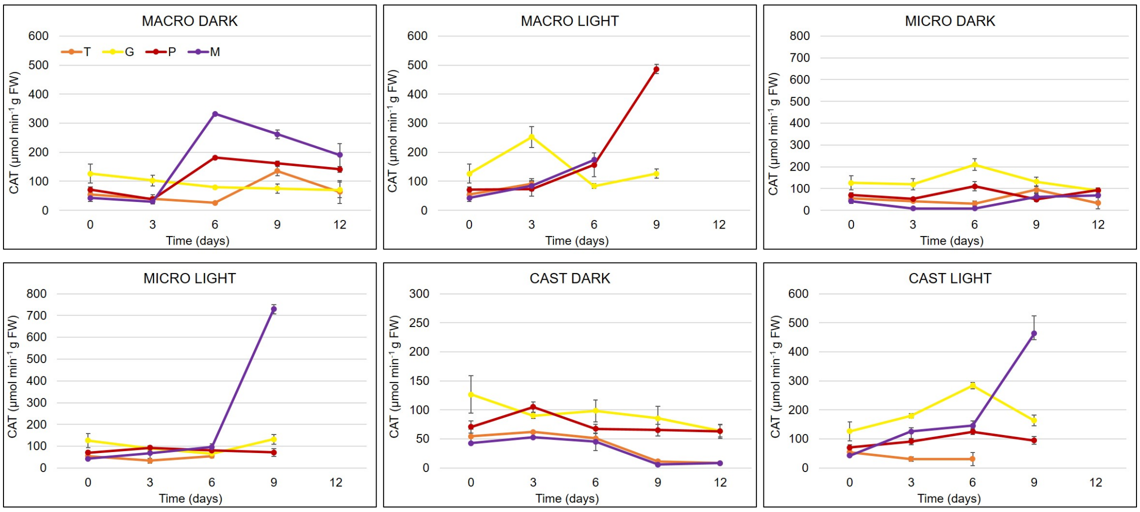

Figure 4. Catalase(CAT) and Superoxide dismutase (SOD) activity in flowers in PP plastic film tested in *Tropaeolum majus* (T), *Glebionis segetum* (G), *Papaver rhoeas* (P) and *Malva sylvestris* (M) at 0, 3, 6, 9 and 12 days.

Looking at the main characteristics of the flowers tested we can conclude that *Glebionis segetum* was the most resistant but it had also the highest weight loss, *Papaver rhoeas* was characterized by extreme fragility, *Malva sylvestris* was not able to tolerate low temperatures and *Tropaeolum majus* changed its color progressively with the time, also due to the characteristics of the species.

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EFFECT OF PACKAGING ON VOLATILE PROFILES OF MULBERRY TEA

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ABSTRACT

This research aimed to investigate the effect of packaging materials and storage time on volatile profiles of mulberry tea. The mulberry tea samples were packaged in linear low-density polyethylene laminated aluminum (AL) bag and polypropylene (PP) bag and stored at 30±1°C for 18 months. The volatile profiles were monitored using a headspace solid phase microextraction/gas chromatography-mass spectrometry. The results showed that storage time had no significant impact on the key volatile compounds of the AL packaged samples. However, some volatiles associated with lipid oxidation like hexanal and 4-oxo-2-nonenal were observed to significantly ($p<0.05$) increase in the PP packaged samples with storage time.

Keywords: herbal tea, mulberry leaf, packaging, SPME-GC/MS, volatile compounds

1. INTRODUCTION

The consumption of mulberry (*Morus alba* L.) leaf tea products is popularly increasing worldwide especially in Asian countries increasing due to its special flavors and health benefits (SÁNCHEZ-SALCEDO *et al*., 2015; SHOAIB ZAFAR *et al*., 2013).

It has been recognized that the sensory quality of herbal tea especially flavor profile is a key factor for quality classification and content. Since the changes in flavor and aroma profile of herbal tea during storage result in the greatly decline in its quality and consumer acceptance, appropriate packaging is crucial for preserving the product's flavors and nutritional quality throughout the storage. HARNNURAK and RIEBROY (2014) reported that the alumimium foil bag could protect the quality attributes of mulberry leaf tea during storage better than paper bag and sachet, respectively. LEE and CHAMBERS (2010) reported that packaging materials significantly ($p<0.05$) affected changes in volatile components of green tea during storage.

To date, several researches have undertaken on the quality attributes and health benefit of mulberry tea. However, scientific information on aroma constituents and quality attributes of herbal tea from mulberry leaves during storage are still limited. Information on the volatile compounds of mulberry tea is beneficial to the herbal tea industry for quality control of products and shelf life evaluation. The purpose of this study was to determine the effect of packaging and storage time on volatile profiles of mulberry leaf tea using headspace solid phase microextraction-gas chromatography/mass spectrometry (SPME-GC/MS).

2. MATERIAL AND METHODS

2.1. Tea preparation and storage condition

Herbal mulberry tea (Thai Silk Products Co., Ltd., Thailand) was prepared using the process of The Queen Sirikit Department of Sericulture as described by RUENGDECH and SIRIPATRAWAN (2019). One hundred grams of mulberry tea were packed into linear low-density polyethylene laminated aluminium (AL) bag (10×15 cm^2; 0.0924 mm in thickness) and polypropylene (PP) bag (10×15 cm^2; 0.0774 mm in thickness), sealed under atmospheric condition, and stored at 30±1°C in a controlled humidity chamber at 75% RH. The samples were randomly retrieved at three-month interval to monitor volatile profiles using SPME-GC/MS.

2.2. Determination of volatile compounds

The tea infusion was prepared by adding 2 g of mulberry leaf tea in 100 ml of hot water at 95±2°C. The samples were infused for 5 min and were then filtered through a Whatman No.4 filter papers.

Volatile compounds of mulberry tea infusion were collected by SPME technique. Briefly, 10 ml of tea infusion were pipetted into a 20 ml glass vial (Chromselection, Brescia, Italy) and 3 g of sodium chloride was added. The vial was then tightly closed with aluminum crimp cap attached with a PTFE silicone septum (Chromselection, Brescia, Italy). After 10 min of sample equilibration at 40°C in an auto-incubator, the 50/30 µm Divinylbenzene/Carboxen/ Polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber (Stableflex; Supelco. Inc., Bellefonte, PA, USA) was exposed to the headspace of the sample vial for 30 min and then immediately inserted into the gas chromatograph (GC)

(AutoSystem XL GC, PerkinElmer, Waltham, MA, USA) injection port with splitless mode at 250°C for 5 min.

The GC column was Stabilwax®-MS capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness; Restek, Bellefonte, PA, USA) was employed for GC-MS analysis. Helium was used as carrier gas with flow rate at 1 ml/min. The GC oven temperature gradient was 50°C (held for 5 min) initially, increased to 125°C (held for 3 min) at 3°C min^{-1}, then ramped to 180°C (held for 3 min) at 2°C min^{-1}, and finally increased to 230°C (held for 5 min) at 6°C min^{-1}. The transfer line temperature was 280°C. Mass spectrometer (MS) (Turbomass MS, PerkinElmer, Waltham, MA, USA) was coupled to GC. The ion source temperature was 230°C and the MS was scanned at 70 eV over 40 to 500 mass range. Compounds were tentatively identified by using the National Institute of Standards and Technology (NIST) database with the reverse match factor more than 800.

Principal components analysis (PCA) was carried out to analyzed volatile compound data. PCA has been used to visualize the hidden trends in a data matrix composed of n (13) samples defined by m (12) selected volatile compounds, which changed in intensity during storage.

3. RESULTS AND DISCUSSION

The effects of packaging materials and storage time on volatile components of mulberry leaf tea stored at 30±1°C were determined using SPME-GC/MS. GC chromatograms of volatile profiles of mulberry leaf tea in PP stored for 0, 6, 12, and 18 months are shown in Fig. 1. Volatile compounds were then identified by comparison of their mass spectra of authentic reference compound with those of the NIST (version 11.0) database. Fifty-three volatile components in mulberry leaf tea include aldehyde, alcohol, acid, ester, and others. Volatile compounds including benzaldehyde; 1-octen-3-ol; 6-methyl-5-hepten-2-one; (E, E)-2,4-heptadienal; nonanal; decanal; 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde; and 6,10-dimethyl-5,9-undecadien-2-one found in our sample were also found in mulberry tea produced in China as report by LIU et al. (2012). Hexanal and 2-hexenal, which have been reported to be responsible for green and grassy odors in mulberry leaves (RUENGDECH and SIRIPATRAWAN, 2019; TANAKA *et al.*, 2009), also present in the mulberry leaf tea in our study. 2(4H)-benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl contributed to sweet, herbaceous, and tea-like odor has been reported as an important aroma constituent of tea and herbal tea (CHEN *et al.*, 2013).

The GC results showed that storage time did not affect volatile components of mulberry the leaf tea. The average concentration of all volatile components of the samples in AL did not significantly change over the whole storage period. However, intensity of volatiles of the PP packaged samples including 2-methyl-butanal; hexanal; 5-hepten-2-one,6-methyl; 6-methyl-3,5-heptadiene-2-one; 3,5-octadien-2-one; heptan-2-one; 3,4-dimethyl-benzaldehyde; 4-oxo-2-nonenal; 3-buten-2-one-4(2,6,6-trimethyl-1-cyclohexen-1-yl); 2(4H)-benzofuranone,5,6,7, 7a-tetrahydro-4,4,7a-trimethyl; 5,9-undecadien-2-one,6,10-dimethyl; and 1,6-octadien-3-ol- 3,7-dimethyl,2-aminobenzoate changed with storage time. The intensities of 2-methyl-butanal and 3,4-dimethyl-benzaldehyde found in mulberry leaf tea in PP bag gradually decreased with increasing storage time, whereas the remaining 10 compounds gradually increased during 12 months of storage and rapidly increased from 15 to 18 months of storage (Fig. 2).

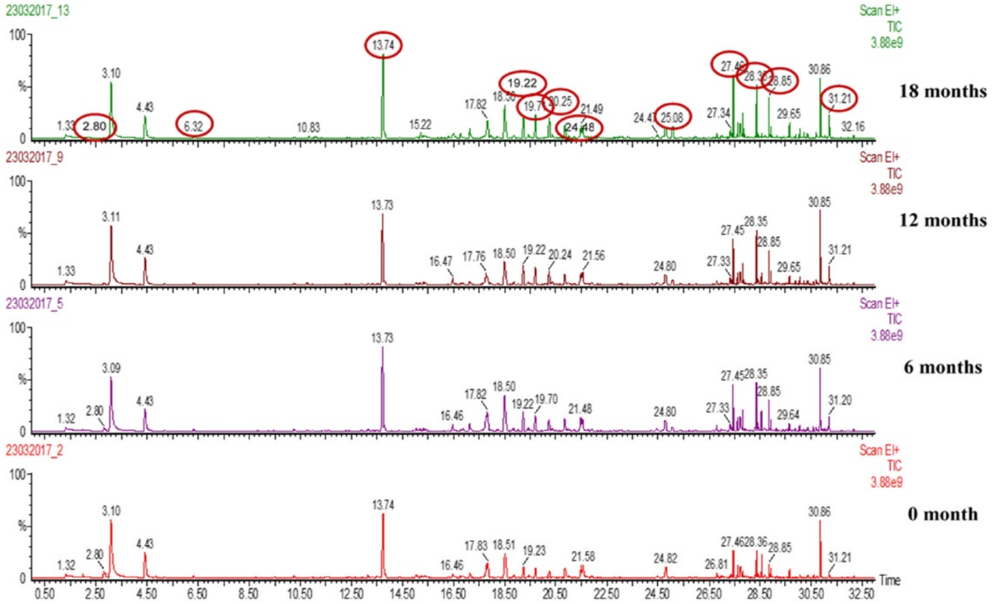


Figure 1. GC chromatograms of volatile compounds of mulberry leaf tea in PP bag during storage at 30±1°C, 75% RH for 0, 6, 12, and 18 months. The peaks of twelve volatile compounds with color circle indicate significant ($p<0.05$) changed in intensity during storage.

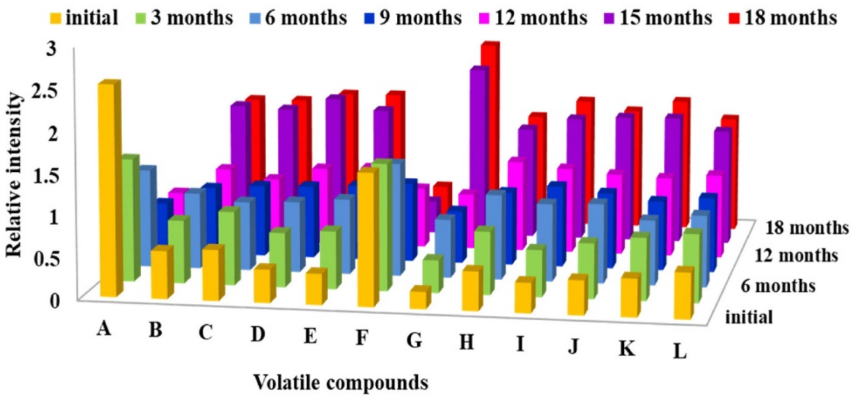
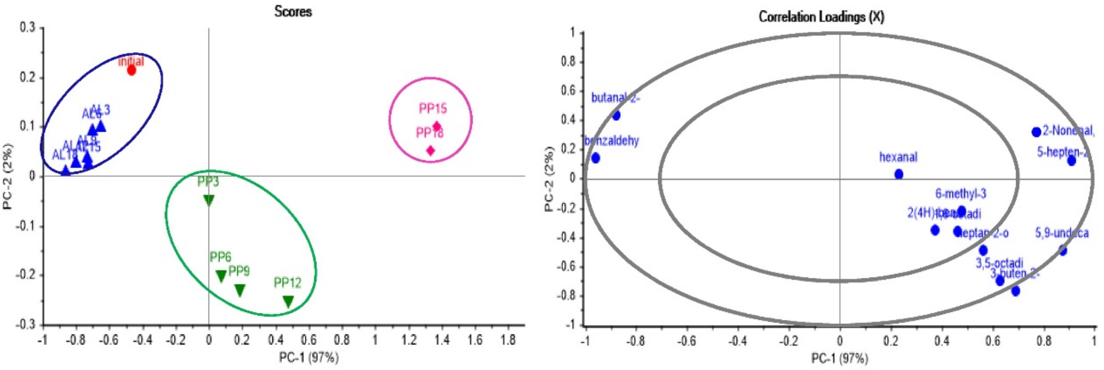


Figure 2. Changing in intensity of twelve volatile compounds found in the mulberry leaf tea packaged in PP during storage at 30±1°C, 75% RH for 18 months.
A: 2-methyl-butanal; B: hexanal; C: 5-hepten-2-one,6-methyl; D: 6-methyl-3,5-heptadiene-2-one; E: 3,5-octadien-2-one; F: 3,4-dimethyl-benzaldehyde; G: 4-oxo-2-noenal; H: 3-buten-2-one-4(2,6,6-trimethyl-1-cyclohexen-1-yl); I: 2(4H)-benzofuranone,5,6,7,7a-tetra- hydro-4,4,7a-trimethyl; J: heptan-2-one; K: 5,9-undecadien-2-one,6,10-dimethyl ; L: 1,6-octadien-3-ol-3,7-dimethyl,2-aminobenzoate.

Changes in the intensity of some volatiles associated with oxidation reaction of components in mulberry leaf tea. Hexanal generated from lipid oxidation contributes to off-flavors such as stale, cardboard, and fishy in foods. Hexanal content is directly related to the deterioration of food quality during storage (MAARSE, 2017). A 4-oxo-2-nonenal is a lipid peroxidation product derived from oxidized omega-6 polyunsaturated fatty acids such as arachidonic acid and linoleic acid. It has been widely used as a marker of lipid peroxidation (LEE and BLAIR, 2000). 2-methyl-Butanal, which is a branched-chain aldehyde, is generally perceived as malty or chocolate-like odor in food products. A decrease of 2-methyl-butanal of mulberry leaf tea in PP during storage may be attribute to the conversion of branched-chain aldehyde compounds to alcohol or acid compounds via reduction or oxidation, respectively (SMIT *et al.*, 2009).

SHOAIB ZAFAR *et al.* (2013) reported that the fresh mulberry leaves contain fat in a range of 0.64 to 1.51 g/100 g dried sample. While in dried mulberry leaves, fat is in a range of 2.09 to 4.93 g/100g dried sample. During storage, UV-light, oxygen, and moisture can all induce lipid oxidation causing off-flavor development in mulberry leaf tea. Since AL has better UV-light, oxygen, and moisture barrier properties than PP, it can protect the product from lipid oxidation better than PP bag. This result is in agreement with the studied of KAACK and CHRISTENSEN (2008), who reported that AL bag had more efficiency to protect the quality of tea processed from flowers of black elder (*Sambucus nigra* L.) than plastic film.

The PCA used to facilitate the data interpretation of various volatile compounds of mulberry tea during storage at different time points. For PCA, data were made up of 78 samples from 13 groups and each group had 6 replicates. Each sample was analyzed using 12 selected volatile compounds, which changed in intensity during storage. The selected volatile compounds of all samples were arranged in a matrix (78 samples×12 volatiles variables). Fig. 3 shows PCA score plot and loading plot of mulberry leaf tea stored at 30±1°C for 18 months using the selected volatile profiles data set. The PCA results allowed for good discrimination among samples, with the PC1 and PC2 can explain 99% of the total variance. The results showed that initial sample and all samples in AL located on the negative region of PC1. The result indicated that volatile compounds of the sample in AL were not significantly different during storage. The samples in PP could divide into 2 groups; one group is the samples store at 3 to 12 months and the other is the samples stored from 15 to 18 months. The PCA results are in accordance with the GC/MS results, which showed that volatile profiles of mulberry leaf tea in AL were not significantly different throughout the storage. Whereas storage time affected volatile intensity of the sample in PP. Twelve volatiles found in mulberry leaf tea in PP of which their intensities gradually changed during 12 months of storage and greatly changed at 15 and 18 months of storage. Thus, the samples could be classified into three groups according to their volatile patterns.



(a) **(b)**

Figure 3. PCA score plot (a) and loading plot (b) on PC1 and PC2 of mulberry leaf tea in AL and PP bags and stored at 30±1°C, 75% RH for 18 months using twelve selected volatile compounds data set.

The loading plot established the relative importance of each selected volatile compounds, and the relationships between the volatiles and the samples. Most of the selected volatile compounds positively correlated to PC1 whereas 2-methyl-butanal and 3,4-dimethyl-benzaldehyde negatively correlated to PC1. From the loading plot, volatiles positively correlated to PC1 were associated with the samples in PP whereas volatiles negatively correlated to PC1 were associated with the samples in AL. The analysis of volatile profiles of mulberry tea suggested that packaging materials used affected its volatile profiles and possibly the odor and flavor of the products.

4. CONCLUSIONS

The results of this study demonstrated that packaging material and storage time affected volatile profiles of mulberry tea. AL bag showed high-effective barrier properties with the quality mulberry leaf tea were not significantly different throughout the storage. Due to a low effective barrier property of PP, the qualities of mulberry leaf tea significantly ($p<0.05$) difference during storage. To protect the product qualities during storage, AL bag is suggested as the packaging of mulberry leaf tea. The information obtained from this study is useful for the selection of suitable packaging material and storage time for mulberry or herbal tea.

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KINETICS OF ALTERATION OF PACKED RED MEAT HAMBURGERS

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ABSTRACT

The objective of this work was to study the oxidation of fresh meat hamburger by selecting the critical quality index of lipid oxidation and quantifying the kinetics of alteration of red meat oxidation. Meat hamburger were stored at 4°C and 10°C for 21 and 17 days respectively. The following quality index were monitored: pH, color (L*, a*, b*), secondary lipid oxidation (TBARS test), and metmyoglobin percentage (Metmio%).
Results showed that among quality indices the colorimetric parameter a* and MDA were the only ones able to discriminate samples as function of time, atmosphere and temperature.

Keywords: lipid oxidation, quality index, red meat, shelf life

1. INTRODUCTION

The work is carried out in the frame of the Healthy Pack project aimed to develop and produce an antioxidant film for improving the shelf life of food. To properly design a new antioxidant film able to extend food shelf life, it is important to define the quality indices related to the alteration mechanism and to quantify the kinetics of alteration at different storage condition.

Lipid oxidation is considered one of the main reactions that affect meat quality in terms of food safety, quality deterioration and the production of off-odour as well (TURGUT *et al.*, 2016). The surface color of the meat, pH, metmyoglobin content and MDA are critical quality indeces monitored for studying the lipid oxidation (DJENANE *et al.*, 2016).

Thus, the objective of this work was to study the oxidation of fresh meat hamburger at different atmospheres and temperatures (i) to select the critical quality index of lipid oxidation and (ii) to quantify the kinetics of alteration of red meat oxidation.

2. MATERIAL AND METHODS

2.1. Materials

Red meats were bought by a local market the day of the experiment and transported to the laboratory of the Department of Agricultural science of the University of Naples in one hour. A multilayer film based on aertop PE was furnished by Coopbox Group Spa, like polistirolo trays was made up by EPS, HIPS and PE. Gas was furnished by SOL spa and the reagents was furnished by Carlo Erba.

2.2. Methods

Hamburgers of red meat (40 g) were packed with a barrier film in modified atmosphere packaging (100% N_2 (MAP1); 60%O_2-40%CO_2 (MAP2)). The samples packed in air were used as control. Samples were stored at 4°C and 10°C for 21 days and 17 days respectively. After 4, 7, 11, 14, 17, and 21 days the following quality index were monitored: pH, color, secondary lipid oxidation (MDA by TBARS test), and metmyoglobin percentage (Metmio%).

The pH of samples was measured using a pH-meter. Instrumental surface colour (CIE L*, a*, b*) of each hamburger was measured using a reflectance spectrophotometer (Minolta Chroma Meter CR-300) (TORRIERI et al..2011). Secondary lipid oxidation analysis (TBARS) was done using method described by MARASCHIELLO et al (1999). Myoglobin redox potentials were analyzed using spectrophotometric method and metmyoglobin percentage (Metmio%) calculation was done with equation:

$$\%\text{MetMioglobina} = \left[-2.51\left(\frac{A572}{A525}\right) + 0.777\left(\frac{A565}{A525}\right) + 0.8\left(\frac{A545}{A525}\right) + 1.098\right]x100$$

2.3. Data analysis

The effect of time, temperature and MAP on quality index and statistical differences between samples were measured by ANOVA and Duncan's test (p<0.05) respectively (SPSS Statistics Inc. v.17). Selected quality indeces were analyzed by linear regression to estimate the constant kinetic of alteration.

3. RESULTS AND DISCUSSION

3.1. pH measurements

pH increases as a function of time from a value of 5.72±0.06 to a value of about 5.9±0.69 in the air after 21 days of storage. Samples packaged in MAP2 show a greater increase up to 6.4±0.17 after 21 days of storage. Similar results are observed at 10 ° C. The pH increases after 17 days up to a value of about 6 for the control sample and 6.4 ±0.17 for the MAP2 sample. The effect of ageing on pH is shown in Table 1.
These results were different to previous studies DJENANE *et al.* (2016) reported that pH values were not affected by storage conditions.

Table 1. Ph (average ± standard deviation) of meat samples stored at different time and under different modified atmosphere packaging.

Temperature	**Sample**	**Time of storage (days)**						
		0	**4**	**7**	**11**	**14**	**17**	**21**
	MAP1	5.72 ± 0.06^{a}	5.90 ± 0.69^{d}	5.79 ± 0.06^{bc}	5.78 ± 0.04^{b}	5.84 ± 0.06^{c}	5.82 ± 0.05^{bc}	5.79 ± 0.09^{bc}
4°C	air	5.72 ± 0.02^{a}	5.78 ± 0.12^{a}	5.90 ± 0.20^{b}	5.80 ± 0.13^{a}	5.92 ± 0.07^{b}	5.94 ± 0.12^{b}	5.94 ± 0.09^{b}
	MAP2	5.72 ± 0.02^{a}	5.77 ± 0.09^{ab}	5.89 ± 0.29^{b}	6.04 ± 0.17^{c}	6.21 ± 0.22^{d}	6.05 ± 0.18^{c}	6.40 ± 0.13^{e}
	MAP1	5.85 ± 0.03^{a}	6.06 ± 0.03^{c}	6.14 ± 0.15^{d}	6.17 ± 0.06^{d}	6.04 ± 0.07^{c}	5.99 ± 0.02^{b}	
10°C	air	5.84 ± 0.06^{a}	6.10 ± 0.11^{b}	6.29 ± 0.13^{d}	6.20 ± 0.05^{c}	6.16 ± 0.06^{c}	6.09 ± 0.04^{b}	
	MAP2	5.86 ± 0.05^{a}	6.33 ± 0.11^{b}	6.43 ± 0.09^{c}	6.43 ± 0.18^{c}	6.50 ± 0.17^{c}	6.32 ± 0.09^{b}	

Means of the same row (between days of display) with different letters differ significantly ($p<0.05$).

3.2. Color measurements and metmyoglobin determination

Surface MetMb did not reach significant values over time at different storage temperature (data not shown). The colorimetric parameter a* was the only one able to discriminate samples as function of time, atmosphere and temperature ($p<0.05$). The a* doesn't change during time for samples stored in air or in 100% N_2. For sample MAP2, the parameter a* decreases over time according to MOHAN *et al.* (2016). The kinetic constant estimated by using the a* parameter was 0.6319 day^{-1} and 0.8623 day^{-1} respectively, at 4°C and 10°C for the samples packed in $60\%O_2$-$40\%CO_2$(Figs. 1 A and B).

3.3. Secondary lipid oxidation

Secondary lipid oxidation analysis or 2- thiobarbituric acid reactive substance analysis (TBARS) changes only for samples packed in $60\%O_2$-$40\%CO_2$ and stored at 10°C by changing from 0.1 mg MDA/kg to 0.4 mg MDA/kg by following a pseudo zero order in the range of time studied with a kinetic constant of 0.018 day^{-1} (Figs. 2 A and B). These results were different to previous studies MOUDACHE et al., 2017, because 2, the TBARS values increased significantly in all samples storage at 4°C.

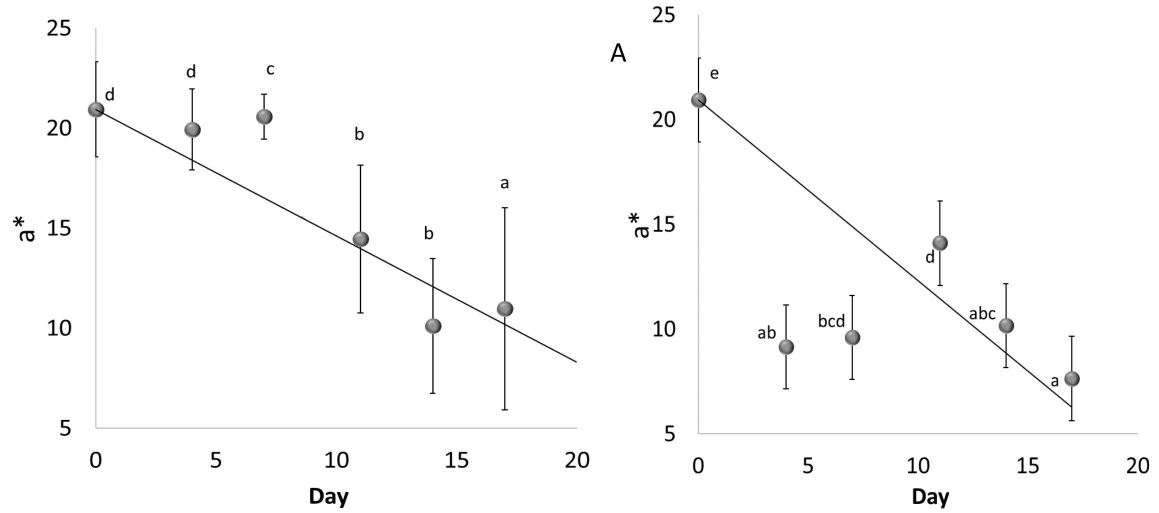

Figure 1. The kinetic constant estimated by using the a* parameter at 4°C (A) and 10°C (B) storage.

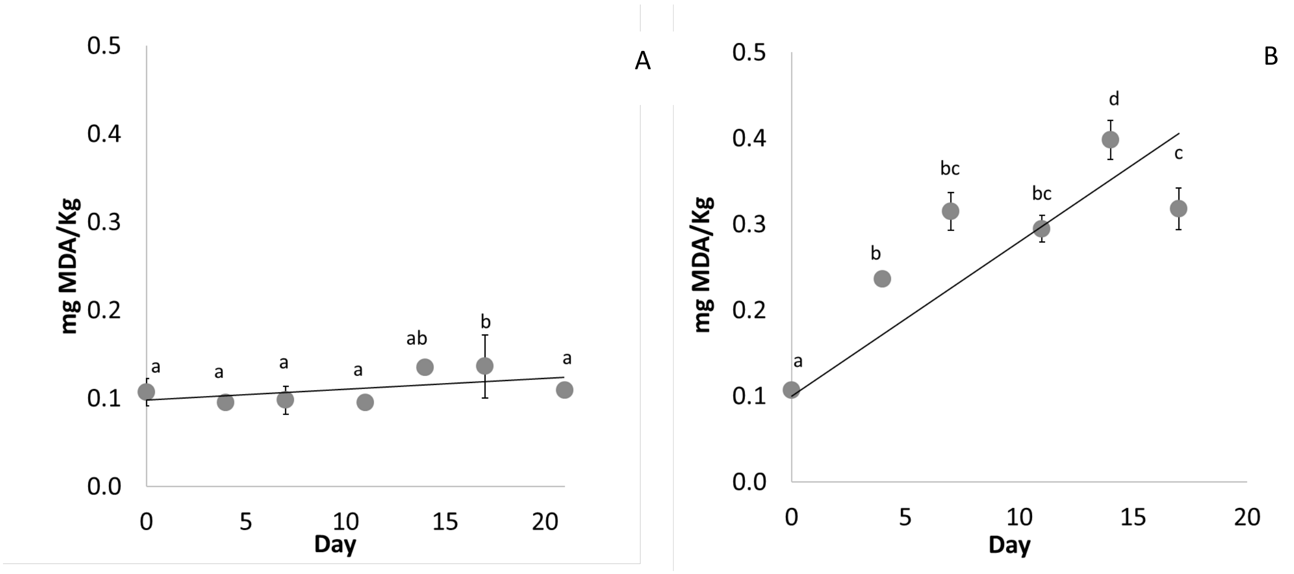

Figure 2. The kinetic constant estimated by using the mg MDA at 4°C(A) and 10°C (B) storage.

4. CONCLUSIONS

The color parameter a* and MDA are the critical quality indices for study red meat oxidation. The parameters a* and MDA followed a pseudo zero order model. More investigations are needed to estimate the kinetic constant at 4°C.

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SENSORY CHANGES OF PACKED *FOCACCIA* DURING STORAGE TIME

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ABSTRACT

The objectives of this work were (i) to identify the sensory attributes of "Altamura" focaccia that change during storage time, by using the check-all-that-apply (CATA) questions, and (ii) to understand which attributes significantly drive the consumer’s liking. Sensory tests were conducted on three different focaccia samples at 7, 22, 40 and 55 days of storage. Results showed that frequency of use of positive attributes decreased with storage time, whereas the contrary occurred for negative ones. The positive attributes that affected consumer's liking belonged to both texture properties of the base and flavor properties of the topping.

Keywords: bakery product, consumer test, liking, quality loss, sensory attributes

1. INTRODUCTION

Focaccia is a typical bakery product of Apulia region, largely appreciated for its sensory properties. Focaccia is made of a few simple ingredients - flour, water, fatty substances (oil, extra-virgin olive oil), yeast, and salt - and can be prepared with several toppings: fresh tomato, tomato sauce, olives or flavoring it with herbs like oregano. During storage time, physico-chemical changes occurred, leading to crumb firming, flavour changes, loss of crispy crust, all of which constitute the global process called staling (AGUIRRE at al., 2011; PASQUALONE *et al.*, 2011). Moreover, since olive oil is one of the essential ingredients of focaccia, it may undergo to oxidative alterations during storage. Thus, for these reasons, the sensory quality decreases over time, with negative effect on the *focaccia* quality perception by consumers.

Several studies have been demonstrated that consumers are the most appropriate tool for determining when a food product reaches the end of its shelf life (GÁMBARO *et al.*, 2004; VOLPE *et al.*, 2017). In particular, Check-all-that-apply (CATA) questions have been used in several studies to determine which attributes might characterise a specific product (ARES & JAEGER, 2015). This approach could be also used to follow the evolution of sensory attributes during storage time and their effects on consumer liking scores (VOLPE *et al.*, 2017). Since focaccia is a niche typical Italian product, no information is reported in literature, neither on sensory attributes that affect the product liking nor on sensory changes during shelf life.

Therefore, the objectives of this study were: (i) to identify and evaluate the sensory attributes of "Altamura" focaccia over time as perceived by the consumers; (ii) to understand which attributes significantly affect the consumers liking.

2. MATERIAL AND METHODS

2.1. Materials

Focaccia with three different toppings (fresh cherry tomatoes and olives (F1), tomato sauce and olives (F2), and olives and oregano (F3)) were gently furnished by OROPAN S.p.A. (Altamura, Bari, Italy). All of them contained around 10% of extra virgin oil and were packaged in a barrier film under modified atmosphere (30% CO_2 and 70% N_2).

2.2. Storage conditions and sample preparation

All the focaccia samples were transported to the laboratory of the Agricultural Department of the University of Naples Federico II, the day after the production. Samples were stored at 20°C and 65% of relative humidity (RH) for 55 days. Sensory evaluation was performed on samples at 7, 22, 40 and 55 days of storage. Immediately before the sensory evaluation, samples were heated at 180°C for 10 min, cut in identical slices and warm served to consumers.

2.3. Consumer test

A preliminary focus group interview was performed with 12 trained judges in order to generate the list of sensory attributes for each focaccia sample. Then, generated attributes were used for the Check-all-that-apply (CATA) questions performed by 80 consumers. They were recruited among staff people and students of the University of Naples Federico II, balanced in gender and ranging in age from 18 to 60 years old. Focaccia samples were identified with a three-digit code number and presented in a monadic and randomized order to consumers. Test was performed in individual booths and consumers were provided with a cup of water to clean their mouth between each sample. For each sample, the judges were asked to evaluate their liking by using Labelled Affective Magnitude scales (LAM) and select all the attributes that applied in each sample.

2.4. Data analysis

The CATA results were evaluated by the elicitation frequency of each sample; statistical differences ($p<0.05$) were assessed by Cochran's Q test. Correlations analysis was applied to correlate the CATA data sets with the liking results (ARES *et al*., 2013). Data were analysed by means of XLSTAT (vers.2016, Addinsoft, Paris, France).

3. RESULTS

From the focus group interview, three different lists of attributes were generated; in particular, the lists were composed of 31, 33 and 22 descriptive attributes for F1, F2 and F3 sample, respectively.

Results from CATA questions are reported in Figs. 1A-3A. In general, significant differences were found in the CATA frequencies between terms used for describing all the focaccia samples at different storage time. Indeed, the frequency of positive attributes elicitation decreased with storage time, whereas the contrary occurred for the negative ones.

In particular, regarding F1, Fig. 1A shows that samples stored for 7 and 22 days were positively related to the positive attributes: *fresh olives* (p=0.005), *fresh tomatoes* (p=0.002), *oregano odour* (p=0.016), *soft* (p=0.04). The samples stored for 40 and 55 days were instead more related to negative attributes such as *dry olives* (p=0.002), *dry tomato odour* (p=0.033), *rancid* (p=0.005), *gummy* (p=0.002) and *stale* (p=0.001).

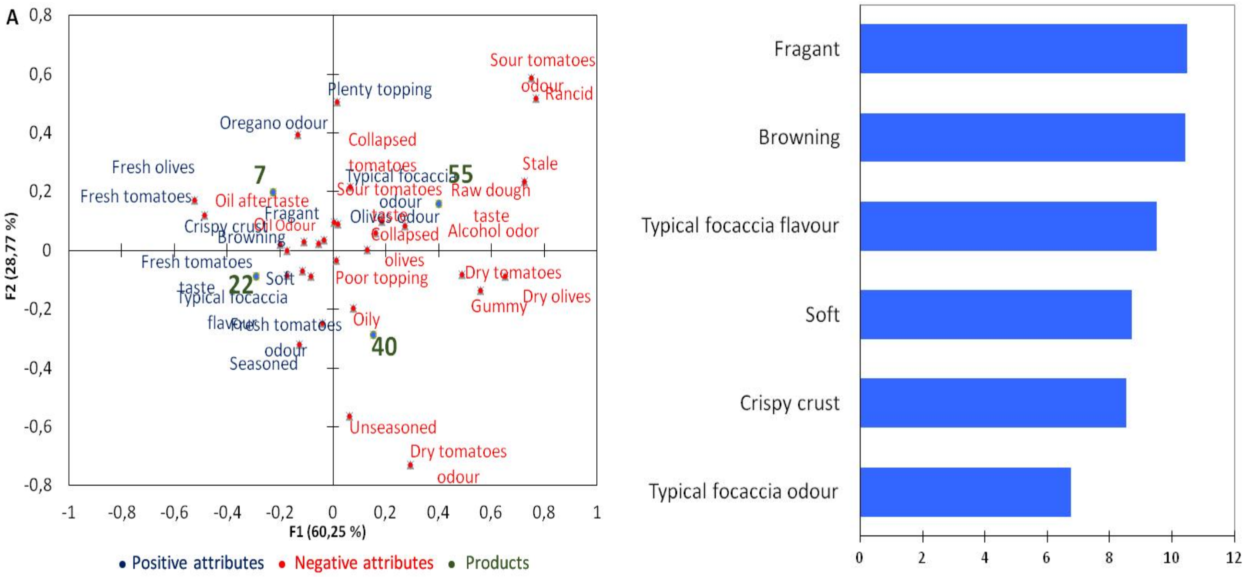

Figure 1. Corresponding analysis from CATA questions (A) and mean effect of attributes on liking (B) for F1.

Fig. 2A shows the biplot for F2. Also in this case, samples could be clustered in two groups. Samples stored for 7 and 22 days were strictly related to positive attributes like *seasoned* (p=0.049), *fresh olives* (p=0.008), *typical tomato sauce odour* (p=0.034) and *typical odour of focaccia* (p=0.011). On the other hand, sample stored for 40 and 55 days were positively related to negative attributes such as *dry olives* (p=0.005), *dry tomato odour* (p=0.015) and *oil odour* (p=0.039).

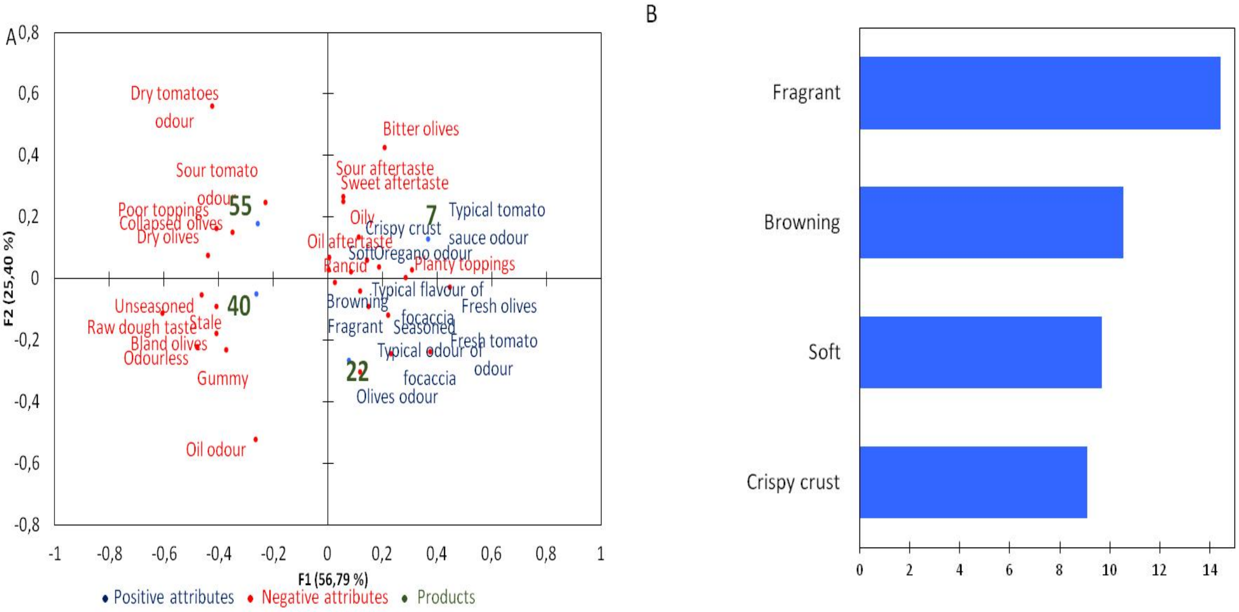

Figure 2. Corresponding analysis from CATA questions (A) and mean effect of attributes on liking (B) for F2.

Finally, Fig. 3A shows the biplot for F3. This *focaccia* sample did not follow the same trend of F1 and F2. It means that it was not possible to discriminate the samples according to the elicitated attributes. Indeed, it was not possible to find a real trend that correlates the positive and/or negative attributes with the storage time. However, the sample stored for 7 days was significantly described by the positive attribute *fragrant* (p=0.001), whereas the negative attribute *gummy* (p=0.001) significantly characterise the sample stored for 40 days.

The second aim of this work was also to verify which attributes drove the consumer's liking. Thus, Figs. 1B-3B show the mean impact of the sensory attributes that significantly affected the liking for each *focaccia* sample, respectively.

Regarding F1 sample, the attributes significantly affecting the liking are shown in Fig. 1B. In particular, it is possible to observe that liking was positively affected by *fragrant* (p<0.0001), *browning* (p<0.0001), *typical focaccia flavour* (p<0.0001), *soft* (p<0.0001), *crispy crust* (p<0.0001), *typical focaccia odour* (p<0.001). Also for F2, the attributes that positively affected the liking were: *fragrant*, *browning*, *soft* and *crispy crust*. Indeed, by increasing the storage time, the elicitation of these attributes decreased. Regarding F3, liking was significantly affected both by positive and negative attributes. The same positive attributes, which affected the liking of F2, affected the liking of F3 as well (p<0.0001). Instead, the negative ones were: *dry olives*, *gummy* and *unseasoned* (p<0.0001). On the base of these results, it can be reasoned that attributes such as *soft, browning* and *fragrant* can be considered a *must-have* of the products, since they had a positive effect on liking scores, for all the evaluated samples.

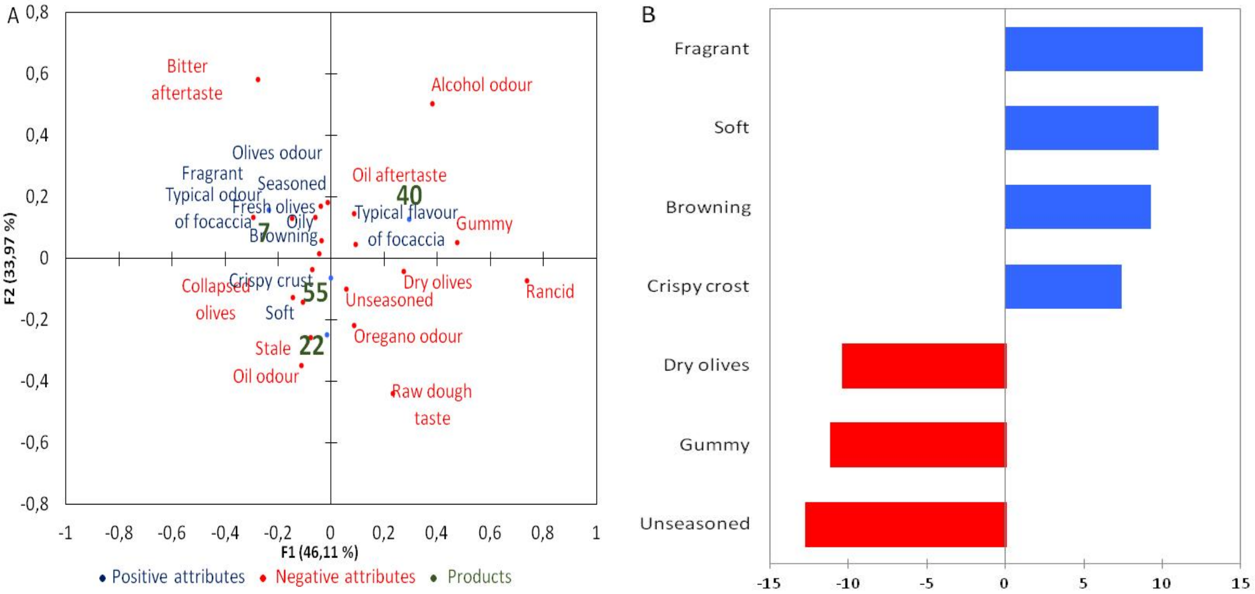

Figure 3. Corresponding analysis from CATA questions (A) and mean effect of attributes on liking (B) for F3.

4. CONCLUSIONS

CATA question method is able to describe the samples and to individuate the attributes that mostly affect the liking of consumers. Moreover it could be a useful tool to evaluate and discriminate the products during the storage time, by looking at the changes of sensory attributes. This knowledge is necessary for the industries, especially in the view of optimizing the products and their storage.

ACKNOWLEDGEMENTS

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SESSION II

New Technologies for Shelf Life Extension

FEATURES OF HEATED CHITOSAN-MALTODEXTRIN SOLUTIONS

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ABSTRACT

In this study, the influence of mixtures concentration and reactants weight ratio on properties and antioxidant activity of Maillard reaction products (MRPs) was investigated. The aqueous mixtures between chitosan and maltodextrin at various concentrations (5, 10 and 15 wt%) and different weight ratios (1:9, 1:24, and 1:49) were heated at 100°C for 60 min and then the resulting reaction products were analyzed. The results suggested that heating of chitosan-maltodextrin mixture solutions at sufficiently high concentration and weight ratio could enhance the intensity of Maillard reaction, which exhibited antioxidant properties and could be used as additive in various food products.

Keywords: antioxidant activity, chitosan-maltodextrin solutions, Maillard reaction products, mixtures concentration, reactants weight ratio

1. INTRODUCTION

Chitosan is a natural-based biopolymer, which is comprised primarily of glucosamine or 2- amino-2-deoxy-β-D-glucose. Its molecule typically composes of amine groups, which can be protonated (-NH3+) by selected acids, since the pKa of this amine is around 6.3 (KLINKESORN, 2013). Chitosan has been used as dietary food additives in many aspects of functions, especially antioxidant activity. However, the antioxidant capacity of native chitosan is reported to be low compare to its derivatives. In order to improve the antioxidant properties of native chitosan, various modifications have been applied. One effective method is conjugating of chitosan with carbohydrate molecules via Maillard reaction. The Maillard reaction is a type of non-enzymatic browning that occurs between an amine group and a carbonyl compound, mainly of a reducing sugar under heated treatment (NURSTEN, 2005). This reaction leading to the formation of brown color called Maillard reaction products (MRPs), which are furan ring and nitrogen-containing brown compounds (KOSARAJU *et al*., 2010). The MRPs can be used as emulsifiers, antibacterial agents and antioxidants (KATO, 2002). Conjugation of chitosan with carbohydrate molecules via Maillard reaction could be controlled by type of reactants, concentration and ratio of reactants, temperature, time and pH (NURSTEN, 2005). Maltodextrin is obtained from the partial hydrolysis of starch by acid- or enzyme-catalyzed reactions with dextrose equivalent (DE) less than 20. Its structure composes of multiple glucose units linked by α-1,4-glycosidic bonds (WANDREY *et al*., 2010). This carbohydrate had been selected because it has carbonyl groups (-CHO), which can react with free amino groups of chitosan. In this study, the influence of mixtures concentration and reactants weight ratio between chitosan and maltodextrin on properties and antioxidant activity of Maillard reaction products was investigated.

2. MATERIAL AND METHODS

2.1. Materials

Powdered chitosan (low molecular weight, 120 ± 98 kDa; deactylation, 75-85%) was purchased from Aldrich Chemical Co. (St. Louis, USA). Maltodextrin (DE 17-19) was a gift from Neo Tech Food Co., Ltd. (Ratchaburi, Thailand). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were products of Sigma-Aldrich (Steinheim, Germany). Analytical grade dimethyl sulfoxide (DMSO; $(CH_3)2_SO$) was purchased from Labscan Asia Co., Ltd. (Bangkok, Thailand).

2.2. Methods

2.2.1 Formation of MRPs

The aqueous mixtures between chitosan and maltodextrin at various concentrations (5, 10 and 15 wt%) and different weight ratios (1:9, 1:24, and 1:49) were prepared in acetate buffer solution (100 mM, pH 3.0). These solutions were heated at 100°C for 60 min and then the resulting reaction products were analyzed.

2.2.2 Measurement of Zeta-potential

The MRPs solutions were diluted at 100 times with an acetate buffer solution. Approximately 1 mL of each solution was placed in a disposable folded capillary cell

(DTS-1070). The zeta- potential of all samples was observed at 25°C by measuring the electrophoretic mobility using a Zetasizer (Nano series Zen 3600, Malvern Instruments Ltd., UK) according to method reported by ANAL *et al.* (2008) with some modifications.

2.2.3 Measurement of color

The color of chitosan-maltodextrin mixture solutions was determined according to the method modified from KOSARAJU *et al.* (2010). Briefly, 20 mL of each mixture solution had been collected into 10 mm path length quartz cuvette (55 mm width × 57 mm height × 10 mm depth). The color was measured using a color measurement spectrophotometer (HunterLab UltraScan PRO, Hunter Associates Laboratory Inc., Virginia, USA) and the b* value was reported to indicate the degree of browning.

2.2.4 Measurement of ultraviolet (UV) absorbance at 294 nm

The intermediate products of Maillard reaction were detected by measuring the absorbance at 294 nm using the method of KOSARAJU *et al.* (2010) with slight modifications. The Maillard solutions were appropriately diluted at 10-fold using acetate buffer solution in 10 mm path length quartz cuvette (12.5 mm path length × 12.5 mm width × 45 mm height). All measurements were obtained using an UV-Vis spectrophotometer (GENESYS 10S, Thermo Fisher Scientific Inc., MA, USA). The acetate buffer solution was used as a blank reference.

2.2.5 Determination of antioxidant activity

The DPPH radical scavenging activity and Trolox equivalent antioxidant capacity were determined according to the methods described by WATTANASIRITHAM *et al.* (2016) with some modifications. Briefly, 30 mL aliquots of MRPs solutions were manually transferred in triplicate to a 96-well flat bottom polystyrene microplate, followed by adding 170 mL of 2 mM of DPPH in DMSO directly in an automated microplate reader (TECAN model Infinite M200, Tecan Group Ltd., Mannedorf, Switzerland). After 30 min incubation in dark at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radicals, which expressed as a DPPH scavenging activity (%) had been calculated using the following equation:

$$\text{DPPH scavenging activity \%} = \frac{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}) \text{ x } 100}{\text{Abs}_{\text{Control}}} \qquad (1)$$

Where AbsControl is an absorbance value of DPPH solution (2 mM), AbsSample is an absorbance value of DPPH solution containing MRPs sample. DMSO solvent was used as the control.

Moreover, Trolox equivalent antioxidant capacity (μg/μL) of MRPs was also reported. A calibration curve ($R^2 \approx 0.994$) was prepared using Trolox solution with concentrations ranging from 20 to 100 mg/L.

2.2.6 Statistical analysis

All experiments were carried out at least twice with triplicate measurements in each experiment. The data was subjected to the analysis of variance (ANOVA) using a statistical software package (SPSS). Duncan's Multiple Range Test comparisons at P value < 0.05 were made to determine significant differences.

3. RESULTS

3.1. Zeta (ζ)-potential

The ζ-potential of all unheated and heated chitosan-maltodextrin mixture solutions at various concentrations and different reactants weight ratios is shown in Fig. 1. We found that all mixture solutions exhibited positive charge. This is probably due to the protonate of amine groups (–NH3+) on chitosan molecules when exposed to environmental pH (~3.0) that lower than its pKa (~ 6.3) (KLINKESORN, 2013). An increase in the magnitude of ζ-potential was observed when the mixtures concentration and reactants weight ratio were increased.

However, the ζ-potential decreased significantly when the mixture solution was heated (P ≤ 0.05). It can be attributed to heating caused condensation between –NH3+ groups of chitosan and –CHO groups of reducing sugars in maltodextrin via the Maillard reaction (PIRESTANI *et al.*, 2017; ZHA *et al.*, 2019).

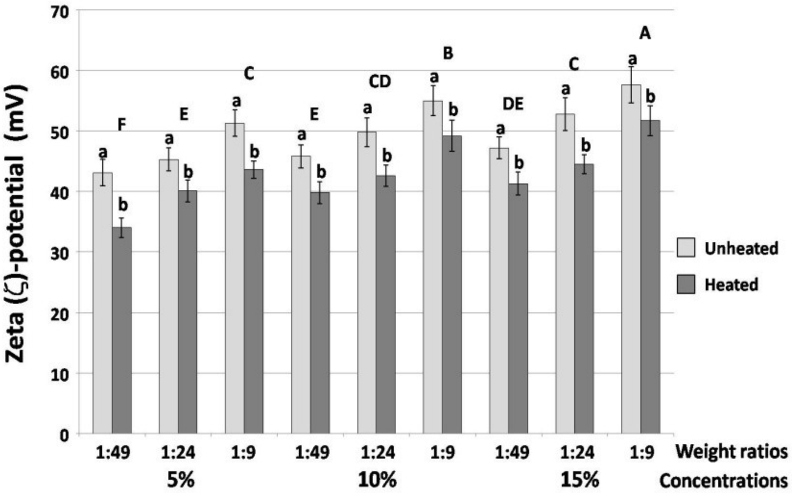

Figure 1. Zeta-potential of unheated and heated (100°C for 60 min) chitosan-maltodextrin mixture solutions at various concentrations and different reactants weight ratios.

3.2. b* value and UV absorbance at 294 nm

The b* values and UV absorbance at 294 nm followed similar trends (Fig. 2). Increasing concentration and reactants weight ratio led to increase of both indices. The heated chitosan-maltodextrin mixture solutions had significantly higher b* values (Fig. 2a) and UV absorbance at 294 nm (Fig. 2b) than unheated which indicated the extent of Maillard reaction between chitosan and maltodextrin (LI *et al.*, 2014).

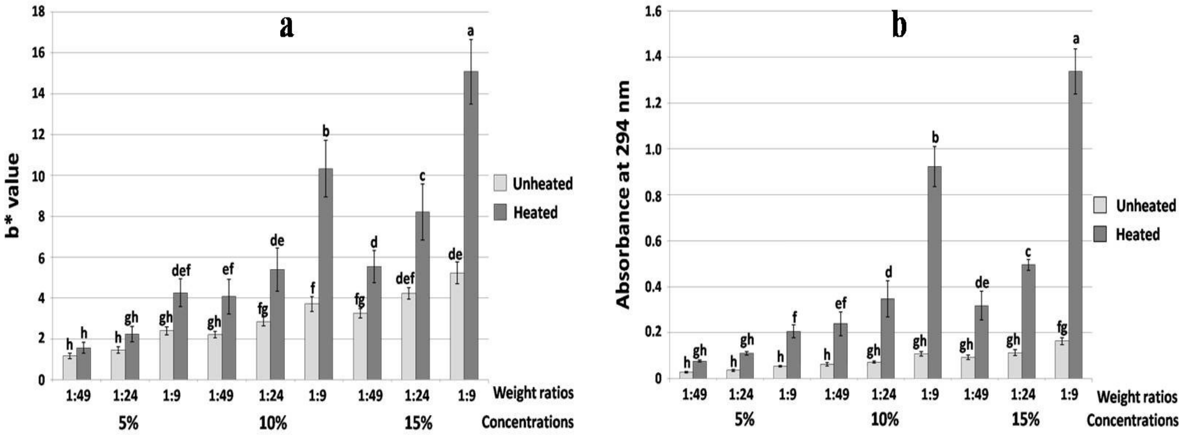

Figure 2. b* values (a) and UV absorbance at 294 nm (b) of unheated and heated (100°C for 60 min) chitosan-maltodextrin mixture solutions at various concentrations and different reactants weight ratios.

3.3. Antioxidant activity

The concentration and reactants weight ratio were found to influence ($P \leq 0.05$) the antioxidant activity of heated chitosan-maltodextrin solutions (Fig. 3). The DPPH radical scavenging activity (Fig. 3a) and Trolox equivalent antioxidant capacity (Fig. 3b) showed similar results, with higher in heated solutions than unheated samples at all concentrations and weight ratios. These results are consistent with previous works, which reported that Maillard reaction can be enhanced the antioxidant activity of native chitosan (KOSARAJU *et al*., 2010; LI *et al*., 2014).

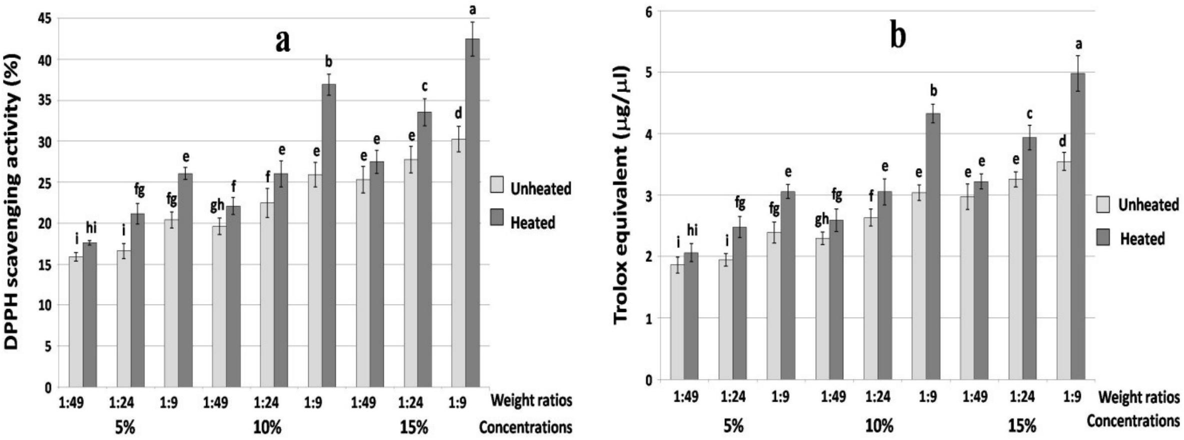

Figure 3. DPPH scavenging activity (a) and Trolox equivalent (b) of unheated and heated (100°C for 60 min) chitosan-maltodextrin mixture solutions at various concentrations and different reactants weight ratios.

4. CONCLUSIONS

Conjugation of chitosan with maltodextrin by heating to induce the Maillard reaction could produce the browning compounds exhibited antioxidant properties, which can be used as additive in various food products. In addition, these chitosan-maltodextrin MRPs may have a potential to be used as encapsulant for entrapping and protecting the labile active compounds.

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SHELF LIFE EXTENSION OF BERRIES USING IN-PACK OZONE

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ABSTRACT

Generation of ozone within a sealed pack of food mitigates previous risks associated with generating ozone for food decontamination. A rapid ozone generation treatment (0.5-3 seconds) creates high concentrations (100-400ppm) of ozone within the pack, allowing for reduction of microbes on the surface of raspberries by 0.8 log and blueberries by 1.4-3.4 logs. This extends the shelf life of berries by 2-3 days, by reducing the number of berries with mould per pack by up to 66%. In-pack ozone offers a novel method for shelf life extension via a rapid, safe and contained treatment.

Keywords: blueberries, ozone, raspberries, shelf life extension

1. INTRODUCTION

Due to the high nutritional value of fresh berries, the daily consumption of berries has been linked to many positive health effects (FOIT *et al.*, 2018; OLAS, 2018). Growing desire for "ready to eat" berry products in supermarkets has led to increasing concerns about the microbial safety of minimally processed fresh fruit. The high sugar content of berries along with their water activity and low pH creates the ideal conditions for microbial spoilage, particularly fungal spoilage (TOURNAS and KATSOUDAS, 2005).

Storage at low temperatures is known to slow down fungal spoilage and dramatically extend the shelf life of soft fruits (MORALES *et al.*, 2014). However, many fungi are still able to grow in chilled conditions, resulting in substantial spoilage throughout shelf life. Additional postharvest technologies can be used along with refrigeration to manage spoilage throughout shelf life. The use of synthetic fungicides and pesticides can reduce spoilage to some extent (CORDOVA *et al.*, 2017). However, there is a growing desire to move away from chemical preservatives in order to reduce environmental pollution and avoid adverse health effects associated with the consumption of such chemicals (WANG *et al.*, 2019). There is therefore a need to develop new, clean technologies of controlling the microbial spoilage of fresh fruit products in order to improve food safety, maintain fruit quality and reduce food waste.

Ozone is a rapid, non-specific germicide and is already used in industry for large-scale decontamination. In the past, ozone has had limitations in food product applications due to its unstable nature, requiring ozone to be generated on-demand for use, which can pose a risk to personnel working on site (PANDISELVAM *et al.*, 2019). However, in-pack ozone technology can generate ozone within a sealed pack of food, mitigating this risk. This is done by converting a small proportion of the oxygen (O_2) within the pack into ozone (O_3) via a high-voltage electrode. The surface of the food product is decontaminated by oxidative cleavage reaction from the O_3 (DE CANDIA *et al.*, 2015; WANI *et al.*, 2015), resulting in decay back to O_2. As this method of ozone generation results in high O_3 concentrations for a short period of time in close contact with the food, this study investigated the use of in-pack ozone technology on fresh berry products to determine whether this mode of O_3 application can result in effective decontamination and shelf life extension.

2. MATERIAL AND METHODS

2.1. Ozone generation

The Anacail ozone system allows an accurately determined proportion of the oxygen in a sealed bag or package to be converted into ozone, without opening or otherwise damaging the pack. An external electrode assembly is brought into contact with the sealed pack, generating intense but localised electric fields within the pack, which convert a small fraction of the oxygen (O_2) into ozone (O_3). Typical treatment times are 0.5-3 seconds with ozone concentrations in the range 100-1000 ppm (www.anacail.com).

2.2. Sample preparation

Two hundred grams of blueberries (*Vaccinium* sp. varieties Kirra/Snowchaser) and raspberries (*Rubus idaeus* variety Driscoll's Maravilla) were purchased from a local retailer and re-packed into unvented K37 punnets and sealed with micro-perforated film. Sealed packs were treated with a two-second ozone generation time using a F250 ozone generator

(Anacail). Packs were set up blind to ensure testers did not know which packs were ozone-treated.

2.3. Decay curves

At selected time points from 0-10 minutes, ozone was sampled from the headspace in pack using an ozone meter 106-MH (2B Technologies) to measure ozone concentration in parts per million (ppm) within the pack. Triplicate packs were tested at each time point.

2.4. Shelf life testing

Raspberries (n = 5 per set) and blueberries (n = 10 per set) were prepared as above, with a two-second ozone generation treatment. Samples were left to naturally decay back to oxygen or left untreated (control). Packs were stored at 8°C in an IC600 temperature-controlled incubator (LTE Scientific) to assess shelf life. Each day, samples were scored for overall pack quality (3 – no spoilage issues; 2 – sellable with marginal spoilage; 1 – unsellable due to spoilage) and the number of fruits per pack with specific spoilage issues was recorded.

2.5. Microbiology testing

Raspberry samples (n = 15 per set) were processed in a stomacher (Seward) and plated onto plate count agar (PCA; Sigma) for total viable counts (TVC) and onto Dichloran Rose Bengal Agar (DRBA; Sigma), supplemented with 50μg/ml chloramphenicol (Sigma) and Rose Bengal Chloramphenicol Agar (RBCA; Sigma) for mould/yeast enumeration. Plates were incubated according to manufacturers' instructions. Blueberry samples (n = 30 per set) were processed by an accredited microbiology laboratory using UKAS accredited methods for TVC (MM01A) and mould/yeast (MM05A).

2.6. Data analysis

Data was analysed using GraphPad Prism (v8.1) for geomean. Shelf life data was analysed for significance using two-way ANOVA with Sidak's multiple comparison test; microbiology data was analysed by Mann-Whitney unpaired t-test for significance.

3. RESULTS AND CONCLUSIONS

3.1. Ozone generation and decay time

A two-second ozone generation treatment on 200g packs of blueberries and raspberries resulted in a peak ozone concentration of 370ppm and 332ppm, respectively (Fig. 1). The ozone rapidly decayed back to oxygen within the pack, with only 32ppm remaining in the blueberries pack after 5 minutes. The ozone in the raspberries pack decayed quicker, with only 2ppm present after 5 minutes. In both experiments, all ozone had decayed back to oxygen (0 ppm) after 10 minutes, meaning the packs were safe to open as the ozone concentration was under the workplace exposure limit (0.02ppm over 15 minutes (HSE, 2014)).

Exposure to ozone in-pack is different from other current ozone generating methods which have shown an improvement to shelf life from ozone, as they tend to generate low concentrations (1-30ppm) of ozone for longer periods of time (e.g. minutes/hours)

(HORVITZ and CANTALEJO, 2012; BERMÚDEZ-AGUIRRE and BARBOSA-CÁNOVAS, 2013; ALWI and ALI, 2014), whereas the exposure time in- pack is at a much higher concentration but for a shorter period of time. As these studies have found that low concentrations of ozone over several hours can extend the shelf life of food products, it was hypothesised that the high concentration of ozone generated in-pack in a short exposure could achieve a similar result.

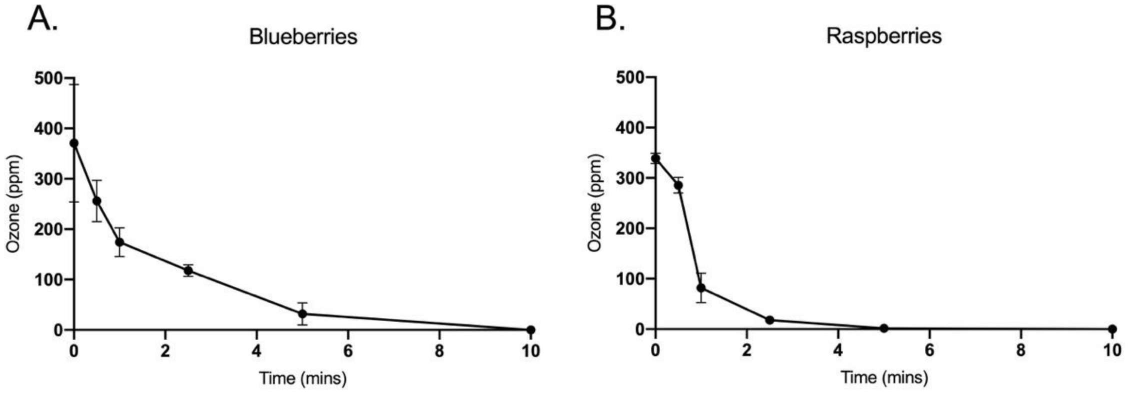

Figure 1. Ozone generation and full decay in sealed packs of blueberries (A) and raspberries (B). All ozone decayed back to oxygen within 10 minutes, therefore the packs were safe to open after this time. Error bars represent standard deviation of the mean.

3.2. Shelf life extension of raspberries and blueberries

Shelf life assessment of raspberries and blueberries following in-pack ozone treatment found that the addition of in-pack ozone significantly improved the quality of both fruits (Fig. 2). Untreated raspberries were all unsellable (quality score of 1) by packing + 3(P+3), whereas in-pack ozone- treated raspberries had an average quality score of 2.6 by this time, out of a potential maximum 3 (Fig. 2A). This quality was significantly improved at P+3 – P+5 in the ozone-treated raspberries ($p<0.05$). This improvement was due to a reduction in the onset of mould (Fig. 2B), with a 66 % reduction in the number of raspberries with mould per pack by the end of the trial (P+6).

A similar result was seen for blueberries, with a higher quality maintained over the course of the experiment and a significant improvement seen at P+6 – P+7 (Fig. 2C; $p<0.05$). As for the raspberries, this improvement in quality and overall shelf life was due to a reduction in moulding, with in-pack ozone treated samples having less blueberries overall with mould per pack for the duration of the experiment (Fig. 2D).

As ozone is a non-specific and fast-acting germicide (KIM *et al.*, 1999; KHADRE *et al.*, 2001), it was proposed that this extension in shelf life and reduction in the onset of visual mould development was due to the in-pack ozone reducing microbes present on the surface of the berries within the 10-minute exposure period.

3.3. Reduction of microbes

To determine the extent of microbial reduction, retail packs of raspberries and blueberries were treated with in-pack ozone and processed for TVC and mould/yeast enumeration. The in-pack ozone dose used to give the extension to shelf life in the previous tests was also found to significantly reduce the presence of bacteria (Figs. 3A; 3C) and moulds/yeasts (Fig. 3B/D) on raspberries and blueberries.

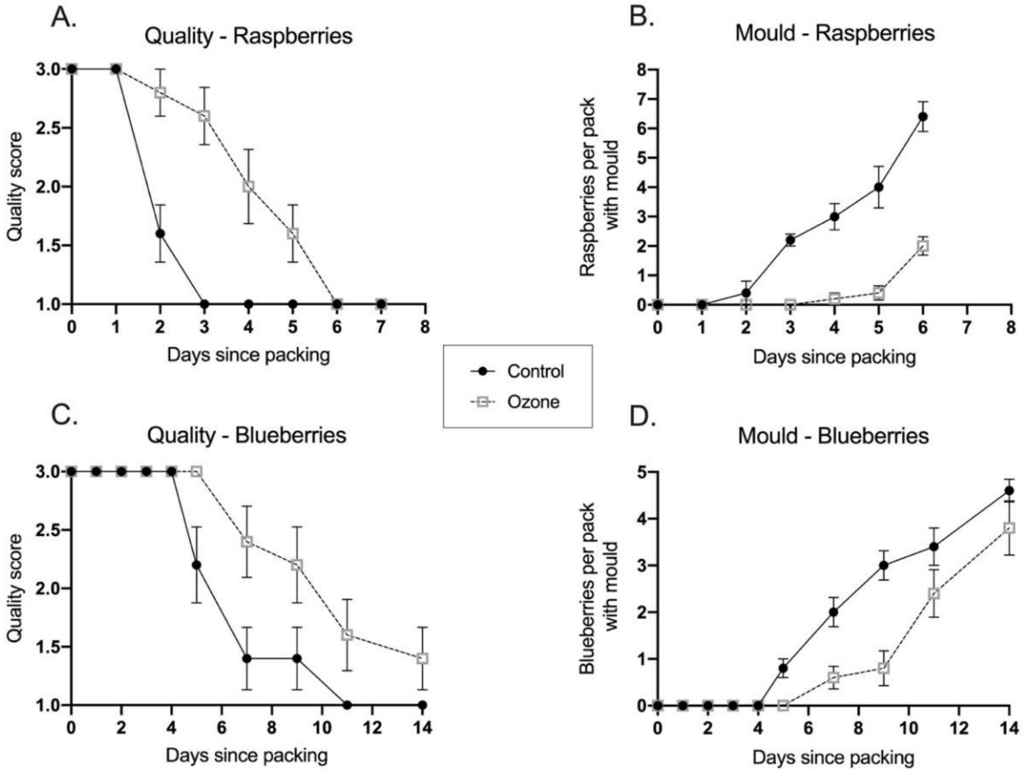

Figure 2. Shelf life extension on raspberries and blueberries, stored at 8°C. Berries were monitored for overall quality (A – raspberries; C - blueberries), scoring from a 3 (no spoilage issues) to a 1 (unsellable due to microbial spoilage). The number of berries per pack for each treatment with mould (B – raspberries; D - blueberries) was recorded daily. Error bars represent standard error of the mean.

A higher overall reduction was seen in the blueberries, with many of the ozone-treated samples brought down to the limit of detection (LOD; <10 cfu/g for TVC; <100 cfu/g for mould/yeast) for each test. In-pack ozone treatment resulted in a 0.8 log reduction in TVC and mould/yeast on raspberries, whereas 3.4 and 1.4 log reductions were observed in blueberries in the same tests, respectively. This could be due to the higher surface area of the raspberries compared to blueberries in an equivalent weighted pack or the presence of potential bleeding fruit present in the raspberries, which may make it more difficult for the ozone to gain access to the microbes.

Overall, the results suggest that the high dose and short exposure time of in-pack ozone is sufficient to reduce the number of microbes, including moulds, present on the surface of raspberries and blueberries, in turn resulting in an extension to shelf life for these products.

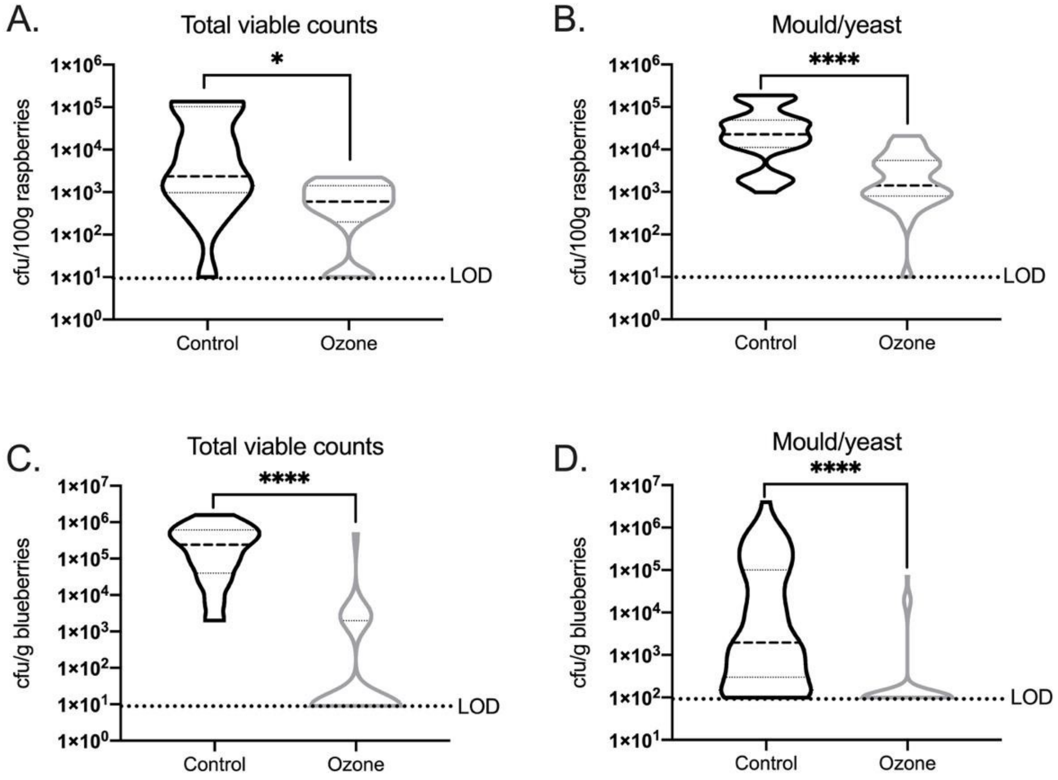

Figure 3. Reduction of TVC and mould/yeast on raspberries (A; B) and blueberries (C; D) by in-pack ozone. LOD represents the limit of detection using standard plating methods. Dotted

This technology could therefore offer a method for improving the life of perishable products such as berries in a rapid treatment, without the risk of re-contamination of the product until the pack is opened by the consumer. This also will have a positive impact on reducing food waste for fresh products throughout the supply route and for the end user.

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STUDY ON EUGENOL LOADED ANTIFUNGAL POLYMERIC COMPOSITES

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ABSTRACT

Antifungal effects of calcium alginate (CA) end-cap treatment and eugenol (EU)-loaded halloysite nanotube (HNT) contents with low-density polyethylene (LDPE) were investigated. Improved thermal stability of EU was achieved by entrapping HNTs with the CA end-cap. EU-loaded HNTs with and without the CA end-cap were incorporated into LDPE using 1, 3, or 5% (w/w) HNTs. The prepared LDPE/HNT composites were characterized by Fourier transform infrared (FT-IR) spectrometer, UV-vis spectrophotometer, and thermogravimetric analysis (TGA). The mechanical and antifungal properties were also evaluated. 3% LDPE/HNTs-EU-CA exhibited a higher thermal stability than the other samples and showed good antifungal property.

Keywords: antimicrobial packaging, calcium alginate, eugenol, halloysite nanotubes

1. INTRODUCTION

Over recent decades, essential oils have emerged as promising antimicrobial agents that can be extracted from natural resources. Biodegradable films as well as coatings are the representative applications of essential oils (ATARÉS *et al.*, 2015). Eugenol (EU) is a member of the phenylpropanoid class of compounds extracted from clove oil, and is responsible for aroma of cloves, which significantly contributes to the antimicrobial activity (VERGIS *et al.*, 2015). Active packaging containing antimicrobial agents can help extend the shelf life of food products while ensuring quality and safety. Halloysite nanotubes (HNTs) are the alumosilicate tubular clay with a diameter of 50 nm, inner lumen of 15 nm, and length of 600-900 nm. The inner surface can be tuned by etching up to 20-30% of the tube volume for loading agents such as antioxidants, anticorrosion or antimicrobial agents (LVOV *et al.*, 2016). Alginic acid is water-soluble and can be ionically cross-linked with a nontoxic divalent cation solution such as calcium chloride. The calcium ions bind the guluronic acid to develop a stable alginate gel, which is applied to cover the edge of HNTs and assist the loading agents (BECKER *et al.*, 2001). The purpose of this study was to investigate the thermal and mechanical properties, and antifungal effects, of LDPE/HNTs-EU composites with a calcium alginate (CA) end-cap in relation to the amount of HNTs.

2. MATERIAL AND METHODS

2.1. Materials

EU (purity 99%) and HNTs were purchased from Sigma-Aldrich (Yongin, Korea). Calcium chloride dihydrate (extra pure) was purchased from Duksan Chemicals (Ansan, Korea), and sodium alginate was obtained from Daejung Chemicals (Siheung, Korea). *Botrytis cinerea* (ATCC 26943) was obtained from the Fungal Genetic Resources Center (Seoul, Korea).

2.2. Preparation of LDPE/HNTs-EU-CA matrix composites

To prepare the LDPE/HNTs-EU-CA composites, 1 g of HNTs was mixed with 1000 mL of EU and sonicated for 30 min (Branson 5800, Emerson, USA) to be evacuated in a vacuum chamber. The chamber was slowly unlocked until atmospheric pressure to load the EU into HNTs. EU replaced air inside the HNTs by capillary forces (JOSHI *et al.*, 2013). After these procedures, the samples were centrifuged, filtered, and washed with distilled water thrice. Separately, a 2% aqueous solution of sodium alginate was mixed with HNTs (w/w, 5:95) in distilled water (100 mL) and stirred for 15 min for end-cap treatment. During stirring, 2% calcium chloride solution was slowly poured into the mixture. After drying the treated and untreated samples at 60°C for 6 h, LDPE/HNT polymeric matrix sheets were prepared using the samples with a hot press machine (TO-200, Test One, Korea) at 120°C and 5 MPa. The composite samples were prepared using 1, 3, or 5% of HNT per LDPE polymer matrix (w/w).

2.3. Characterization of the prepared samples

To evaluate the loading capacity of EU in the HNTs, absorption spectra were recorded in the wavelength range of 250 to 300 nm using a UV-vis spectrophotometer (V-640, Jasco, Japan). To determine the amount of EU in HNTs, 1 g of extract from the sample was obtained using 99.9% ethanol at 95°C for 30 min; this was followed by centrifugation at 5000 rpm for 5 min. The UV absorbance of the supernatant was tested at 283nm. The thermal stability of EU in the HNTs and CA was investigated by TGA (TGA-4000 system, PerkinElmer, USA). The samples were heated under nitrogen atmosphere from 30 to 400°C at a heating rate of 10°C min^{-1}. FT-IR spectrometer (PerkinElmer 1760X, Perkin Elmer, USA) was employed to observe the structural interactions of among samples. The FT-IR spectra were obtained with 16 scans over a wavenumber range of 400-4000 cm^{-1}. An antifungal test was conducted by a modified well diffusion method (JUNQUEIRA-GONÇALVES *et al*., 2013). *B. cinerea* was grown in potato dextrose broth (PDB) for 5 days at 25°C. Subsequently, 1 mL of PDB was injected in potato dextrose agar (PDA) at five different spots surround the center. The prepared samples in PDA were incubated for 72 h at 25°C. Each sample was replicated ten times. LDPE composites were prepared according to ASTM D638-91. The tensile strength (TS) and elongation (% EL) were measured with a universal material testing machine (DTU-900, Daekyung Tech, Korea) at a test speed of 50 mm min^{-1} according to the ASTM D882.

2.4. Statistical analysis

Data analysis was performed using IBM SPSS statistics for Windows, Version 24.0, Armonk, NY, IBM Co. USA. Duncan's multiple range tests were conducted to verify the significant differences at $p=0.05$. All data are expressed as means with standard deviations.

3. RESULTS AND DISCUSSION

3.1. Characteristics of EU-HNTs samples

Fig. 1 shows that EU underwent major weight loss in the range of 110-210°C (SCREMIN *et al*., 2018), which corresponds to EU degradation or evaporation. Also, EU content from HNTs-EU sample underwent a significant weight loss of more than 10% at 180°C. The incorporation of HNTs-EU and CA significantly affected its thermal stability compared with the HNTs-EU. The result demonstrates that CA can delay the loss of EU in HNTs during high-temperature process of the polymer fabrication. As the polymer fabrication process involves highly elevated temperatures, it may be difficult to maintain the thermal sensitive bio-organic materials in polymer composite. Therefore, EU retention in the fabrication of LDPE composite is very important to determine the antimicrobial activity in polymeric matrixes.

Fig. 1 shows the FT-IR of HNTs-EU, HNTs-EU-CA, and HNTs-CA. Bands characteristic to EU peaks at 1510 cm^{-1} (C=C) and 1270 cm^{-1} (C–O) were presented due to the successful loading of EU into HNTs (SCREMIN *et al*., 2018). Bands at 1029 and 906 cm^{-1} were assigned to Si–O stretching and Al–O bending, which are characteristic of HNTs from all samples (BERAHMAN *et al*., 2016). It can show the method to load EU into HNTs by capillary force with a vacuum chamber was appropriate. However, HNTs-EU-CA showed CA peaks like HNTs-CA at 1595 and 1420 cm^{-1}, which are attributed to the asymmetric and symmetric stretching vibrations of free carboxyl groups, respectively (VOO *et al*., 2015). EU from HNTs-EU-CA was detected by UV-Vis; however, it is assumed that EU peaks of HNT-EU-CA sample were overlapped by CA peaks from this result (MURATORE *et al*., 2018). Table 1 shows the amount of extracted EU from 1 g of HNTs with and without CA end-cap treatment. The average amount of EU from HNTs before treatment was 263±23.8 μL;

however, after treatment, it decreased to 113.7±10.1 μL. As a result, the CA end-cap treatment did show a loss in EU concentration.

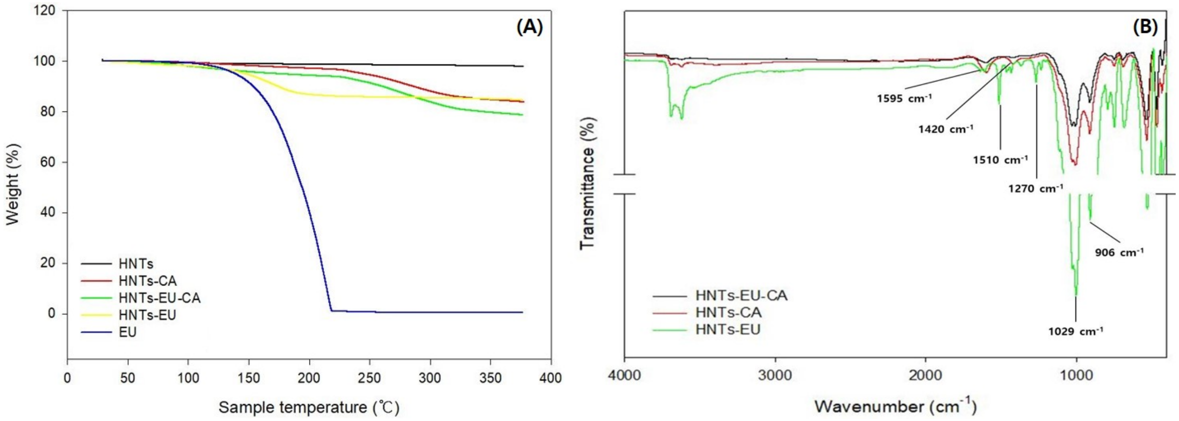

Figure 1. TGA (A) and FT-IR spectra (B) curves of the respective prepared materials

3.2. Antifungal test

Fig. 2 shows that 1, 3, and 5% LDPE/HNTs-EU didn't show the inhibition of the microbial growth failed to inhibit growth for anti-fungal test. Meanwhile, 3% and 5% LDPE/HNTs-EU-CA was represented an inhibition of the microbial growth among the end-cap samples.

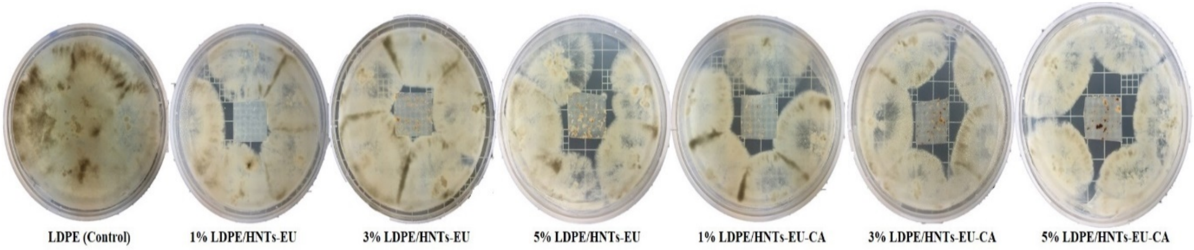

Figure 2. Antifungal effects of EU loaded LDPE composites after 72 h incubation at 25 °C

As shown in Table 1, the contents of EU on LDPE/HNTs-EU and LDPE/HNTs-EU-CA were 263±23.8 and 121±10.8 μL/g HNT. The result showed that the end-cap treatment reduces the amount of EU, while the TGA and antifungal test demonstrated appropriate thermal stability and antifungal property. As EU is the only material to exhibit antifungal property, it is considered that EU from the untreated samples was lost during hot pressing.

3.3. Mechanical properties of LDPE composites

The tensile strength (TS) and elongation (EL) of the LDPE composites containing HNTs-EU-CA were evaluated (Table 1). The average thickness of the prepared LDPE composites was approximately 0.58 mm. The LDPE composites containing HNTs-EU-CA or HNTs-EU had lower TS and EL than their corresponding control LDPE. Increase in the HNTs-EU-CA or HNTs-EU contents in the LDPE composites resulted in decrease in the TS and EL in comparison to those of control LDPE.

Table 1. Mechanical properties and EU content in antifungal LDPE composites.

Samples	Control LDPE	LDPE/HNTs-EU			LDPE/HNTs-EU-CA		
		1%	3%	5%	1%	3%	5%
Eugenol content (μl/g HNT)	-		263±23.8			121±10.8	
Tensile strength (N)	45.46±2.20^{a}	29.32±2.19^{b}	27.07±1.49^{cd}	26.69±2.71^{d}	30.34±2.06^{bc}	28.17±2.32^{d}	27.12±1.28^{d}
Elongation (%)	574.98±49.66^{a}	67.14±6.67^{b}	29.53±7.24^{c}	17.96±1.08^{c}	65.29±11.44^{b}	29.53±7.72^{c}	18.06±1.12^{c}

$^{a-d}$Mean in the same column with different letters are significantly different ($p \leq 0.05$).

This is probably because of the poor dispersion of HNTs in the LDPE polymer matrix in our experiments (SOHEILMOGHADDAM *et al.*, 2013). This implies that the HNTs may be affected by the lack of the network structure in the LDPE composite when incorporated into the LDPE. In this experiment, there were no significant differences in the TS and EL between HNTs-EU-CA and HNTs-EU at different contents in the LDPE composites.

4. CONCLUSIONS

This work shows that LDPE polymeric matrixes incorporated with a CA end-cap for the EU compound into HNTs particles exhibit the better antifungal effect despite the high temperature processing conditions. Hence, CA end-cap treatment can provide better thermal stability of heat sensitive EU and maintain the antifungal activity without losing its functional efficacy. The results of LDPE composites containing HNTs-EU-CA indicate that it has good potential for the functional food composite films.

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RELEASING OF *MICHELIA ALBA* COMPONENT AGAINST MOULD

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ABSTRACT

This study aimed to determine the optimum condition for inhibiting the growth of *Aspergillus flav*us on brown rice using Tyvek® sachet containing *Michelia alba* essential oil (EO) by using response surface methodology. Brown rice (100 g, moisture content [MC] 14%, 16%, and 18%) was inoculated with *Aspergillus flavus* and packed in a plastic bag (250 ml) containing *M. alba* oil (0–300 μl), then stored at 25°C and 40°C for 60 days. The effect of temperature (25°C and 40°C) and relative humidity (RH, 75% and 100%) on releasing of antifungal volatiles were investigated in the headspace using SPME-GC-MS. Results indicated that the addition of the sachet containing *M. alba* EO (300 μl) in brown rice (14% MC) and storage at 25°C was the optimal condition for inhibiting *A. flavus* on brown rice with the highest antifungal activity. Furthermore, linalool and caryophyllene were found to be increased with temperature and RH increase.

Keywords: antifungal volatiles, brown rice, *Michelia alba* EO, response surface methodology

1. INTRODUCTION

Michelia alba essential oil (EO) in the vapour phase has been proven to inhibit the growth of many significant moulds in post-harvest agricultural products (SONGSAMOE *et al.*, 2017). A recent study demonstrated an effective application of *M. alba* EO vapour in the packaging system of brown rice and brown rice products for inhibiting spoilage and mycotoxigenic moulds (SONGSAMOE *et al.*, 2017; SUHEM *et al.*, 2017). The effect of *M. alba* EO vapour on the spore germination and mycelium growth of mould in brown rice during storage has been investigated. However, the important conditions, including moisture and temperature for inhibiting mould growth on brown rice during storage under the *M. alba* EO vapour needed to be optimised. In addition, the effect of moisture and temperature on the releasing behaviour of the antifungal volatile compound of *M. alba* EO in vitro also needed to be investigated. Therefore, the present study aims to optimum condition for inhibiting *Aspergillus flav*us on brown rice using Tyvek® sachet containing *M. alba* EO and observe the releasing behaviour of the antifungal volatile compound of *M. alba* EO in the packaging system.

2. MATERIAL AND METHODS

2.1. Optimisation of brown rice storage conditions for inhibiting *A. flavus*

Brown rice (100 g) with adjusted moisture content (MC) (14%, 16%, and 18%) was inoculated with *A. flavus* (6 $\log_{10}$ CFU g^{-1}) and packed in a plastic bag (LLDPE, 220 µm thick and 250 ml volume). Dried fibers from water hyacinth were used as absorbent material containing five different amounts of *M. alba* EO (0–300 µl). These fibers were packaged inside porous Tyvek® sachets used as active packaging system in the plastic pouch containing the rice. After that, all samples were stored at 25°C and 40°C for 60 days. The mould growth on the brown rice surface was observed, and the period of inhibition (day) was reported. The three independent variables including temperature (25–40°C, MC (14–18%) and quantity of *M. alba* EO (0–300 µl), and the dependent variable (period of inhibition, day) were optimized using a factorial design (2x3x5) and analysed by response surface methodology.

A second-order polynomial quadratic equation was fitted to the data to relate the period of inhibition (day, Y) to the storage temperature (X_1), rice MC (X_2) and quantity of *M. alba* EO (X_3). The quality of fit was checked with the coefficient of determination R^2, and its statistical significance was determined by the *F*-test. The statistical analysis was performed by using Statistica software (StatSoft, USA).

2.2. Releasing behaviour of the antifungal volatiles from the sachet as affected by temperature and relative humidity (RH) in vitro

The releasing behaviour of the main volatile compounds of *M. alba* EO released from the Tyvek® sachet in the headspace as affected by temperature and RH was investigated in vitro using the SPME-GC-MS technique. An SPME fibre coated with PDMS-DVB-CAR phases with 50/30 µm (divinylbenzene/carboxen) film thickness (Supelco, Bellefonte, PA, USA) was used. The setting up of SPME conditions was performed as follows: RH inside the glass vessel (1 L) was adjusted at 75% and 100% using a saturated salt solution (NaCl) and distilled water. After getting the targeted %RH, the Tyvek® sachet containing *M. alba* EO (75 µl) was placed into the glass vessel. Then, the vessel was tightly sealed with a screw cap containing rubber septa. The sample vessel's temperature was maintained at 25°C and 40°C. At specified time intervals, the SPME fibre was manually exposed to the headspace of the vessel with the extraction time of 20 min to extract the sample of the volatile compound that was released from the sachet. Desorption was carried out in the gas chromatograph injection port for 10 min. A Perkin Elmer Autosystem XL gas chromatograph equipped with a DB-5MS (30 m, 0.25 mmID, a column with a film thickness of 0.25 µm), a Merlin Microseal™ Septum Kit installed on the Capillary Inlet system and a Turbomass mass spectrometer was used. The injection was carried out at 250°C in the splitless mode. The GC program was as follows: an initial temperature of 40°C was maintained for 2 min. The temperature was then increased to 170°C at a rate of 4°C min^{-1} and held for half a minute. It was then further increased to 240°C at a rate of 15°C min^{-1} and maintained for an additional 2 min. Total run time was 43 min. The injector temperature was 250°C. Helium was used as the carrier gas (flow rate: 1.2 ml min^{-1}). The mass spectrometer was operated in electron impact mode (70 eV), and masses were scanned over an m/z range of 40–350 m/z. Compounds were identified, by matching their mass spectra with the US National Institute of Standards and Technology (Gaithersburg, MD, USA) commercial library. The concentrations of main antifungal volatile compounds were determined using the standard curves for each compound. All experiments and analyses were carried out in duplicate. The releasing curves of the main antifungal volatile compounds were constructed.

3. RESULTS AND CONCLUSION

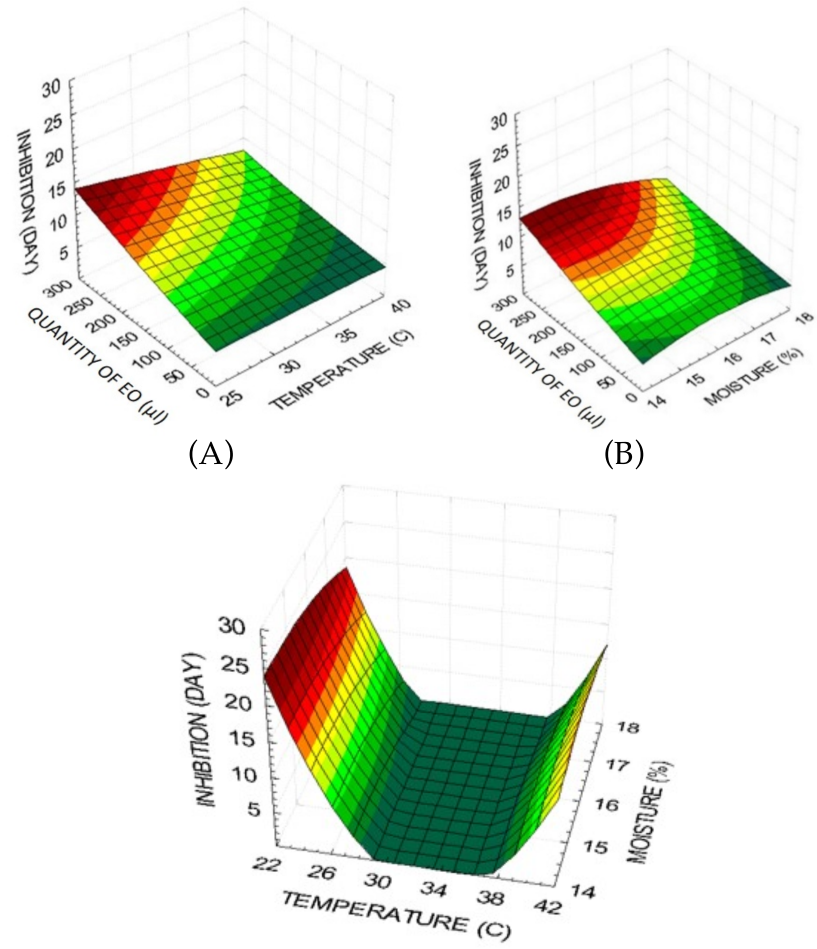
3.1. Optimisation of brown rice storage conditions for inhibiting *A. flavus*

The effects of storage conditions are shown in Fig. 1. The results indicated that stored brown rice grain at a low MC (14%) under high quantity of *M. alba* EO in the packaging(300 µl) at 25°C was the optimal condition for inhibiting *A. flavus* growth with the highest antifungal activity (>4 times compared with the control). In addition, the period of inhibition of *A. flavus* in brown rice (day) is best predicted by the second-order polynomial quadratic equation as follows:

$$Y = -40.866667 - 1.44X_1 + 9.30X_2 + 10.533333X_3 - 0.3875X_2^2 + 0.083333X_3^2 + 0.085X_1X_2 - 0.13X_1X_3 - 0.3X_2X_3$$

WhereY is the predicted period of inhibition (day), X_1 is storage temperature (°C), X_2 is the rice MC (%) and X_3 is the quantity of *M. alba* EO (µl).

The coefficient of determination (R^2) was 0.92, which indicated that the model equation adequately fit the data. Magnitudes of the model coefficients showed that the quantity of *M. alba* EO (X_3) was the most important factor in the growth of *A. flavus* in brown rice followed by the temperature (X_1) and the rice MC (X_2) (Table 1). The MC of rice grain and storage temperature are significant factors of rice spoilage. GENKAWA *et al.* (2008) reported that the moulds were observed on the brown rice surface stored at 25°C with an MC of more than 14.4% on a wet basis, but no mould was observed on brown rice surface with an MC of less than 12.8% wet basis. Therefore, the results in the present work indicated that the *M. alba* EO vapour treatment could inhibit the growth of mould on brown rice surface in accelerated storage conditions.



(A)

(B)

(C)

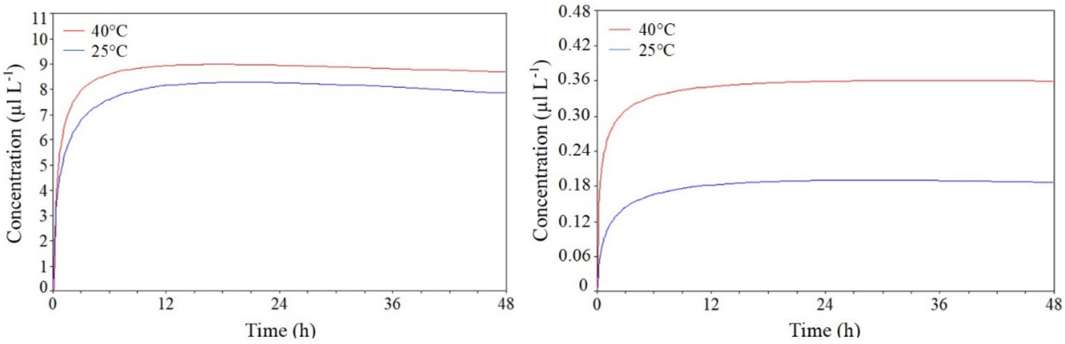
Figure 1 The response surface of the period of inhibition; quantity of *M. alba* EO vs temperature, A, quantity of *M. alba* EO vs moisture, B, and temperature vs moisture, C.

Table 1. Analysis of variance (ANOVA) for response quadratic model from experimental designs. *($p<0.05$).

	Degree of freedom	**Prob. > *F***
X_1	1	0.0018*
X_2	1	0.0160*
X_3	1	0.0000*
$X_1 * X_1$	0	-
$X_1 * X_2$	1	0.0025*
$X_1 * X_3$	1	0.0016*
$X_2 * X_2$	1	0.0002*
$X_2 * X_3$	1	0.0026*
$X_3 * X_3$	1	0.4983

3.2. Releasing behaviour of the antifungal volatiles from the sachet as affected by temperature and RH in vitro

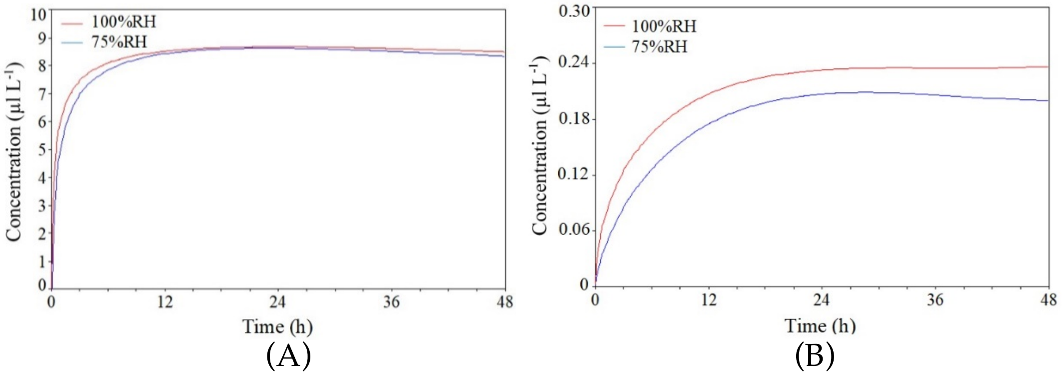
The effect of temperature on the release of linalool and caryophyllene from the Tyvek® sachet containing *M. alba* EO is shown in Figs. 2A, B. The temperature could significantly affect the vaporization of linalool and caryophyllene. The high temperature (40°C) stimulated the release of linalool and caryophyllene from the sachet into the air. It is in contrast to the lower temperature (25°C), at which the releasing rate of both volatile compounds was lower. At high temperature, the molecules of linalool and caryophyllene had high kinetic energy that resulted in the increased vaporization of both volatile compounds.



(A) (B)

Figure 2. The releasing behaviour of linalool (A) and caryophyllene (B) in a closed system as affected by temperature (25°C and 40°C) at 75% RH.

The releasing behaviour of linalool and caryophyllene from the Tyvek® sachet containing *M. alba* EO at different RH (75%–100%) is shown in Figs. 3A, B. The result indicated that only the releasing rate of caryophyllene was affected by RH and its concentration at equilibrium in the headspace was lower if compared with the concentration of linalool. Therefore, the ratio of the two compounds in the headspace did not change significantly when the RH increased. The relative humidity of the environment could have favoured the release of the EO's compounds absorbed onto the hyacinth fibres. Since the dried plant absorbent is characterized by a certain porosity and hydrophilicity, the water vapour could interact stronger with the active sites of the absorption than linalool and caryophyllene. Therefore, in the system that is rich in water vapour, the release of linalool and caryophyllene were activated from the substitution of the water vapour on the absorbent's surface.



(A)

(B)

Figure 3. The releasing behaviour of linalool (A) and caryophyllene (B) in a closed system at 75% RH and 100% RH, 25°C.

This result indicated that the Tyvek® sachet containing *M. alba* EO (300 µl) could inhibit the mould growth in a wide range of food storage conditions such as normal or high temperature and low or high RH.

ACKNOWLEDGEMENTS

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DEVELOPMENT OF A BRINE FOR MOZZARELLA PRESERVATION

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ABSTRACT

Mozzarella shelf life is highly influenced by spoilage microorganisms, which are responsible of off-flavour, texture deterioration and chromatic alteration. The effect of 24 different preserving brines on the chemical, microbiological and sensory characteristics of the product during storage was investigated. Both the brine and product compositions changed over time, because of mass transfer phenomenon. Most of the brines tested caused worsening of the characteristics, whereas that containing 1% calcium lactate at pH 3.50 gave very interesting results. The product stored in such brine had lower bacterial counts and better sensory characteristics than the control, and did not undergo to chromatic alteration.

Keywords: mozzarella, shelf-life, brine, spoilage microorganisms, blue discoloration

1. INTRODUCTION

Mozzarella is a fresh cheese with soft body and milky flavor that is sold packaged in a liquid for preserving freshness. Shelf life is very short, and commonly ranges from a few days to 2 weeks, depending on the type of milk (raw or pasteurized), product (buffalo or cow) and preserving liquid (pot water or brine). In the case of buffalo mozzarella, the liquid is an acidic brine that consists of salt and "stretching process water" or salt added with citric or lactic acid (PAONESSA, 2004). Such brines cannot be used for the bovine type that presents too delicate skin: for this product, pot water is commonly used that, in the case of industrial manufacturing, can be also pasteurized. A possible alternative is the use of diluted NaCl solutions (less than 2.0 g L^{-1}). Deterioration of mozzarella is caused both by spoilage microorganisms and by interactions between cheese and preserving liquid (LAURIENZO *et al.*, 2008). Among spoilage microorganisms, psychrotrophic bacteria play a primary role since the cheese is maintained under refrigeration until consumption. *Pseudomonas* spp. is one of the most feared psychrotrophic microorganism, since it causes off flavours and chromatic alterations. A number cases of "blue discoloration" defect in bovine mozzarella recently occurred in Europe, which was ascribed to *Pseudomonas fluorescens*, a bacteria that also induces fluorescence (CHIESA *et al.*, 2014; CARRASCOSA *ET AL.*, 2015; DEL OLMO *et al.*, 2018). Very interestingly, this defect is very rare in buffalo mozzarella. Recently, an innovative strategy has been proposed to counter the activity of *Pseudomonadaceae* in bovine mozzarella, involving addition of antimicrobial compounds to the milk or preserving liquid (DEL NOBILE *et al.*, 2009; GAMMARIELLO *et al.*, 2010; GORRASI *et al.*, 2016). Unfortunately, almost all the active principles that have been proposed cannot be adopted by the dairies, due to impact on the sensory characteristics or incompatibility with the EU legislation. The present study reports the results of an experimentation that aimed to evaluate the effect of several brines, made only with low-compatible compounds, on mozzarella shelf life.

2. MATERIALS AND METHODS

Mozzarella knots weighing about 70 grams were manufactured in a local dairy from pasteurized milk by direct acidification and packaged immersed in 24 different brines (Table 1) or water (as control).

Table 1. Composition of the brines used in the experimentation.
*= present; - = absent

	pH	Citric acid	Lactic acid	Calcium chloride	Calcium lactate	Polypropylene glycol	Sorbic acid	Sodium chloride
A1	4.50	*	-	1.0	5.0	-	-	-
A2	4.50	-	*	1.0	5.0	-	-	-
A3	4.50	*	-	2.0	5.0	-	-	-
A4	4.50	-	*	2.0	5.0	-	-	-
A5	4.50	*	-	1.0	10.0	1.0	5.0	-
A6	4.50	-	*	1.0	10.0	1.0	5.0	-
A7	4.50	*	-	2.0	10.0	1.0	10.0	-
A8	4.50	-	*	2.0	10.0	1.0	10.0	-
A9	4.00	*	-	1.0	-	-	-	4.0
A10	4.00	-	*	1.0	-	-	-	4.0

A11	4.00	*	-	2.0	-	-	-	8.0
A12	4.00	-	*	2.0	-	-	-	8.0
A13	4.00	*	-	4.0	-	-	-	4.0
A14	4.00	-	*	4.0	-	-	-	4.0
A15	4.00	*	-	4.0	-	-	-	8.0
A16	4.00	-	*	4.0	-	-	-	8.0
A17	3.50	*	-	-	5.0	-	-	-
A18	3.50	-	*	-	5.0	-	-	-
A19	3.50	*	-	-	5.0	-	-	-
A20	3.50	-	*	-	5.0	-	-	-
A21	3.30	*	-	-	10.0	-	-	-
A22	3.30	-	*	-	10.0	-	-	-
A23	3.30	*	-	-	10.0	-	-	-
A24	3.30	-	*	-	10.0	-	-	-

All ingredients were food grade and were allowed by legislation in dairy manufacturing as additive or as adjuvant. Storage of the packages took place at 8 ± 1 °C (average conditions of commercial distribution) for 3 weeks (3 replicates). Samples were taken at 0, 7, 14 and 21 days storage and subjected to the following determinations: pH, moisture (oven drying method), NaCl (Volhard method), organic acids (ZEPPA *et al.*, 2001), counts of spoilage microorganisms (total viable, Enterobacteriaceae, *Pseudomonas* spp., yeasts and molds) as reported in a previous paper (FACCIA, 2019), and Qualitative Descriptive Sensory Analysis (TRANI *et al.*, 2016). All analytical data were processed using Statistica 7.1 for Windows program. Least significant different analysis was used to determine differences between control and experimental samples. For the sensory analysis, the means of the scores were calculated.

3. RESULTS AND DISCUSSION

Both the brine and product compositions changed over time, because of mass transfer phenomenon. In most cases, the brine caused sloughing of the cheese skin and fast decrease of the sensory characteristics. This effect was much more pronounced when citric acid was present, due to the strong calcium sequestering property. Maintaining an intact skin is of pivotal importance for shelf life of mozzarella, not only under the sensory point of view (appearance of the product), but also for limiting the mass transfer that causes enrichment of the brine in nutrients. The most suitable brine for shelf life extension proved to be that containing the pair calcium lactate/lactic acid, at pH 3.50. At the end of the storage period, the samples kept in such brine had lower pH (5.21 versus 5.98), and better microbiological and sensory characteristics than the control. In particular, the growth of total viable, *Pseudomonas* spp. and Enterobacteriaceae was significantly delayed, whereas yeasts and molds were not affected (Fig. 1).

Very interestingly, the samples kept in the experimental brine did not undergo to chromatic alteration, differently from the control sample that evidenced fluorescence of the liquid after 2 weeks (Fig. 2) and, in some cases, blue discoloration at the end of the storage period (not shown). The antimicrobial effect was confirmed by the pattern of the organic acids: citric acid was not degraded, and both lactic and acetic acid remained almost constant with time. The reason of the antimicrobial activity observed should be attributed both to the low pH and to the effect of lactate anion (SHELEF, 1994). We had already found a weak antimicrobial effect of calcium lactate in a previous work but, in that study, the brine had not been acidified (FACCIA *et al*., 2013).

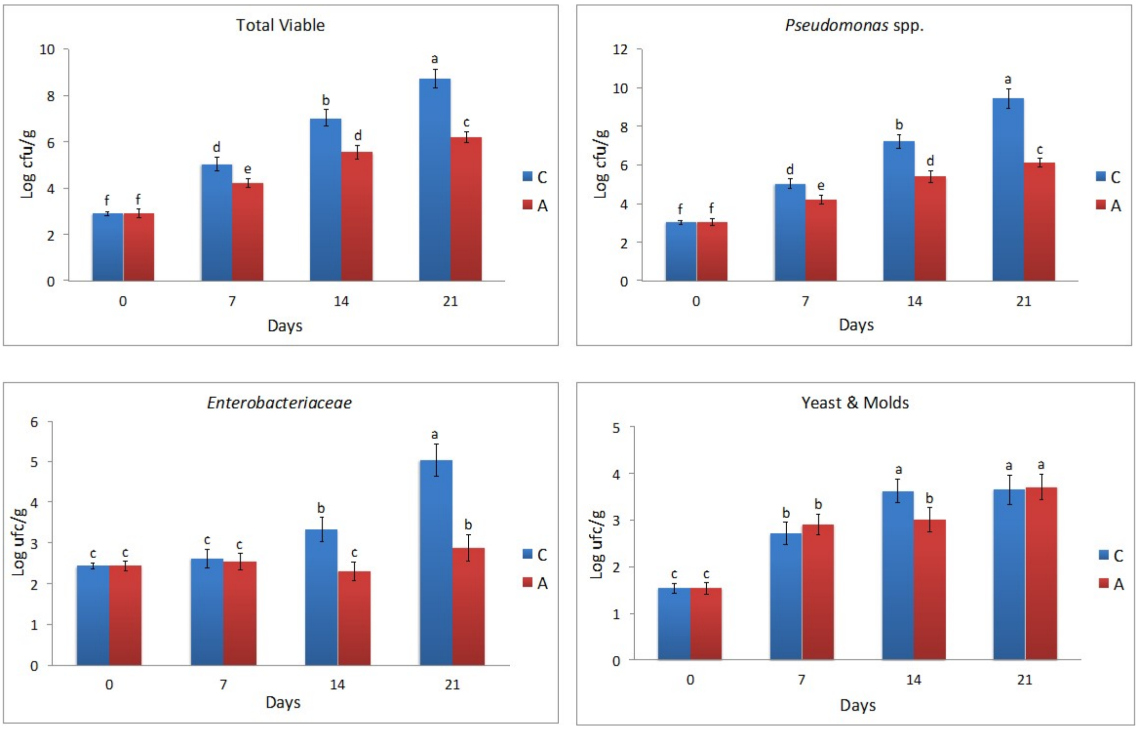

Figure 1. Average counts of the spoilage microorganisms during mozzarella storage.
A= experimental brine; C= control (in water).

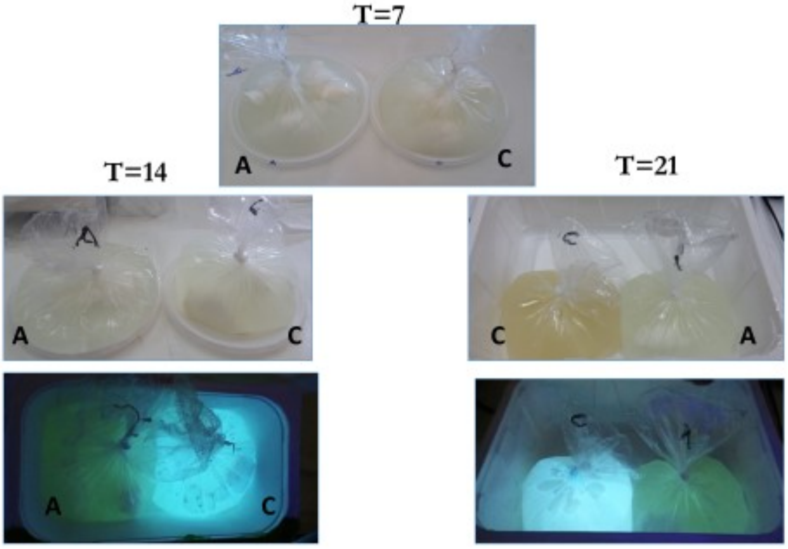

Figure 2. Occurrence of fluorescence in the preserving liquid of mozzarella during storage. A= calcium lactate brine; C= control (in water)

The experimental brine did not influence the sensory characteristics of the product (Fig. 3), which were preserved for long time. Moreover, many texture and taste parameters received better scores than the control: it is likely to be connected to the formation of a thin but elastic skin on the cheese surface, which exerted a sort of "barrier effect" against the pseudo-osmotic exchanges.

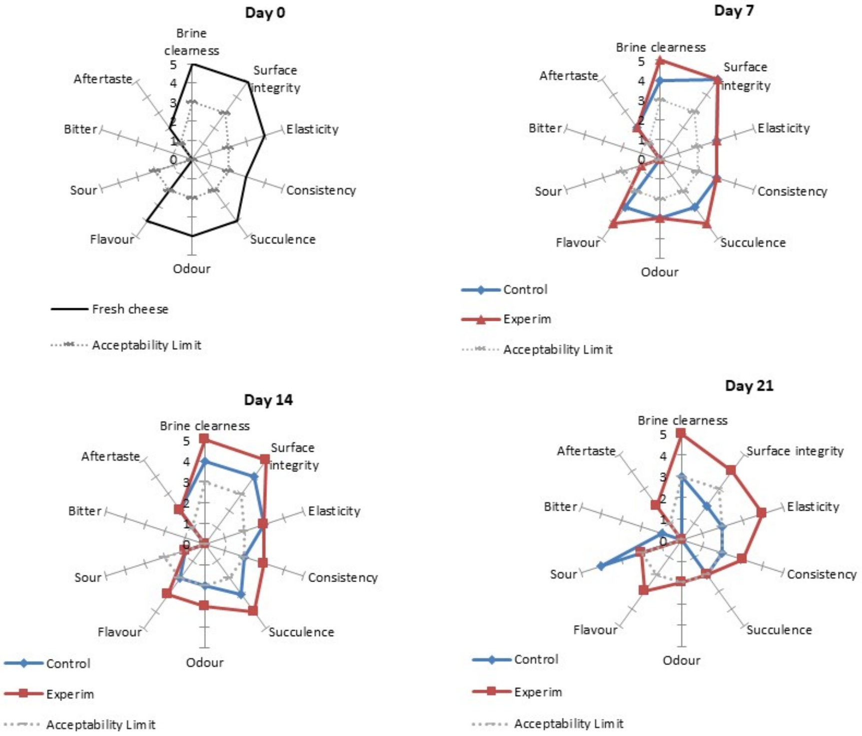

Figure 3. Quantitative Descriptive Analysis of mozzarella samples stored in water (Control) or Experimental brine.

The higher concentration of NaCl in the experimental sample at 21 days (3.2 $g.kg^{-1}$) with respect to control (1.4 $g.kg^{-1}$) gave an evidence of the reduction of such exchanges. The results obtained were slightly better than those reported in a previous paper (FACCIA *et al*., 2019), of which the present experimentation represent the continuation.

4. CONCLUSIONS

The use of calcium lactate/lactic acid brine at pH 3.50 as preserving liquid allowed delaying the growth of spoilage microorganisms in bovine mozzarella and to counter the occurrence of discoloration without impairing the sensory characteristics. The brine has low economic impact, is easy to prepare and is fully compatible with the EU legislation

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ALTERNATIVE PRE-TREATMENT FOR ORGANIC TRIMMED AROMATIC COCONUT

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ABSTRACT

The objective of this research was to study effect of alternative pre-treatments on quality of organic trimmed aromatic coconut. The fruits were dipped in acidified sodium chlorite (ASC; 0.1% sodium chlorite and 0.6% citric acid) followed by combination of 5% NaCl and 10% citric acid and vice versa, in comparison with 3% sodium metabisulfite and control (no dipping). Visual quality and surface color and color change were evaluated during storage at 2 °C for 8 weeks. The results showed that pre-treatment with a combination of 5% NaCl and 10% citric acid could extend shelf life of organic trimmed aromatic coconut up to 7 weeks by maintaining surface color and visual quality. Other pre-treatments including ASC, ASC followed by NaCl/citric acid, and NaCl/citric acid followed by ASC resulted in yellowness immediately after dipping and could only extend shelf-life up to 6 weeks.

Keywords: organic trimmed aromatic coconut, acidified sodium chlorite, citric acid, NaCl, shelf-life

1. INTRODUCTION

Aromatic coconut is one of the most well-known fruit from Thailand. After trimming, browning usually develop on fruit surface. Quality of trimmed coconut could be maintained by 1-3% of sodium metabisulfite (SMS) or dipping in chlorine solution. However, sulfite agents and chorine were reported to cause allergy to human (POINTING, 1960). For organic produce, FDA has allowed the application of acidified sodium chlorite (ASC) (FOOD AND DRUG ADMINISTRATION, 1986). Citric acid and NaCl were safe and widely used additives, which were reported to help prolonging quality of fresh produce (PARK *et al.*, 2011). The objective of this study was to study the effect of alternative pre-treatments (citric acid, NaCl and ASC) on quality of organic trimmed aromatic coconut.

2. MATERIALS AND METHODS

2.1. Raw material

Seven months old organic aromatic coconuts from Samutsakorn, Thailand, were used in this study

2.2. Pre-treatment for organic trimmed aromatic coconut

ASC (0.1% sodium chlorite and 0.6% citric acid) and combination of 5% citric acid and 5% NaCl, were used as pre-treatment in this study. After trimming, coconuts were immediately dipped each pre-treatment for 10 min (ASC or NaCl/citric acid or ASC followed by NaCl/citric acid or NaCl/citric acid followed by ASC) at room temperature. 3% sodium metabisulfite (SMS) and control (no dipping) were used for comparison. Visual quality and surface color values (L*, a*, b*, browning index (BI), and color change (ΔE)) were evaluated during storage at 2 °C for 8 weeks.

2.3. Color analysis

Surface color (L*, a* and b* values) at 15 different readings position of trimmed coconut was directly measured using a colorimeter (Ultra Scan PRO Hunter Lab spectrophotometer, USA). Color difference (ΔE*) and browning index (BI) were calculated according to equation 1 and 2, respectively (HUNTER and HAROLD, 1987).

$$BI = \frac{x-0.31}{0.172} \times 100 \qquad (1) \qquad \text{When } x = (a - 1.75L)(5.645L + a - 3.012b)$$

$$\Delta E = \sqrt{\Delta a^2 + \Delta b^2 + \Delta L^2} \qquad (2)$$

2.4. Visual quality evaluation

Visual quality was evaluated using method modified from CABEZARD-SERRANO *et al.* (2009). The evauation was conducted by a group of 3 judges, using a photographic reference scale with brief descriptions. The quality rating scale was 5 = excellent (white color and free of decay), 4 = good (10% browning and free of decay), 3 = fair (limit of marketability, 25% browning and free of decay), 2 = poor (10% browning and less than or equal to 25% decay) and 1 = very bad (more than 25% browning and more than 25% decay).

2.5. Statistical analysis

The experimental design was a complete randomized design. The data were analyzed by SPSS version 16 software (IBM Corp., USA). Differences among means were analyzed by two-way analysis of variance (ANOVA) and mean comparison was evaluated using Duncan's News Multiple Range Test. Differences between means at 5% ($p<0.05$) level was considered significantly different.

3. RESULTS AND DISCUSSIONS

3.1. Influence of treatments on visual quality of trimmed organic aromatic coconut

The results showed that beside SMS, combination of 5% NaCl and 10% citric acid was the only pre-treatment that could maintain visual quality score of the fruits above marketability limit up to 7 weeks while other pre-treatments including ASC, ASC followed by NaCl/citric acid, and NaCl/citric acid followed by ASC could maintain visual quality score up to 6 weeks (Fig. 1). SMS acted as bleaching and sanitizing, which caused damage to the samples (POINTING, 1960). In contrast, NaCl/citric acid maintained visual quality as well as controlling microbial growth without causing damage to coconut tissues. NaCl and citric acid were reported to have protective effect against enzymatic activities, which caused browning in tomato puree (PLAZA *et al.*, 2003). However, in further study, higher concentration of NaCl and citric acid may be applied in order to obtain desired shelf life at 8 weeks at 2°C.

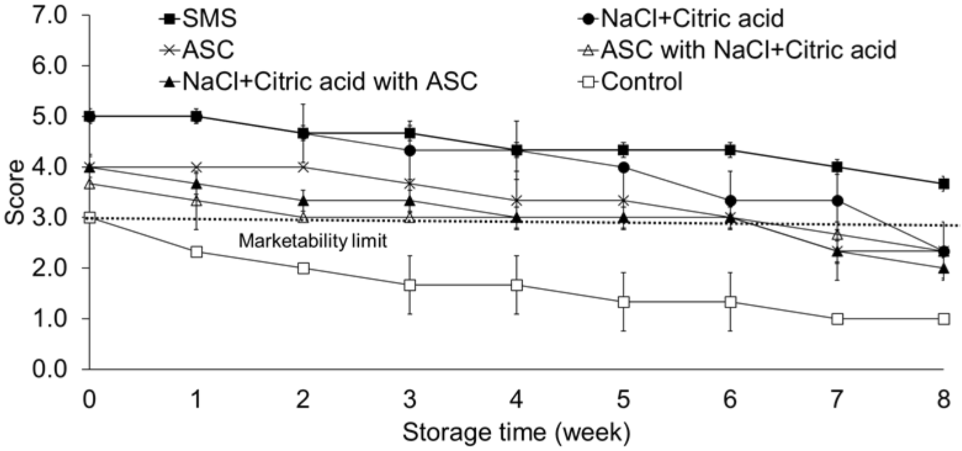

Figure 1. Visual quality of organic trimmed coconut dipped in different chemical treatments during storage at 2°C.

3.2. Influence of treatments on color of organic trimmed aromatic coconut surface during storage

During 8 weeks of storage at 2°C, coconut treated with NaCl/citric acid has slightly lower L*value when compared to SMS (Fig. 2A). The results also showed that beside SMS, NaCl/citric acid could control BI values of coconut throughout the storage time ($p \geq 0.05$), while BI of other treatments increased after 6 weeks (Fig. 2B). Treatment with NaCl/citric acid samples also had lowest ΔE when compared to other treatments and this color change was quite stable during 8 weeks of storage at 2°C ($p \geq 0.05$) (Fig. 2C). In contrast, ΔE of other treatments increased significantly until the end of storage ($p<0.05$). SMS is bleaching agent, which cause color lost on samples surface (POINTING, 1960). The anion chloride contributed to the inhibition effect of NaCl in the combination with citric acid (JANOVITZ-KLAPP *et al.*, 1990). Citric acid did not only inhibit enzyme nonspecifically due to pH lowering, but also the act as chelating agents which modified enzymes structure (REICHEL *et al.*, 2017).

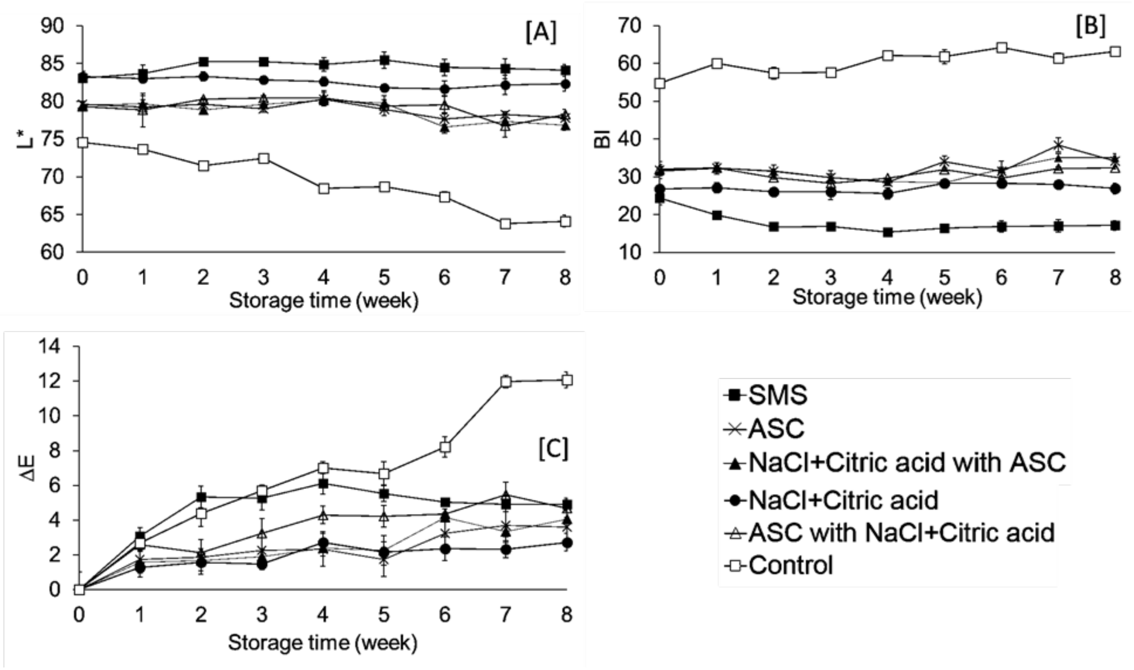

Figure 2. Lightness (L*) values (A), Browning index (BI) values (B) and Color change (ΔE) values (C) of organic trimmed aromatic coconut during storage at 2°C.

4. CONCLUSIONS

This study showed that combination of 5% NaCl and 10% citric acid could be used as an alternative pre-treatment for organic trimmed aromatic coconut. However higher concentration may be applied in order to maintaining on quality of throughout coconut export supply chain.

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EFFECT OF PACKAGING ON QUALITY OF FRESH-CUT KIWI

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ABSTRACT

This study determined the effect of PP1 packing film permeability of fresh-cut kiwifruit. Kiwifruit packed in an atmosphere of 17% O_2 and 3.6% CO_2 reached equilibrium and gave the most acceptable quality after 2 days of storage at 5°C. Longest shelf life was 8 days with lowest ethanol concentration and weight loss, highest firmness and total soluble solids. Concentration of ethanol was determined as a limiting factor for fresh-cut kiwifruit. Microorganisms grew more rapidly during storage but did not exceed the shelf life microbiological limit for fresh-cut fruit product in all treatments.

Keywords: fresh-cut, kiwifruit, permeability, modified atmosphere packaging, shelf life

1. INTRODUCTION

Demand for various fresh-cut fruits is increasing; however, their shelf life is limited by the cutting process, packaging, and storage temperature. Anaerobic respiration enhances microbial spoilage and softening is caused by increased enzymatic activities. Packaging is one of the most important factors to prolong the shelf life of fresh-cut fruits. Shrink film is commonly used for fresh-cut produce in Thailand to decrease O_2 concentration but this does not stop the accumulation of CO_2 inside the package. One technology to reduced respiration and deterioration, and extend shelf life of fresh-cut fruits is modified atmosphere packaging (MAP). The effectiveness of MAP has been reported by many previous studies. Kiwifruit is one of the most popular Thai imported fruits. It is high in vitamin C and other nutrients and can be sold as the whole fruit or fresh-cut. Kiwifruit production of at least 70% comes from a single cultivar, *Actinidia deliciosa* 'Hayward' ("green" kiwifruit). A new commonly traded yellow-fleshed kiwifruit is *A. chinensis* 'Zesy002' (Gold3), sold as Zespri® SunGold Kiwifruit. Kiwifruits have high market demand; however, the phrase "eat within 1 day" on the label often impacts purchase response. The objectives of this study were to determine the effect of film permeability in modified packaging atmospheres with a view to extending the shelf life of fresh-cut SunGold Kiwifruit under storage at 5°C.

2. MATERIALS AND METHODS

Fruit sample "Gold3" or 'Zesy002' a popular variety of Kiwifruit (*Actinidia chinensis*), sold as Zespri® SunGold Kiwifruit, was harvested from commercial orchards in New Zealand at optimal maturity.

Experimental methods Kiwifruit was washed with NaOCl solution (150 ppm) for 2 min and then drained. The fruit was peeled and cut into cubes with a sharp stainless steel knife. One hundred and thirty grams of fresh-cut kiwifruit were packed in polypropylene (PP) cups and sealed with various highly permeable films (PP, PP-1 and PP-2) as shown in Table 1. Fresh-cut kiwifruit was evaluated during storage for respiration rate, gas composition, weight loss, firmness, total soluble solids (TSS), titratable acidity (TA), ethanol concentration and microbiology. Headspace gas samples were taken using a gas-tight syringe inserted through an adhesive septum fixed on the film and analyzed by gas chromatography (GC-TCD) (Hewlett-Packard, USA). Firmness was evaluated using a texture analyzer (Testometric Micro 350, UK) by measuring the force value obtained during the test using a stainless steel probe with 4.77 mm diameter to penetrate the fruit 4 mm at 1 mm/s. Total soluble solid contents of fresh-cut fruit were measured using a hand refractometer. Titratable acidity was determined by titration of fruit juice with 0.1 N NaOH to pH 8.1, using phenolphthalein as the endpoint indicator. Ethanol concentrations were determined by immersing frozen fruit juice in a water bath (50 °C) for 20 min. Headspace gas samples were taken and analyzed using a gas chromatography-flame ionization detector, GC-FID) (column DB-WAX 30 m × 0.25 mm, injection temperature 100 °C; oven temperature 50 °C; detector temperature 150 °C). Fresh-cut kiwifruit was assayed for bacteria, yeasts and mold counts according to the methods described by TOURNAS et al. (2001). All experiments were performed in triplicate. The marketable shelf life of fresh-cut fruit was determined according to ethanol concentration and microbiology.

Table 1. Thickness, oxygen, carbon dioxide and water transmission rates of films.

Film	Thickness (μm)	$O_2TR^{1/}$ ($cc.m^{-2}.day^{-1}$)	$CO_2TR^{2/}$ ($cc.m^{-2}.day^{-1}$)	$WVTR^{3/}$ ($g.m^{-2}.day^{-1}$)
PP	30.0±0.0	1,417±390	3,709±233	35.4±1.00
PP-1	30.0±0.0	24,573±2532	25,500±2,121	-
PP-2	30.0±0.0	>100,000	>100,000	-

Means±Standard Deviations.

$^{1/,2/,3/}$ at 23°± 2°C.

3. RESULTS AND CONCLUSIONS

The respiration rate of fresh-cut kiwifruit was measured in a closed system stored at 5°C. Average respiration rate was 28 mgCO_2/kg/h, classified as high-class along with avocado, strawberry, blackberry and raspberry (KADER, 2002). Respiration rates of fresh-cut fruits rapidly increase after storage for 12 hours and reach equilibrium at 24 hours. Damage of kiwifruit tissues through peeling, cutting and trimming results in higher respiration rates that cause quality changes compared to the whole fruits. These changes can be reduced by proper harvesting, processing, packaging, distribution, and storage (CHONHENCHOB *et al*., 2017). Gas compositions inside packaged fresh-cut kiwifruit are shown in Fig. 1. These results showed the relation between fresh produce respiration and gas exchange through the packaging. PP-1 and PP packaged were reach equilibrium in 2 and 3 days. In-package gas compositions of fresh-cut kiwifruit packed in PP-1 and PP were 3% CO_2, 17% O_2 and 11% CO_2, 5% O_2, respectively. KITSIOU and SFAKIOTAKIS (2003) reported that CO_2 concentrations higher than 7% can be damaging to kiwifruit. Therefore gas compositions in PP-1 are optimal conditions for fresh-cut kiwifruit.

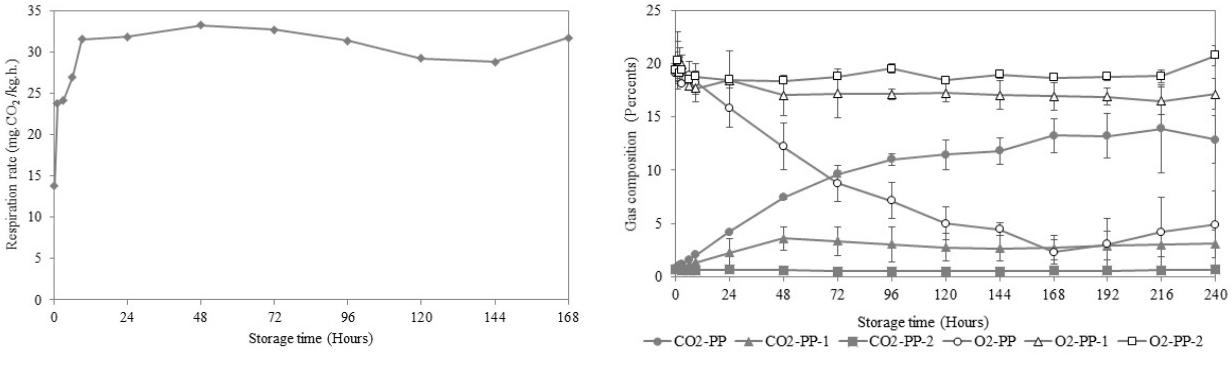

Figure 1. Respiration rate and gas composition (%CO_2, %O_2) inside packages of fresh-cut kiwifruit cv. Gold3 stored at 5°C.

Changes in weight loss and firmness of fresh-cut kiwifruit during storage are shown in Fig. 2. Fresh-cut kiwifruit packed in PP showed the highest weight loss. Fruit texture or firmness is indicative of the ripeness and also used to determine the suitability of fruit for critical steps in postharvest performance. Firmness of fresh-cut fruit obviously decreased after 2 days of storage. Fresh-cut kiwifruit packed in PP-1 showed significantly highest firmness than the other packaging films after storage for 10 days. This result demonstrated the important influence of polymeric film on the delay of flesh softening.

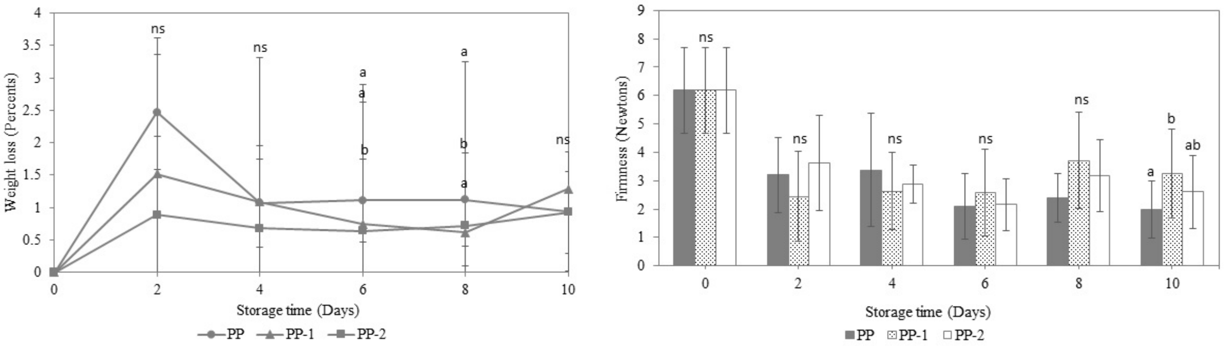

Figure 2. Weight loss and firmness of fresh-cut kiwifruit cv. Gold3 stored at 5°C.

Total soluble solids (TSS) and pH of fresh-cut kiwifruit stored at 5°C were not significantly different during storage (Fig. 3). The initial selection of harvest index was 6.2% TSS for New Zealand-grown "Gold3" and other cultivars for storage and export. Initial increase in sugar possibly affected the ripening process. In addition, TSS values here concurred with BURDON *et al*. (2016) who reported that maximum TSS occurred when the fruit was almost fully ripe. Titratable acidity of fresh-cut kiwifruit decreased with time in all treatments but PP-1 packaging showed the highest value, which significantly changed after 10 days of storage. Ethanol concentration of fresh-cut kiwifruit packed in PP film increased during 10 days of storage at 5°C. Higher ethanol concentration was found in fresh-cut kiwifruit stored under low O_2 composition in continuous film (PP) compared to fruit stored in microperforated films (PP-1 and PP-2). Inside the PP film packaging, gas transmission rates did not match well with the respiration rate of fresh-cut kiwifruit. Moreover, accumulations of CO_2 and O_2 inside these packagings were higher than in other treatments and caused anaerobic respiration. AGAR *et al*. (1999) reported that major fermentative metabolism products in fruits are ethanol and acetaldehyde, and their accumulation correlated well with off-flavor development.

Minimally processed fruit or fresh-cut fruit are generally impacted by bacteria and fungi, which can cause quality loss. Grey mold is caused by *Botrytis cinerea*, a common disease that causes loss in kiwifruit. Initial microbial counts on fresh-cut kiwifruit were 2.00 log cfu/g. After storage for 10 days, bacteria increased to 4.03 log cfu/g in PP-1, whereas yeasts and molds were 5.25 log cfu/g which did not exceed the microbiological limit of 6 log cfu/g (DEPARTMENT OF MEDICAL SCIENCES, 2017) (Fig. 4).

Sensory assessment of fresh-cut kiwifruit packed in various highly permeable films was evaluated by their appearance. Fresh-cut kiwifruit packed in PP-1 gave the best appearance and was not characterized by texture abnormalities until 8 days of storage, while fresh-cut kiwifruit packed in PP and PP-2 films had a dry texture with pale and brown colors in the middle of the core, respectively.

It can be concluded that PP-1 created optimal conditions inside the packaging and extended the shelf life of fresh-cut kiwifruit to 8 days.

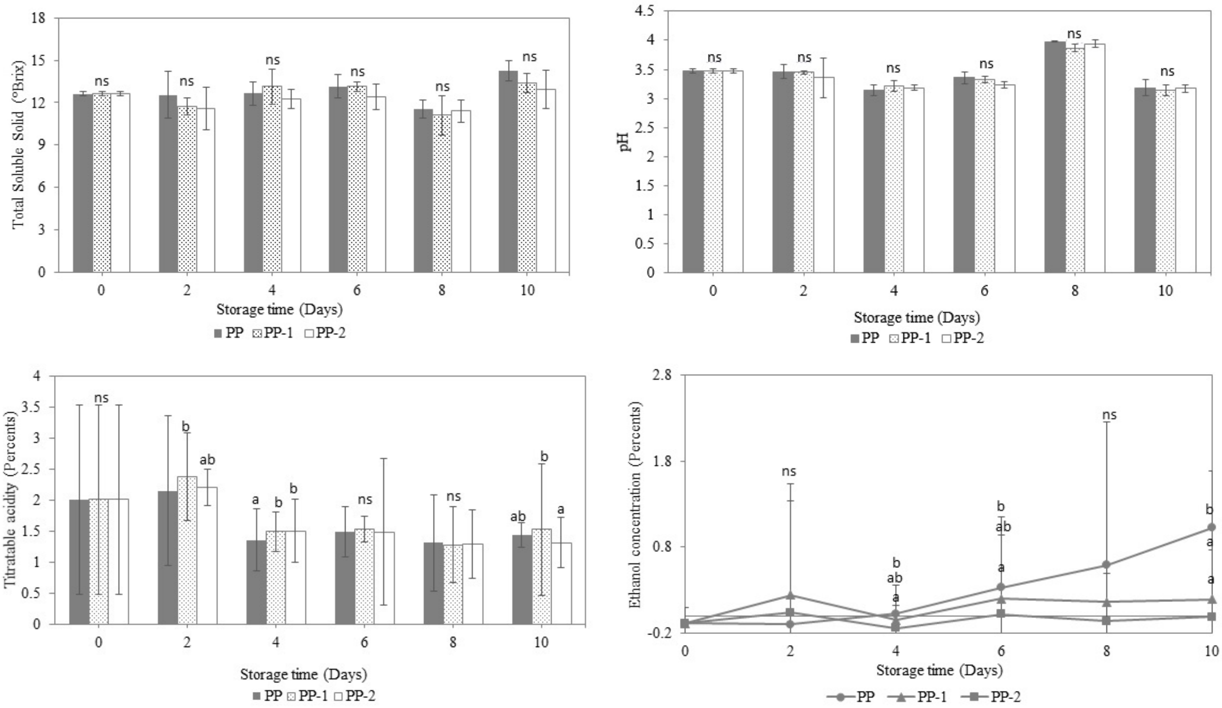

Figure 3. Total soluble solids, pH, titratable acidity and ethanol concentration of fresh-cut kiwifruit cv. Gold3 stored at 5°C.

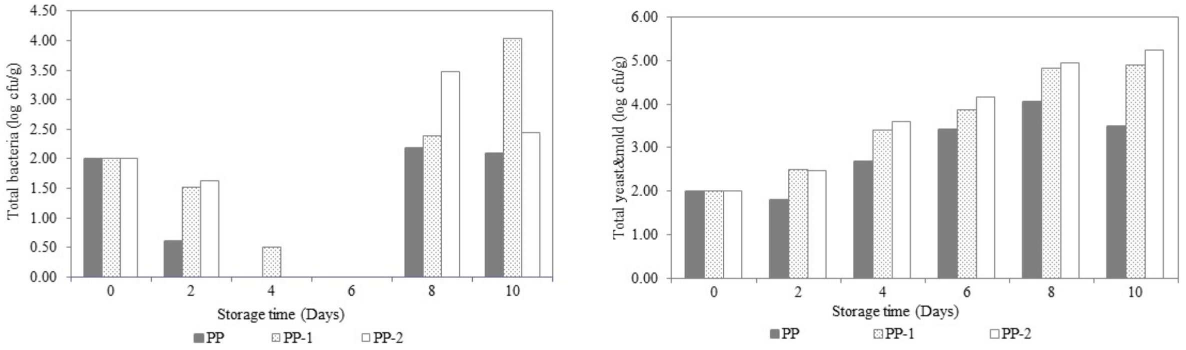

Figure 4. Total bacteria, yeast and mold of fresh-cut kiwifruit cv. Gold3 stored at 5°C.

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POTENTIAL OF FORWARD OSMOSIS ON CONCENTRATED LIME JUICE

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ABSTRACT

Lime juice is very sensitive to thermal processing, therefore Forward osmosis may be used for concentration of lime juice. Forward osmosis (FO) is membrane technology, consisting of feed solution, membrane and draw solution (osmotic agents such as salts). In this study, a nanofiltration membrane was used as semipermeable material in FO system. The concentration of feed and draw solution were measured in the term of total dissolved solid (TDS). The draw solutions were prepared by sodium chloride (NaCl) at 97 ppt and 194 ppt TDS and mixed salts at 97 ppt TDS. Lime juice (3.45 ppt TDS) was used as feed solution, in comparison with distilled water (0 ppt TDS) and citric acid (0.72 ppt TDS). Efficiency of FO determined by measuring water flux taking out from feed was monitored during 30 minutes of operating time. The results showed that increasing concentration of draw solution (NaCl) from 97 ppt TDS to 194 ppt TDS could increase water flux drown out from lime juice from 0.33 L/m^{2}h to 0.58 L/m^{2}h due to osmosis phenomenon. In this system, the highest water flux came out of lime juice (0.83 L/m^{2}h) was obtained at 10 min. The results also showed that at 97 ppt TDS, mixed salts could draw water out of lime juice better than NaCl.

Keywords: draw solution, forward osmosis, lime juice concentration, nanofiltration membrane, water flux

1. INTRODUCTION

Forward osmosis (FO) is a potential membrane process to concentrate juice at ambient temperature and pressure without significant product deterioration. FO, also known as the direct osmosis phenomenon of water permeate across a selectively permeable membrane from high chemical potential region (draw solution) to low chemical potential region (feed solution) under osmotic pressure driving force (GARCIA-CASTELLO *et al*., 2009). FO has several advantages compared to conventional food industrialization techniques, such as: low pressure for operation which reduces the cost for electrical energy, low temperature operation which avoids the thermal degradation of food quality factors and conserves energy and high product recovery and low discharge of brine to the environment (VOLTAIRE *et al*., 2012). To solve lime shortage removal of water content is used to extend shelf-life of lime juice. Lime juice concentration process such as vacuum evaporation is carried out under high temperatures, condition caused significant flavor aromatic and nutritional change of lime juice these characteristics are conferred by volatile components and vitamins C, which are thermosensitive compounds therefore FO may be used for concentration of lime juice (JESUS *et al*., 2007). The objective of this research is to investigate of possibility of FO application on concentration of lime juice by study the effect of concentration and type of draw solution on efficiency of FO for lime juice concentration in lab scale. Efficiency of FO was determined by water flux that exhibit the flow rate of permeate per membrane surface area.

2. MATERIAL AND METHODS

2.1. Raw Material

Lime (*Citrus aurantifolia*) juice was obtained, mixed and stored in 1 L plastic container at -18 °C until use. Lime juice (3.45 ppt TDS), distilled water and 0.71 ppt TDS citric acid solution were use as feed solution. The draw solution was NaCl of 97 ppt and 194 ppt TDS and mixed salts of 97 ppt TDS.

2.2. Forward osmosis module

The forward osmosis experiments were carried out with a polyamide thin-film composite membrane (98% NaCl rejection). Experiments were performed using a 2 sides membrane with 90 cm^2 surface area FO as shown in Fig. 1. NaCl and mixed salts 20 ml as draw solution were injected in the FO module via syringe. Distilled water, citric acid and lime juice 1000 mL as feed solution were put in 100 mL beaker where the FO module had been set up inside.

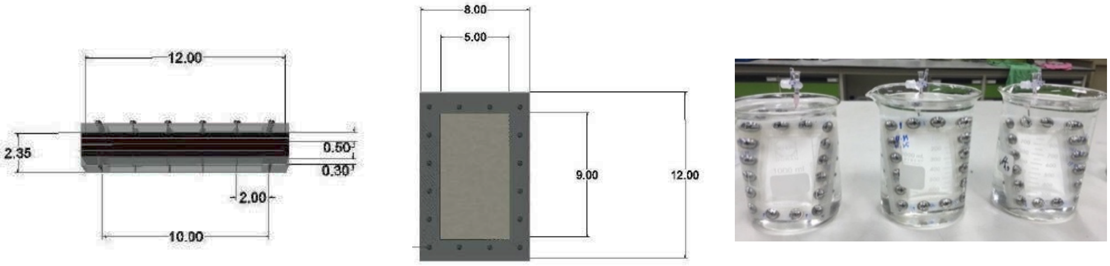

Figure 1. Forward osmosis module in experiments (unit; centimeter).

2.3. Forward osmosis experiments

Forward osmosis experiments were carried out in two experiments. First, effect of draw solution concentration on FO of lime juice 97 ppt and 194 ppt NaCl draw solution study. Second, effect of draw solution type of on FO of lime juice with NaCl solution and mixes salts solution at 97 ppt TDS draw solution. The experiments monitoring were performed at 10, 15, 20, and 30 minutes water flux evaluation and concentrated pH, Total dissolved solids, Salinity, Conductivity and °Brix in the concentrated product. The average temperature was maintained around 25°C.

2.4. Water flux

Water flux (CHETAN *et al.*, 2011) was calculated according to the following equation:

$$J = \frac{V_2 - V_1}{A \times t} \qquad (1)$$

in which J is water flux. V_1 and V_2 are the draw solution volume at initial and monitoring times, respectively. A is the membrane surface area and t is the osmosis times (operating time).

2.5. Differences of total dissolved solids

Differences of total dissolved solids was calculated according to the following equation:

$$\Delta TDS_{D,F} = TDS_D - TDS_F \qquad (2)$$

in which $\Delta TDS_{D,F}$ is the differences of total dissolved solids between draw solution and feed solution. TDS_D and TDS_F are total dissolved solids of draw solution and feed solution, respectively (CHETAN *et al.*, 2010).

2.6. pH, Total dissolved solids, salinity and conductivity analysis

The pH, TDS, salts and conductivity of the sample were measured by pH meter; AMT03 AMTEST, Amtrast USA Inc., UAS.

2.7. Brix analysis

The °Brix of draw and feed solution were measured by Brix refractometer; PAL1, ATAGO, Saitama, Japan.

3. RESULTS AND DISCUSSION

The result of the experiment 1 (Fig. 2) found that 194 ppt NaCl got water flux higher than 97 ppt NaCl from all experimental tests; such as, operating time at 10 minutes with lime juice as feed solution found that 97 ppt and 194 ppt NaCl obtained $\Delta TDS_{D,F}$ were 93 and 190 ppt respectively and water flux were 333.33 mL/m^{2}h and 566.67 mL/m^2 respectively, due to at concurrent time, 194 ppt NaCl got $\Delta TDS_{D,F}$ higher than 97 ppt NaCl. The results showed that water flux was increased by the higher concentration of draw solution that reason to decreased volume of feed solution and increased concentration of feed solution because of driving force from osmosis phenomenon. More concentration of draw solution resulted the higher efficiency of FO system because increasing of concentration of draw solution ($\Delta TDS_{D,F}$ increased) that resulting the difference of osmosis pressure on the system increased Water flux depend on $\Delta TDS_{D,F}$ at ambient temperature. (ROY *et al*., 2016)

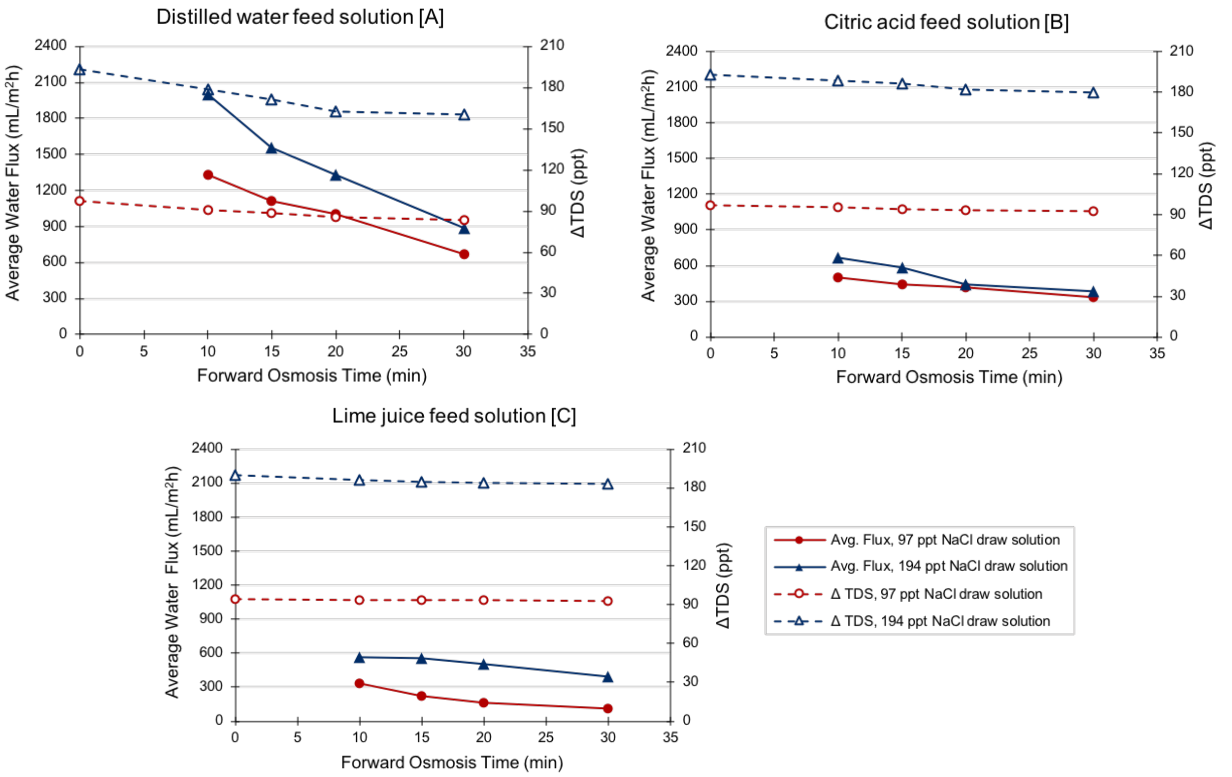

Figure 2. Effect of draw solution in different concentration (97 ppt and 194 ppt NaCl) at 10, 15 20 and 30 minutes; Average water flux (solid line) and $\Delta TDS_{D,F}$ (dashed line). Feed solution were distilled water [A], Citric acid [B] and lime juice in lab scale.

3.2. Effect of type of draw solution of forward osmosis for lime juice concentration

Even though 194 ppt NaCl was able to draw water out form lime juice greater than 97 ppt NaCl but the experiment 2, we selected to use 97 ppt NaCl due to the draw solution. Mixed salt type got the saturated solution at 97 ppt NaCl at ambient temperature. Fig. 3 showed effect of type of draw solution on forward osmosis. The result found that mixed salts showed °Brix of lime juice and water flux higher than NaCl from all experimental tests; such as, we used lime juice as feed solution at 10 minutes and found that 97 ppt mixed salts and 97 ppt NaCl were obtained by water flux 833.33 mL/m^2h and 566.67 mL/m^2h respectively. Due to the mixed salts are consisted by molecule of various substance which those types represented individual and different osmosis pressure whenever they were mixed by the solution that caused such solution obtained higher osmosis pressure than the single solution at the same concentration (YANG *et al*., 2009).

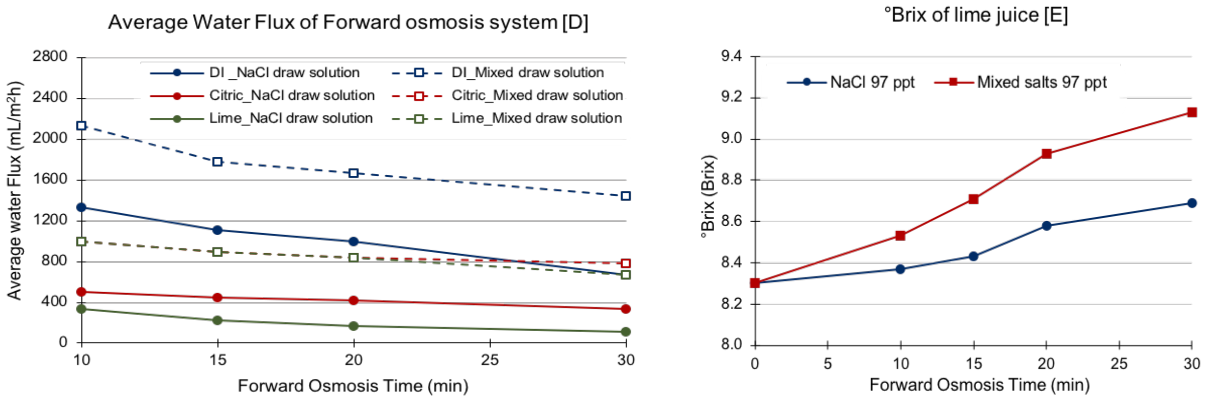

Figure 3. Effect of different type of draw solution (97 ppt NaCl and 97 ppt Mixed salts) at 10, 15 20 and 30 minutes. Average water flux [D] and °Brix of lime juice [E] of FO module. Feed solution were distilled water, Citric acid and lime juice in lab scale.

4. CONCLUSIONS

Effect of draw solution on efficiency of forward osmosis (FO) for lime juice concentration found that the concentration and type of draw solution was influential to the efficiency of FO. More concentration of draw solution resulted the higher efficiency of FO system because difference of osmosis pressure ($\Delta TDS_{D,F}$) on the system increased. Mixed solution type (mixed salts), that resulted the higher efficiency of FO system than single solution (NaCl) with the identical concentration so the mixed salts was the most efficiently to draw water out from the lime. Lower operating time that the efficiency of the system was higher due to difference of osmosis pressure wasn’t a lot of change. Forward osmosis system of concentrated lime juice is the membrane technology with potential efficiency to efficiently increase the concentration of lime juice. However, continuous system should be considered for higher capacity and efficiency of forward osmosis system.

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CALCIUM IMPACT ON XANTHAN GEL AND FROZEN COCONUT QUALITY

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ABSTRACT

The objective of this investigation was to understand the impacts of xanthan matrices with various strength degrees of gels with/without calcium ions on the quality preservation of frozen coconut pulp. Xanthan without Ca^{2+} protected textural firmness, drip loss, and browning of the freeze-thaw coconut pulp. However, once the Ca^{2+} was present, the Ca^{2+}-xanthan matrices improved the firmness but increased the drip loss and browning development. These phenomena were likely related to the gel strength of the xanthan and Ca^{2+}-xanthan matrices. These findings suggested that the addition of ions has a strong impact on the structural arrangement, gel-like degree and cryoprotectability of hydrocolloids.

Keywords: xanthan, calcium, freeze, thaw, gel, coconut

1. INTRODUCTION

Ice crystallization and re-crystallization cause frozen fruit tissues to rupture and lose their capacity to hold cellular liquids and the fruit texture becomes soggy (Boonsupthip and Lee, 2003). There is also a color change (normally darker) due to enzymatic browning reactions and with coconut pulp the color alters from opaque white to dark yellow or pink (THAISAKORNPHAN *et al*., 2014).

The incorporation of hydro-dynamically active ingredients to protect fruits against the detrimental effects of freezing and thawing has been described by many researchers (MAITY *et al*., 2013). Hydrocolloids (e.g., xanthan, alginate, pectin, carboxyl methyl cellulose) are ones of the most used functional ingredients. They improve the rheological and textural characteristics of frozen food by enhancing viscosity, creating a gel structure, holding water, networking with divalent ions and reducing the glass transition temperature (DICKINSON 2003).

Xanthan gum is a unique hydrocolloid, having a substantial yield stress value at a low concentration, and resistance to heat, acid and freeze-thaw damage (PETRI, 2015). Xanthan is a large polysaccharide molecule with an average molecular weight in the range 1 × 106 to 20 × 106 mol/g, depending on the biosynthesis conditions and interchain association. Its backbone is cellobiose (two units of b,1-4-D-glucose) as a repeating unit. Every alternate glucose unit contains a tri-saccharide side chain of mannose–glucuronic acid–mannose. The mannose linking to the backbone carries an acetyl group, and the terminal mannose has a pyruvate group. The degrees of acetylation and pyruvylation depend on the fermentation conditions. At pH > 4.5, xanthan behaves as a polyanion due to the deprotonation of *O*-acetyl and pyruvyl residues. The structure of xanthan is controlled by temperature and ionic strength and xanthan builds up physical networks with bivalent cations. At calcium concentrations up to the stoichiometric value (e.g., Ca^{2+}:COO– ratio = 1:2), intermolecular cross-linking of Ca^{2+} ions between pairs of carboxyl groups (*O*-acetyl and pyruvyl residues) on separate xanthan helices promotes intermolecular association and strengthens the Ca^{2+}-xanthan "weak gel" networks (more gel-like state) (Mohammed *et al*., 2007). On the other hand, at higher calcium concentrations up to twice the stoichiometric equivalence between Ca^{2+} ions and the carboxyl groups of xanthan (e.g., Ca^{2+}:COO– ratio = 1:1), the intermolecular association is reduced by partial replacement by binding of Ca^{2+} ions to individual carboxyl groups. The degree of Ca^{2+}-xanthan intermolecular complexation and network formation is decreased; leading to a more liquid-like state.

It is common to apply Ca^{2+} ions as a pre-treatment step to fruits prior to freezing. Application of xanthan as a cryoprotectant would interact with the Ca^{2+} ions. The objective of this investigation was to evaluate the impact of xanthan networks in various gel-like states with different calcium concentrations on their ability to improve the freeze-thaw tolerance of frozen coconut pulp. The different concentrations of Ca^{2+} ions were applied in a range below the stoichiometric value to create a gel-like network. A higher concentration toward a liquid-like state is also of interest and should be further investigated. Different degrees of Ca^{2+}-xanthan intermolecular complexation in the gel-like structure were expected to have different molecular permeability and then different cryoprotectant ability.

2. MATERIALS AND METHODS

2.1. Sample preparation and pre-treatment

Young, aromatic coconut fruits grown in Rachaburi province, Thailand, were harvested at approximately 200-210 days after blooming. At this stage, the fruit had meat about 3-4 mm thick and water at 7-9°Brix. The green outer coat and coir of each fruit was peeled off, exposing the white hard shell. The fruit samples were stored at 10°C. At the time of use, the hard shell was broken with care and the coconut pulp was collected. Its testa (brownish purple layers) was peeled off to expose the white pulp. The pulp was sliced into 5 x 5 cm-pieces and stored in an ice bath prior to use (within 20 min).

Samples were dipped in a solution of calcium chloride ($CaCl_2$) and left for 10 min at room temperature. Then, they were dipped in a 1% xanthan gum solution, and left for 10 min prior to freezing. Individual slices of coconut were stored in a sealed plastic bag (6.5 x 7.6 cm), frozen and stored overnight at -20°C. A freeze-thaw cycle was performed by freezing at -20°C for 14 h, thawing at room temperature for 2 h, freezing at -20°C for 6 h and thawing at room temperature for 2 h. Ten freeze-thaw cycles were applied. Twenty samples were prepared for each treatment and measurement.

2.2. Texture measurement

Textural firmness was evaluated as the maximum resistance to penetration. Samples were evaluated using a texture analyzer (TA.XT plus, Stable Micro Systems Ltd., Godalming, UK) with a 49 N load cell. A slice of coconut was placed on a flat platform. A cylindrical Perspex probe (6 mm diameter, 35 mm length) was used to puncture through the coconut tissue from the outer endosperm toward the inner endosperm, penetrating to 80% of the total fruit width. The crosshead speed was set at 2 mm/s. The textural parameter measured on the resulting force- distance curves was recorded as the firmness or maximum resistance to penetration. The mean values of 10 replicates, expressed in g force, were reported.

2.3. Rheological measurement

Rheological measurements were carried out using a Physica MCR300 (Anton Paar GmbH, Stuttgart, Germany) with a cone-and-plate geometer (50 mm diameter, 1° cone angle, 0.05 mm gap) under controlled stress and temperature. The samples consisted of a mixture between 1 % xanthan gum and various concentrations of calcium lactate from 0.3 to 1 % at 40°C. The temperature was then controlled at 5°C with the measurement of consequent change at 0.5 % strain and 1 rad s-1. After vibration for 5 min at 5°C, the frequency dependence was measured at a fixed strain of 0.5 % and recorded at a fixed frequency of 1 rad s-1.

2.4. Drip loss measurement

Samples were weighed before freezing (m1), and after undergoing 10 freeze-thaw cycles and then laid over absorbent papers at room temperature until a constant weight was reached (m2). A Mettler AT 100 analytical (New ClassicMF. JS13203C. Mettler Toledo, Switzerland) balance with a precision of 0.00001 g was used. Measurements were made in triplicate per sample. The drip loss (DL) was calculated using equation (1):

$$DL = [(m1 - m2) / m1] \times 100 \qquad (1)$$

2.5. Color measurement

Color measurements (Hunter L*, a* and b* values) were performed on the surface of the samples using a Hunter Lab DP 9000 (Hunter Associates Laboratory Inc., Reston, VA). The browning index (BI) was calculated as follows:

$$BI = [100(x-0.31)]/0.17 \qquad (2)$$

$$x = (a^*+1.75L^*)/(5.645L^*+a^*-3.012b^*) \qquad (3)$$

2.6. Statistical analysis

The experiment was arranged using a completely randomized design with three replications. An analysis of variance (ANOVA, 95% significance level) was carried out using the statistical analytical software package SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). Differences between means were assessed using Duncan's multiple range test with differences considered significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Rheological property

Calcium ions had a strong impact on the storage modulus (G') of the xanthan solution (Fig. 1).
As the concentration increased, G' increased exponentially. On the other hand, the loss modulus (G'') only slightly increased and had lower values than G''. The tangent loss (d) was below 1 and was lower with increasing calcium concentration. These phenomena suggested that an increase in calcium ions provided a more gel-like xanthan network.

3.2. Textural firmness

Fresh young coconut pulp at this maturity level had a strong and firm texture (Fresh, 1612 ± 222 N) (Fig. 2a). However, without any treatment it did not withstand the severity of 10 freeze- thaw cycles. Its textural firmness (None) was extremely reduced compared to fresh coconut. Five freeze-thaw cycles are commonly used to represent mishandling of frozen food over storage period (BENJAKUL and BAUER, 2001). In the current study, we exaggerated the treatment as xanthan was expected to have a high level of cryoprotectibility. The results showed that coating coconut pulp with 1% xanthan (X) slightly enhanced the firmness. Xanthan is reported to reduce textural damage by ice crystals due to its ability to reduce water mobility (SAE-KANG and SUPHANTHARIKA, 2006.) and decrease the glass transition temperature (MAITY *et al.*, 2013). Incorporation of calcium chloride into xanthan significantly increased the tolerance of coconut pulp for freeze-thaw damage, especially when the concentration of calcium chloride was high. This was due to the fact that the Ca^{2+} ions interacted with xanthan forming a Ca^{2+}-xanthan complex leading to a stronger gel. This strong gel potentially protected coconut tissue against damage. The Ca^{2+}-xanthan association changed the solution rheology as illustrated in Fig. 1. The values of G‘‘ and G‘‘‘‘ increased with incremental calcium concentration. The increase in G‘‘ was at a higher rate than that of G‘‘‘‘; therefore, tan d was lower, meaning that the resultant product was more gel-like or less liquid-like. This gel-like state was presumably induced by the site-binding of Ca^{2+} ions between pairs of carboxyl groups on the separate helices of xanthan molecules. The binding promoted intermolecular association and strengthened the "weak gel" network (MOHAMMED *et al*., 2007). This indicated that a more gel-like Ca^{2+}-xanthan association was more powerful in textural firmness protection.

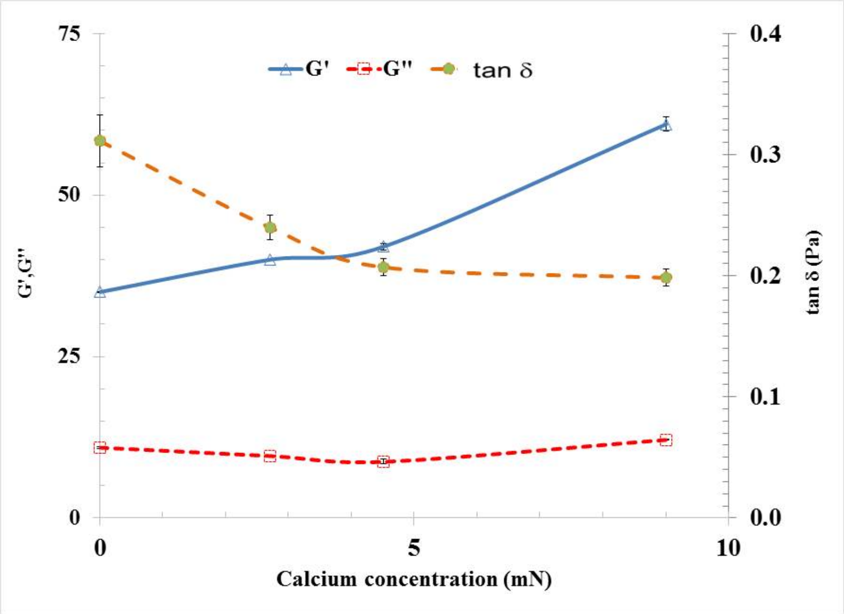

Figure 1. Variation of G‘‘, G‘‘‘‘ and tan d, measured at 1 rad/s, 0.5% strain and 5°C with increasing concentration of Ca^{2+} in 0.1 % xanthan solution).

3.3. Drip loss

Drip loss tended to increase with incremental calcium concentration (Fig. 2b). Xanthanwithout calcium ions slightly reduced the drip loss of coconut pulp. Unexpectedly, the presence of calcium ions inhibited the ability of xanthan. The inhibition was possibly due to the Ca^{2+}-xanthan association, which forms a rigid/firm gel network in a way that enhances the diffusion of small molecules through it. Without Ca^{2+} ions, the xanthan matrices were more extensively swollen (BAUMGARTNER *et al*., 2008). This swollen form retarded molecular diffusion. As a result, the coconut liquid leaking due to the freeze-thaw damage did not promptly pass through the swollen xanthan-coated film on the coconut pulp surface. On the other hand, the stiffer and less swollen Ca^{2+}-xanthan matrices enhanced liquid diffusion. Some hydrocolloids have shown a positive relationship between swelling degree and diffusion enhancement. For example, the presence of Ca^{2+} ions in higher concentration in a cellulose-ether solution was reported to form a less swollen gel, which allowed lower molecular diffusion (BAUMGARTNER *et al.*, 2006). This kind of gel system would likely provide better texture and drip loss protection.

3.4. Brown index

The freeze-thaw cycles caused browning to the frozen coconut pulp (None) to an extensive degree compared to the fresh coconut (Fig. 2c).

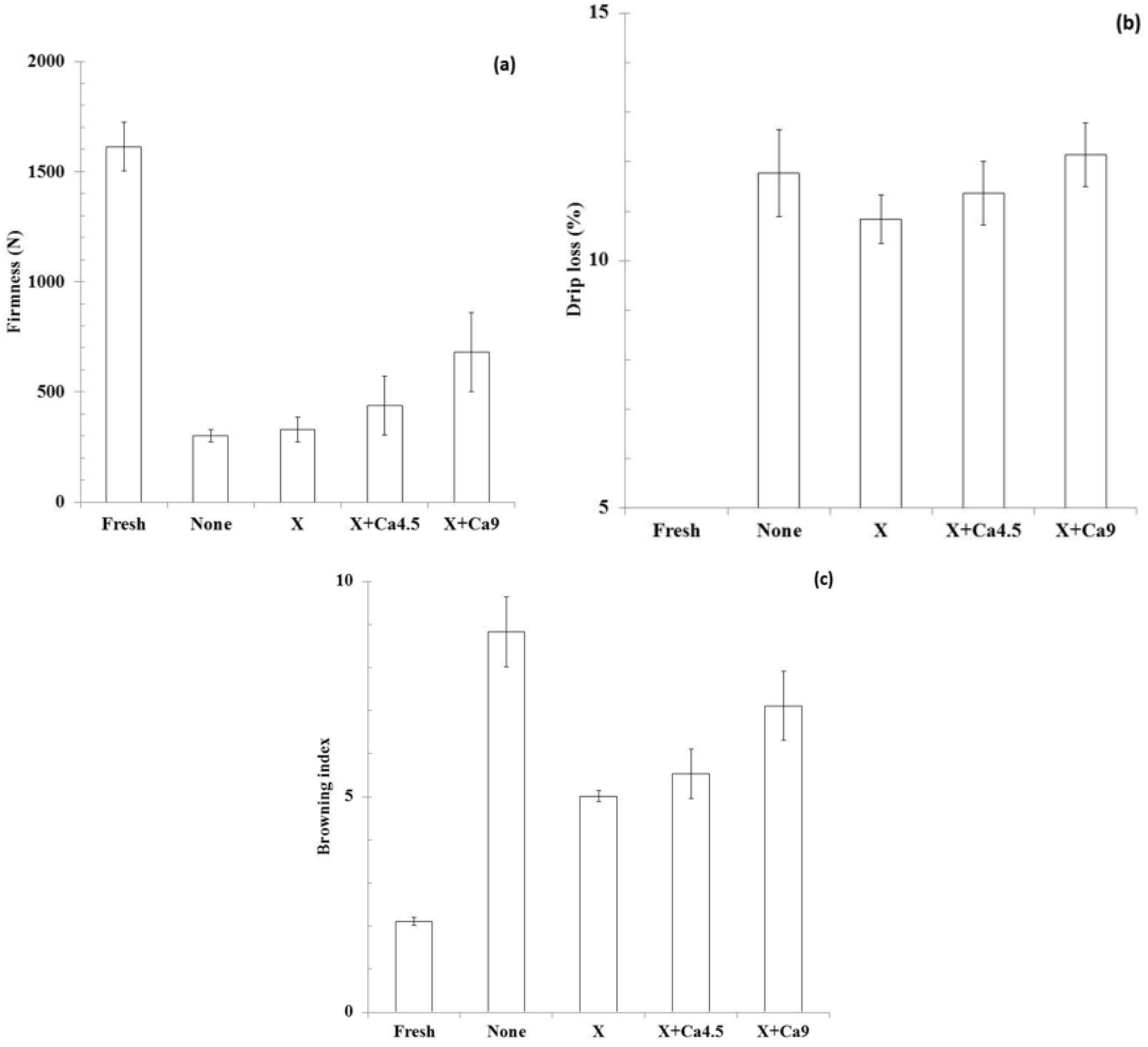

Figure 2. Effect of calcium concentration (0 (X), 4.5 (X+Ca4.5) and 9 (X+Ca9) mN) on cryoprotectability of xanthan network toward coconut pulp quality, (a) textural firmness, (b) drip loss and (c) browning index, compared to fresh (Fresh) and no added compound (None) coconut pulp samples.

When coated with xanthan, the coconut pulp underwent significantly lower browning. This effective browning protection was possibly due to diffusion retardation by the swollen structure of the xanthan making it more difficult for browning reaction substrates (oxygen, phenolic compounds, phenolase) to move closer to one another and interact. On the other hand, the rigid/firm network of the Ca^{2+}-xanthan matrices enhanced diffusion of the browning substrates, leading to rapid browning reactions. As a result, an increase in the calcium concentration resulted in higher browning development.

4. CONCLUSIONS

The calcium ion concentration played an important role in the gel strength and cryoprotectability of xanthan against damage to frozen fruits. Calcium-derivative compounds are commonly used in pre-treatments to frozen fruits prior to freezing. The application of a hydrocolloid as a cryoprotectant should be performed with this in mind. It is likely that Ca^{2+}-hydrocolloid matrices can protect the frozen texture. However, protection against drip loss and discoloring was uncertain. An increase in the calcium ion concentration in the xanthan solution could better protect the frozen texture while it had an adverse impact on drip loss and discoloring. This was likely related to the higher calcium ion concentration creating a more gel-like network in the Ca^{2+}-xanthan matrix (G“ > G”). The higher calcium concentration resulting in the Ca^{2+}-xanthan matrix having a more liquid-like state was also interesting and should be further investigated. Different degrees of Ca^{2+}-xanthan intermolecular complexation in the gel-like structure were expected to have different molecular permeability and hence different cryoprotectant ability.

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SESSION III

Safety Issues of Packaging Materials

MIGRATION TESTING OF LOW TEMPERATURE SURFACE HARDENED STAINLESS STEELS

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ABSTRACT

Austenitic stainless steels qualify for processing and packaging applications in the food and beverages industry due to their excellent corrosion resistance and hygienic properties. Nevertheless, poor tribological behavior has limited the use in applications where corrosion and wear resistance are required. Low temperature carburizing (LTC), named Kolsterising®, offers a solution to enhance the tribological properties by increasing the surface hardness, without altering the corrosion resistance. In order to verify the harmless use of LTC for stainless steel components, metal migration tests according to the guideline of the Council of Europe (CoE) have been carried out on surface hardened AISI 316L sheets. The tests confirm the safe use of LTC, since all measured migrated metal values are far below the Specific Release Limits (SRLs) stated in the guideline. In addition, dry-sliding ball-on-disc tests were carried out, which prove the enormous increase in wear resistance by more than a factor of 10.

Keywords: austenitic stainless steel, corrosion, food and beverage applications, migration testing, surface hardening

1. INTRODUCTION

Austenitic stainless steels are widely used in food and beverage applications due to their excellent corrosion resistance, non-toxicity and cleanability. These properties are based on the formation of a protective chromium-rich oxide layer on the surface, which remains chemically inert under different environments. Even if it is damaged, the passive film will heal itself rapidly in the presence of oxygen. Additionally, stainless steel surfaces can be disinfected and sterilized easily (PARTINGTION, 2006; TESSDA, 2009). **Error! Reference source not found.**Nevertheless, poor tribological behavior of non-hardened austenitic stainless steels, especially low wear resistance, which negatively impact cleanability, and a tendency to fretting, has limited the use of these materials in applications where both corrosion and wear resistance are required (COSSP, 1978).

Classic methods such as coatings or conventional heat treatment techniques can be used to improve the hardness and therefore the wear resistance. But applied coatings have the potential to detach from the base material, as the example of a flaked-off hard chrome coating in Fig. 1 shows (BODYCOTE, 2017). Contaminations by chipped-of material are a substantial risk in processing and packaging food and beverages (COSSP, 1978).

Figure 1. Piston with chipped coating; coating flakes can lead to contamination of food and beverage (BODYCOTE, 2017).

Although this is not the case for conventional heat treatments, which introduce carbon and/or nitrogen into the case structure of the base material by diffusion processes, the corrosion resistance of stainless steels is drastically reduced. This is caused by the formation of precipitations such as carbides or nitrides due to excessive, high process temperatures. The heat treated material is depleted of chromium and more susceptible to attack by corrosive media such as acidic foods (COSSP, 1978). A diffusion based process below the critical heat treatment temperature of 500°C, named Kolsterising®, hardens the surface of stainless steel and preserves corrosion resistance unlike conventional heat treatment techniques.

1.1. Surface hardening with LTC Kolsterising®

Low temperature carburizing (LTC) increases the surface hardness of stainless steels based on diffusion processes, as shown in Fig. 2. After temporarily removing the oxide layer, the diffusion of massive amounts of carbon (red dots) into the austenitic lattice (white dots represent the elements Fe, Cr, Ni or Mo) is enabled, leading to high compressive stresses against the surface (green arrow) and the base material (blue arrow) and therefore improves the surface hardness. The carbon atoms in the built-up diffusion zone (light gray area), also called S-phase or expanded austenite, are interstitially embedded into the metallic structure. Within this zone the carbon content and therefore the hardness has a gradual progress into the material, thereby maintaining the ductility so no embrittlement occurs. Due to the low process temperatures (<500°C), the formation of unwanted chromium carbides can be prevented. The corrosion properties remain unchanged. (GÜMPEL, 2012)

Depending on the material and the treatment, a surface hardness between 800 and 1300 $HV_{0.05}$ is possible. The diffusion depth can range from 10 to 40 µm. As a result, machine components in food and beverages industry processing equipment achieve longer lifetimes, especially due to lower wear rates and the elimination of stainless steel's tendency to galling. Furthermore, since the carbon-enrichment process does not apply a coating on the surface, the risk of delamination is eliminated. Compared to coatings, no changes in shape, size, color and surface roughness occurs, thereby no further treatment of the surface hardened parts is necessary (BODYCOTE, 2002 and 2014).

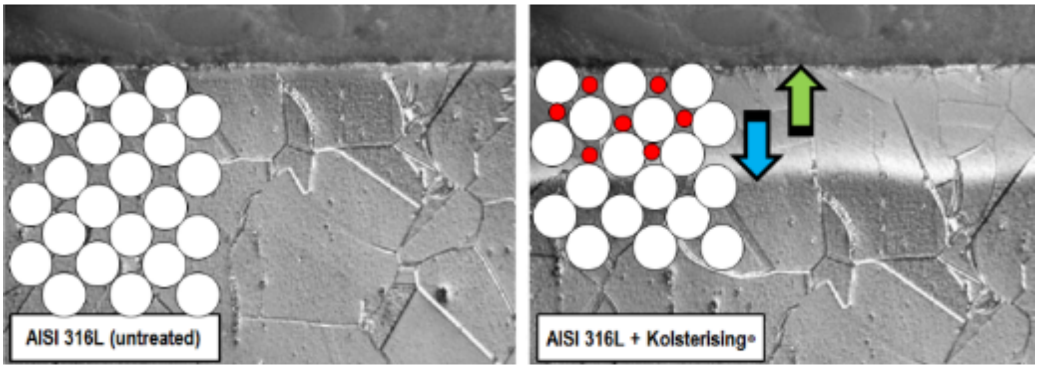

Figure 2. Left: Microstructure of untreated stainless steel AISI 316L; right: Microstructure of AISI 316L after LTC; a homogeneous, precipitation-free and hard diffusion zone is formed (S-Phase/expanded austenite) due to the introduction of interstitial atoms (red dots) into the austenitic structures (white dots) (BODYCOTE, 2014).

1.2. Testing of LTC food contact stainless steel materials

In 2013, a new guideline for food contact materials was published by the Council of Europe (CoE). The values for metal transference in food contact applications were updated and clearly define lower specific release limits (SRLs) for alloy elements of stainless steel: iron, chromium, nickel, manganese and molybdenum. Moreover, a new, more aggressive test, applying citric acid as the food simulant, was introduced (COE, 2013). In order to prove compliance of LTC for stainless steel components with the CoE 2013 guideline, an untreated (non-hardened) AISI 316L metal sheet was tested for its migration level against LTC AISI 316L metal sheets with a passivated and an electro-polished surface finish. The test is intended to prove the safe use of LTC for stainless steel components in the food and beverage industry. Additional, to show the greatly improved mechanical resistance, dry sliding ball-on-disc test according ASTM G99-95a were carried out on untreated and low temperature carburized 316L test samples.

2. MATERIALS AND METHODS

2.1. Material

100x100x2mm metal sheets out of AISI 316L austenitic stainless steel with 2B surface finish were low temperature carburized (LTC) and afterwards either passivated or electro-polished with 2 μm removal depth to obtain an optimal cleanable and non-reactive surface. Surface morphologies are comparable to each other: The LTC and passivated surface (condition 2; Fig. 3 middle) optically has no different appearance than the untreated surface (condition 1; Fig. 3, left). The nickel-plated surface is a bit shinier (condition 3; Fig. 3, right). The surface hardness measurement results in Table 1 show an enormous increase in surface hardness after LTC. In addition, according to EN 1672-2 (EN, 2009) and EHEDG Guideline No. 8 (EHEDG, 2018), a suitable surface topography and roughness play a key role for the cleanability within food and beverage applications and should be below Ra < 0.8 μm, which is the case for all three conditions.

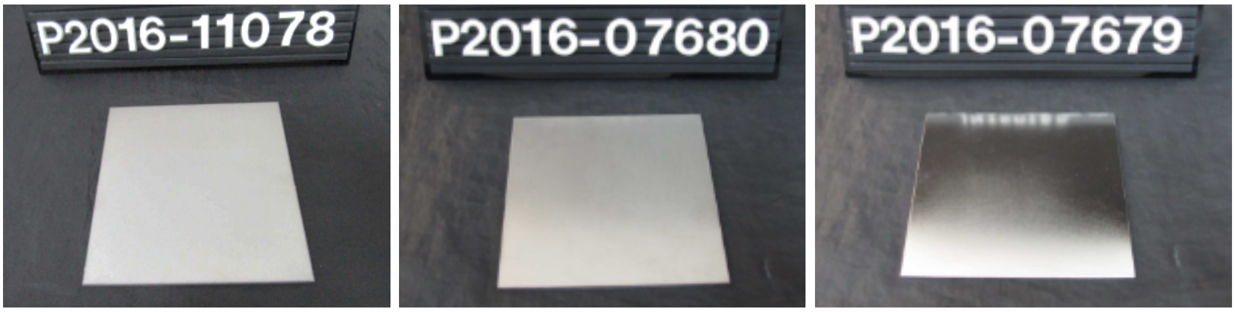

Figure 3. Tested AISI 316L metal sheets in untreated state (left), LTC+passivation (middle) and LTC+electro-polishing (right).

Table 1. Overview of AISI 316L stainless steel migration testing samples in different conditions, showing an enormous increase in surface hardness after LTC and only slight changes in surface roughness.

Condition	LTC	Surface finish after treatment	Surface hardness HV$_{0.05}$	Diffusion depth μm	Surface roughness μm
1	no	-	206	-	0.31
2	yes	Passivation	1153	43	0.43
3	yes	Electro-polishing	1043	41	0.25

2.2. Migration test

A total geometric testing surface area of approximately 60 cm² per condition is used for migration testing. As required by the CoE guideline, the specified metal surface area is in contact with 100 ml test solution of 5 g/L citric acid (pH < 4.5) via a single migration cell test set-up. One migration cycle corresponds to 2 h contact of the metal sheet with the 70°C heated up test solution (simulate hot filling), followed by 24 h at 40°C (simulate storage of food). In total 3 migration cycles has to been performed (COE, 2013). After the first and second cycle the migrated solutions were mixed to achieve an average value after both cycles. The mixed solutions as well as the third migration cycle solution were analyzed by inductively coupled plasma-optical emission spectrometry (ICP-OES).

2.3. Ball-on-Disc test

The enhanced wear behavior of the surface hardened samples was evaluated by using dry sliding ball-on-disc tests in accordance with standard ASTM G99-95a (ASTM, 2000) in order to analysis the wear depth and wear width after a constant distance of 500 m (0.6 miles). The tribological tests were carried out on AISI 316L metal sheets in untreated and LTC (with no further treatment) condition in ambient air with a rotating tribometer test set-up and a constant speed of 100 rpm (0.052 m/s). A ball of Al_2O_3 was used as counterpart, which is pressed with 2 kg (equal to 20 N) onto the stainless steel surface.

3. RESULTS AND DISCUSSION

3.1. Migration test results

Migration levels of the two LTC conditions are compared to the untreated condition. The measured released amounts of cobalt, chromium, copper, iron, molybdenum and nickel of the untreated (condition 1) and surface hardened metal sheets (conditions 2 and 3) are presented in Table 2. All three tested conditions pass the migration test since no Specific Release Limit (SLR) value was exceeded. It is worth noting that the migration levels of the surface hardened metal sheets (conditions 2 and 3) after the first and second migration cycles are even lower than those for the untreated metal sheet (condition 1). After the third migration cycle the values decrease for all metal sheet conditions to indeterminable levels or values far below the SRLs. No differentiation can be made, whether a subsequently passivated surface (condition 2) or an electro-polished surface (condition 3) provides better results. Generally, the results confirm that the surface hardening of 316L stainless steel by low temperature carburizing does not deteriorate the migrations characteristics, compared to the untreated condition. Therefore, it is expected that the corrosion resistance of the austenitic stainless steel remains or even increases. Based on this assumption, the low temperature carburized stainless steel grade 316L can safely be used for components in the food and beverage industry. (INTERNAL, 2016).

Table 1. SRLs in µg/kg and released amounts of different elements from 316L in three different conditions into 5 g/L citric acid (pH < 4,5) after 2 h exposure at 70°C followed by 24 h exposure to 40°C; n.d.: not determinable, below the quantification limit of the measurement unit (INTERNAL, 2016).

	Spec. release limits (SRLs)		Condition 1		Condition 2		Condition 3	
Elements (in µg/kg)	**Average 1st & 2nd Migration**	**3rd Migration**	**Average 1st & 2nd Migration**	**3rd Migration**	**Average 1st & 2nd Migration**	**3rd Migration**	**Average 1st & 2nd Migration**	**3rd Migration**
Cobalt	140	20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chromium	1750	250	240	n.d.	126	57	n.d.	n.d.
Copper	28000	4000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Iron	280000	40000	4000	n.d.	n.d.	n.d.	n.d.	n.d.
Molybdenum	840	120	60	n.d.	n.d.	n.d.	n.d.	n.d.
Nickel	980	140	300	29	52	n.d.	42	n.d.
Result			**Test passed**		**Test passed**		**Test passed**	

3.2. Ball-on-Disc test results

Normally stainless steel grades show a very low wear resistance, which is confirmed by dry-sliding ball-on-disc tribometer tests. The untreated 316L metals sheet (Fig. 4, left) shows clear signs of abrasive wear, unlike the surface hardened specimen (Fig. 4, right) which shows only a slight indentation of the surface. The wear depth was measured tactilely and is only 3.3 μm for the LTC specimen, whereas a more than 10-time higher value is measured for the untreated sample with 42.4 μm. In addition to the wear depth, the wear width is significantly greater for the untreated surface than for the LTC surface. This measurement is summed up by calculating the wear volume, which is around 3.7 mm^3 for the non-hardened and only 0.06 mm^3 for the carburized sample. Qualitatively at least 98% lower wear volume for the low temperature surface hardened sample can be assumed under the test parameters mentioned above. (INTERNAL, 2018)

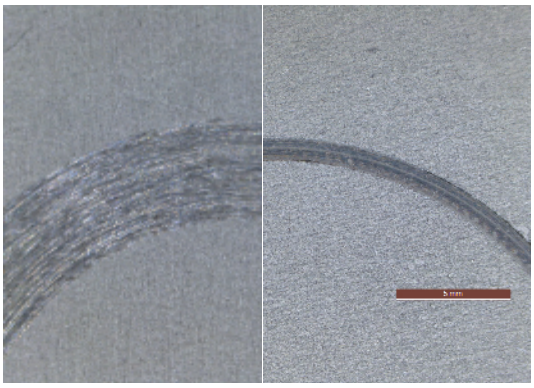

Figure 4. Surface conditions of untreated (left) and low temperature surface hardened (right) 316L metal sheets after 500 m (0.6 miles) dry sliding ball-on-disc testing against Al_2O_3-ball with 2 kg load and 100rpm (INTERNAL, 2018)

Figure 5. Wear tracks of untreated (black) and low temperature surface hardened (grey) 316L metal sheets after dry sliding ball-on-disc testing against Al_2O_3-ball; up to 98% reduced wear volume with low temperature surface hardening compared to untreated condition (INTERNAL, 2018).

4. CONCLUSION

The Council of Europe (CoE) updated its guideline for metals and alloys in food contact applications in 2013 and clearly define lower specific release limits (SRLs) for iron, chromium, nickel, manganese and molybdenum – the main alloying elements of austenitic stainless steels. The objective of this study was to quantifiably determine whether LTC AISI 316L metal sheets with different post-surface-treatments comply with the given migration levels compared to an untreated AISI 316L in citric acid (5 mg/L, pH<4,5) following the test instruction according to the CoE guideline. Additional dry-sliding ball-on-disc tribometer tests have been performed to examine the increased wear resistance after surface hardening.

The LTC AISI 316L metal sheets, which were additionally passivated or electro-polished (2 μm), show in both cases, that the low migration levels of austenitic stainless steels are maintained. It can be assumed that the corrosion resistance against acids and less aggressive media is maintained or even improved compared to the untreated surface state. Furthermore, the wear test presents high abrasion resistance of the surface hardened samples by showing the significantly reduction in wear volume compared to the non-hardened condition. With this unique technique, existing applications like weighing units and moving parts in general can be surface-hardened without any changes in shape or size, but with increased wear and galling resistance. Additional new design possibilities for packaging applications will arise: Metal-on-metal sliding bearings for dosing pumps can replace polymeric sealing rings. The geometrical complexity can be reduced to avoid the accumulation of undesirable microorganisms.

In summary, low temperature carburizing (LTC) allows to create robust, wear and corrosion resistance surfaces, resulting in a variety of applications for food and beverage industry. No general statement can be made as to whether a subsequently passivated or electro-polished surface is better. This is a question of the application area and its requirements. Restrictions may exist in the selection of material.

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CoSSP Committee of Stainless Steel Producers. 1978. American Iron and Steel Institute. Review of the wear and galling resistance characteristics of stainless steels. Washington. 9-12:22-26.

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SESSION IV

New Materials for Shelf Life Extension

‘PHULAE’ PINEAPPLE LEAF MOLDED PULP TRAY WITH ZNOPS

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ABSTRACT

The main chemical compositions of ‘Phulae’ pineapple leaf fiber (PALF) were holocellulose (85% d.b.) and α-cellulose (40.8% d.b.). This fiber can be used to make a molded pulp tray (MPT). The objective of this research was to study the effect of chitosan combined with ZnO nanoparticles on the properties of PALF molded pulp tray (MPT). Blending with 4% (w/v) chitosan and 10% (w/v) ZnO nanoparticles (ZnONPs) improved mechanical properties, increased tensile strength, elongation at break, water wetting time, and reduced thickness swelling of PALF-MPT. Adding ZnONPs increased antimicrobial activities of PALF tray against *Escherichia coli*, *Staphylococcus aureus*, and *Penicillium dijitatum*.

Keywords: pineapple leaf fiber, molded pulp tray, chitosan, zinc oxide nanoparticles, properties

1. INTRODUCTION

The packaging has an important to maintain the shelf life of the fruit and vegetable. It is made from natural materials which will not result in harm to the products and consumers. Packaging material from natural fiber can reduce agricultural waste and also decrease environmental impact. The one that is abundantly available and is used very little is pineapple leaf fiber (PALF) (KENGKHETKIT and AMORNSAKCHAI, 2012). After fruit harvesting, the leaves are disposed of by burning or decomposing. Although methods for fiber extraction are known for a long time and have been using to the present day, the use of PALF is still very limited to the textile and handcrafted paper (SENA-NETO *et al.*, 2013). Products packaged in easily recycled, biodegradable and compostable material such as molded pulp which made from natural fiber (e.g PALF) tend to be perceived as coming from a source that values sustainable practices. Chitosan is obtained by deacetylating chitin comprising copolymers of beta(1,4)-glucosamine and N-acetyl-d-glucosamine which is the second most abundant polysaccharide found in nature after cellulose and its extremely good binder for cellulosic fiber structures (BAJPAI, 2015). Thus, the properties of molded pulp trays from PALF pulp with chitosan blending with ZnO nanoparticles were studied.

2. MATERIAL AND METHODOLOGY

2.1. PALF preparation and alkaline-AQ pulping process

Pineapple leaves (cv. 'Phulae') from orchard in Chiang Rai province was washed to clean the soil and cut to small piece around 1 inch. The sample was dried in a tray dryer at 70°C for overnight. The pulping process was prepared by immersion a pineapple leaves (oven-dry weight) with 15% NaOH at 1:10 (w/v) ratio and 0.2% AQ charge (based on oven-dry fiber), autoclave at 121°C for 1 hour, then washing with water for neutralizing.

2.2. Chemical composition analysis of PALF

Analysis of chemical compositions of PALF was investigated. The chemical compositions such as holocellulose, alpha-, beta- and gamma-cellulose of the dried-PALF pulp were examined according to TAPPI T 19 m-54 standards.

2.3. Synthesis of Zinc oxide nanoparticle

Zinc oxide nanoparticles were synthesized by using pineapple peel extract as described in a previous study (SAEKOW *et al.*, 2019).

2.4. Chitosan and ZnO nanoparticle incorporation and Molded Pulp Tray Production

Prior weighing 18 g (w/v) of PALF (dried weight) base on volume was added water at a ratio of 1:10 (w/v) into a blender and blended for 1 min, and then pour into pre-form. The 4% (w/v) chitosan solution and 4% (w/v) chitosan solution with 10% (w/v) ZnONPs were prepared. After that, the PALF preformed sheet was dipped into chitosan solution for 2 min (MUNAWAR *et al.*, 2008) and then placed into a mold. The molding process was done with a hydraulic hot compression machine (Lab Tech Engineering Company LTD) under the condition of pressure 250 psi for 10 min at 130°C.

2.5. Molded Pulp Tray Characterization

The PALF molded pulp trays were characterized and determined the color with colorimeter, functional groups with FTIR spectroscopy, thickness, water wetting time (WWT) (RAZAK *et al.*, 2015), thickness swelling (ASTM D 1037-99), mechanical properties (tensile strength (TS), % elongation at break (%EB) according to ASTM D 1037-06a method, and modulus of rupture (MOR) by using a modified ASTM D 1037-99), and antimicrobial activities by the disc diffusion method.

2.6. Statistical analysis

All data were analyzed using the SPSS program (version 20.0). Analysis of Duncan and Tukey's using for determining the significance between treatment at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Chemical Analysis

The content of holocellulose in PALF was in the range of 80-87.56%. The yield of the pulping process with 15% NaOH and 2% AQ was showed to 35 %.

3.2. Characterizations of Molded Pulp Tray

3.2.1 Color

The appearance of color is the first sensation that human perceives and uses as a tool to evaluate the product of molded pulp tray. In order to carry out a detailed characterization of the image of a molded pulp tray, the color measurement was conducted to compare the differences in each sample of MPT as well as commercial MPT. As shown in Table 1, control has the highest L* value compared to other samples. Meanwhile, L* value was decreased as the 4%chitosan and %4chitosan with ZnONP but b* were increased, which represented more yellowish of MPT. However, L* value of chitosan-treated and chitosan with ZnONP MPT were lower than control due to the slight yellowish of chitosan.

Table 1. Average color of PALF tray.

Sample	L*	a*	b*	c	h
Control	73.58±1.5^{a}	2.02±0.2^{a}	15.08±0.5^{ab}	15.22±0.5^{ab}	82.26±0.8^{b}
Tray+4% chitosan	72.8±2.0^{ab}	1.58±0.2^{b}	16.08±0.8^{a}	16.12±0.9^{a}	84.40±0.4^{a}
Tray+4% chitosan+10%ZnONPs	70.46±2.5^{b}	1.78±0.3^{c}	16.18±2.0^{a}	13.32±2.8^{b}	82.04±1.8^{b}

3.2.2 FTIR analysis

Fig. 1 showed the FTIR spectrum comparison of each sample. The OH vibration bands of PALF-chitosan and PALF-chitosan-ZnONP trays were shifted from 3331.89 to 3349.13 cm^{-1} due to an interaction of hydroxyl groups in PALF and the amino group of chitosan (XU *et al.*, 2005). The band at 1578 cm^{-1} was NH bending for amide II exhibiting peak intensity as

4%chitosan and 4%chitosan with ZnONP. The C=C stretching was present at peak 2320 cm^{-1} in 4%chitosan and 4%chitosan with ZnONP PALF MPT.

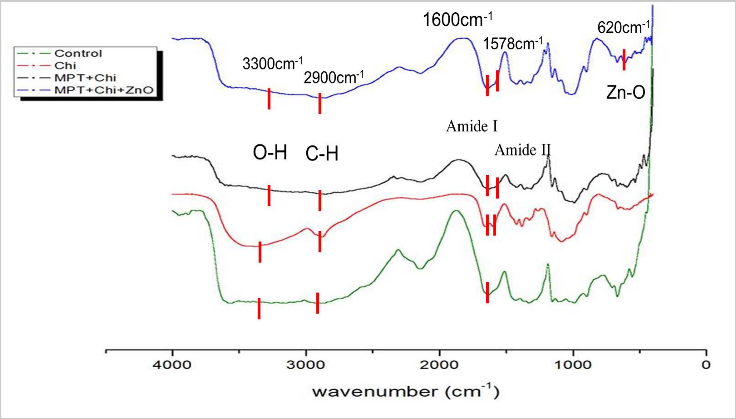

Figure 1. FTIR spectrum of control, chitosan powder, tray+4%chitosan, and tray4%chitosan+ ZnONP.

3.2.3 Thickness Swelling and Water Wetting Time

Thickness swelling (TSW) was determined the ability of the product to uptake water and moisture, while water wetting time (WWT) was determined the time needed for water to absorb into the surface of the product (RAZAK *et al*., 2015). Fig. 2 showed that 4% chitosan and 4% chitosan with ZnONP coating reduced the moisture uptake of the tray as the reducing of TSW and increasing of water wetting time. This is due to hydrophobic features of chitosan molecules that cover the surface of PALF. Furthermore, the utilization of chitosan biopolymer on the PALF reduced the degree of water uptake.

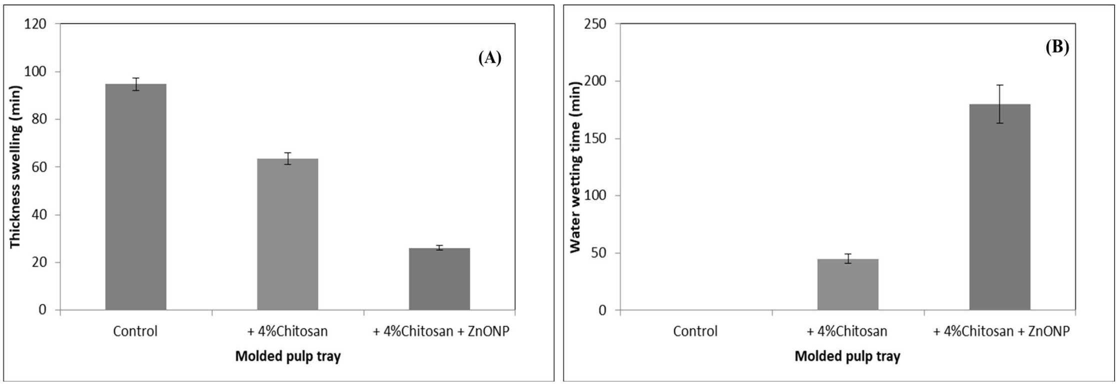

Figure 2. Thickness swelling (A) and water wetting time (B) of control, tray+4%chitosan and tray4%chitosan+ ZnONP.

3.2.4 Mechanical Properties of Molded Pulp Tray

The mechanical properties of PALF molded pulp tray are given in Fig. 3. PALF-4%chitosan with ZnONP gave the highest tensile strength (14.45 MPa) as well as elongation at break (7.64 MPa) compared to other samples. This value affected by the contribution of the chitosan layer in the surface of PALF as reported by MUNAWAR *et al.* (2008). ZnONPs also gave the strength improvement of the PALF molded tray.

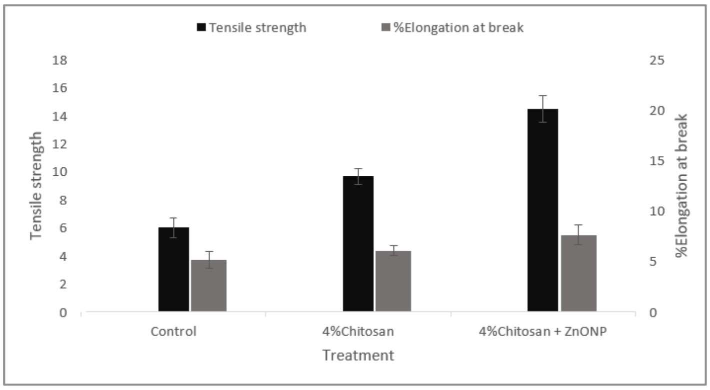

Figure 3. Tensile strength and Elongation at break of control, tray+4%chitosan, and tray4%chitosan+ ZnONP.

3.2.5 Antimicrobial activities

The antimicrobial activities of PALF molded pulp tray are given in Table 2 PALF-4%chitosan with 10%ZnONP can inhibit the growth of *S. aureus*, *E. coli*, and *P.dijitatum* as shown with the diameter of clear zones 11, 12, and 11 mm, respectively.

Table 2. Antimicrobial activities of the tray from PALF.

Sample	**The diameter of clear zone (mm)**		
	E. coli	***S. aureus***	***P. dijitatum***
Control	0	0	0
Tray+4%chitosan	0	0	0
Tray+4%chitosan+10% ZnONPs	12	11	11

4. CONCLUSION

Molded pulp tray dipping with chitosan and ZnO nanoparticle can increase tensile strength, elongation at break, water wetting time, and antimicrobial activities, and reduce thickness swelling. PALF Molded pulp tray with chitosan and ZnO nanoparticles can be one alternative of active packaging that can be used for food and fresh produce.

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ANTIMICROBIAL NANOFIBERS IN FOOD ACTIVE PACKAGING

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ABSTRACT

Among encapsulation techniques of antimicrobial substances, electrospinning outstands for enabling the synthesis of polymeric nanofibers that act as micro- or nanocapsules, and release systems. A myriad of polymers has been successfully electrospun to obtain ultra-thin fibers, including synthetic polymers, biomacromolecules, and their composites. In the context of food packaging, these materials have advantages such as mechanical resistance, large surface areas/porosities, and responsiveness to external stimuli. Ultra-thin fibers have been used to incorporate antimicrobial substances. The resulting functionalized nanofibers have been proved as promising devices in a shelf-life extension of various food models. The target microorganisms against which these materials have been tested comprise *S. aureus*, *E. coli*, *Salmonella* spp., *Pseudomonas* sp., *Rhizoctonia*. In this review, a brief literature examination of the experimental evidence of electrospun nanofibers containing antimicrobials as food shelf life extenders is presented

Keywords: nanofibers, electrospinning, food active packaging, antimicrobial compounds

1. INTRODUCTION

Active packaging materials and devices are designed in order to have a relevant role in the conservation of food, beyond being a barrier between the product and the external environment (MAJID *et al.*, 2016). Essential oils, polyphenol-rich plant extracts, silver nanoparticles, and antimicrobial polymers and peptides are among antimicrobial agents that have been used for active packaging purposes. These agents inhibit microorganism cellular processes, thus contributing to the improvement of food shelf life, and might represent an alternative to the use of preservatives or even thermal processing; however, many of these substances are themselves prompt to deterioration (ZHANG *et al.*, 2018).

The use of nanomaterials to encapsulate antimicrobial substances represents a step forward in the design of packaging systems with controlled release of food protective agents. In this context, electrospinning is an effective and versatile electrohydrodynamic technique, used to manufacture fibers on a sub-micron to nano-scale from various polymeric materials (FUENMAYOR and COSIO, 2016). For the elaboration of these structures, a solution containing the dissolved polymer is pumped at a controlled flow rate towards a needle (spinneret) where a high voltage is applied (Sullivan *et al.*, 2014). In this polymer solution, antimicrobial agents can be incorporated, in order to obtain functionalized polymer nanofibers. Thus, structures with hydrophilic or hydrophobic character will be obtained, depending on the features of the polymer and bioactive compounds incorporated in the electrospinning solution/dispersion, offering an advantage in terms of controlled release of active agents (SOARES *et al.*, 2018). The principle and mechanism of the technique have been widely studied (ZHANG *et al.*, 2018; WEN *et al.*, 2017), and are beyond the scope of this review. Therefore, this article presents current developments in the field of antimicrobial compounds encapsulation in nanofibers intended for food shelf life extension.

2. ENCAPSULATION AND RELEASE OF NATURAL ANTIMICROBIALS

Antimicrobial substances, especially those of natural origin, such as natural essential oils, absolutes, essences, extracts, resins, infusions, etc. are of great interest for the active packaging industry. Nevertheless, their efficient encapsulation and release represent a major challenge, considering the fact that they are very sensitive to heat, oxygen, and light. Because of their submicron to nano-scale diameter and very large surface area, electrospun fibers may offer additional advantages compared to film and sheet carriers, as they are more responsive to changes in the surrounding atmosphere, which enhances a tunable release of the entrapped compounds (VEGA-LUGO and LIM, 2009). Moreover, since the electrospinning process takes place at ambient conditions, the produced fibers are more suitable to encapsulate thermally-labile substances than fibers prepared by conventional processes, or other encapsulation methods, such as spray drying and fluid bed coating (LESMES and MCCLEMENTS 2009; XU *et al.*, 2006). In this framework, electrospun nanofibers of PVA have been used to encapsulate allyl isothiocyanate (AITC) (AYTAC *et al.*, 2014). Pullulan and β-cyclodextrin emulsions in water have also been electrospun for the encapsulation of volatile antimicrobials limonene (FUENMAYOR *et al.*, 2013) and perillaldehyde (MASCHERONI *et al.*, 2013), allowing for a controlled release of the antimicrobial, triggered by humidity. Other examples include electrospun zein, which was used to encapsulate rosehip seed oil (REO). Electrospun REO-loaded zein fibers improved the shelf-life of peeled and segmented bananas, demonstrating its potential as active packaging material (YAO *et al.*, 2016).

3. ELECTROSPUN NANOFIBERS WITH *IN VITRO* EVIDENCE AS ANTIMICROBIAL FOOD PACKAGING MATERIALS

Most of the existing evidence on the potential of electrospun nanofibers as encapsulation systems for food preservation against microbial spoilage, relies on *in vitro* antimicrobial testing, as presented in Table 1.

Interestingly, most of the electrospun polymer materials within this group of reports are edible or food-grade biopolymers. *S. aureus* and *E. coli* are the most common target microorganisms for testing the antimicrobial activity of electrospun nanofibers, being representative of Gram-positive and Gram-negative bacteria, respectively (Jenab *et al.*, 2017). The *in vitro* analytical methods for evaluating the antimicrobial activity of electrospun nanofibers are diverse semi-quantitative tests aimed at measuring the microbial growth after contact with the antimicrobial packaging material.

The disk diffusion method is a technique often selected. However, according to Espitia and Andrade (2015), this method might present slight variations from one experiment to another due to target microorganisms, and experimental variables, such as incubation time and temperature. Other microbiological techniques include the optical density (OD) measurement, the colony counting method and the broth microdilution method for determining inhibitory minimum concentration (MIC). In other studies, viability and bacterial adherence have been determined qualitatively in terms of biofilm formation capacity (CLAVIJO-GRIMALDO *et al.*, 2019).

Table 1. Electrospun nanofibers intended as antimicrobial food packaging tested *in vitro*.

Polymeric nature of nanofibers	Incorporated compounds	Target microorganisms	Antimicrobial test*	Reference
Chitosan nanofibers	Poly(ethylene oxide) and silver nitrate, as a co-electrospinning polymer and silver nanoparticle precursor	*E. coli*	Disk diffusion method	(Annur *et al.*, 2015)
Blend of Poly(lactide-co-glycolide) (PLGA) and Chitosan	The nanofiber was functionalized with graphene oxide decorated with silver nanoparticles	*E. coli* *Pseudomonas aeruginosa* *S. aureus*	Colony counting method	(Faria *et al.*, 2015)
Chitosan–polyethylene oxide	zeolitic imidazolate framework nanoparticles	*S. aureus* *E. coli*	Colony counting method - AATCC test method 100–2004	(Kohsari *et al.*, 2016)
PLA and glycidyl methacrylate	Cellulose nanocrystals Lignin nanoparticles	*Pseudomonas syringae pv. tomato*	Colony counting method	(Yang *et al.*, 2016)
Cellulose	Lysozyme	*Listeria monocytogenes* *Yersinia enterocolitica*	OD measurement	(Bayazid *et al.*, 2018)
Pullulan	Palindromic peptide Lfcin B	*E. coli* *S. aureus*	Disk diffusion method to determinate the MIC	(Román *et al.*, 2019)
Tragacanth	Peppermint oil	*E. coli* *S. aureus*	Disk diffusion method	(Ghayempour and Montazer, 2019)
Zein	Curcumin	*E. coli* *S. aureus*	OD measurement	(Wang *et al.*, 2019)

*OD: Optical density; TSB broth: Tryptic soy broth; Minimum Inhibitory Concentration (MIC).

4. ELECTROSPUN NANOFIBERS FOR FOOD SHELF LIFE EXTENSION

The application of these materials in food matrixes is more important, but still limited, as presented in Table 2. Target microorganisms include Gram-positive such as *L. monocytogenes* and *S. aureus*, as well as Gram-negative such as *E. coli* and *Salmonella* (Erbay *et al.*, 2017). Antimicrobial studies in food matrixes also include the electrospun nanofibers effect against fungi, such as *Aspergillus niger* and *Penicillium* (Table 2).

Table 2. Electrospun nanofibers potential applications in food preservation.

Polymeric nature of the electrospun nanofiber	Incorporated substances	Target microorganisms	Food matrix	Reference
Polyvinyl alcohol	cinnamon essential oil /β-cyclodextrin inclusion complex	*E. coli* *S. aureus*	Strawberries	(Wen *et al.*, 2016b)
Poly(ε-caprolactone)	*Urtica dioica* L. extract incorporated into Whey Protein Isolated complex at different concentrations	Total aerobic mesophilic bacteria lactic acid bacteria	Rainbow trout fillets	(Erbay *et al.* 2017)
Blend of polyvinyl alcohol and β-cyclodextrin	Cinnamon essential oil and lysozyme	*Penicillium* *Salmonella enteritidis* *Aspergillus niger*	Strawberries	(Feng *et al.* 2017)
Gelatin	Rosemary essential oil	*Campylobacter jejuni*	Chicken	(Lin *et al.*, 2018)
Chitosan	Chrysanthemum essential oil	*L. monocytogenes*	Meat	(Lin *et al.* 2019)
Gelatin	Moringa oil	*L. monocytogenes* *S. aureus*	Cheese	(Lin *et al.* 2019)
Silk fibroin	Thyme essential oil	*Salmonella typhimurium*	Poultry meat	(Lin *et al.* 2019)

Results from the application of antimicrobial nanofibers in packaging with food matrixes ensure the potential application of these materials in food preservation. However, more studies are needed in order to elucidate the release mechanism of antimicrobial agents from the packaging material to the food matrix, as well as the molecular interaction between the antimicrobial agent, the target microorganisms and the macro and microstructural components of the food matrix.

CONCLUSIONS AND FUTURE PERSPECTIVES

There is substantial evidence on the potential of antimicrobial nanofibers for food preservation. However, further experimental evidence on wider variety of food models is needed. Moreover, studies on the use of nanofibers for bioactive peptides and beneficial microorganism encapsulation is limited, which highlights a future trend. Finally, the lack of actual evidence on the toxicity of nanofibers as food contact materials, as well as the availability of electrospinning setups for industrial applications, are important challenges of this technology.

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FEATURES OF CHITOSAN-GELATIN FILMS LOADED WITH PLANT EXTRACTS

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ABSTRACT

The main aim of this study was to characterize the physical, optical and antimicrobial properties of chitosan-gelatin blend films enriched with cinnamon, nutmeg and thyme essential oils. Microstructure determined by scanning electron microscopy showed that active films had heterogeneous surface. The presence of new bands and changes in the FT-IR spectra confirmed intermolecular interactions between the chitosan-gelatin matrix and essential oils. The active films proved as effective barriers against UV light and inhibited the growth of four major food bacterial pathogens, showing potential as environmentally friendly antimicrobial packaging material.

Keywords: chitosan-gelatin blend film, disk diffusion assay, essential oil, microstructure

1. INTRODUCTION

Recently, biodegradable films enriched with natural extracts have been considered as attractive alternatives to synthetic plastic packaging. Among biopolymers, chitosan (CS) and gelatin (GL) have shown excellent film forming properties and could be used for substitution of non-biodegradable synthetic plastics. Our previous study showed that blending CS and GL could combine the advantages of these two biopolymers as well as minimize their disadvantages (HAGHIGHI *et al.*, 2019b). In addition, the enrichment of packaging materials with certain additives is an innovative concept aimed at improving food safety and quality. Among active compounds, natural extracts and plant essential oils (EOs) have been recognized as valid alternatives to synthetic food additives due to their proved wide-spectrum antimicrobial capacity (BURT, 2004; ALOUI *et al.*, 2014). In this sense, combination of CS and GL films with EOs to create bio-based active films could be a promising strategy for packaging applications. Therefore, the purpose of the present work was to characterize CS-GL films enriched with cinnamon, nutmeg and thyme EOs to evaluate physical, optical, barrier and antimicrobial properties for food packaging application.

2. MATERIALS AND METHODS

CS with a molecular weight of 100–300 kDa was obtained from Acros Organics™ (China). GL (bloom 128- 192°) was purchased from AppliChem GmbH (Darmstadt, Germany). cinnamon, nutmeg and thyme EOs were purchased from Solime S.r.l (Cavriago, Reggio Emilia, Italy).

2.1. Preparation of film-forming solutions (FFSs) and films

Preparation of films was adapted from (Bonilla and Sobral 2016) as described by HAGHIGHI *et al*. (2019a). CS FFS (2%, w/v) was prepared by dissolving CS in an acetic acid solution (1%, v/v) under continuous stirring at 55°C for 30 min. GL FFS (2%, w/v) was prepared by dissolving GL in distilled water, first being allowed to swell at 7°C for 15 min and then stirred at 55°C for 30 min. Glycerol (25% w/w of CS or GL) was then added as a plasticizer into both FFS, followed by additional stirring for 30 min. CS- GL blend solution was prepared by mixing CS and GL FFS at 1:1 ratio. Moreover, EOs (1%, v/v) together with Tween 80 (0.2%, v/v EO) were added to FFS, followed by stirring at 55°C for additional 30 min. Films were obtained by casting 20 mL of the FFS into Petri dishes (14.4 cm in diameter) and drying at 25±2°C overnight in the chemical hood at ambient relative humidity (RH) of 45%.

2.2. Scanning electron microscopy

Scanning electron microscopy (SEM) from cross section images of the films were obtained with the use of a scanning electron microscope (FEI, Quanta 200, Oregon, USA). Film samples were fixed on a stainless- steel support with a double side conductive adhesive. The analysis was conducted in low vacuum (0.6 Torr) at an acceleration voltage of 20 kV.

2.3. Attenuated Total Reflection (ATR)/Fourier-Transform Infrared (FT-IR) Spectroscopy

The infrared spectra of different films were obtained using an ATR/FT-IR spectrometer (type Alpha, Bruker Optik GmbH, Ettlingen, Germany). Spectra were collected from two different locations from the top and bottom of the same samples in the 4000-400 cm^{-1} wavenumber range by accumulating 64 scans with a spectral resolution of 4 cm^{-1}.

2.4. UV barrier and light transmittance

The barrier properties of films against UV and visible light were determined at the UV (200, 280 and 350 nm) and visible (400, 500, 600, 700 and 800 nm) wavelengths onto square film samples (2×2 cm^{2}) using a Jasco V – 550 UV/Vis spectrophotometer (Jasco **Corporation, Tokyo, Japan).**

2.5. *In vitro* antimicrobial activity

Antibacterial activity test on films was assessed against four typical food bacterial pathogens including *Listeria monocytogenes* (UNIMORE 19115), *Escherichia coli* (UNIMORE 40522), *Salmonella typhimurium* (UNIMORE 14028) and *Campylobacter jejuni* (UNIMORE 33250) using the disk diffusion assay according to HAGHIGHI *et al.* (2019b).

3. RESULTS AND DISCUSSION

3.1. Microstructure

The surface and cross-section images of CS-GL film (control) and CS- GL film enriched with different EOs (active films) are presented in Fig. 1. The surface of control films was smooth and homogenous and did not show pores or cracks (Fig. 1a) indicating the formation of an ordered matrix. Active films showed heterogenous surface with remarkable pores that resulted from oil droplets after drying (Fig. 1c, e and g). This might be attributed to the high volatility of these EOs during the drying process (YAO *et al.*, 2017). A compact and continuous structure without phase separation can be observed in the cross-section of the control film (Fig. 1b) indicating high compatibility among CS and GL to form a blend. The cross-section of active films showed discontinuities and heterogenous structure with air bubbles (Fig. 1d, f, and h).Bonilla *et al.* (2018) also reported that CS-GL blend film containing eugenol and ginger EOs had uncompact texture with sponge-like structure due to the uneven dispersion of EOs with hydrophobic nature from the aqueous phase during the film drying process.

3.2. Attenuated Total Reflection (ATR)/Fourier-Transform Infrared (FT- IR) spectroscopy

The FT-IR spectra of control and active films are shown in Fig. 2. The control film spectrum showed three types of amide by characteristic bands at 1636 cm^{-1} (amide-I), 1545 cm^{-1} (amide-II) and 1245 cm^{-1} (amide III) (Bonilla and Sobral 2016). The broad absorption band between about 3600 and 3200 cm^{-1} corresponds to ν(O-H) and ν(N-H) stretching. The band doublet at 2927/2874 cm^{-1} can be assigned to antisymmetric and symmetric νas(CH3/CH2)/νs(CH3/CH2) stretching vibrations. The ATR/FT-IR spectra of the active films showed partly characteristic additional bands of the incorporated EOs. In Fig. 2 the spectra have been arranged (from top to bottom) in the order of increasing ν(C=O) bands in the wavenumber range 1720-1740 cm^{-1} that can be assigned to ester, aldehyde or ketone functionalities of the EO admixtures. Thus, it can be assumed that the admixed C=O and OH functionalities of the EOs contribute to intermolecular interactions with the hydroxyl and amino groups of the CS-GL film network.

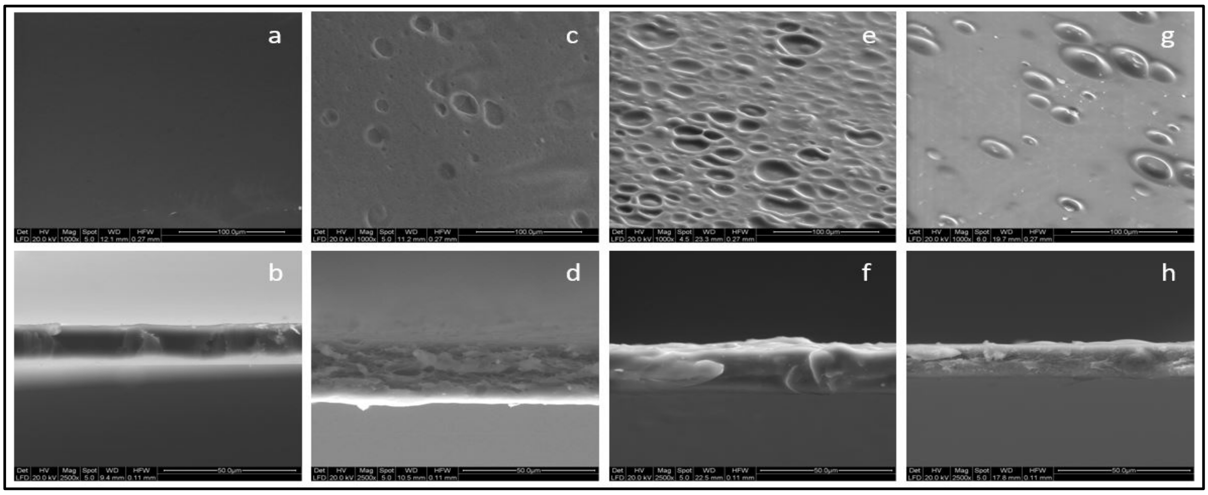

Figure 1. SEM images on the surface and cross-section of films based on: Chitosan-Gelatin blend (CS-GL) as a control (a and b); CS-GL-Cinnamon (c and d), CS-GL-Nutmeg (e and f) and CS-GL-Thyme (g and h).

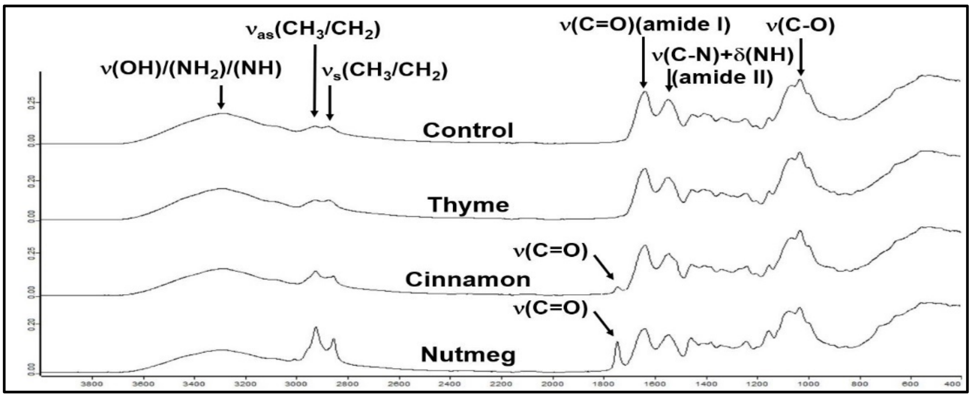

Figure 2. ATR-FT-IR spectra of films based on a: Chitosan-Gelatin blend (CS-GL) as a control and those enriched with EOs.

3.3. UV barrier and light transmittance

The UV barrier and visible light transmittance in the wavelength range 200-800 nm of control and active films are shown in Fig 3. Active films were effective against UV, since transmittance value was below 10% at 280 nm for these films. Therefore, films containing these EOs are able to retard lipid oxidation and preserve the organoleptic properties of the packaged food and prolonging food shelf-life. Active films showed lower transmittance in the visible range (350-800 nm) than the control film indicating that the incorporation of EOs into the film matrix reduced the transparency of the films, allowing to minimize photo-oxidation of organic compounds and degradation of vitamins and other pigments.

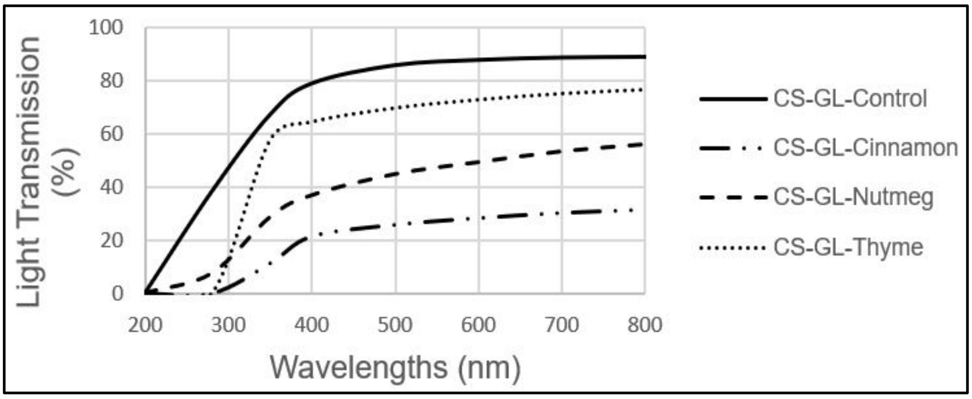

Figure 3. UV and visible light transmittance (T%) of the films based on chitosan-gelatin blend (CS-GL) as a control and those enriched with EOs.

3.4. In vitro antimicrobial activity

The details of antimicrobial activity of the active films against *C. jejuni, E. coli, L. monocytogenes and S. typhimurium* are shown in Fig. 4. The control film did not show an inhibitory effect against any of the tested microorganisms. Incorporating EOs into the films revealed an antimicrobial effect. Thyme EO was the most effective ($p<0.05$). Thymol and carvacrol are two main phenolic compounds (monoterpenoids) represented in thyme EO, and their high antimicrobial activity has been attributed to structural and functional damages to the bacterial cytoplasmic membrane and to the inhibition of intracellular metabolic pathways (CAO *et al.*, 2018).

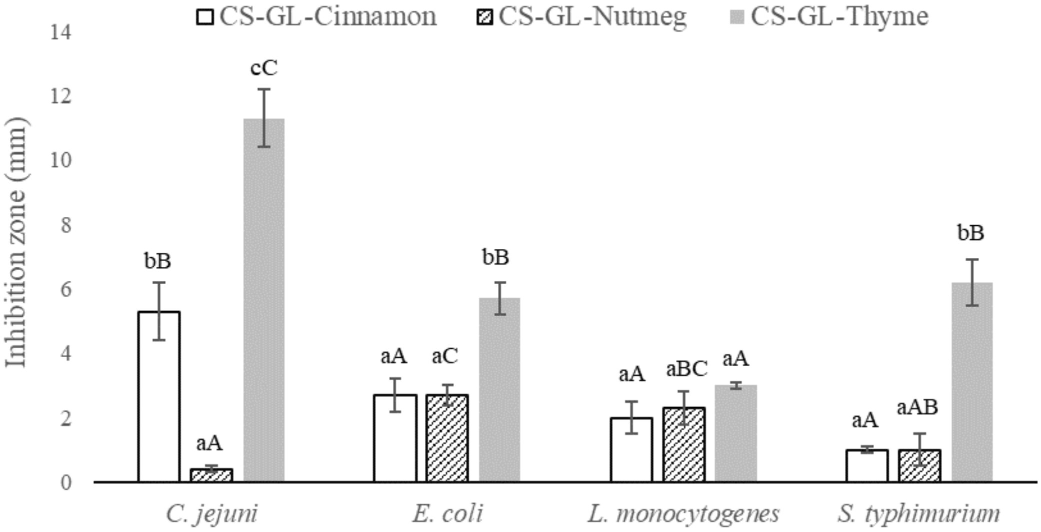

Figure 4. Inhibition zone diameters of the chitosan-gelatin blend (CS-GL) film disks (22 mm diameter) enriched with EOs. Values are mean±SD (n = 3). Different lowercase letters indicate significant differences ($p<0.05$) among treatments for each target microorganism. Different capital letters indicate significant differences ($p<0.05$) among target microorganisms for each treatment.

4. CONCLUSIONS

The results showed that the incorporation of different EOs could significantly improve the UV barrier properties of CS-GL film. The developed films, with special regards for those including thyme EO, possessed noticeable antimicrobial activity against common bacterial food pathogens, thus suggesting that the CS-GL films enriched with different EOs could be used as environmentally friendly, active food packaging with antimicrobial properties.

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EFFECT OF COCONUT PAD WITH LIME OIL ON VEGETABLES

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ABSTRACT

The aims of this study were to develop an antimicrobial pad from a coconut fibre containing lime oil emulsion (0.3%, 0.5%, and 0.7% w w^{-1}), and to study the effect of heat curing (60°C, 80°C and 100°C) and various times (30, 60 and 90 min) on the antimicrobial activity of lime oil. Response surface methodology was employed to find the optimum condition. Next, the antimicrobial pad was placed inside a polypropylene bag (20x30 cm) with vegetable (50 g) and kept inside a refrigerator at 4°C±2°C for 7 days. The results indicated that mixed vegetable packed with the coconut pad containing lime oil at 0.5% w w^{-1} and heat at 100°C for 90 min showed the highest antimicrobial activity against psychrophilic bacteria and mesophilic bacteria on the fresh-cut vegetables during cold storage ($P < 0.05$). In addition, mixed vegetable packed with an essential oil pad retarded browning appearance and visual quality of mixed vegetables than the control (without lime oil in a pad).

Keywords: Coconut fibre pad, heat curing, lime oil, mixed vegetables

1. INTRODUCTION

In Thailand, coconut production plays an important role in the national economy. The coconut tree (*Cocos nucifera*) is advantageous for its wood, leaves and fruit, from which water, pulp and fibres are extracted for different parts of the industry. However, coconut husks are difficult to dispose in environment thus the easy way to destroy them as burned, then becoming to be a source of pollution. As previously mentioned, coconut coir has beneficial purposes for use as food packaging as it is cheap and easily obtainable due to its porous properties (HARISH *et al.*, 2009; KHALIL *et al.*, 2007). Fibre from coconut is found to be a good source to create coconut pad or packaging.

In this research, lime oil was selected to develop the antimicrobial pad by incorporating it into the coconut fibre pad. Because lime oil is one of the essential oils that have good antimicrobial function. Heat curing was selected to enhance antimicrobial activity and to reduce the concentration of lime oil. Therefore, the objectives of this study was to study the antimicrobial effect of lime oil in combination with heat curing for extending the shelf-life of mixed vegetables during cold storage.

2. MATERIALS AND METHODS

2.1. Mixed vegetables

A mixture of cut green oak lettuce, shredded carrot and cherry tomatoes were purchased from a local market at Thasala, Nakhonsithammarat, Thailand. Vegetables were cleaned using water and dried before using.

2.2. Lime oil preparation

Lime oil was obtained from Thai China Flavors & Fragrances Co. Tween-80 was purchased from Merck, Thailand. Lime oil emulsion was prepared with essential oil (0.3, 05 and 0.7% w w^{-1}) and 2% tween 80, which was mixed in distilled water. This emulsion was mixed with a homogeniser (Speed 20,000 rpm min^{-1}) for 10 minutes.

2.3. Antimicrobial pad preparation

Coconut fibre from a coconut husk was used in this study. The husk was cut into small pieces and mashed to order, before being dried in a hot air oven for 1 hour at 75°C and storage in a desiccator before using. The antimicrobial pads were produced by incorporating a known concentration of lime oil emulsion in the coconut fibre. Then, the coconut fibre pad was prepared from fibres (5 g), tapioca starch (6 g), glycerol (2 g) and distilled water (7 ml). All materials were mixed together at 100°C for 5 min. After that, the paste was formed by rolling and was dried in the hot air oven at 60°C for 24 hours. All samples were kept in the desiccator.

2.4. Effect of heat curing on coconut fibre pad containing with lime oil emulsion on mixed vegetables

Coconut pad (5x5 cm) containing lime oil emulsion at 0 (control), 0.3, 0.5 and 0.7% w w^{-1} were prepared. Next, the pad was placed on water bath at 60, 80 and 100°C for 30, 60 and 90 min, respectively. Then, the pad was put inside a polypropylene bag (20x30 cm) with vegetables (25 g) and kept inside a refrigerator at 4°C±2°C for 7 days. Mesophilic and psychrophilic bacteria were studied. All results were investigated as $\log_{10}$ cfu g^{-1} (colony forming units per gram of sample).

3. RESULTS AND CONCLUSIONS

The results of the log reduction of antimicrobial pad based on coconut fibre and mixed vegetables with and without lime oil emulsion at 0.3, 0.5 and 0.7% w w^{-1} are presented in Fig. 1. Lime oil in coconut fibre pad at 0.3, 0.5 and 0.7% w w^{-1} can reduce mesophilic bacteria for 0.7 and 1.2 $\log_{10}$ cfu g^{-1}. In addition, they can decrease psychrophilic bacteria for 0.6 and 0.8 $\log_{10}$ cfu g^{-1}. On the other hand, the coconut fibre pad with lime oil after heat curing showed a higher reduction of both bacteria (mesophilic and psychrophilic) for 2.1 and 1.3 $\log_{10}$ cfu g^{-1} at 0.5 and 0.7% w w^{-1} for 1.3 and 1.9 $\log_{10}$ cfu g^{-1},respectively (Fig. 2). Moreover, heat curing demonstrated that the effect of high temperature enhanced the essential oil in the fibre pad, extending the shelf life of the mixed vegetables inside. Heat curing supported the reduction of mesophilic and psychrophilic bacteria mixed vegetables.

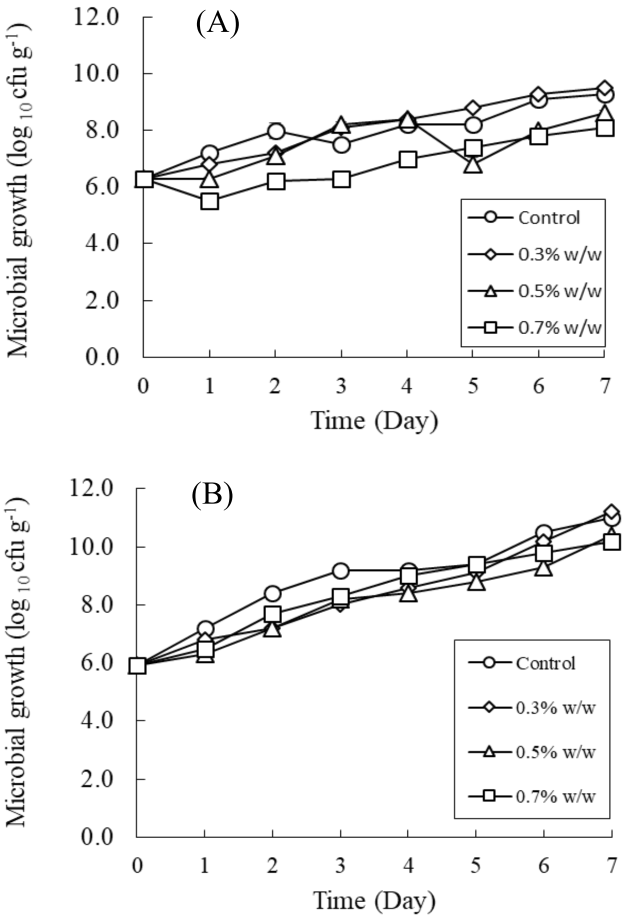

Figure 1. Mesophilic bacteria (A) Psychrophilic bacteria (B) population dynamics in the mixed vegetables kept with coconut fibre pad containing lime oil before heat curing during cold storage at 4-7°C for 7 days.

In conformity with SUHEM *et al*. (2018) reported that the effect of high temperature at 100 °C for 12 hours combined with *Litsea cubeba* oil more than 300 mg g^{-1} can against moulds. Moreover, MATAN *et al*. (2013) investigated the heat curing effect on antifungal activities of lime oil and found that Heat curing at 70°C best enhanced antifungal activity of lime oil against *A. niger* both in medium and on sedge. The temperature for enhancement of lime oil at 100°C can increase antimicrobial activities. ESPINA *et al*. (2012) reported that citrus fruit essential oil can enhance by heat and use with low concentrations of citrus fruit extract with a heat treatment at 54°C for 10 min showed synergistic lethal effects to the inactivation of a bacterial cell with more than 5 $\log_{10}$ cfu ml^{-1}. Consequently, the efficacy of some citrus oil constituents (a-pinene, b-pinene and p-cymene) could be improved when combining them with mild heat. This approach could allow the use of very low doses of antimicrobials. The compositions showed possible synergistic effects in combination with mild heat (AIT-OUAZZOU *et al*., 2011).

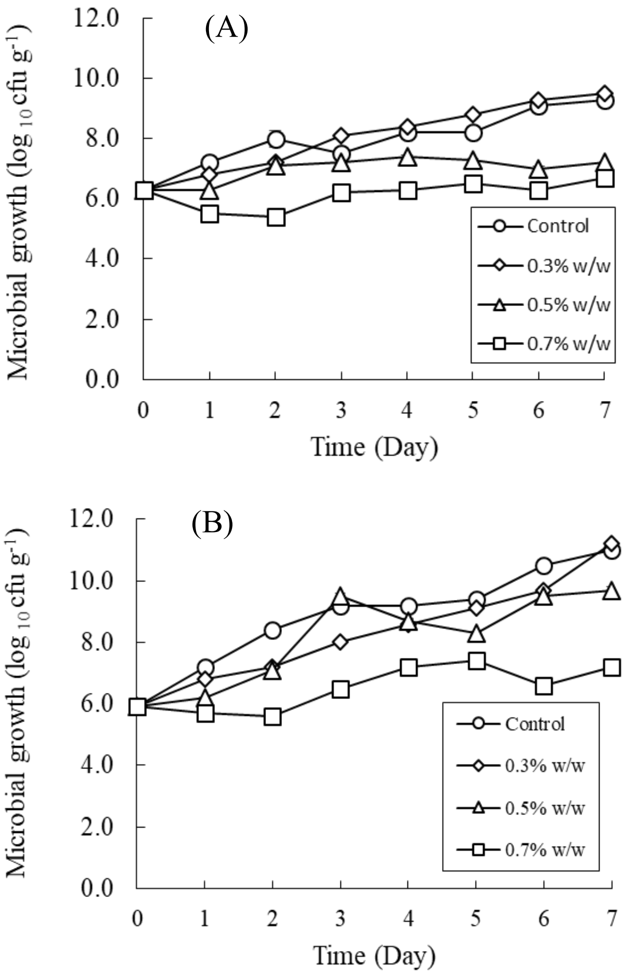

Figure 2. Mesophilic bacteria (A) Psychrophilic bacteria (B) population dynamics in the mixed vegetables kept with coconut fibre pad containing lime oil after heat curing at 100°C for 90 min during cold storage at 4-7°C for 7 days.

Certainly, the use of antimicrobial pad based on the release of volatile antimicrobials compounds and heat curing combined with coconut fibre pad is an alternative technology to improve the microbiological of mixed vegetables. The results from this experiment demonstrated that the combine effect of heat curing and lime oil in coconut fibre pad can increase the efficacy of antimicrobial activity, especially with regard to growth inhibition of mesophilic bacteria and psychrophilic bacteria at the beginning of storage. However, this method have to improve for increasing the antimicrobial activity.

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PHLOROTANNINS CONTENT OF *GRACILARIA* SPP. HYDRO-ETHANOLIC EXTRACTS

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ABSTRACT

In today's world, seaweeds are used by the food industry as food additives because of their technological properties. In the particular case of *Gracilaria* spp., this seaweed is mainly used for the extraction of agar-agar. Phlorotannins are phenolic compounds, which are produced as secondary metabolites by plants for their natural defense against external stimuli. Therefore, the main objective of this study was to produce hydroethanolic extracts from *Gracilaria* spp. and measure their content in phlorotannins. The results showed that the extracts obtained from the freeze-dried *Gracilaria* spp. with 75 % ethanol, presented the highest content in phlorotannins and they are a potential candidate to be used to produce antioxidant active food packaging.

Keywords: algae, extracts, food additives, *Gracilaria*, ghlorotannins

1. INTRODUCTION

Phenolic compounds are secondary metabolites produced by terrestrial and aquatic plants for their natural defense against external stimuli, such as radiation and predators (Andrade, Ribeiro-Santos, Costa Bonito, Saraiva, & Sanches-Silva, 2018). They have proven powerful biological activities such as antioxidant and antimicrobial activities, which are very important for the food industry. Food additives are applied to foods to prevent or delay their natural degradation and microbial spoilage. The most used food additives by the food industry are synthetic ones, because of their easy application, economic value and chemical stability. However, there has been evidence of adverse effects on human health, such as carcinogenesis promotion and neurodegenerative diseases (CAROCHO *et al*., 2015; PEREIRA DE ABREU *et al*., 2010; SANCHES-SILVA *et al*., 2014). Natural extracts and essential oils can be a valid alternative for these synthetic additives since they have a high content of phenolic compounds and, consequently, possess powerful antioxidant and antimicrobial activities.

Seaweeds are used by the human kind since the very beginning of civilization, especially in Asian cultures. Nutritionally, these type of plants possess a higher mineral content than terrestrial plants and a low fat content, being a good subject to nutritional supplements to overcome some nutritional deficiencies (AGREGÁN *et al*., 2017; BALINA *et al*., 2016; CHAPMAN and CHAPMAN, 1980; NISIZAWA *et al*., 1987). *Gracilaria* spp. is actually used by the food industry in the extraction of agar-agar, a polysaccharide with important technological properties. *Gracilaria* spp. is a red seaweed (Rhodophyta) with proven anti-viral and antimicrobial activity (BIANCO *et al*., 2015).

The objective of this study was to produce hydroethanolic extracts from dried and freeze-dried *Gracilaria* spp. and to evaluate their content in phlorotannins.

2. MATERIALS AND METHODS

Fresh *Gracilaria* spp. (2 kg) was acquired from ALGAPlus™ in October 2018. Seaweed were washed three times with tap water in order to remove impurities and separated in half. One of the halves was placed in an oven at 30 ºC for 10 days, protected from the light. The other half was freeze at -80 ºC and freeze-dried in a Heto PowerDry PL 9000 from Thermo Scientific for 3 days. Then, the samples were grinded using a granulator mill Grindomix GM200 (Haan, Germany) and kept at 4 ºC, protected from the light, in vacuum conditions. For the extraction process, 5 g of the dried or freeze-dried seaweed were mixed with 50 ml of solvent (100 % ethanol, 75 % ethanol (v/v), 50 % ethanol (v/v), 25 % ethanol (v/v), 100 % water). The mixtures were homogenized for 30 min in a compact stirrer Edmund BühlerTM Digital Compact Shaker KS 15 A (Hechingen, Germany) at 350 rpm and, centrifuge at 11952 g, for 15 min at 15 ºC. The supernatant (Fig. 1) was removed to a plastic container, which was placed in a kiln, at 37 ºC for days, protected from the light. The powder was removed with the aid of a spatula and diluted at 5 mg/ml. The extracts obtained with 100 % ethanol, 75 % ethanol and 50 % ethanol were diluted in dimethyl sulfoxide (DMSO) and the extracts obtained with 25 % ethanol and 100 % water were diluted in water.

For the determination of the total content in phlorotannins (TPC), two-calibration curves were drawn using phloroglucinol as a standard diluted in water and DMSO. The method performed for the TPC was described by WANG *et al*. (2012) and the results are expressed in mg of phloroglucinol equivalents per g of extract (mg PGE/g).

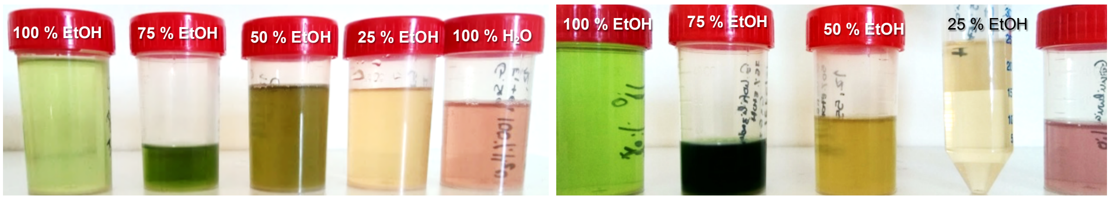

Figure 1. Hydroethanolic extracts from the freeze-dried and the dried *Gracilaria* spp.
Extracts from the dried *Gracilaria* spp.(left). Extracts from the freeze-dried *Gracilaria* spp.(right)

Briefly, to 1 ml of sample, 5 ml of Folin-Ciocalteu regent diluted in water (10 %, v/v) were added and the solution homogenized. After 5 min, 4 ml of a sodium carbonate aqueous solution (7.5 %, w/v) were added. The solutions were homogenized and kept in the dark for 120 min at room temperature (23 ºC ± 1). The absorbance was read in a ThermoScientificTM Evolution 300 LC (serial no EV3-155003) spectrophotometer at 725 nm.

3. RESULTS

The hydroethanolic extracts are compared according to their content in phlorotannins content. Regarding the calibrations curves, the calibration curve using phloroglucinol dissolved in water was y = 6.8357x - 0.067 with a work range between 0.010 and 0.125 mg/ml (r^2=0.999), and the calibration curve drawn using phloroglucinol dissolved in DMSO was y=6.2931x + 0.0312 with a work range between 0.010 and 0.150 mg/ml (r^2=0.9998). As can be observed in Fig. 2, the extract obtained from the freeze-dried seaweed with 75 % ethanol presented the highest value of phlorotannins. Contrary to what was expected, the 100 % ethanolic extract obtained from the freeze-dried seaweed presented the second lowest phlorotannins content. The extracts obtained from freeze-dried *Gracilaria* spp. with 75 % and 50 % ethanol showed the highest content in phlorotannins and have potential to be used by the food and food packaging industries. Although they are used since ancient times for health purposes, such as anticoagulant and antimicrobial activities, red seaweeds are largely used by industries as a source of agar, a typical phycocolloid of red algae (TORRES *et al*., 2019).

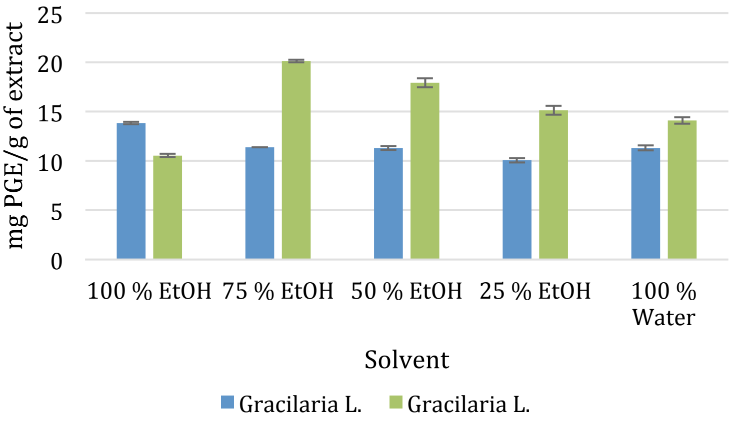

Figure 2. Results of the determination of the phlorotannins content (the results are expressed as mean ± standard deviation).

However, other studies shall be carried out to confirm the antioxidant potential of hydroethanolic extracts of *Gracilaria* spp. and to evaluate their antimicrobial potential. Also, due to their high content in polysaccharides, *Gracilaria* spp. can be used in the manufacture of food packaging applications, contributing to minimize the accumulation of plastics in the environment.

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NEW ANTIOXIDANT LDPE FILMS CONTAINING APPLE AND GINGER EXTRACT

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ABSTRACT

In recent years, a new type of packaging that merges usual food packaging materials with antioxidant substances to extend shelf-life is gaining interest in the packaging industry. Various natural antioxidant compounds can be combined with different types of packaging materials to replace the chemical additives in food industries. In this work, the most suitable fruit extract between apple/ginger and apple/ginger/carrot/beet in term of antioxidant activity has been determined. LDPE-based films have been loaded with apple/ginger (50/50) extract by a melt mixing and compression process. Structural and functional properties of the obtained active films have been investigated together with their antioxidant activity.

Keywords: bioactive film, food packaging, natural antioxidants

1. INTRODUCTION

The interest in the development of antioxidant packaging materials containing natural fillers is growing due to consumers concerns towards the importance of healthy food consumption and improvement of life quality and about health-related issues, such as the use of synthetic antioxidant agents. The production of antioxidant films containing fruit extract also helps to overcome the issue related to the growing quantity of by-products (BP) from food industry. The increasing awareness of consumers towards the importance of healthy food consumption in disease prevention and improvement of life quality has promoted the consumption of fruits and vegetables with consequent generation of more by-products. The waste generated by the food industry is currently an environmental concern at global scale. In fact, fruits processed for the production of juices and pulps generate about 40 to 50% of agro-industrial waste.

2. MATERIALS AND METHODS

2.1. Materials

The antioxidant packaging system was obtained by directly incorporating the fruit extract ginger/apple 50:50 extract supplied by Frubaça - Cooperativa de Hortofruticultores, CRL, Portugal, into the polymeric matrix (LDPE supplied by Polimeri Europa, Italy) through mixing and compression steps.

2.2. Film preparation

Active films were produced by preparing an active masterbatch using a counter-rotating mixer, where a known amount of apple/ginger extract (5%w/w) was loaded into the polymeric matrix. The mixing is carried out by loading the two components into the hopper at the processing temperature of 180°C at a rotation speed of 50 rpm, for about 5min. The obtained mixture was collected and subsequently submitted to the compression molding process at a temperature higher than its melting point, then the melted polymer is processed using a Collin P300P press under a pressure of 50 bar. The obtained active films (thickness around 100 micron) were characterized to study the physico-chemical and structural properties of the material and the effect of the antioxidants on the mixing.

2.3. Methods

The thermal stability of the samples was investigated by using a TGA Q5000 TA Instuments and a DSC TA Instrument. Measurements were performed using 10 mg circa of sample, at a heating rate of 10°C/min, between 40 and 800°C in a nitrogen atmosphere.
The Attenuated Total Reflectance (ATR) spectra of all samples were collected with a FT-IR Frontier Dual Range Perkin Elmercon instrument with a 4 cm^{-1} resolution, in a wavelength range between 4000-400 cm^{-1} and with 32 scan. Mechanical properties were investigated by performing tensile tests using an Instron Machine.
By-products extraction: Fifty ml of ethanol were added to 5 g of each sample. The mixture was homogenized at room temperature (±23ºC) and centrifuged. The extract was evaporated till dryness in a rotary evaporator.
The Total Phenolics Content (TPC) and Total Flavonoids Content (TFC) were evaluated for ethanolic extracts of both by-products. The TPC method was adapted from the method of

ERKAN *et al*. (2018) and the TFC method was adapted from the method of YOO *et al*. (2008).

3. RESULTS AND CONCLUSIONS

In this work, in order to choose the most suitable extract for the development of active polymeric films, the antioxidant capacity of two different industrial fruits by-products, BP1 (apple and ginger (50:50, w/w)) and BP2 (apple, carrot, beet and ginger (50:29:20:1, w/w/w/w), both frozen and freeze-dried, have been evaluated and compared. Results, reported in Fig. 1, show that the apple/ginger extract presents higher antioxidant capacity than the apple/ginger/carrot sample, in both the fresh and freeze-dried samples, probably due higher amount of the ginger. Indeed, it contains the bioactive compounds, such as pyrogallol p-hydroxybenzoic acid, ferulic acid and p-coumaric acid, which are reported to have high antioxidant activity. In both assays, the freeze-dried sample BP1(A) presented higher antioxidant capacity than the corresponding extract obtained from the fresh sample, proving that freeze-drying is an effective method of preserving this by-product. This effect was not observed in the BP2(B). On the basis of these results, freeze-dried apple/ginger (50/50) extract has been selected as the best additive to confer antioxidant activity to the developed active polymeric films.

The obtained films show good thermal properties, it is possible to observe a small increase in stability of the film containing the filler, in particular a slight increase in the initial degradation temperature of the polymer (Fig. 2).

The loaded and pristine LDPE films were analyzed by ATR and their spectra were compared (Fig. 3). Active film has the same characteristic peaks of pristine LDPE. In fact, it is possible to notice the presence of two very intense peaks in the area of 3000 cm^{-1}, in particular there are two peaks at 2915 e 2848 cm^{-1} that indicate respectively a strong asymmetric and symmetrical stretching of the CH_2 group. While at 1471 and 1463 cm^{-1} peaks are characteristic of bending of the CH groups. Moreover, there is also a lower wavelength peak, at 718 cm^{-1}, which is typical of LDPE and represents the rocking of the C-C group.

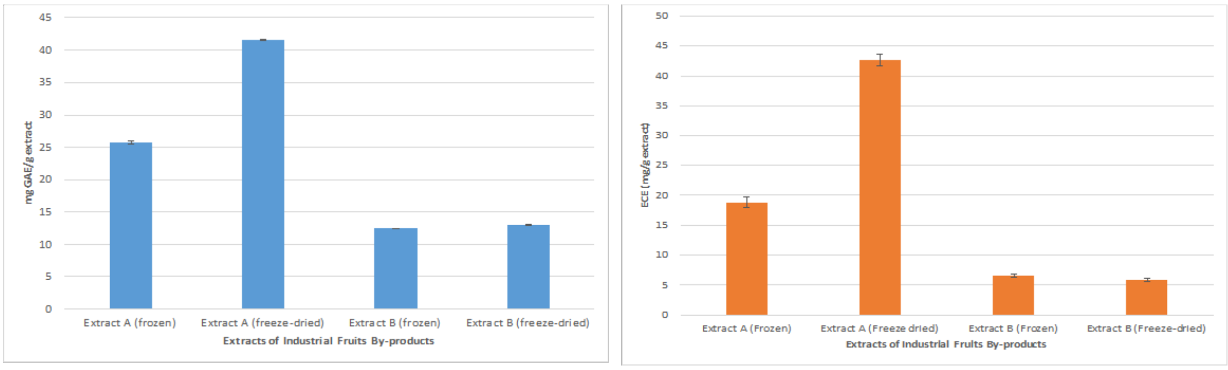

Figure 1. A. Total Phenolic Contents (TPC); B. and Total Flavonoids Contents (TFC) for by-products, A= BP1 and B= BP2, frozen and freeze-dried, respectively.

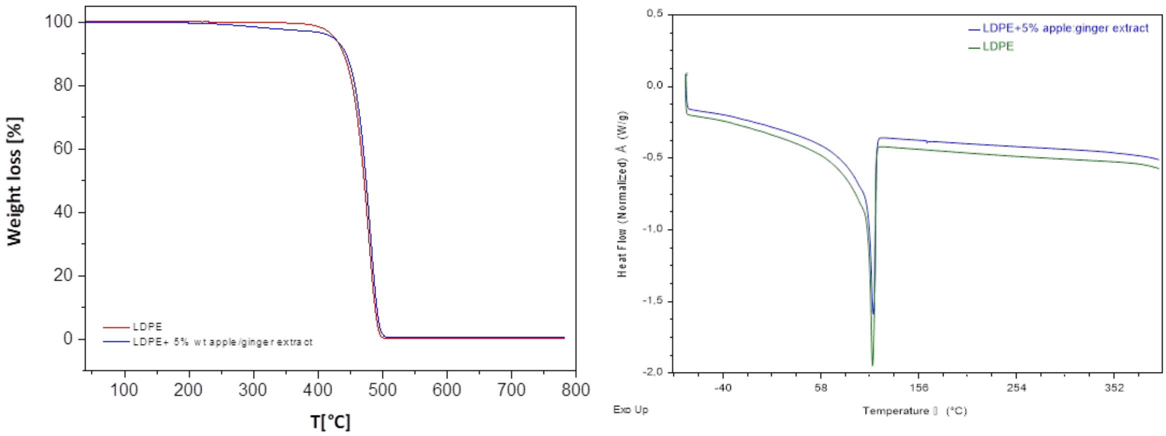

Figure 2. TGA and DSC curves of pristine and loaded LDPE films.

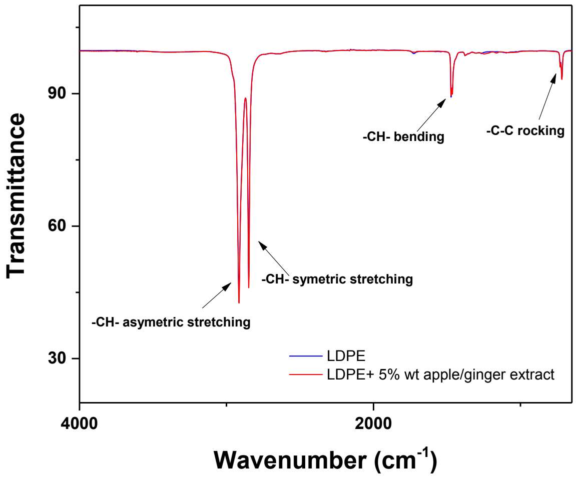

Figure 3. ATR analysis of pristine and loaded LDPE films.

Permeability tests show a slight increase of water permeability values when apple/ginger extract is added to the polymer. This result does not significantly affect the performances of the resulting material. On the contrary, an improvement in mechanical properties can be observed from data reported in Table 1.

Table 1. Tensile strength, elongation at break, elastic modulus of pristine and loaded film.

Film	σ [MPa]	ε [%]	E [MPa]
LDPE	10.57	253	37.5
LDPE/Apple:Ginger	10.60	274	111

Active film was characterized through antioxidant tests (Fig. 4) and showed an increase in the antioxidant activity (AO) of the film when the fruit extract is added to the polymer matrix.

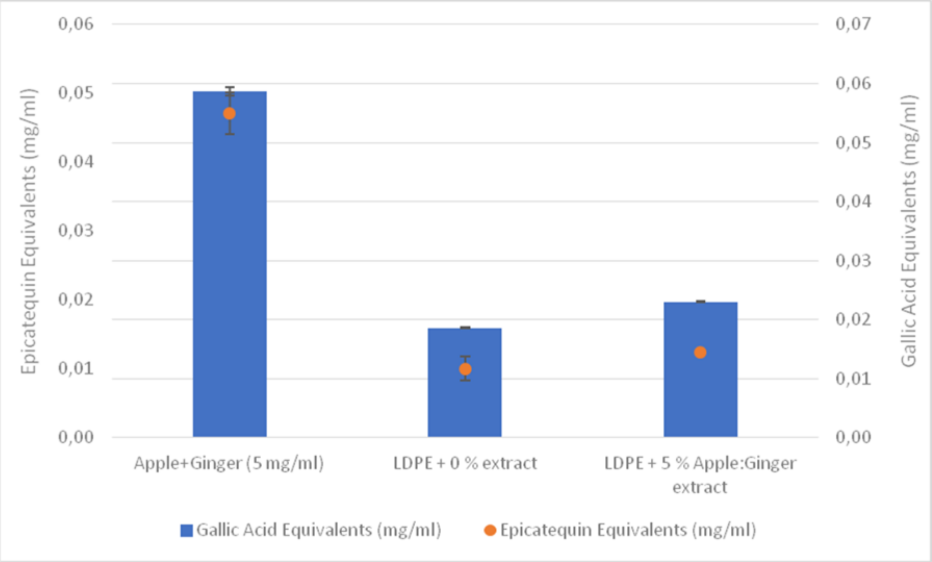

Figure 4. Total Phenolic Contents (TPC) and Total Flavonoids Contents (TFC) of pristine LDPE and LDPE+apple/ginger

Results show that the total phenolic and flavonoid content in individual fruits was highly correlated with antioxidant activity. According with this, it possible to say that the apple/ginger extract present good antioxidant capacity and that its presence imparts a very good AO activity to the LDPE film. In conclusion, industrial fruit by-products containing phenolics compounds, namely flavonoids, can be extracted an used to develop active films, which exhibit good structural and functional properties, to be further used and tested for the extension of shelf-life of real packaged foodstuff.

ACKNOWLEDGEMENTS

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DEVELOPMENT OF CUTIN-BASED ANTIOXIDANT FILMS

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ABSTRACT

Polyphenolic-rich and/or carotenoid-rich extracts from tomato possess potential biological properties, such as antioxidant activity, extremely valuable for the food, cosmetic and pharmaceutical industries. Cutin, a component of cuticle of tomato peels, was blended with thermoplastic polymers such as low-density polyethylene and amorphous vinyl alcohol with low environmental impact to produce active flexible food packaging films by using conventional manufacturing processes. The polymer formulations containing the polyphenolic-rich and/or carotenoid-rich extracts were characterized to study the physicochemical, structural properties and the additive induced effects on the functional properties of the obtained active films.

Keywords: antioxidants, active food packaging

1. INTRODUCTION

In recent years, the concern to provide consumers with high quality foodstuffs has led to the adoption of measures to limit the oxidation phenomenon during the processing and storage of products (VILARHINO *et al.*, 2018). The combination of active and biodegradable packaging represents an interesting solution to promote the shelf life extension of packaged foodstuff and to reduce environment pollution related to the accumulation of plastic wastes. Active packaging is a widely accepted term indicating packaging systems that directly interact with the enclosed food by delaying or inhibiting those phenomena, responsible for food quality decay. Among commercially available polymeric matrices, the low density polyethylene (LDPE), a traditional polymer with low environmental impact, has attracted great interest and, recently a new biodegradable polymeric material (Nichigo G-Polymer) has received considerable interest from both scientific and industrial point of view due to its superior gas barrier properties, quickly dissolution in water even at low temperature, compostability and easy processability by all production techniques.

The search of natural antioxidant substances to be added as fillers to polymeric matrices is attracting a great interest in the field of food packaging due to its advantages compared with the addition of antioxidants generally in contact with the food. Protection against abiotic stress factors (i.e. water loss, high density of UV and visible radiation or extreme temperatures) and abiotic threats (i.e. the attack of pathogens or herbivores) is generally the most fundamental issue concerned with fruit and/or tomato surfaces (XIA *et al.*, 2010). The cuticle, with its composite and heterogeneous structured layer plays a crucial role in the physiological interaction with the cell wall underneath (DOMÍNGUEZ *et al.*, 2011).

In the present work, antioxidant active films, based on LDPE and G-Polymer, were manufactured and characterized in order to study the physicochemical and structural properties, investigating the cutin loading effect on the functional properties of the films.

2. MATERIAL AND METHODS

2.1. Materials

Cutin extract was supplied by the Institute National de Recherche et d’Analyse Physico-chimique (INRAP, Tunisia). G-Polymer OKS 8049 (GP), amorphous vinyl alcohol, was supplied by Nippon Gohsei. Low density polyethylene (LDPE) was purchased by Polimeri Europa (Italy).

2.2. Cutin extraction

Tomato pomace collected from a tomato processing industry (Sidi Thabet, Tunisia) was immersed into 3 wt. % NaOH solution and autoclaved at 120°C for 2 h. The resulting liquid phase was collected by filtration and then acidified using a 6 M HCL solution till pH 5-6 for cutin precipitation. The obtained suspensions were centrifuged (30 min, 4000 rpm) and freeze-dried to recover cutin monomers.

2.3. Active film preparation and characterization

The process variables for the production of films containing active substances have been identified and optimized. The LDPE and G-Polymer films containing the active substance (5 % wt. of cutin) were prepared by direct melt processing by following two steps procedure: melt mixing and hot compression. In detail, the hot mixing was characterized by rotation speed of 10 rpm and subsequent to 50 rpm both for about 5mins; the compression stage was carried out at 50 bar pressure on the molten polymer placed between two flat plates of a Collin P300P press. Active films with a thickness of about 100 microns containing the active substances were produced. Pristine GP and LDPE based film were manufactured by using the same process for comparison purpose.

Thermogravimetric analysis (TGA) was carried out by using a TGAQ500, TA Instruments. The samples were heated in nitrogen flow up to 800°C with a heating rate of 10°C/min.

Fourier transform infrared spectroscopy (FT-IR): FTIR spectra were recorded at room temperature by using a Nicolet FT-IR spectrometer (Thermo Scientific, Italy) in Attenuated Total Reflectance (ATR) mode from 400 to 4000 cm^{-1} operating in single reflection.

Cutin release tests were carried out by determining the amount of cutin released from LDPE and GP active films in 96% v/v ethanol, used as food simulants for migration tests[6]. The amount of cutin released from the polymeric films at 25°C was determined by using an Agilent Technologies Cary 60 UV-Visspectrophotometer. The calibration curve was obtained by plotting the peak height of cutin at 284 nm versus cutin concentration of standard solutions from 10 to 100 ppm.

Water vapour permeability was measured by using infrared sensor technique by means of a PermatranW3/31 (Mocon, Germany). Samples with a surface area of 5 cm^2 were tested at 25°C. Permeation tests were performed by setting the relative humidity at the downstream and upstream sides of the film to 0% and to 50% respectively. A flow rate of 100 ml/min of a nitrogen stream was employed. Each test was carried out in duplicate.

Contact angle measurements were carried out by using a DataPhysics OCA 20 apparatus. Water was dropped into at least 10 different sites on each sample, and the static contact angle was reported as the average value from the measured ones.

Mechanical tests in simple tension were performed by using an Alpha Technologies Tensometer Mod. 2020, equipped with a 5kN load cell according the standard ASTM D882-02.

Antioxidant activity was determined using DPPH and ABTS free radical scavenging assays. The DPPH and ABTS scavenging activities were calculated against a calibration curve established with Trolox and expressed as μmol Trolox equivalent/g film according to DOU *et al*. (2018) and CHENG *et al*. (2015), respectively.

3. RESULTS

Manufactured films were characterised to investigate the effect of loaded cutting over thermal stability and diffusion properties of the hosting polymer. Interesting results were revealed by the experimental data. Fig. 1 reports the temperature dependent mass loos behaviour of the two polymers and corresponding loaded systems. In both cases, the effect of the loaded cutin is very negligible. In details, cutin slightly reduces the degradation time of GP based film, whereas in LPDE this effects if only reported at $T>T_{onset}$ (~ 410°C).

FTIR spectra (Fig. 2) highlight that in both cases cutin characteristic peaks are present in both active films. In particular, for GP/Cutin (Fig. 2b), strong features of long chain aliphatic compounds (i.e. bands assigned to asymmetric and symmetric CH_2 stretching at 2,918and 2,849 cm^{-1} and CH_2 bending at 1,462 cm^{-1}) could be identified. Whereas in the case of LDPE based film additionally, the presence of ester functional groups assigned to cutin was revealed by the 1,732-cm^{-1} weak band and by the partially masked vibrations at 1,159and 1,104 cm^{-1} (asymmetrical and symmetrical C-O-C stretching, respectively). The band at 1,034 cm^{-1} (medium intensity) was assigned to glycosidic bonds typical of polysaccharides. The band appearing at approximately 1,688 cm^{-1} was associated with free carboxylic acid functional groups. Vibrations around 1,640 and 1,515 cm^{-1} were assigned to the stretching of C=C bonds and the stretching of aromatic rings, respectively.

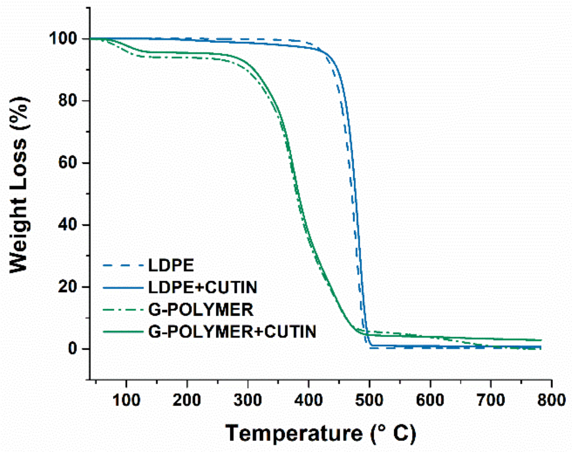

Figure 1. TGA curves respectively for LDPE, LPDE/Cutin, GP and GP/Cutin films.

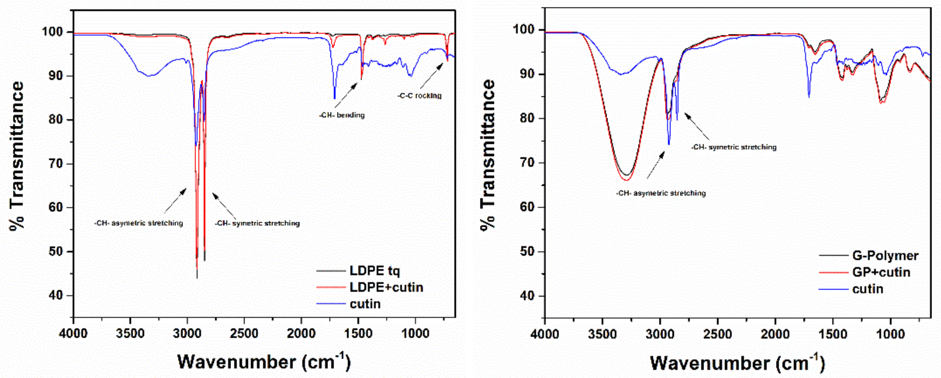

Figure 2. FTIR spectra of LDPE, LPDE/Cutin, GP and GP/Cutin films.

Fig. 3 shows the results of water vapour permeability data for the pristine and active films along with the corresponding contact angle images. The presence of cutin does not modify significantly the water permeability of the LDPE based film as the obtained values are within the error rage of the data, whereas the presence of active filler slightly increases the barrier properties in the case of GP based film. It is worth noting that water contact angle values of pristine LDPE and LDPE /Cutin films are respectively 81.1°, and 83.8°, these values confirm that the presence of cutin does not modify significantly the characteristic hydrophilicity behaviour of LDPE.

In the case of GP/Cutin film the contact angle value is 80.5°, which is higher than the corresponding pristine GP film value (72.8°) and, thus, showing a more hydrophobic character of the active films. Likely hydrophobicity of GP-based active films plays a role in the improvement of water barrier properties by repulsing water molecules (BRAS *et al.* 2007).

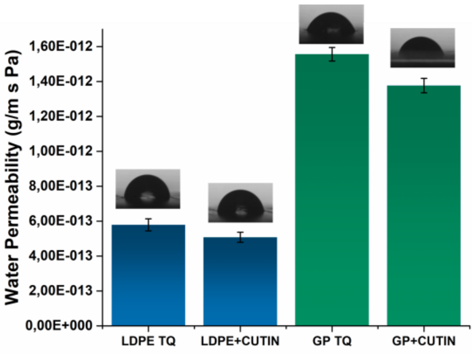

Figure 3. Water permeability and contact angle images of LDPE, LPDE/Cutin, GP and GP/Cutin films.

Experimental data for the release kinetics are reported in Fig. 4. It is clearly shown that cutin is poorly not released by the LPDE films over the whole time period, up to 200 hours, likely due to chemical bonds with polymer structure. Instead, from GP films cutin is progressively released up to ≈ 80% of the total amount.

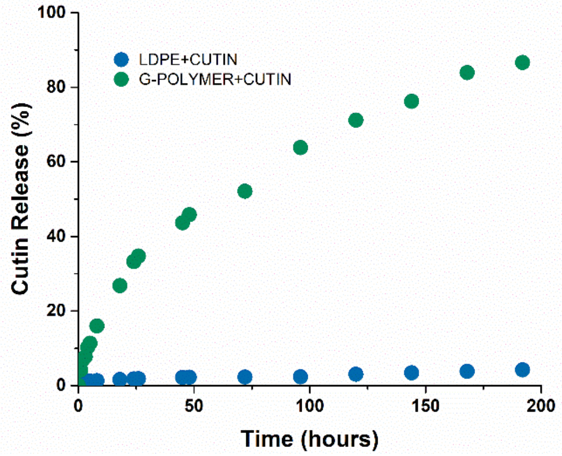

Figure 4. Kinetics of Released cutin curves for LDPE, LPDE/Cutin, GP and GP/Cutin films.

The effect of the cutin was also investigated by testing in tensile configuration mode pristine (LPDE and GP) and active (LPDE/Cutin and GP/Cutin) films. Results are summarised in Table 1, reporting the measured values respectively for tensile strength, elongation at break and Young modulus.

Table 1. Mechanical parameters from tensile test for (LPDE, GP) and active (LPDE/Cutin, GP/Cutin) film.

	Tensile Strength (Mpa)	Elongation at break (%)	Young Modulus (Mpa)
Pristine LPDE	11,51	282,11	292
LPDE/Cutin	9,93	69,61	299
PristineGP	8,35	419,18	266
GP/Cutin	6,50	307,93	240

The main effect induced by the cutin loading on the produced films is a tensile and elongation decrease with a reduced level of plasticity, being the value of the modulus almost constant in the case of the LPDE and reduced of about 10 % for the GP active films. For both LDPE and GP active films, the change in tensile strength is of the order of 15-20%. Decrease of tensile strength and elongation at break could indicate that the filler in the matrix probably acts as a weakening constituent making the polymers less capable of sustaining the applied load.
Kinetics curves of the cutin release indicate that the filler is completely frozen into the hosting LDPE with a very negligible percentage of filler loss thus antioxidant activity analysis was performed only on the GP based samples, as indicated in Table 2.

Table 2. Antioxidant activity index for GP-based films.

	DPPH - scavenging activity (μM trolox equivalent/g film)	**ABTS - scavenging activity (μM trolox equivalent/g film)**
Pristine GP	2.34±0.03	5.72±0.83
GP+Cutin	3.32±0.21	8.93±0.20

The activity analysis of cutin loading GP films highlight that the cutin acts as a potential antioxidant agent, increasing the DPPH and ABTS index respectively of 42% and 56% compared to the corresponding unfilled polymer.

4. CONCLUSIONS

Results highlighted that LDPE based films do not show any interesting antioxidant activity due to the fact that cutin is not released toward the food simulant. On the contrary, the good antioxidant activity of GP-based films has been shown through in vitro antioxidant assays. Thus, natural antioxidant cutin can be employed as filler for G-Polymer matrix to develop active food packaging systems potentially able to extend the shelf-life of real packaged foodstuff.

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ANNEALING OF ACID-MODIFIED RICE STARCH TO USE AS A THICKENING AGENT

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ABSTRACT

Acid-modified rice starch (AMRS) was annealed to improve rheological properties. High, medium and low-amylose rice starches were hydrolyzed using methanol in 0.36% HCl at 25°C for 0, 16 and 40 h and were annealed at 50-60°C for 72 h. Annealing improved thermal & shear stability and reduced retrogradation. AMRS paste exhibited non-Newtonian flow. The consistency coefficient (K) and apparent viscosity (ηa,100) decreased with hydrolysis time. High-amylose starch hydrolyzed for 16 and 40 h had a flow behavior index (n) and K comparable to commercial chili and tomato sauce.

Keywords: annealing, acid-modified, rice starch, steady shear viscosity, thickening agent

1. INTRODUCTION

Native rice starch is poor in thermal, shear and acid stability; moreover, its paste is easily retrograded. For commercial application, rice starches are often chemically modified to promote such properties (JAYAKODY and HOOVER, 2008). Hydrothermal modification of acid-modified rice starch (AMRS) was proposed in our study to obtain rice paste with proper rheology, flow behavior and stability for thickening purposes. Acid-modified starch has greater hot water solubility, lower hot paste viscosity, and higher gelatinization temperature, and undergoes less retrogradation. These properties result from a decrease in chain length of starch polymers and an increase in the crystalline portion (OTT and DAY, 2000). Acid attacks the non-specific types of linkages, especially in the amorphous regions. Acid attacks the semi-crystalline shell more easily due to its less crystalline organization (GALLANT *et al*., 1997). A change of D-glucopyranosyl conformation from chair to half-chair is more difficult for a closely packed helical structure (KAINUMA and FRENCH, 1971; FRENCH, 1984; ROBIN and MERCIER, 1974). Highly crystalline dextrin has been prepared from hydrolyzed rice starches with 2-4% hydrochloric acid in 70%, 85% and 95% ethanol solutions for 5 h. (CHUN *et al*., 1997).

Acid modification changes the physicochemical properties of starch without destroying its granular structure (SHI and SEIB, 1992). The recovery of starch granules after acid-alcohol treatment can be as high as 90% in potato starch and 88% in waxy maize starch (CHANG *et al*., 2004; FOX and ROBYT, 1992; LIN *et al*., 2003). The solubility of acid-treated starch increases, while the swelling power, pasting viscosity and gel strength decrease (CHANG *et al*., 2004; LIN *et al*., 2003; SODHI *et al*., 2009). The temperature range of the gelatinization endotherm was broadened when hydrolysis increased, but its enthalpy was only slightly affected (CHUN *et al*., 1997; JENKINS and DONALD, 1997). The average degree of polymerization (DP) of starch progressively decreased in the order of methanol>ethanol>2-propanol>1-butanol (MA and ROBYT, 1987). Annealing can change the physicochemical properties of starch by improving its crystalline perfection and facilitating interactions between starch chains (JACOBS and DELCOUR, 1998). Annealing was reported to increase the pasting temperature and thermal stability and decrease peak viscosity and setback (JACOBS *et al*., 1996; HOOVER and VASANTHAN, 1993). The viscosity reduction and shear stability improvement of annealed starch contribute to the reduction in granular swelling and amylose leaching, together with an increase in the interaction between starch chains during annealing (HOOVER and VASANTHAN, 1993; JACOBS *et al*., 1995). The effect of various molecular sizes of rice starch on annealing has been studied; it was found that the onset and peak temperatures (T_o and T_p) of acid-treated starches were increased after annealing (LIN *et al*., 2008). This suggested an increase in the thermal stability of starch granules after a combination of acid-methanol and annealing treatment.

2. MATERIAL AND METHODS

2.1. Rice flour preparation

Three rice cultivars Chai Nat 1 (CN 1), Khao Dawk Mali 105 (KDML 105) and Rice Division 6 (RD 6) were obtained from the Rice Department of Thailand. Rice flour was prepared by wet milling using a double-disk stone mill and dried in a hot-air oven at 40°C.

2.2. Acid modification

Starch (25 g) was suspended in 99% methanol (100 ml), HCl (36% w/w) (1 mL) was added, stirred and maintained at 25°C for 0, 16 and 40 h. The reaction was stopped by neutralizing with 1 M $NaHCO_3$ (14 mL) and cooling in ice bath. AMRS was centrifuged at 3500 *g* for 5 min, washed with 50% ethanol four times. The precipitates were oven-dried at 40°C until the moisture content reached 10-12%.

2.3. Preparation of annealed starch

AMRS (100 g) was suspended in water (300 mL) containing 0.02% sodium metabisulfide to prevent spoilage. The annealing process was conducted in a water bath for 72 h at 60°C for CN 1, and 50°C for KDML and RD 6, centrifugation (3500*g*, 5 min), washed with distilled water and dried at 35°C until MC 10-12%.

2.4. Gelatinization thermal properties

Thermal properties of native and annealed starches were determined using DSC (micro DSC VII, SETARAM Instruments, Japan). The samples were heated from 25 to 95°C heating rate 1.0°C/min.

2.5. Pasting properties

Pasting properties of all native, AMRS and anneal-AMRS were determined using a rapid visco analyzer (model RVA-3D; Newport Scientific, Australia). Using rice starch profile (AACC, 2000).

2.6. Steady shear viscosity measurements

Native and anneal-AMRS slurries (8% w/w) were stirred continuously at 220 rpm for 10 min, heated to 95°C, held at 95-98°C for 30 min, and cooled down to 40°C, centrifuged 190*g* for 2 min. Steady shear viscosity was measured using rotational mode of a stress-controlled rheometer (Physica MCR 301, Anton Paar, Stuttgart, Germany) equipped with 25 mm diameter cone-and-plate geometry, 2°cone angle and 0.21 mm gap, shear rate 0.0003-100 s^{-1} at 25°C. Shear viscosity of commercial sauces was determined under the same condition.

2.7. Statistical analysis

Completely randomized design was used as an experimental design. Statistical analysis was carried out using SPSS version 12. Differences in means were calculated using ANOVA at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1. Thermal properties of annealed acid methanol modified rice starch

Thermal properties showed that T_o and T_p of all rice starches increased after annealing (Fig. 1). The positive values of dT_o and dT_p indicated that annealing could improve the thermal stability of both native and acid–methanol-treated rice starches. However, ANOVA results showed an insignificant difference in thermal properties changes before and after annealing of starches with different acid methanol treatment times. There was no obvious difference in enthalpy change (dΔH) for KDML 105, whereas the difference was more pronounced for CN 1.

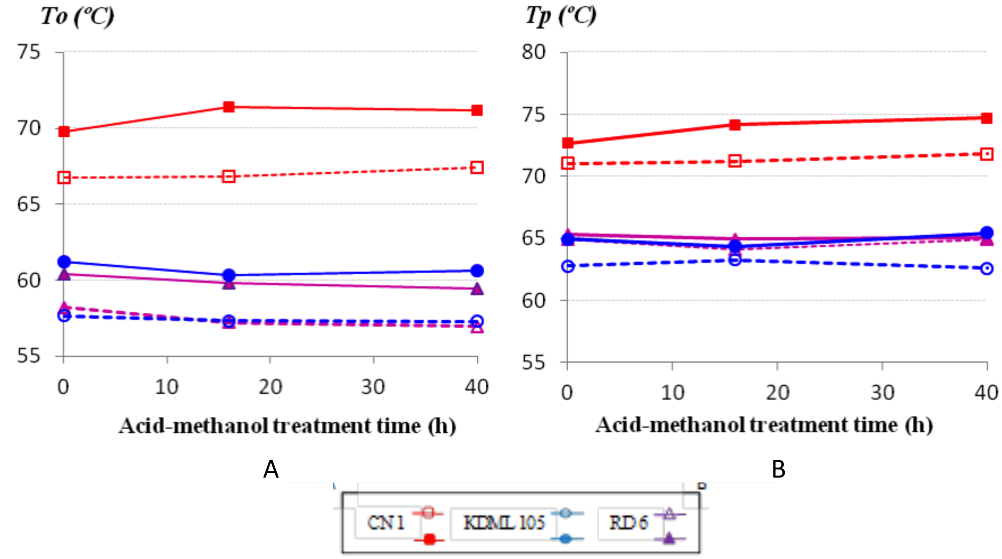


Figure 1. Gelatinization onset temperature (A) and gelatinization peak temperature (B) of acid-methanol-treated rice starches before (open symbols) and after annealing (closed symbols).

3.2. Pasting properties of annealed acid methanol modified rice starch

Breakdown of high-amylose rice starch (CN 1) was less susceptible to acid methanol treatment (Fig. 2). The decrease in peak viscosity with increasing hydrolysis time could be attributed to the degradation of amylose and amylopectin long chains, leading to a decrease in swelling power and increase in solubility. The decrease in setback of starch treated by acid methanol indicated less recrystallization of gelatinized starch during cooling. Pasting temperature of KDML 105 and RD 6 treated by acid methanol for 40 h was nearly undetectable. This was similar to the results of LIN *et al*. (2003) who found that RVA parameters of acid modified maize and potato starches could not be observed after 24 h under similar conditions.

Annealing increased the pasting temperature of all starches. The strengthening of intragranular bonds resulted in more heat being required for structural disintegration before paste formation occurred (GOMEZ *et al*., 2004; ADEBOWALE *et al*., 2009). However, the peak viscosity and hot paste viscosity of all native and AMRS starches decreased after annealing (Fig. 2). This accounted for the reduction in granular swelling and amylose leaching (HOOVER and VASANTHAN, 1993; STUTE, 1992). The decrease in breakdown of annealed starch gel during heating and mechanically applied shear indicated high stability of the starch. The improved shear stability after annealing was enhanced by an increase in the interaction between starch chains (HOOVER and VASANTHAN, 1993). The setback of annealed starch was decreased because of the decrease in retrogradation after annealing. Annealing promoted the reorganization of starch molecules, causing a more stable conformation and hence a decrease in amylose leaching (GOMEZ *et al*., 2004).

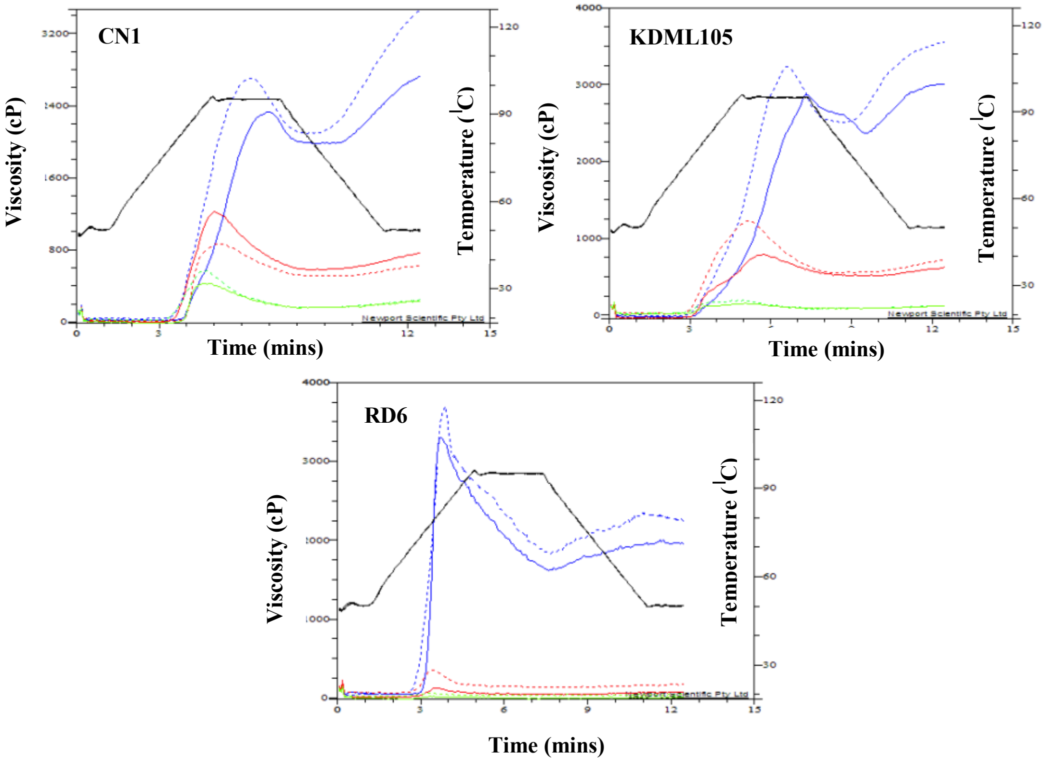


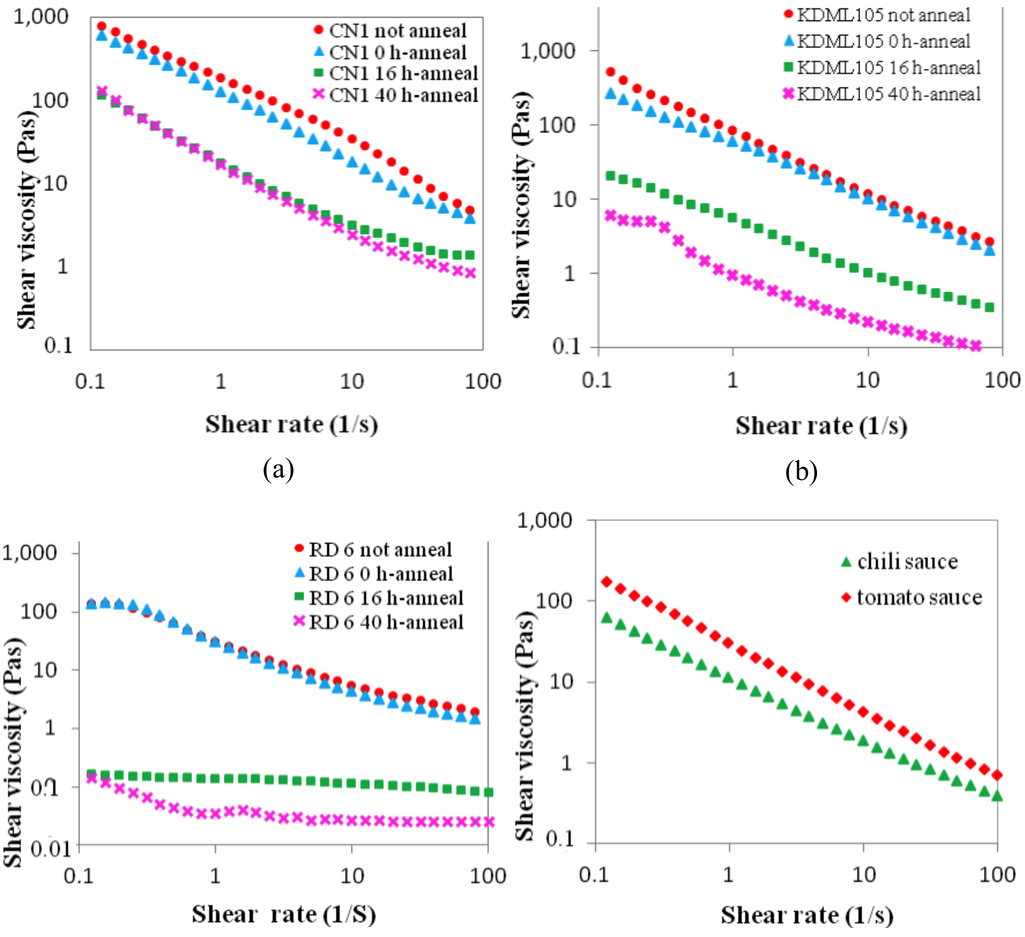
Figure 2. RVA of native starches (blue lines) and acid methanol treated rice starches for 16 h (red lines) and 40 h (green lines) before (dotted lines) and after annealing (solid lines).

3.3. Steady shear viscosity of annealed acid–methanol-modified rice starch

The results of shear viscosity as a function of shear rate are shown in Fig. 3. At a constant temperature (25°C), the shear rate depended on the steady shear viscosity of gelatinized starch. All rice pastes (8% w/w) still showed shear-thinning flow behavior, except for waxy rice starch hydrolyzed for 16 and 40 h, which was close to Newtonian flow. The apparent viscosity of rice starch paste decreased as hydrolysis time increased.

The power law model was utilized to describe the flow behavior of selected rice starch pastes, since the determination coefficients (R^2) were higher than 0.9 (Table 1). A flow behavior index or power law index (n) that is close to 1 reflects the closeness to Newtonian flow. A lower n value indicates a higher degree of pseudoplastic properties of the fluid (RAO, 1999). All pastes applied in this study exhibited non-Newtonian pseudoplastic flow, with a flow behavior index (n) ranging from 0.18 to 0.91 (n<1). The n of all samples increased with hydrolysis time, resulting in pastes with less pseudoplastic. The apparent viscosity ($\eta_{a,100}$) and consistency coefficient (K) of all pastes were lower than those of native starch, indicating lower consistency and a less viscous nature.

For application purposes, the steady shear viscosity of commercial chili and tomato sauces were determined under the same conditions (Table 1). The flow behavior of chili and tomato sauces indicated a higher degree of pseudoplastic properties (n = 0.17 and 0.23, respectively) as a result of their higher total solids.



(a) (b)

(c) (d)

Figure 3. Shear viscosity as a function of shear rate a) CN 1 b) KDML c) RD 6 d) commercial sauce.

Table 1. Power law parameters of native and annealed acid–methanol-treated starch.

Sample	Time (h)	Apparent viscosity	Power law		
		$\eta_{a,100}$ (Pa s)	K (Pa.s^n)	n (-)	R^2
CN 1	Native	5.01^b	173.67^c	0.23^a	0.99
	0	2.86^b	119.26^b	0.19^a	1.00
	16	0.71^a	19.55^a	0.28^b	0.98
	40	0.46^a	18.41^a	0.20^a	0.99
KDML	Native	2.02^d	88.15^c	0.18^a	1.00
	0	1.84^c	58.10^b	0.25^b	1.00
	16	0.24^b	5.27^a	0.33^c	0.99
	40	0.04^a	0.86^a	0.35^c	0.96
RD 6	Native	1.24^c	32.57^b	0.29^b	0.99
	0 h	0.86^b	31.07^b	0.22^a	0.98
	16 h	0.09^a	0.14^a	0.91^d	0.86
	40 h	0.02^a	0.05^a	0.79^c	0.69
Chili sauce		0.34	11.82	0.23	0.99
Tomato sauce		0.65	29.85	0.17	0.99

Mean values in the same column with different superscript letters are significantly different ($p \leq 0.05$).

These commercial sauces had *K* of 11.8 and 29.9 Pa.s^n, respectively. High-amylose rice starch hydrolyzed for 16 and 40 h had *n* of 0.20 and 0.28 and *K* of 18.4 and 19.6 Pa.s^n, respectively.

4. CONCLUSIONS

The thermal, pasting and rheological properties of annealed hydrolyzed rice starch indicated the potential application of high-amylose rice starch hydrolyzed for 16-40 h as a thickening agent in further studies.

ACKNOWLEDGEMENTS

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TRANSGLUTAMINASE CROSSLINKING TO IMPROVE QUALITY OF RICE FLOUR GEL

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ABSTRACT

Rice flour gel quality was improved by protein crosslinking using transglutaminase (TGase) altogether with pea protein isolate (PPI) for lysine enrichment. High, medium and low amylose rice flour were prepared with 1% and 2% TGase and 5% PPI (w/w). TGase exerted more effect on gel forming properties than PPI did. PPI showed a synergistic effect with TGase especially in high amylose rice flour gel. TGase treatment in the presence of PPI altered textural property of rice flour gel especially hardness. The polymerization of rice proteins with TGase occurred only in globulin and albumin fractions in both disulfide and non-disulfide crosslinking.

Keywords: crosslinking, transglutaminase, difulfide bond, pea protein isolate

1. INTRODUCTION

Rice noodle products based on rice are usually in the form of gel or gel sheets. Rice flour has poor viscoelastic property comparing to wheat flour. Gel quality improvement is widely explored in starch, and rarely in protein. Rice protein is existed in small amount and mostly is water soluble hence has less effect on textural or rheological property. The addition of PPI, which is high in lysine did not only to increase its nutritional value but also to promote crosslinking possibility. Crosslinking of rice proteins by TGase were studied in a reducing and non-reducing condition in order to investigate the presence of disulfide bond, which effect the changes in physicochemical properties of rice flour gel.

Protein crosslinking is covalent bonds formed between the side chains of amino acid residues of a polypeptide chains. It occurs in both within (intramolecular crosslink) and between protein (intermolecular crosslink). Protein crosslinking can be performed by either physical, chemical, or enzymatic means. Protein crosslinking such as disulfide bond alters protein structure, resulted in changes of functional and nutritional properties of food products (GERRARD, 2002; GUJRAL and ROSELL, 2004; MOTOKI *et al.*, 1998). Enzyme Transglutaminase (TGase) (EC 2.3.2.13) is a GRAS enzyme and has been used widely to improve quality of foods (GERRARD *et al.*, 2001; GUJRAL and ROSELL, 2004). TGase catalyses the acyl-transfer reaction between γ-carboxylamide groups of protein-bound glutamine amino residues and ε-amino groups on protein-bound lysine residues. This leads to a covalent crosslinking by the formation of ε-(γ-glutamyl) lysine bond (G-L bond) (RASTALL, 2007; GERRARD, 2002; JOYE *et al.*, 2009). The action of TGase also led indirectly to a conversion of soluble proteins to insoluble high-molecular-weight protein polymers through formation of disulfide covalent crosslinking in wheat protein (GUJRAL and ROSELL, 2004). Protein crosslinking could modify dough rheology. Protein crosslinking by enzymes also affected crumb hardness (GUJRAL and ROSELL, 2004). HUANG *et al.* (2010) found that 1.5% TGase could increase cooking stability and reduced tan δ of oat dough. LIN *et al.* (2009) found that both E′ and E″ of rice gel increase with an increase in TGase. TGase also found to increase hardness, gumminess and adhesiveness of rice sheet.

2. MATERIAL AND METHODS

2.1. Rice flour preparation

Thai rice cultivars; Chainat 1 (CN 1; amylose 28.5%, protein 6.8%), Khao Dok Mali 105 (KDML105; amylose 16%, protein 6.3%) and waxy rice (RD6; amylose 5.5%, protein 5.7%) were obtained as paddy rice from the Department of Rice, Thailand. The paddy rice was dehusked, dehulled and milled using Rotor Mill (Retsch SR300, Germany) to obtain 100 mesh particle size.

2.2. Rice flour gel preparation

Rice flour was dry mixed with 1%, 2% (w/w) TGase (Activa® STG-M; Ajinomoto Co., Ltd., Thailand) and 5% PPI (w/w) (Pisane® C9; Nutrition Sc. Co., Ltd., Thailand). Rice flour suspension (flour:water 1:3; w/w) was stirred for 30 min, preheated in water bath at 52°C for 2 min. Rice paste (15 g) was poured into 2.5 cm diameter can and steamed at 95°C for 15 min, cooled at room temperature and stored in plastic bag (CHAM and SUWANNAPORN, 2010).

2.3. Extraction of rice protein

Defatted rice flour was extraction using Osborne method based on the solubility of protein into 4 fractions. Albumin, globulin, glutelin, and prolamin were obtained by sequentially extracting with deionized water, 5% NaCl, 70% ethanol and 0.1M NaOH, respectively. The slurry was adjusted pH using 0.1M HCl to the isoelectric pH, centrifuged at 3000g for 30 min and the sediment was used for next fractionation. The supernatant for each stage was collected. The precipitated protein was washed twice with distilled water, freeze-dried and stored at -21°C in plastic bag.

2.4. Analysis of rice proteins

SDS-PAGE analysis was carried out according to Laemmli's buffer system under reducing and non-reducing conditions, using 10% acrylamide running gel (LAEMMLI, 1970). For the reducing sample, β-mercaptoethanol (βME) was added as a reducing agent. Sample solution (10 μL) containing 5-10 mg protein was loaded into the gel and was run at 15 mA for about 2.5 h.

2.5. Viscoelastic properties of rice flour gel

Rice gel was cut into 20x20x20 mm. Viscoelastic property of rice flour gel was analyzed using Dynamic Mechanical Analyzer (DMA Eplexor, Gabo, Germany). Linear viscoelastic region (LVR) of static and dynamic force using static dynamic sweep mode with force range 0.5-2.0 N. Frequency sweep test (0.5-10.0 Hz) using compression mode, prism geometry, 1.0 N static and 0.5 N dynamic load. Static and dynamic max strain was 10% and contact force was 0.5 N.

2.6. Textural properties of rice flour gel

Texture Profile analysis of rice gel (10 mm height) was analyzed by Texture Analyzer (Stable Micro Systems TA.XT Plus, UK). The puncture test used cylindrical probe (6 mm diameter), test speed 1 mm/s, compression distance 5 mm, and holding time 5 s (VANDEPUTTE *et al.*, 2003).

2.7. Statistical analysis of data

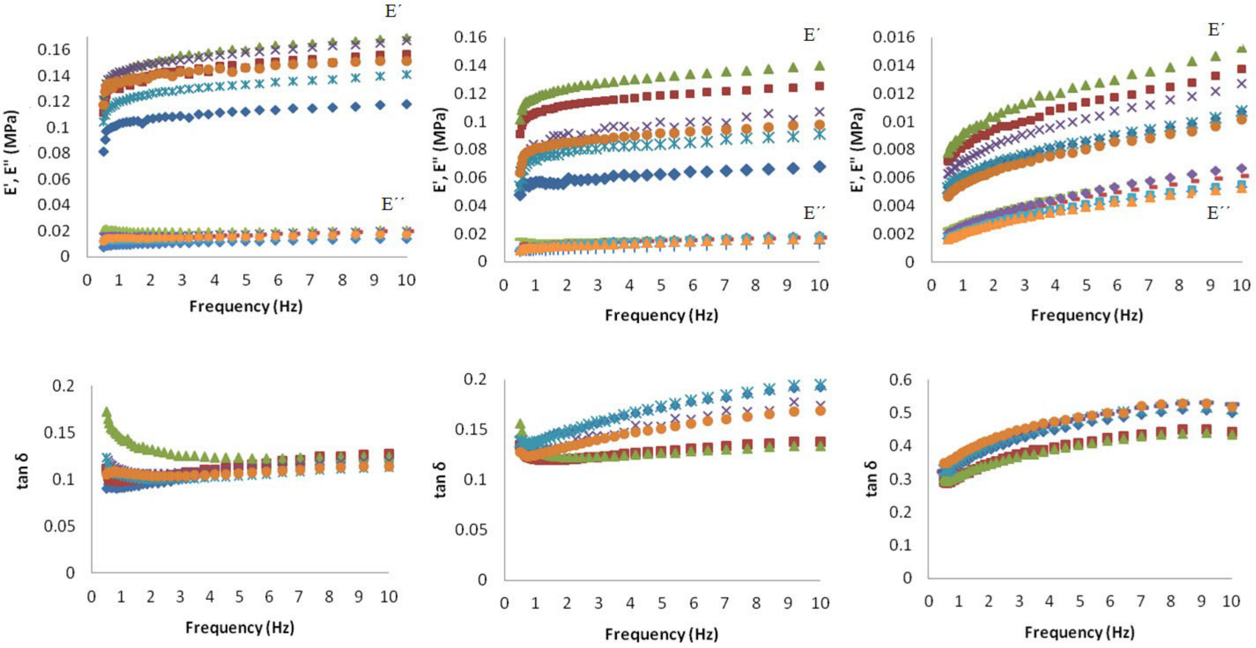
The experiment was designed using Factorial in CRD. Data was analysed using one-way ANOVA by SPSS software version 16.0 (IBM Corporation, USA).

3. RESULTS

3.1. Viscoelastic properties of rice flour gel

The elastic-liked behavior was found in all treated gels indicated by the higher E' than E'' (Fig. 1) and agreed well with works of GUJRAL and ROSELL (2004) and LIN *et al.* (2009).

E' increased slightly with an increase in frequency in Chainat 1 and KDML 105 whereas RD6 had less effect. TGase and PPI could also shift E' and E'' in all cultivars. Amylose plays more role against structure disruption than protein crosslinking. Upon rupture, the network had high chance to break at the cross-link point (MAKRI *et al.*, 2006). 2% TGase showed the highest shift of E′. MARCO and ROSELL (2008b) and HUANG *et al.* (2010) also found that TGase could increase both E' and E'' in rice flour and oat dough respectively. The effect of TGase on E''was found to have more effect than PPI. Tan δ remained unchanged in high amylose flour but decreased in low amylose and waxy rice flour gel. This supported previous statement of the stronger effect on structure disruption of amylose. GUJRAL and ROSELL (2004) and HUANG *et al.* (2010) obtained similar result of the decrease in Tan δ with the increasing level of TGase. TGase could led to the more structured and solid-liked gel when use together with PPI (LIN *et al.*, 2009; RIBOTTA *et al.*, 2012). Viscoelastic property of rice gel was associated with the disruption of protein phase in gel phase. MARCO and ROSELL (2008b) investigated rice flour dough structure using SEM and found a disrupted structure where starch granules were hold together by the proteins.



(a) Chainat 1 (high amylose) **(b) KDML105 (low amylose)** **(c) RD6 (waxy)**

Control, 1% TGase 2% TGase

PPI 1% TGase + PPI 2% TGase + PPI)

Figure 1. Storage modulus (E'), loss modulus (E'') and tan δ of rice flour gels.

3.2. Textural properties of rice flour gel

Hardness and gumminess increased with the increase in TGase concentration in all cultivars particularly at 2% level, ($p \leq 0.05$) (Table 1). LIN *et al.* (2009) found that the addition of TGase increased hardness and gumminess in rice gel sheet. But cohesiveness and adhesiveness were not affected by the enzyme. TGase promoted higher gel hardness than PPI. This could be related to the gel formation of PPI. Two types of gel could be formed in the mixture of polysaccharides and protein isolate, which are phase-separated and interpenetrating network gel which depended on the procedure, time, mixing temperature, and protein concentration (MAKRI *et al.*, 2006). As PPI concentration was relatively low compare to starch, an interpenetrating network gel or filled gel was expecting to occur. The proximity of the cross-linked polypeptide chains that bring amino acids closer to each other was expected to play a crucial role in crosslinking. The synergistic effect of TGase and PPI was more pronounced in high amylose gel (Table 1). High amylose gel was comparatively firm and compact so the accessibility of TGase to lysine and glutamine residues was easier.

Table 1 Texture Profile Analysis (TPA) of three cultivars rice flour gel treated with TGase and PPI.

Cultivar	Treatment	Hardness	Adhesiveness	Springiness	Cohesiveness	Gumminess
Chainat 1 (high amylose)	Control	267.8±40.5^{c}	-134.5±45.4^{a}	0.6±0.1^{b}	0.7±0.1^{a}	181.1±33.0^{bc}
	TG 1%	308.7±21.6^{ab}	-82.3±71.1^{a}	0.6±0.1^{b}	0.7±0.1^{a}	219.7±20.7^{a}
	TG 2%	325.6±24.4^{a}	-126.1±61.8^{a}	0.6±0.1^{b}	0.7±0.1^{a}	225.1±20.3^{a}
	PPI	281.6±32.2^{bc}	-154.7±10.7^{a}	0.6±0.0^{b}	0.6±0.1^{a}	171.7±42.0^{c}
	TG 1% + PPI	280.8±31.8^{bc}	-82.5±64.1^{a}	0.8±0.1^{a}	0.7±0.0^{a}	196.4±12.1^{abc}
	TG 2% + PPI	316.4±34.6^{ab}	-122.7±44.7^{a}	0.7±0.1^{a}	0.7±0.1^{a}	215.6±32.7^{ab}
KDML105 (low amylose)	Control	49.9±7.6^{c}	-33.6±14.6^{a}	0.7±0.1^{a}	0.7±0.1^{a}	33.9±1.8^{c}
	TG 1%	79.5±7.9^{a}	-47.2±16.5^{a}	0.8±0.0^{a}	0.7±0.0^{a}	51.2±3.7^{a}
	TG 2%	72.8±11.2^{ab}	-44.8±22.9^{a}	0.8±0.1^{a}	0.7±0.1^{a}	46.9±6.5^{ab}
	PPI	50.8±7.9^{c}	-24.4±13.0^{a}	0.7±0.1^{a}	0.7±0.1^{a}	34.3±4.1^{c}
	TG 1% + PPI	62.9±7.4^{b}	-37.5±20.8^{a}	0.7±0.1^{a}	0.7±0.1^{a}	43.3±6.5^{b}
	TG 2% + PPI	68.4±6.0^{b}	-37.7±18.0^{a}	0.7±0.1^{a}	0.7±0.1^{a}	45.3±4.0^{b}
RD6 (waxy)	Control	12.7±2.9^{c}	-4.5±1.9^{a}	0.6±0.1^{a}	0.6±0.1^{ab}	7.7±1.2^{b}
	TG 1%	16.7±1.8^{ab}	-8.1±1.3^{bc}	0.6±0.1^{a}	0.6±0.1^{ab}	9.7±0.6^{a}
	TG 2%	18.5±2.2^{a}	-11.2±2.0^{d}	0.7±0.1^{a}	0.5±0.1^{b}	9.8±0.9^{a}
	PPI	12.9±1.7^{c}	-6.5±2.8^{ab}	0.6±0.1^{a}	0.7±0.1^{a}	8.6±1.1^{ab}
	TG 1% + PPI	14.4±2.1^{bc}	-9.3±1.4^{cd}	0.6±0.2^{a}	0.7±0.1^{a}	9.3±0.9^{a}
	TG 2% + PPI	16.2±1.5^{ab}	-10.9±1.4^{d}	0.6±0.1^{a}	0.6±0.1^{ab}	9.7±1.1^{a}

$^{a-c}$ lowercase superscript letter in the same column were not different using at $p \leq 0.05$.

3.3. Protein patterns of rice protein fractions treated by TGase

For reducing condition, protein band shift in globulin and albumin fractions was observed in all cultivars. Band intensity of less than 50 kDa was decreased after TGase treatment. Bands at the boundary between stacking and running gels, and/or at the top of stacking gel, were increased clearly in globulin fraction (Fig. 2). These suggest the polymerization of proteins through intermolecular crosslinking (MARCO *et al.*, 2007). RENZETTI *et al.* (2012) found that no new polypeptides were detected in the TGase-treated flour, and the products of the polymerization were larger than 230 kDa. Band shift of proteins to high molecular weight by TGase were also observed in globulin and albumin fractions of buckwheat proteins (RENZETTI *et al.*, 2012). TGase did not induce band shift in glutelin and prolamin. The cross-linking is dependent on the availability and accessibility of glutamine and lysine residues (RENZETTI *et al.*, 2012). Glutelin and prolamin fractions are rather hydrophobic, so TGase was difficult to access into those proteins. Rice globulin and albumin were susceptible to TGase but not glutelin and prolamin. HUANG *et al.* (2010) found that globulin and gliadin (avenalin) were the desirable substrates for TGase.

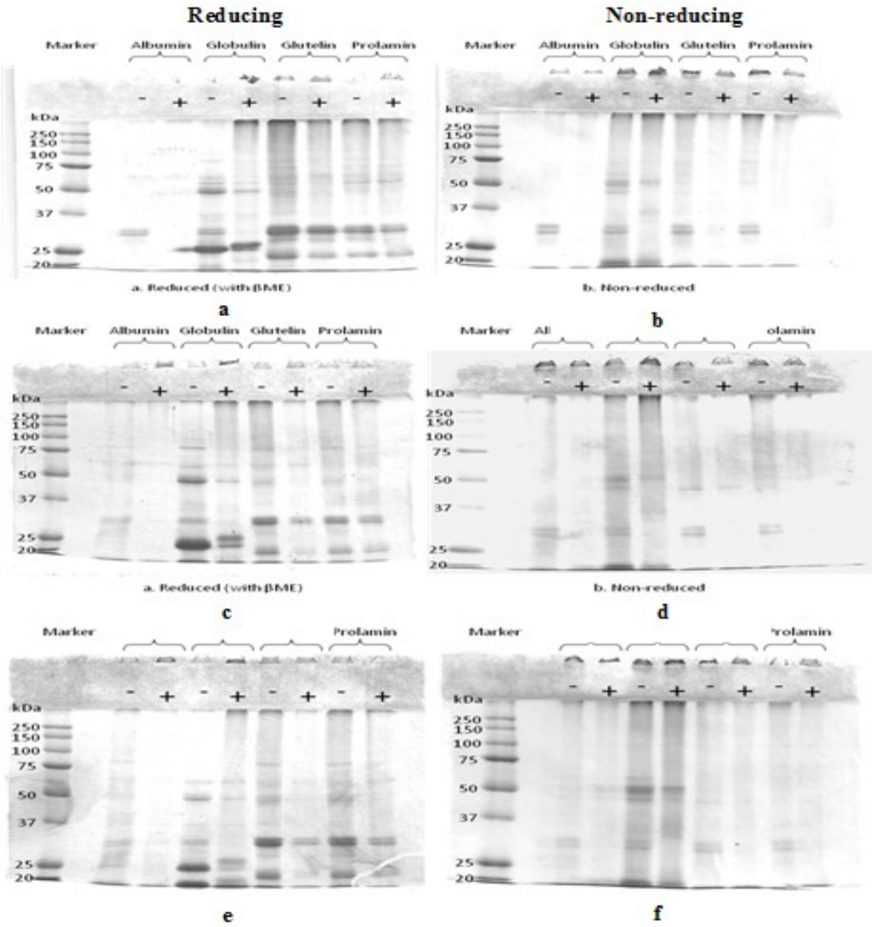


Figure 2. SDS-PAGE patterns of rice protein fractions from three cultivars treated with TGase. Chainat1 (a, b), KDML105 (c, d) and RD6 (e, f); (+) represents sample with TGase and (–) without TGase, under reducing (a, c, e) and non-reducing condition (b, d, f).

For non-reducing condition, SDS-PAGE showed dense protein bands at the boundary between stacking and running gels and/or at the top of stacking gel in all fractions. This indicated disulfide crosslinking during the incubation and it happen independently of TGase. However, disulfide crosslinking was more noticeably in TGase treated samples. The formation of disulfide bond by TGase action was also reported by GUJRAL and ROSELL (2004), LARRÉ *et al.* (2000), and MARCO *et al.* (2007, 2008a). The formation of disulfide bond was most likely to favor the proximity of the crosslinked polypeptide chains. The crosslinking reaction may bring some amino acids closer to each other as the protein molecules become more compact. Thus, the sulfur-containing amino acids may come close to each other leading to the formation of disulfide bonds by oxidation process (GUJRAL and ROSELL, 2004). MARCO *et al.* (2007) mentioned that the crosslinking reaction may expose the sulfur-containing amino acids, facilitated the formation of these bonds. TGase gave two effects to rice proteins; one is the direct effect of TGase, that is, crosslinking reaction between glutamine and lysine residues, and the other the indirect effect of the formation of intermolecular disulfide bonds.

4. CONCLUSIONS

TGase induced the polymerization of rice proteins, in which globulin and albumin fractions are mainly involved. Polymerization of rice protein induced by TGase promoted the alteration of physicochemical properties of rice flour gel especially during aging of gel. PPI showed a synergistic effect with TGase particularly in high amylose rice flour gel. TGase treatment in the presence of PPI altered textural property of rice flour gel especially hardness. Thus rice protein crosslinking using TGase could improve certain textural and rheological properties of rice flour. Its application appropriateness depended on different final product characteristics.

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ACTIVE ANTIMICROBIAL COLLAGEN CASING

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ABSTRACT

The active antimicrobial collagen casing by chitosan impregnation was investigated. The optimum condition of the impregnation process was revealed. The antimicrobial casing obtained from this condition decreased *L.* monocytogenes and *S. aureus* by 2.42 Logs (>99% reduction) and 3.90 Logs (>99.9% reduction), respectively. Accordance with the concentration of chitosan released from the impregnated collagen casing was 0.068% v/v. Thus, the active antimicrobial casing has the potential to be used in the meat industry to protect the sausage from post-process contamination instead of pasteurization in a package which is more practical and cost saving for the food industry.

Keywords: antimicrobial activity, chitosan, casing, impregnation, foodborne pathogen, sausage

1. INTRODUCTION

Chitosan has reactive amphiphilic polymers with very different chemical, physical and biological properties (RAAFAT *et al.,* 2010). The interesting characteristics of chitosan are not only that it is nontoxic, biodegradable, biofunctional and biocompatible, but also that it presents a wide range antimicrobial activity against human pathogens as well as foodborne organisms (TANTALA *et al.,* 2012). Regarding the shelf-life extension of meat product, the using of chitosan as a natural food preservative in order to reduce the outgrowth of foodborne and food spoilage microorganism is the most outstanding (Campos *et al.,* 2011). The incorporation of chitosan into meat products can be achieved using several methods by coating chitosan to the sausage casing or by dipping meat and meat product into chitosan solution (KANATT *et al.,* 2013; LEKJING, 2016). Recently, vacuum impregnation (VI) has been applied in many food industries as this technique can incorporate the solution into the structure of the food and the characteristics of the food are improved (FITO *et al.,* 2001). Our previous research developed an antimicrobial agent *via* VI technology for use in an antimicrobial casing. These casing incorporated chitosan into cellulose casing (KAOWKUM *et al.,* 2012) and also produced the antimicrobial collagen casing impregnated with nisin (BATPHO *et al.,* 2017), which aid foodborne pathogens inhibition. Thus, the purpose of this study was to produce antimicrobial collagen casing by using VI technique and investigated the penetration ability of chitosan including its antimicrobial activity.

2. MATERIALS AND METHODS

2.1. Production of antimicrobial collagen casing

Chitosan (low molecular weight, 22-218 KDa; Sigma-Aldrich, USA) solutions at a concentration of 1 and 2% (w/v) in 1% (v/v) acetic acid were prepared. The collagen casing tubes (2 cm in length) was immersed in a chitosan solution and placed in a vacuum chamber. A maximum condition of vacuum pressure at -680 mmHg varied holding a vacuum time (V) from 30 to 300 s and an atmospheric time (A) from 0 to 240 s. At the end of the VI step, samples were suddenly removed from the chitosan solution. The casing was dried in an oven at 50 °C for 30 min and the casing was kept in a nylon pouch until use. A control sample was casing soaked in chitosan solution at room temperature for 24 h, which is simply to ensure that the casing is saturated with the chitosan.

2.2. Antimicrobial activity of collagen casing impregnated with chitosan

The effectiveness of the VI conditions used to produce antimicrobial casing was measured by evaluation of the results of the survival cell count method (BATPHO *et al.,* 2017) against *L. monocytogenes* Scott A and *S. aureus.* Three independent replications of this experiment were conducted.

2.3. Determination of chitosan concentration released from the antimicrobial casing

The ninhydrin assay was used to determine chitosan concentration which released from impregnated or soaked collagen casing as adopted from KAOWKUM *et al.* (2012). Three independent replications of this experiment were conducted.

3. RESULTS AND DISCUSSION

3.1. Antimicrobial activity of impregnated casing

The Log reduction of cell number (Log N_0/N) in contact with either chitosan-impregnated casing or chitosan-soaked casing is presented in Fig. 1.

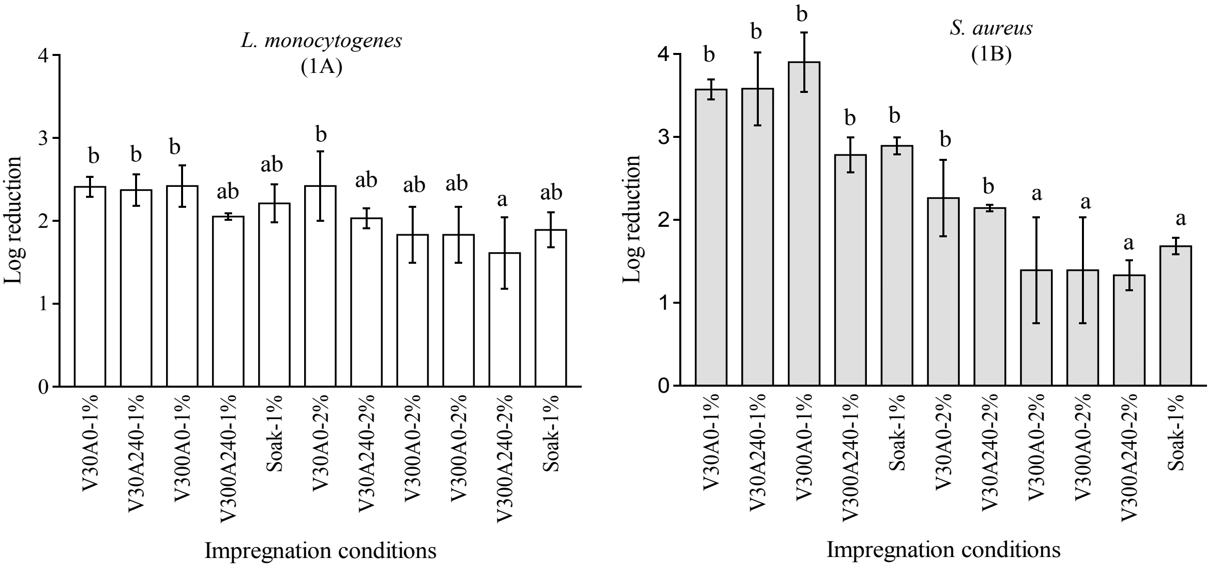

Figure 1. Antibacterial activity of the collagen casing combined with chitosan at different technique against *L. monocytogenes* (1A) and *S. aureus* (1B) within 24 h. Error bars represent±standard deviation. Bars of the same period in each tested bacterium followed by different letters indicate significant differences ($p \leq 0.05$).

The most active antimicrobial casing was achieved at the condition of 1% chitosan solution, the vacuum pressure was 300 s and atmospheric pressure was 0 s (V300A0-1%) which decreased *L.* monocytogenes and *S. aureus* by 2.42 Log (>99% reduction) and 3.90 Log (>99.9% reduction), respectively. While a control sample, collagen casing immersed in 1% chitosan solution for 24 h (Soak-1%)) decreased those bacteria by 2.21 and 2.89 Logs, respectively. When increased vacuum time and atmospheric time, chitosan concentration in impregnated casing and number of Log reduction decreased. The high vacuum and atmospheric time provided the high flowing gas out and the liquid flow in but this condition make the natural constituents diffuse flow out more rapidly than the chitosan from the osmotic solution to flow in (FITO *et al.,* 2001) leading to the lower chitosan concentration and the number of Log reduction.

3.2. Quantitative of chitosan impregnated into a collagen casing

Impregnated casing at V300A0-1% contained the highest concentration of chitosan than other casings (0.068% v/v) (Table 1), which related to its strongest antibacterial activity. The higher concentration of chitosan solution did not increase the chitosan concentration in the casing. This phenomenon was due to the 2% chitosan is more viscous than 1% chitosan leading to the difficulty to transfer into the porous of the casing.

Table 1. Quantitative of chitosan concentration released from the antimicrobial casing for 24 h.

Chitosan concentration (% w/v)	Impregnation condition	Chitosan concentration in casing (% w/v)
Control	-	0.003±0.00a
1	V30A0-1%	0.054±0.15cdef
	V30A240-1%	0.065±0.01ef
	V300A0-1%	0.068±0.07e
	V300A240-1%	0.043±0.02bcd
	Soak-1%	0.027±0.005b
2	V30A0-2%	0.040±0.04bcd
	V30A240-2%	0.049±0.05cde
	V300A0-2%	0.038±0.08bc
	V300A240-2%	0.030±0.05b
	Soak-2%	0.047±0.03cde

Values are given as mean±standard deviation. Different letters in the same column indicate significant differences between the means obtained in Duncan's test ($p \leq 0.05$).

4. CONCLUSIONS

The impregnation technique can deliver chitosan into collagen casing leading to antimicrobial casing production. The antimicrobial casing produced at V300A0-1% decreased *L. monocytogenes* and *S. aureus* by 2.42 and 3.90 Logs, respectively. Vacuum impregnation can be produced the active antimicrobial casing which is a cost and time saving, simple process and economical to acquire. Furthermore, it has the potential to be used in the meat industry to protect the sausage from post-process contamination instead of pasteurization in the package, which is more practical and cost saving for the food industry.

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RECENT APPLICATIONS OF MONTMORILLONITE IN FOOD PACKAGING

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ABSTRACT

In the last year's researchers have dedicated great attention to the development of bionanocomposites based on chitosan/montmorillonite incorporating essential oils or plants extracts, and the assessment of its performance as primary packaging, to evaluate their possible use as active or intelligent food packaging. The aim of this work was to compile the recent papers, regarding this subject. In the compiled studies different techniques of characterization of the films were applied to evaluate the mechanical, barrier and optical properties. A common conclusion is the fact that they are promising materials to be used as an alternative to the conventional packaging materials originating from fossil resources.

Keywords: chitosan, essential oils, food packaging, bionanocomposites, montmorillonite, active packaging, intelligent packaging

1. INTRODUCTION

Currently, the society lives in an accelerated changing environment, which has a huge impact on the food industry. Therefore, the global food market is being requested to respond to new demands and needs, also related with the better assurance of food safety and quality. Consumers prefer foods with natural food additives once these are not related with adverse health effects. Thereby, there is an increased interest in looking for natural compounds with bioactive properties to guarantee the quality of different types of foods. Biopolymers based films could be a possible solution for some of the problems that appear from the application of conventional food plastic packaging materials (e.g., very low biodegradability, obtained from fossil resources, limited ability for bioactive behavior) (SOUZA and FERNANDO, 2016).

Chitosan (CH) is a modified, linear cationic polysaccharide composed of glucosamine and acetylglucosamine, with a high molecular weight and is obtained through chitin deacetylation (BUTNARU *et al.*, 2019). In fact, it is the second most abundant polysaccharide found in nature after cellulose (GIANNAKAS *et al.*, 2016). Barrier and mechanical properties of pure chitosan films are suitable for food packaging and active packaging, but in order to improve the intrinsic properties of these biopolymer-based packaging materials, it is necessary the incorporation of nanoscale reinforcements (CAZÓN and VÁZQUEZ, 2019). The nanoparticles of montmorillonite (MMT) are the layer silicate mineral clay that is the most commonly combined with polymeric materials to form nanocomposites (SINHA RAY and OKAMOTO, 2003). The nanoparticles of MMT are composed of silica sheets stacked in a plate form, with a high surface area (700-800 m^2/g) and a thickness of approximately 1 nm (RHIM and NG, 2007). In the last years, the European Authority for Food Science (EFSA) evaluated several nanomaterials for plastics and the updated list of approved compounds to be used in the manufacture of plastic packaging in the EU includes the use of some nanoforms, most of them with restrictions (Regulation EU No 10/2011, 2011). In this positive list is included an MMT clay modified by dimethyldialkyl (C16-C18)ammonium chloride (VILARINHO *et al.*, 2019). However, the use of this modified MMT is only approved to be used up to 12 % (w/w) in polyolefins in contact with dry foods (Commission Regulation EU 2017/752, 2017)

Active packaging is designed to deliberately incorporate components that can be released or absorbed into or from the packaged food or the environment surrounding the food (PRASAD and KOCHHAR, 2014). In this line, the improvement of the antioxidant properties of the chitosan films may depend on the incorporation of antioxidants, such as plant extracts or essential oils (EO) (SIRIPATRAWAN and HARTE, 2010). EO present antioxidant and antimicrobial properties, assigned to the presence of phenolic compounds, which are responsible for the inhibition of the lipid oxidation reactions and for the microorganisms growth in food (SIRIPATRAWAN and HARTE, 2010), aiming to increase the shelf life of the food without affecting its nutritional and sensorial attributes (RIBEIRO-SANTOS *et al.*, 2017).

Intelligent packaging monitors a condition "of packaged foods to give information about the quality of the packaged food during transport and storage" (PRASAD and KOCHHAR, 2014). Anthocyanins are secondary metabolites of some vegetables such as black carrot, red cabbage and grapes. These natural pigments can be added directly to food packaging materials and to function as a colorimetric pH indicator, to monitor the changes of acidic or basic components of the food (PRIETTO *et al.* 2017).

The aim of this work was to compile some of the most recent papers, which describe the development of bionanocomposites based on chitosan and MMT.

2. METHOD

A literature review was carried out focused on the most recent papers (from 2018 and 2019) dealing with the development of bionanocomposites based on chitosan and MMT.

3. RESULTS AND CONCLUSIONS

The effectiveness and the fit purpose of the chitosan-based bionanocomposites films depend on several parameters that must be defined during their development, such as nanoclays' type, method and level of incorporation of the nanoclay. Table 1 summarizes some of the most relevant research papers published in 2018 and 2019 dealing with the development of bionanocomposites films based on chitosan and MMT.

According to the results of the different papers compiled in Table 1, the presence of nanoparticles of MMT improves the mechanical and barrier properties of the chitosan/MMT bionanocomposites. Furthermore, BUTNARU *et al.* (2019) reported that the addition of rosehip seed oil (RSO) is responsible for the improvement of the flexibility of the chitosan-based films. Besides that, the RSO acts also as antibacterial, antioxidant and plasticizer. The conclusions of this study reported that the chitosan films exhibited potent antioxidant and antibacterial activity, and can be considered acceptable for materials proposed for applications in bioactive food packaging.

In its turn, KOOSHA and HAMEDI (2019) demonstrated that the developed chitosan/PVA/anthocyanins/MMT film can be potentially applied in intelligent and active food packaging, once by incorporation of black carrot anthocyanins, pH-sensitive films were prepared and the color change was detected after pH variation. They also evidenced that the addition of bentonite and anthocyanins can effectively inhibit undesired bacterial growth.

In a paper published by Pires, de Souza, and Fernando (2018), the efficacy of two different MMTs (Cloisite*Na^{+}, Cloisite*Ca^{+2}) and two different essential oils (rosemary essential oil, REO and ginger essential oil, GEO) was evaluated when these were incorporated in chitosan, to test them as primary active packaging for fresh poultry meat. They concluded that no significant differences were observed between the two types of chitosan-MMT films or between the two EOs. They observed that the chitosan acts as a chelator of transition metal ions, interrupting the lipid oxidation chain reactions once the meat is packaged in the active films and showing lower microbiological contamination when compared with the non-packaged meat. They concluded that the reinforcement with MMTs improved chitosan barriers properties and that the supplementation with EOs does not improve the antimicrobial barrier of chitosan. This paper demonstrated the potential of application of these films in food packaging.

In a recent study, Souza *et al.* (2018) evaluated the efficacy of three different bionanocomposites based on chitosan, reinforced with Cloisite*Na^{+}, Cloisite*Ca^{+2} and Cloisite*20, respectively, and in all of them, REO was incorporated. They concluded that the chitosan films produce with MMTNa were the most hydrophilic. For this reason, they were chosen to incorporate REO, enabling the improvement of bionanocomposite functional properties. Souza *et al.* (2018) reported that the MMT enhanced the mechanical properties of the material while the REO acted as a plasticizer. They concluded that these films represent an interesting alternative for packaging food matrices with high fat content.

Table 1. Chitosan-based bionanocomposite films.

Biopolymer	Type of MMT	Incorp. method	Level of incorp.	Characterization methods	Ref.
CH/Tween 80/ RSO/MMT	Cloisite*30B	Emulsion/ solvent casting	3 wt%	DLS; ATR-FTIR; SEM; Mechanical Testing; Gas Permeability Test; Dynamic Moisture Sorption; Antibacterial Test; DPPH Radical Scavenging Assay	(Butnaru *et al.* 2019)
CH/PVA/ anthocyanins/ MMT	Bentonite	Casting method	3 wt% 5 wt%	UV/Vis spectroscopy; SEM/EDX; XRD; FTIR; TGA; Mechanical properties; Color changes of pH-sensitive films; Colorimetric analysis; WVTR; Antimicrobial activity	(Koosha and Hamedi 2019)
CH/MMT/REO	Cloisite*Na^+	Casting method	3 wt%	SEM; ATR-FTIR; WVP; OP; *in vitro* active characterization; *in situ* shelf life assessment of fresh poultry meat	(Souza *et al.* 2019)
CH/MMT/REO and CH/MMT/GEO	Cloisite*Na^+ Cloisite*Ca^{+2}	Casting method	2.5 wt% MMTNa 2.5 wt% MMTCa	Meat characterization: Moisture and ash content; pH and total acidity; lipid oxidation; color; microbiological analysis	(Pires, de Souza, and Fernando 2018)
CH/MMT/REO	Cloisite*Na^+ Cloisite*Ca^{+2} Cloisite*20	Casting method	2.5 wt%	X-ray diffraction; Thickness; Mechanical properties; Optical properties; Solubility and swelling degree; contact angle; DSC	(Souza *et al.* 2018)

ATR-FTIR- Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy; Bentonite- Montmorillonite 60% a 80% CH- Chitosan; Cloisite*20- Bis-(hydrogenated tallow alkyl) dimethyl, salt with bentonite; Cloisite*30B- MMT-Na^+ modified with bis-(2-hydroxyethyl) methyl tallow alkyl ammonium cations; Cloisite*Ca^{+2}- Calcium montmorillonite; Cloisite*Na^+- Sodium montmorillonite; DLS- Dynamic Light Scattering; DPPH- 2,2-diphenyl-1-picrylhydrazyl; DSC- Differential Scanning Calorimetry; FTIR- Fourier Transform Infrared Spectroscopy; GEO- Ginger Essential Oil; MMT- Montmorillonite; OP- Oxygen Permeability; PVA- Poly(vinyl alcohol); REO- Rosemary Essential Oil; RSO- Rosehip Seed Oil; SEM- Scanning Electron Microscopy; SEM/EDX- Scanning Electron Microscopy/Energy Dispersive X-ray Scattering; TGA- Thermogravimetric analysis; Tween 80- Polyoxyethylene sorbitan monooleate; WVP- Water Vapor Permeability; WVTR- Water Vapor Transmission Rate; XRD- X-ray Diffraction.

In general, bionanocomposites are a good alternative to conventional materials, because they can improve barrier, mechanical and optical properties of original biomaterials. Moreover, the incorporation of essential oils or plants' extracts can improve the shelf life of packaged foods due to their antioxidant or antimicrobial properties or can also allow monitoring a condition of the packaged film such as pH changes.

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PACKAGING OF PLA FOR MINIMALLY PROCESSED CARROTS

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ABSTRACT

Qualitative characteristics of minimally processed carrots packaged in PLA film were monitored at 4°C and 7°C up to verify a possible shelf life extension respect to the commercial one, attested at 7 days packaged in PP pouches. Acids stabilized the colour of cut carrots and reduced the total microbial growth up to 14 days. The best results were obtained in carrots treated with the highest citric acid % at 4°C. Acidic dipping contributed to extend the shelf life of minimally processed carrots. Moreover, PLA can be used as packaging material for carrots, as option to conventional plastics.

Keywords: carrots, citric acid, dipping, PLA

1. INTRODUCTION

Due to concerns about the environmental impact and health, consumers are avoiding the use of petroleum-based conventional packaging, which takes hundreds of years to decompose, and food products containing synthetic additives or preservatives (NAMPOOTHIRI *et al.*, 2010). One of the most promising bio-based polyesters aimed for food packaging is polylactic acid (PLA) (PANSERI *et al.*, 2018), a biopolymer chemically synthesized using monomers obtained from agro-resources like wheat, corn, and cassava. At the moment, PLA is one of the most used bio-polyesters due to its availability on the market, its low price and its mechanical and barrier properties (AVÉROUS, 2008; BORDES *et al.*, 2009). Considering that PLA is also classified as GRAS (Generally Recognized As Safe) by the American Food and Drug Administration (FDA) and it is authorised by the European Commission (Commission Regulation No 10/2011), this polymer might be used in contact with food (BISHAI *et al.*, 2014). Moreover, the use of some methods for sanitize the minimally processed vegetables is questioned since it has been shown that the habitual use of chlorine in the industries involves the formation of halogenated by-products, correlated to environmental and health risks in processing areas. Other typologies of agents have to be then considered that can be show multiple effect: sanitizing and inhibitor of oxidative and enzymatic reactions. In particular, among minimally processed vegetables, carrots often manifest a characteristic defect: the discolouration (also named whitening), due to the enzymatic reactions, which follow the cut of tissues. In a recent research the quality maintenance of carrots packed in conventional plastic material was supported by the dipping in acidic solutions that reduced the visual deterioration and slowed down the microbial growth during the storage (PISCOPO *et al.*, 2019a). Different studies were conducted on the evaluation of the packaging with PLA for different food matrixes: orange juice (HAUGAARD *et al.*, 2002), cheese (HOLM *et al.*, 2006; PISCOPO *et al.*, 2019b), yoghurt (FREDERIKSEN *et al.*, 2003), spinach (BOTONDI *et al.*, 2015) but on minimally processed carrots it has not been yet considered. The aim of this work was to test the PLA as packaging material for minimally processed carrots treated with acidic dipping as healthy alternative to chlorine.

2. MATERIAL AND METHODS

Minimally processed carrots were produced in a farm located in Calabria. To sanitize the shredded carrots, the farm applied the dipping in chlorinated water (300 mg L^{-1}) and packaged the carrots in Polypropilene (PP) microforated pouches at normal atmosphere. Two acidic solutions were used as dipping alternative to chlorine: 1% (B) and 1.5% citric acid (C), in comparison to a control in tap water (A). After dipping, carrots were centrifuged for 2′ and packed in BoPLA pouches (NATIVIA ® NTSS, 25 cm x 20 cm of size; 30 μm of thickness; OTR: 730 cm^3 m^{-2} 24 h^{-1} atm^{-1}; WVTR: 270 g m^{-2} 24 h^{-1}) at normal atmosphere and stored at 4°C and 7°C. The principal qualitative analyses were monitored up to 14 days to verify a possible shelf life extension respect to the commercial one, attested at 7 days. Total bacterial count of carrot aqueous extract was measured on PCA Plant Count Agar- growth land (Oxoid) at 26°C for 48 h and expressed as Log Colony Forming Units g^{-1} (Log CFU g^{-1}). Colour measurement was monitored in each carrot sample (15 replicates) using a tristimulus colorimeter (model CM-700d, Konica Minolta, Osaka, Japan) calibrated with a standard white plate with reference to CIELab colour space. Results were expressed as whitening index score (WI) according to the formula (BOLIN and HUXSOLL 1991): $WI = 100 - [(100-L^{*2}) + a^{*2} + b^{*2}]^{0.5}$. AOAC methods (1980, 2000) were performed for measurements of the pH extracts (pH meter Crison GLP, Barcelona, Spain) and the titratable acidity, expressed as % of citric acid. Dry matter (% d.m.) was evaluated by loss weight in an oven at 70°C until a constant weight was reached.

3. RESULTS AND CONCLUSIONS

In Table 1 the results of total microbial charge of carrots packed in PP pouches are reported, indicating a shelf life of 7 days at both storage temperatures. The initial count of 4.9 Log CFU g^{-1} tended to increase with high significance ($p<0.01$) to 6.7 and 6.8 Log CFU g^{-1} (inside the safety threshold of 7 Log CFU g^{-1}).

Table 1. TBC on minimally processed carrots dipped in chlorine during storage at two temperatures (PISCOPO *et al*., 2019).

Temperature/Days	1	3	7	Sign.
4°C	4,91c	5,97b	6,70a	**
7°C	4,91c	6,21b	6,88a	**

** Significance at P < 0.01.

Carrots dipped in acidic solutions manifested lower total microbial charge than the control samples during the storage at 4°C (Fig. 1a).

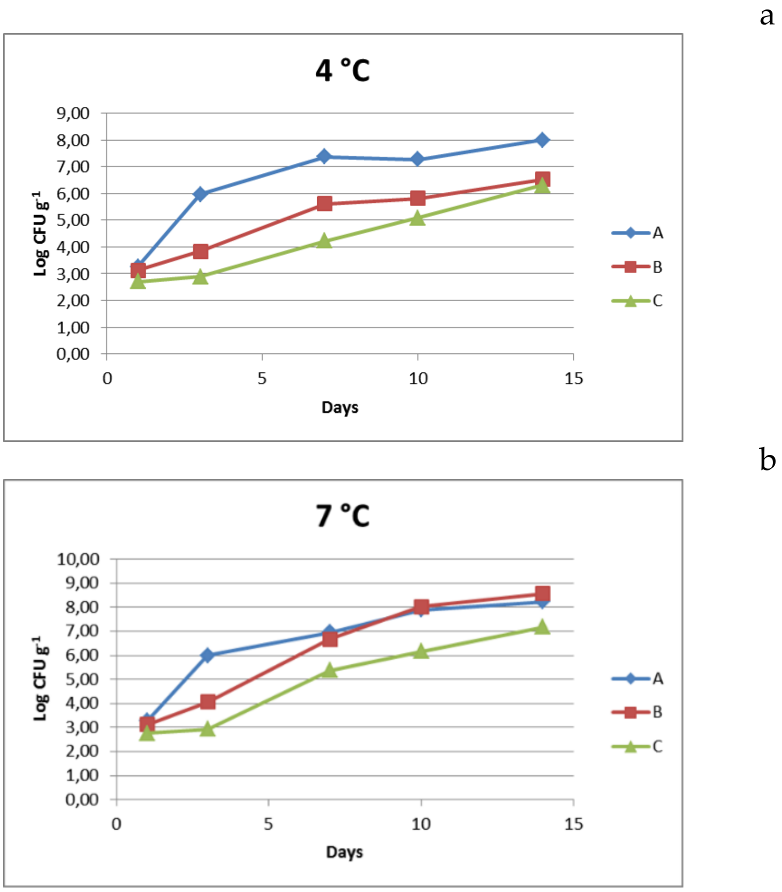

Figure 1. Total Bacterial Count during storage at 4°C (a) and 7°C (b).

At the end of monitoring both the treated samples were similar for microbial count (inside the safety threshold of 7 Log Colony Forming Units g^{-1}). In particular, the 1.5% citric acid dipping was the most effective to 10 days at both temperatures. These results were confirmed by pH and total acidity determinations (Table 2).

Table 2. Titratable acidity (TA), pH and dry matter of carrot samples during storage at two temperatures.

		Samples/days	1	3	7	10	14	Sign.
4 °C	TA (g% citric acid)	A	0,07a	0,04ab	0,03b	0,06ab	0,05ab	*
		B	0,22a	0,13b	0,10b	0,13b	0,10b	**
		C	0,35a	0,24b	0,19c	0,26b	0,17c	**
	pH	A	6,46c	6,48c	6,74a	6,55bc	6,65ab	**
		B	4,92d	5,08c	5,38ab	5,35b	5,53a	**
		C	4,28d	4,63c	4,89ab	4,77bc	5,01a	**
	d.m. (%)	A	9,32	8,70	9,67	10,30	8,64	n.s.
		B	8,87ab	8,47b	9,13ab	10,03a	8,47b	*
		C	9,39ab	8,34b	9,11b	11,13a	8,25b	**
7 °C	TA (g% citric acid)	A	0,07a	0,05b	0,05b	0,04c	0,03c	**
		B	0,22a	0,25a	0,09b	0,14b	0,13b	**
		C	0,35a	0,12d	0,18c	0,26b	0,17c	**
	pH	A	6,46b	6,53b	6,78a	6,78a	6,58b	**
		B	4,92d	5,19c	5,63a	5,52ab	5,40b	**
		C	4,28d	5,18a	4,97bc	4,79c	5,09ab	**
	d.m. (%)	A	9,32	9,00	9,30	9,75	7,91	n.s.
		B	8,87ab	9,17ab	8,73b	9,80a	8,35b	*
		C	9,39ab	8,28b	8,93ab	11,11a	8,31b	*

Results are presented as the mean value ± standard deviation (n=2). ** Significance at P < 0.05.
* Significance at P<0.01; n.s. not significant.

At higher storage temperature only the carrots dipped in 1,5% of citric acid controlled the microbial growth, allowing to extend their shelf life to 14 days (Fig. 1b). The colour parameters did not significantly (p<0.05) change during the storage, as illustrated in Fig. 2 by Whitening index, showing that the two tested concentrations of citric acid can stabilize the colour of carrots and do not involve further discolouration. This event could be possible when the treatment is not efficient for the vegetables and does not stop the lignin formation by enzymatic activity, as just observed for a mixed solution (ascorbic and citric acid) in a previous study (PISCOPO *et al.*, 2019). In conclusions, PLA proved to be a valid packaging material for minimally processed carrots and it can be considered an alternative and eco-friendly proposal for the food industry. The successful use of acids in this experimentation can propose an option to chlorine in the carrot washing step. The tested concentrations of citric acids for the carrot washing step, in fact, in combination to the packaging in PLA, allowed to extend the commercial shelf life from 7 to 14 days at 4°C. At 7°C only the dipping in 1.5% citric acid maintained the quality of carrots up to 14 days. These results can be proposed to the food industry as a healthy and simple operations to produce ready to eat carrots of good quality.

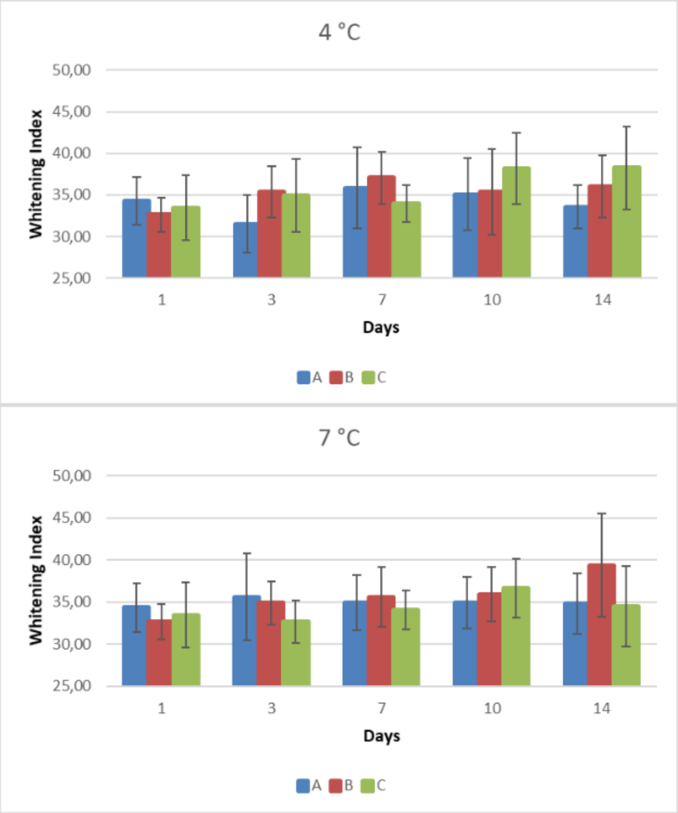

Figure 2. Whitening index during storage at two temperatures.

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SESSION V

Sustainability and Shelf Life

OLIVE LEAF EXTRACT AS NATURAL PRESERVATIVE

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ABSTRACT

Olive leaves, a waste from olive oil production, represent a good source of bioactive compounds, exploitable as natural preservatives in foods for their antioxidant and antimicrobial activities. In this frame, olive leaf extract (OLE) was added in non-thermally stabilized olive-based-paste at two different concentrations (0.5 and 1 g kg^{-1}), and the samples were stored for 90 days. Antioxidant and antimicrobial activity were evaluated by means of ABTS-TEAC assay and microbiological analyses. The samples added with OLE showed the highest value of antioxidant activity. The main microbial groups registered a significant loss (of about 0.5-1 logarithmic cycles) when OLE was added.

Keywords: olive leaves, antioxidant, polyphenols, shelf-life

1. INTRODUCTION

Shelf-life is usually defined as the time during which a food product remains safe, comply with label declaration of nutritional data and retain desired sensory, chemical, physical, and microbiological characteristics when stored under the recommended conditions (IFST, 1993).

Modified atmosphere packaging (MAP) is an efficient means of extending the shelf-life of foods as well as the use of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated-hydroxytoluene (BHT), propyl gallate (PG), and *tert*-butylhydroquinone (TBHQ), although recent reports reveal as these compounds may be implicated in many health risks, including cancer and carcinogenesis (UMEMURA *et al.*, 2001) causing a general consumer rejection of synthetic food additives. Among natural antioxidants, extracts rich in phenolic compounds have been reported as good alternatives since they are readily available as industrial wastes and maintain a potential preservative effect (LORENZO *et al.*, 2014) and present many positive effects on human health including anti-inflammatory, anti-carcinogenic, cardioprotective, and vasodilatory properties (BONILLA *et al.*, 1999). Olive leaves are a waste from olive oil industry (10% of the total weight of the olives) and accumulate during pruning of the olive trees (TABERA *et al.*, 2004). The most abundant phenolic component of olive leaves extract (OLE) is oleuropein (DIFONZO *et al.*, 2017), which along with the bitter taste given to olives and olive oil, shows an in vitro inhibitory effect against many foodborne pathogens such that it could be suitable for use in the food industry as natural preservative.

In this framework, the aim of this work was to investigate the effect of OLE addition in modulating the antioxidant activity and the microbiota of non-thermally stabilized olive-based paste stored in MAP at refrigerated conditions.

2. MATERIAL AND METHODS

2.1. Formulation and manufacture of olive-based paste

Olive-based paste was produced with 840 g kg^{-1} of fermented table olives and 160 g kg^{-1} of extra-virgin olive oil. All ingredients were mixed using a homogenizer (WFP16SE, Waring Commercial, Torrington, USA) for 5 min to produce a homogeneous creamy paste. Three kinds of olive-based paste were produced: (i) control olive paste without any supplementary antioxidant (CTR); (ii) OLE 0.5, added at the concentration of 0.5 g kg^{-1}; (iii) OLE 1, added at the concentration of 1.0 g kg^{-1}. After homogenization, approximately 70 g of each mixture was transferred into plastic trays (95×10 mm), and a stainless steel heat sealer (VGP 25n, Orved, Musile di Piave, Veneto, Italy) was used to pack under argon-based atmosphere for a total of 96 samples. After packaging, the products were stored at 4°C, and then sampled after 1, 15, 30, 45, 60, 75, and 90 (T0, T15, T30, T60, T90). Three independent production trials were carried out for each sampling time and for each batch.

2.4. Antioxidant activity evaluation and microbiological analyses

The antioxidant activity was assayed by means of ABTS-TEAC as reported in DIFONZO et al. (2018). The results were expressed as μmol TE g^{-1} of olive-based paste.

Microbiological analyses were carried out as reported by COSMAI et al. (2017) and CAPONIO et al. (2019). Briefly, olive-based paste samples (5 g) were diluted with 45 mL of sterilized physiological solution, homogenized using a Stomacher 400 lab blender (Seward Medical, London) for 3 min, serially diluted and plated in triplicate in selective media according to methods previously described (De Angelis et al., 2015; Difonzo et al., 2019). Counts were expressed as log cfu g^{-1}. The microbiological counts were preliminary confirmed by taking representative colonies for each medium which were analyzed for morphology, motility Gram staining reaction and catalase test.

2.5. Statistical analysis

Analysis of variance (one-way ANOVA) was carried out on the experimental data and significant differences among the values of all parameters were determined at $p<0.05$. All data were processed by Minitab (Minitab Inc., State College, PA, USA).

3. RESULTS AND CONCLUSIONS

Fig. 1 shows the effect of OLE addition on the antioxidant activity measured by ABTS-TEAC. The samples OLE showed values significantly higher than CTR ($p<0.05$), and only the samples added with OLE at the highest concentration (1 g kg^{-1}) kept this trend until T60.

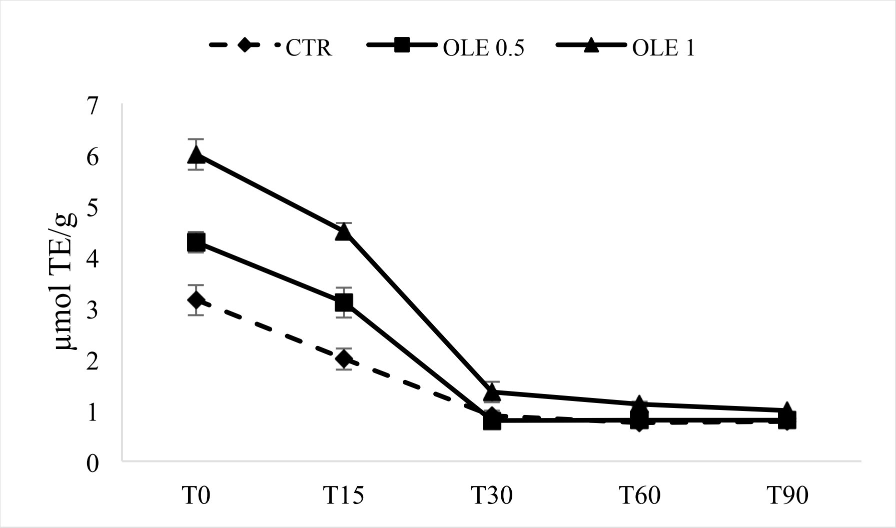

Figure 1. Trend of antioxidant activity assayed by ABTS-TEAC during storage. CTR: control samples; OLE 0.5: olive-based paste added of 0.5 g/kg of olive leaves extract; OLE 1:olive-based paste added of 1 g/kg of olive leaves extract; T0: production day; T15, T30, T60, and T90: 15, 30, 60, and 90 days of storage.

Several studies have reported the application of OLE in improving the antioxidant activity of olive oil (BOUAZIZ *et al*., 2008; DIFONZO *et al*., 2017), cooked meat products (HAYES *et al*., 2009), and bakery products (DIFONZO *et al*., 2018). The antioxidant activity improvement could result in stability oxidative improvement, thus the extension of shelf-life and in some cases is also related to the improvement of the nutritional value of foods (SHAHIDI *et al*., 2015).

Fig. 2 reports the results of the microbiological analysis related to the growth of microorganisms in olive-based pâté under study. Spoilage and pathogen bacteria were not detected. The cultivable bacteria, yeasts and moulds gradually decreased in all samples during storage. The addition of OLE and the refrigeration storage affected the cultivable microbiota, as reported in previous research activities (CAPONIO et al., 2019) using the olives leaf extract.

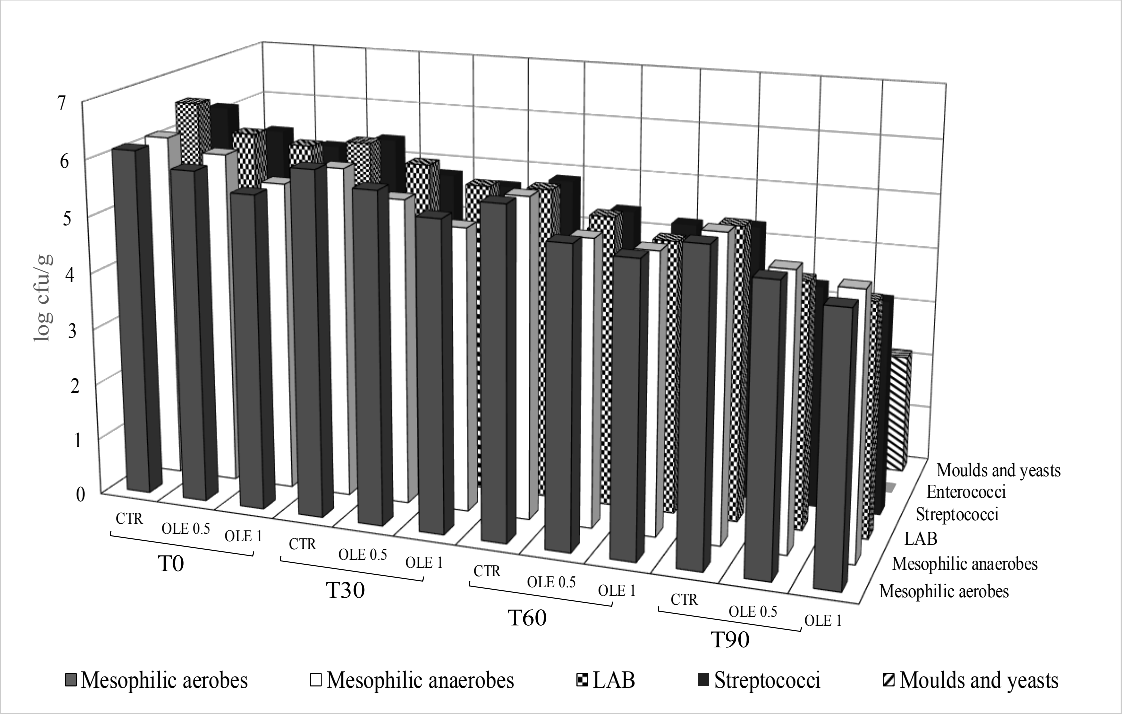

Figure 2. Cell densities (log cfu g^{-1}) of microbial groups detected in olive-based paste during storage time. CTR: control samples; OLE 0.5: olive-based paste added of 0.5 g kg^{-1} of olive leaves extract; OLE 1: olive-based paste added of 1 g kg^{-1} of olive leaves extract; T0: production day; T30, T60, and T90: 30, 60, and 90 days of storage.

The authors reported that the OLE addition affected the fermentative and oxidative processes of table olives and their nutritional properties. When 1 g kg^{-1} of OLE (OLE 1) was added to olive-based paste, the main microbial groups registered a loss ($p<0.05$) of ca. 0.5-1 logarithmic cycles. DIFONZO *et al*. (2019) showed that olive leaves are a waste of the olive oil processing industry and represent a good source of phenolic compounds. Those ones possess potential anti-microbial properties that can inhibited the microorganisms (HURTADO *et al*., 2012).
To conclude, the results of the present study indicate the potential use of OLE as natural preservative in non-thermally stabilized foods.

ACKNOWLEDGEMENTS

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PRODUCTION OF CALCIUM-RICH SNACK FROM SALMON BONE

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ABSTRACT

The salmon bone, byproduct generated from fish processing operations, was a good calcium source and could be produced as a value-added product through pretreatment with sodium hydroxide and thermal processing. The pretreatment with 4-8% NaOH for 30-60 min could decrease lipid content of salmon bone to 4.26-8.41%. After pretreatment process, salmon bones were boiled by a gas retort and following dried by a hot air drying. Increasing drying temperature and time (140-180°C for 15-45 min) resulted in an increase in crispness of salmon bone product, whereas a decrease in values of hardness and moisture content. Drying at 180°C for 15 min contributed the physical and chemical properties of the salmon bone product including; 84 count peaks of crispness, 13.16 N of hardness, 2.96% moisture content, and 19.23 g calcium 100 g^{-1} sample. This result indicated that the sodium hydroxide pretreatment and thermal processes had the potential to promote salmon bone as a calcium-rich product with crispy texture like a snack.

Keywords: fish bone, calcium, utilization, byproduct, drying, food waste reduction

1. INTRODUCTION

In Thailand, a rising demand for importing of frozen salmon fish had been estimated at 16,000 tons in 2018 (MINISTRY OF COMMERCE, 2018), since consumption of salmon meat is one of the popular food commodities. During fish processing, salmon bone around 1,600 tons was generated as a byproduct with valueless and had been paid attention for utilization. It was because the bone byproduct contained abundant nutrient, including 13.50% calcium mineral, 16.75% protein, and 18.40% lipid (SYAHIDAWATI and LIMPISOPHON, 2017; TOPPE *et al.*, 2007). This byproduct can be considered as a value-added product, which is one of profitable topics for fish industry. According to rich in calcium content of salmon bone, it can be utilized as a calcium food or ingredient. Therefore, in this study, a calcium-rich product from salmon bone as a crispy snack has been investigated by using alkaline pretreatment and thermal processes. The objectives of this research were to pretreat salmon bone by sodium hydroxide (NaOH) in order to reduce the lipid content and to improve texture of edible salmon bone product by the thermal process.

2. MATERIAL AND METHODS

Atlantic salmon bone, provided from Thai Union Group PCL, was kept at -18°C until use. Frozen bone was thawed, and cut as a cube-like specimen with length 0.8-1.5 cm. The bone was soaked in 1% (w/v) sodium chloride solution for 10 min, and then soaked in 4-8% (w/v) NaOH solution for 30-60 min. NaOH-treated bone was neutralized by 0.25 M citric acid for 30 min, and then washed with tap water. Ratio of bone to each solution at 1:5 (w/v) was used for all steps. Chemical compositions of NaOH-treated bone were analyzed, including moisture, lipid, protein and ash according to AOAC (2016). Pretreated salmon bone was boiled in water at 121°C for 25 min by a gas retort, and then dried at 140-180°C for 15-45 min by a hot air drying. After drying, the product was called as salmon bone product. Physical and chemical properties of the salmon bone product, including crispness and hardness following method of NATH and CHATTOPADHYAY (2007), moisture content (AOAC, 2016), and color (CIE L*a*b* system) using a spectrophotometer (Ultra Scan Pro, Hunter Lab, USA) with D65 illuminant were analyzed. Calcium contents of fresh bone, NaOH-treated bone, pretreated bone and the product were evaluated following ISO 6869 (2000). A difference between means in each treatment was performed using one-way ANOVA and the significant difference between means was tested by Duncan's Multiple Range test ($p \leq 0.05$) (SPSS version 17).

3. RESULTS AND DISCUSSION

The salmon bone with high lipid content may contribute unpleasant odor. Prior to the drying process, the lipid content in salmon bone was required to reduce, since it may result in rancidity of a final product (JANNAT *et al.*, 2008). In this study, the fresh salmon bones had high lipid content at 22.98 % that it was reduced by soaking with NaOH solution as shown in Table 1. The salmon bones were treated with 4-8% NaOH for 30-60 min, the lipid content was dramatically decreased as compared with the control ($p \leq 0.05$). The result indicated that NaOH solution can remove lipid and organic component in salmon bones because the NaOH reagent helped to increase polarity and water-soluble ability of lipid during washing (TECHOCHATCHAWAL *et al.*, 2009). However, sample, treated with 8% NaOH for 60 min, was easily broken, not uniform after drying. Therefore, 4% NaOH-treated bone for 60 min was used for further study due to a low level of chemical use. Furthermore, calcium content, a majority of ash component in fish bone approximately 30% by ash content (TOPPE *et al.*, 2007), might be increased. Since the lipid ratio was reduced, it resulted in an increase in proportion of calcium in the product.

Table 1. Effect of NaOH pretreatment on chemical compositions of salmon bone[1].

Sample/Pretreatment conditions	Chemical compositions (% wet basis) Moisture	Lipid	Protein	Ash
Fresh salmon bone	$47.28^{ab}\pm1.99$	$22.98^{a}\pm0.83$	$14.82^{d}\pm0.35$	$15.58^{b}\pm0.08$
0% NaOH, 60 min	$48.49^{a}\pm1.41$	$19.07^{a}\pm0.76$	$14.33^{d}\pm0.16$	$15.63^{b}\pm0.47$
4% NaOH, 30 min	$43.45^{c}\pm0.57$	$8.41^{b}\pm0.01$	$16.85^{ab}\pm0.18$	$30.66^{a}\pm0.83$
4% NaOH, 60 min	$45.18^{bc}\pm0.18$	$6.46^{bc}\pm1.08$	$16.45^{bc}\pm0.52$	$28.73^{a}\pm1.80$
8% NaOH, 30 min	$44.32^{c}\pm0.33$	$7.22^{bc}\pm3.55$	$15.95^{c}\pm0.02$	$29.92^{a}\pm0.19$
8% NaOH, 60 min	$45.70^{abc}\pm1.10$	$4.26^{c}\pm0.67$	$17.24^{a}\pm0.19$	$30.82^{a}\pm0.64$

[1] Data are expressed as mean ± standard deviation from two treatment replications.
Different superscripts in the same column are significantly different means ($p \leq 0.05$) by Duncan's multiple range tests.

According to preliminary experiment, fresh salmon bones had excessive hardness for human consumption. The pretreated salmon bone was boiled at 121°C for 25 min using the retort for improving softening and edible texture. A compact collagen fiber in fish bone structure might be destroyed by using retort at high temperature, resulted in lower hardness of fish bone (ZHANG *et al.*, 2016). When increasing drying temperature and time, a count peak of salmon bone product, indicating crispness, was increased, whereas values of hardness and moisture were decreased ($p \leq 0.05$) as shown in Table 2.

Table 2. Effect of drying condition on characteristics of salmon bone product by a hot air drying[1].

Temperature (°C)	Time (min)	Crispness (number of peak)	Hardness (N)	Moisture (%)	L*	a*	b*
140	15	$18^{d}\pm0.00$	$44.79^{a}\pm4.79$	$24.36^{a}\pm0.08$	$60.37^{bc}\pm0.93$	$3.74^{c}\pm0.02$	$18.54^{c}\pm1.12$
	30	$72^{c}\pm7.07$	$21.60^{b}\pm3.47$	$2.35^{cd}\pm0.53$	$62.40^{ab}\pm1.07$	$3.11^{c}\pm0.21$	$18.13^{c}\pm0.26$
	45	$90^{a}\pm4.95$	$17.50^{bc}\pm0.08$	$2.09^{cd}\pm0.62$	$62.90^{ab}\pm1.48$	$3.57^{c}\pm0.07$	$19.28^{bc}\pm0.45$
160	15	$74^{bc}\pm3.54$	$22.14^{b}\pm3.33$	$5.67^{b}\pm0.93$	$64.80^{a}\pm1.82$	$3.10^{c}\pm0.11$	$18.28^{c}\pm0.25$
	30	$87^{ab}\pm2.83$	$14.83^{c}\pm4.14$	$1.62^{d}\pm0.13$	$63.12^{a}\pm0.96$	$3.84^{c}\pm0.86$	$19.85^{bc}\pm1.56$
	45	$82^{abc}\pm5.66$	$14.01^{c}\pm1.34$	$1.85^{cd}\pm0.33$	$59.26^{c}\pm0.23$	$5.86^{ab}\pm0.24$	$21.22^{b}\pm0.77$
180	15	$84^{abc}\pm4.95$	$13.16^{c}\pm1.73$	$2.96^{c}\pm0.73$	$62.71^{ab}\pm1.36$	$3.73^{c}\pm0.28$	$19.20^{c}\pm0.23$
	30	$78^{abc}\pm12.02$	$12.08^{c}\pm0.63$	$1.49^{d}\pm0.01$	$60.29^{bc}\pm0.94$	$5.55^{b}\pm0.34$	$24.18^{a}\pm0.10$
	45	$80^{abc}\pm0.71$	$10.72^{c}\pm1.47$	$1.42^{d}\pm0.05$	$56.02^{d}\pm0.11$	$6.64^{a}\pm0.24$	$23.34^{a}\pm1.22$

[1] Data are expressed as mean ± standard deviation from two replications.
Different superscripts in the same column are significantly different means ($p \leq 0.05$) by Duncan's multiple range tests.

The moisture content of the product should be lower than 4% by following crispy fish skin standard in Thailand because it can be kept at room temperature (THAI COMMUNITY PRODUCT STANDARDS, 2004). All drying conditions could provide the uniform shape of product (Fig. 1). Drying at higher temperature and longer time resulted in lower lightness (L*), higher redness (a*), and higher yellowness (b*) as shown in Table 2. Increasing drying temperature and time provided darker brown color on the product as shown in Fig. 1, since carbonyl compound from lipid oxidation during a hot-air drying process could interact with amino group in fish bone. As a result, the dark brown product was generated (HIDALGO and ZAMORA, 2000). Drying at 180°C for 15 min by a hot air drying was selected to process the salmon bone product according to short drying time and low cost production.

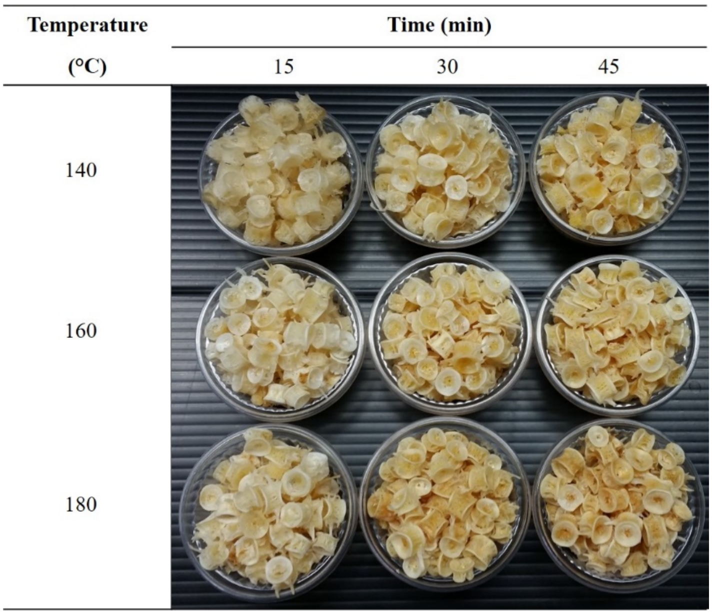

Figure 1. Effect of drying condition on appearance of salmon bone product by a hot air drying.

In Table 3, the calcium content of pretreated bone by NaOH and citric acid was decreased to 11.03% dry basis as compared with sample after washing with NaOH solution (NaOH-treated bone) because the citric acid solution can dissolve calcium in the pretreated bone (SRIUTTHAI *et al*., 2014). After drying, the calcium content of the final product was increased to 19.81% dry basis and was higher amount than from salmon bone (15.70% calcium content) as report of MALDE *et al*. (2010b). Calcium intake from salmon bone might be help bone metabolism and blood clotting. MALDE *et al*. (2010a) reported that calcium absorption from salmon bone (22.5%) in healthy men was not significantly different from that from calcium carbonate supplement (27.4% absorption) ($p > 0.05$). Majority of protein type in bone structure was collagen. The collagen could support calcium intake in rat as beneficial effect on bone metabolism (WU *et al*., 2004). The calcium mineral was the main mineral in the salmon bone product due to great number (39.15%) of calcium content calculated by ash basis. Therefore, fish bone with high levels of protein content and calcium mineral as shown in Table 3 has potential to be used as a good calcium-source for human consumption. Since calcium content of the salmon bone product was 19.23 g 100 g^{-1} sample; therefore, intake of the product only 5 g could provide enough calcium content following FAO/WHO (2001) recommendation at 1,000 mg of daily calcium intake. However, seasoning process of the product will be further studied before sensory evaluation.

Table 3. Chemical compositions and calcium of bones after treatment processes and salmon bone product[1].

Characteristics	Sample		
	NaOH-treated bone	Pretreated bone (After washing with citric acid)	Salmon bone product (After hot air drying)
	Chemical compositions (%wet basis)		
-Moisture	$44.66^{b}\pm0.69$	$54.60^{a}\pm1.12$	$2.96^{c}\pm0.73$
-Lipid	$5.33^{b}\pm0.99$	$5.52^{b}\pm0.41$	$9.92^{a}\pm2.00$
-Protein	$16.94^{c}\pm0.07$	$19.30^{b}\pm0.48$	$23.92^{a}\pm1.17$
-Ash	$31.40^{b}\pm1.02$	$22.91^{c}\pm0.43$	$49.11^{a}\pm0.17$
Calcium content (% dry basis)	$23.17^{a}\pm0.89$	$11.03^{c}\pm0.04$	$19.81^{b}\pm0.13$
Calcium content (% by ash)	$40.84^{a}\pm1.27$	$21.87^{c}\pm0.34$	$39.15^{b}\pm0.39$
Calcium content (g 100 g^{-1}sample)	nd	nd	19.23 ± 0.12

[1] Data are expressed as mean ± standard deviation from two treatment replications.
Different superscripts in the same row are significantly different means ($p \leq 0.05$) by Duncan's multiple range tests. nd means not determined.

4. CONCLUSIONS

Lipid content of salmon bone was decreased to 6.46% after pretreatment with 4% NaOH solution for 60 min. Drying condition at higher temperature and longer time increased the crispy texture of salmon bone product, whereas decreased its hardness as well as moisture content. The salmon bone product with crispy texture was created by a hot-air drying process at 140°C for 45 min, 160°C for 30-45 min, and 180°C for 15-45 min without statistically significant difference between means ($p > 0.05$). The crispness, moisture and calcium content of the salmon bone product, dried at 180°C for 15 min, were 84 count peaks, 2.96% (wet basis) and 19.23 g 100 g^{-1} sample, respectively. The salmon bone product from byproduct of fish processing had potential to be a source of natural calcium.

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ANTIOXIDANT ACTIVITY OF BIO-COATINGS FOR FOOD PACKAGING

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ABSTRACT

Poly (lactic acid)-based coatings, containing a hydroxytyrosol-rich olive extract and two different surfactants (Tween 80 and Span 85), suitable for food packaging applications, were developed and characterized. *In vitro* release of hydroxytyrosol in Ethanol 95% simulant was quantified by HPLC analysis, and 2-diphenyl-1-picrylhydrazyl (DPPH) spectroscopic assay was applied to investigate the antioxidant capacity. Results pointed out the effectiveness of the formulated bio-coatings in releasing antioxidant molecules and the key role of the surfactant in determining the release kinetics suggesting the possibility to design and develop tailor-made active packaging solutions according to the requirements of the target food.

Keywords: active packaging, antioxidant, biodegradable coating, controlled release, hydroxytyrosol, olive extract

1. INTRODUCTION

Active packaging systems play a crucial role in improving food quality and shelf life, thanks to the capability of continuously modify of the environment inside the packaging, through the release or the absorption of specific substances (APICELLA *et al*., 2018a). In active technologies field, the controlled release strategy paves the way for a wide range of applications, by tailoring and tuning packaging systems according to the specific requirements (PIOTTO *et al*., 2013)

In case of oxygen-sensitive foods, the release of biocompatible molecules from the polymer to the food matrix represents an innovative approach, overcoming the addition of synthetic antioxidants such as butylhydroxytoluene (BHT) and butylhydroxyanisole (BHA), that can accumulate in the human tissues, increasing the risk of toxicity to the consumer. Natural antioxidants can be successfully addressed to this purpose. Many phenolic compounds such as carvacrol, thymol or sesamol, as well as flavonoids such as quercetin and catechin, have been reported to possess multiple biological effects including antioxidant activity (LOPEZ-DE-DICASTILLO *et al*. 2012). Addition of alpha-tocopherol in polymeric packaging films acted as oxygen scavenger by autoxidation (SCARFATO *et al*., 2015) while green tea, rosemary and barley husks extracts were effective in slowing down lipid hydrolysis by scavenging free radicals and increasing lipids oxidative stability (SANCHES-SILVA *et al*., 2014). Among the biological molecules, hydroxytyrosol, a catechol derivative originating from oleuropein metabolism and highly abundant in olives, showed high antioxidant potential and health benefits (ACHMON and FISHMAN, 2014). Nowadays, hydroxytyrosol is mostly extracted from the olive fruits, as well as leaves, pomace and milling wastewaters. In this latter cases, the recovery of phenolic compounds from wastes represents not only an innovative route for the valorization of industrial by-products, but also solves a burdensome problem of environmental impact (APICELLA *et al*., 2019).

The above considerations motivated the selection of a hydroxytyrosol-rich olive extract for the realization of biodegradable active coatings with the aim of extending the shelf life of oxygen-sensitive foods. Multilayer films were produced by spreading a polylactide (PLA) coating layer, containing 3% w/w of active phase and 1% w/w of surfactant, on a corona treated bi-oriented polyethylene-terephthalate (BOPET) substrate. PLA was selected thanks to its good optical properties, suitability for food contact and good processability, in addition to the environmental benefits (SCARFATO *et al*., 2017). Moreover, amorphous PLA provides biodegradable sealant layer for improving the functional properties of the polyester film, while corona treatment on BOPET surface promotes the coating adhesion strength (APICELLA *et al*., 2018b). Two different releasing systems were developed by using two different surfactants, with different HLB values (Tween 80 and Span 85, respectively), to prepare the coating mixture. *In vitro* release tests in a proper fatty food simulant and DPPH radical scavenging measurements were carried out to evaluate the effectiveness of the produced systems as antioxidant carriers. In particular, the surfactants effect on the hydroxytyrosol release profiles and on the antioxidant capacity of the films was investigated.

2. MATERIAL AND METHODS

2.1. Materials

Liquid olive extract Hydroliv-Plus, with a minimum content of 15% in hydroxytyrosol was supplied from Bioresearch life (Spain). PLA 4060D (d-isomer content 12%) was supplied from Natureworks (Minnetonka, USA). Corona treated BOPET film was gently supplied from Nuroll S.p.A. (Italy). Tween80, Span85, free radical 2,2-diphenil-1-picrylhydrazyl (DPPH·) and ascorbic acid were supplied from Sigma-Aldrich. All the solvents were analytical grade.

2.2. Active coatings preparation

The biodegradable antioxidant coatings were realized by precipitation, induced by solvent evaporation, of a PLA 4060D coating solution (with mass ratio 20:80), incorporated with 3% w/w of Hydroliv-Plus extract and 1% w/w of surfactant under magnetic stirring. To avoid the active agent precipitation and to produce an homogeneous casting solution, non-ionic surfactants Tween 80 and Span 85 (HLB values equal to 15 and 1.8, respectively) were separately added to the polymeric mixture, which was prepared by using acetone and chloroform as solubilizing solvents, respectively. Before the coating deposition, the solution was sonicated using an ultrasonic bath to eliminate the air bubbles formed during the stirring process. Afterwards, the casting mixture was spread on the BOPET substrate by means of a K Hand Coater (RK, Printocoat Instruments Ltd., Litlington, UK), equipped with stainless steel closed wound rod, with wire diameter equal to 0.64 mm, yielding final coatings with comparable thicknesses, as listed in Table 1. Solvent was evaporated at room conditions overnight, then the coated films were stored under vacuum sealing in aluminium bags before analysis. The coating layer thickness was evaluated as difference among the total thickness of the coated films and the BOPET substrate thickness.

Table 1. List of the prepared films with their composition and thicknesses.

Sample	HT concentration [%wt]	Surfactant used	Total Thickness [μm]	Coating layer thickness [μm]
BOPET	0	-	23±0.9	0
BOPET-PLA4060D	0	Both	30±1.1	7±0.2
BOPET-PLA4060D-A	3	Tween 80	30±1.3	7±0.4
BOPET-PLA4060D-C	3	Span 85	30±1.4	7±0.5

2.3. Evaluation of coated films release kinetics and antioxidant activity

The release of hydroxytyrosol was evaluated as suggested by CHEN *et al.* (2012) with some modifications. Active films with 95 cm^2 area were placed into a single-side migration cell, with only the active side exposed to 100 ml of Ethanol 95% v/v, acting as fatty food simulant. The cell was kept in darkness at room conditions for 20 days. One millilitre of the food simulant was periodically sampled. The concentration of hydroxytyrosol released into the simulant was quantified using a UV-Vis spectrophotometer Perkin Elmer Lambda 800 (Perkin Elmer, Waltham, MA, USA) at 280 nm, on the basis of a calibration curve built with tyrosol as external standard, and values were expressed in mg of phenol per litre. The antioxidant activity released into the simulant solution was then quantified by DPPH method, using the stable radical 2,2-diphenyl-1-picrylhydrazyl as previously reported by BYUN *et al.* (2010). 100 μL of simulant solution was mixed with 3.9 mL of DPPH methanolic solution (6 × 10−5 M) in a capped cuvette. The mixture was shaken vigorously and kept at room temperature in the dark for 30 min. The absorbance was measured at 517 nm with UV–Vis. All analyses were performed in triplicate, and the obtained values were reported using a calibration curve of ascorbic acid as AEAC (Ascorbic acid Equivalent

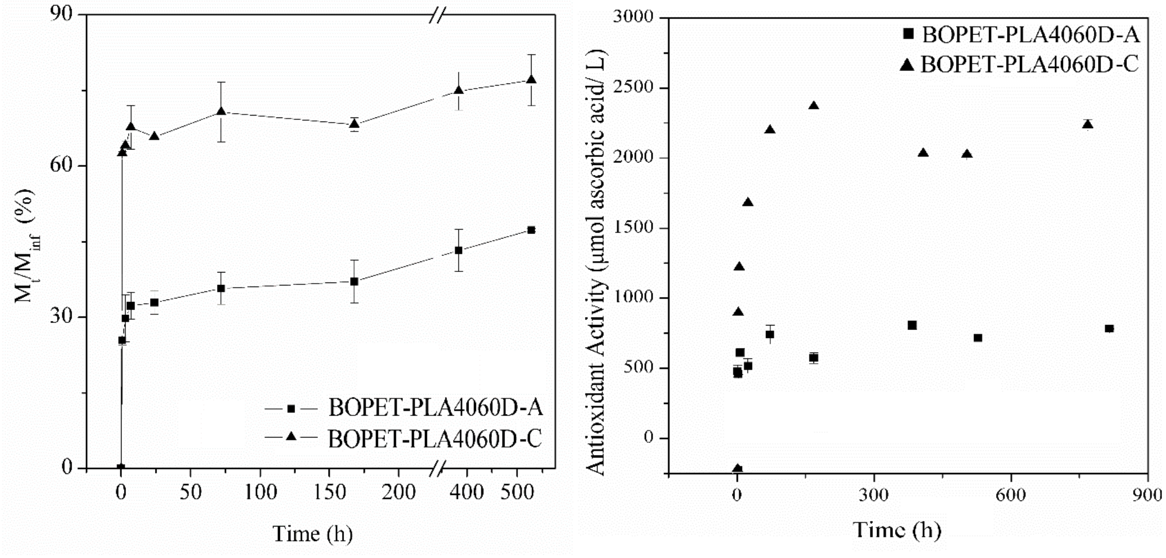
Antioxidant Capacity). Release studies and DPPH measurements were also conducted on control films (BOPET and BOPET-PLA4060D), and no significant absorbance at 280 nm and 517 nm was observed.

3. RESULTS

3.1. Release tests and antioxidant activity

The release profiles of hydroxytyrosol from the active films into the food simulant are reported in Fig. 1 (a), expressed as M_t/M_∞, where M_t is the concentration of antioxidant into the release solution at time t and M_∞ is the equilibrium concentration of the antioxidant in the release solution.

Results display the effectiveness of both the developed active systems in releasing the antioxidant molecules, exhibiting an initial faster kinetic, with a burst effect, followed by a sustained release, with a typical Fick's curve. An immediate release of ca. 65% of the antioxidant was achieved for the BOPET-PLA4060D-C sample during the first 24 hours, up to reach ca. 70% maximum release after 550 h. On the other hand, BOPET-PLA4060D-A sample displayed an initial hydroxytyrosol release of approximately 33%, followed by a maximum release of ca. 47% after 550 h. Therefore, the film containing Tween80 surfactant presented lower release profile than the Span 85 based film.



(a) (b)

Figure 1. Release profiles of hydroxytyrosol from active multilayer films into the food simulant (a) and antioxidant activity [μmol ascorbic acid/L] evaluated on the release simulant after contact with active multilayer films, by DPPH assay (b).

It is worth to note that the significant difference in diffusion mechanisms among the active films is the result of single interactions of a complex, quaternary system including the polymer matrix, the surfactant, the active agent and the food simulant. Some of the factors affecting the release kinetics include the polymer morphology and molecular weight distribution, the chemical interactions, the polarity, the polymer swelling capability, the temperature, as well as the antioxidant diffusivity into the matrix and the surfactant solubility into the simulant (APICELLA *et al*., 2019).

In this case, it is clear that the surfactant plays a crucial role in the release kinetics, because it affects both the coating polarity and the mobility of macromolecules.

Relevant effects of surfactants on *in vitro* release kinetics from antioxidant films were also found by other Authors. In fact, MARTELLI *et al*. (2017) reported an hindering effect of Tween 80 on the release mechanism of alpha-tocopherol from carboxymethylcellulose matrix, while lecithin-based films showed a maximum tocopherol release five times higher, and higher values of antioxidant activity. In the same way, BOONNATTAKORN *et al*. (2016) reported a significant increase of the release rate of mangiferin from ethylene-vinyl acetate (EVA) films after Span 20 (HLB=8.6) addition.

The results of antioxidant capacity of the films are shown in Fig. 1 (b). These values follow a similar trend as those of the release assays, i.e. higher values of antioxidant activity were found when Span 85 is added to the active film (higher amount of phenols are released from the PLA coating).

The maximum antioxidant activity displayed by BOPET-PLA4060D-C sample was equal to 2350 μmol ascorbic acid/L after 150 hours, while BOPET-PLA4060D-A reached a maximum antioxidant activity equal to 750 μmol ascorbic acid/L after ca. 75 hours. The slight decrease in the antioxidant activity occurring in BOPET-PLA4060D-C sample after 200 h could be attributable to the degradation of the phenolic components reacting with the oxygen dissolved in the medium, leading to the formation of superoxide radicals (MARTELLI *et al*., 2017).

4. CONCLUSIONS

In this research, active biodegradable coatings based on an hydroxytyrosol-rich olive extract and two different surfactants were successfully produced and characterized.

The results pointed out the effectiveness of the produced active bio-coatings in releasing the antioxidant agent into a proper food simulant. Results also highlighted that, modifying the chemical and morphological composition of the coating matrix, it is possible to modulate the release of the active agent. Therefore, this technology offers the possibility of tailoring the films performance according to the shelf-life requirements of the target food.

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ENZYMATIC STUDY FOR VALORIZATION OF PLANT WASTES

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ABSTRACT

Plant wastes represent an interesting source of natural polymers such as cellulose. An enzymatic study was carried out with hydrolytic enzymes in order to obtain a high quality fibers from tomato stalks. The total sugars released in the incubation solution were evaluated with Dubois Method. Cellulose, hemicellulose and lignin percentages of obtained products were determined by Van Soest method. SEM was used to evaluate the quality of fibers. The results demonstrated that tested enzymes can be applied in the tomato stalks pulp processing, to improve lignocellulosic material, which can be used as starting material in different industrial sectors.

Keywords: hydrolytic enzymes, lignocellulosic material, tomato stalks, plant wastes

1. INTRODUCTION

Agro-food industries produce a huge amount of plant wastes, which must be disposed. Among these, tomato stalks represent an interesting source of natural polymers that are increasingly popular products on the market for a wide variety of applications in several different industrial sectors, including the food industry. Biotechnological treatment of wood pulps provides great potential for the reduction of energy consumption and greenhouse gas emissions (ŽNIDARŠIÈ-PLAZL *et al.*, 2009). Therefore, it demands the need of the most appropriate and novel extraction method to extract the biopolymers from plants. Advances in technology for enzyme preparations have led to an increasing use of enzymes in industrial processes in the last two decades (ŽNIDARŠIÈ-PLAZL *et al.*, 2009). Hydrolases are used for protein and polysaccharides degradation to produce low molecular weight fragments from natural polymers that show new biochemical and technological properties. In the plant cell wall various polysaccharides, such as cellulose, hemicellulose, lignin and pectin, are present in large amount (ACOSTA-ESTRADA *et al.*, 2014; AJILA *et al.*, 2011). Enzyme assisted extraction of biopolymers from plants is a potential alternative to conventional chemical extraction methods and is gaining increasing attention as an efficient, sustainable and eco-friendly extraction technology. Enzymes such as xylanase, amylase, pectinase and hemicellulase are also used as a pretreatment step before extraction in order to maximize the extraction yield (SOWBHAGYA and CHITRA, 2010). Operational conditions such as temperature of reaction, time of extraction, pH, enzyme concentration, and substrate availability are critical for the extraction process (M'HIRI *et al.*, 2014). Excessive enzyme treatment might erode the fiber surface and reduce the strength of pulp (ŽNIDARŠIÈ-PLAZL *et al.*, 2009). The mixture of carbohydrate hydrolysing enzymes such as pectinase, hemicellulase and xylanase might provide better opportunity to adequately disrupt the cell wall structure and improve the extraction of the polymers (HAMMED *et al.*, 2013; PURI *et al.*, 2012; SOJITRA *et al.*, 2016). Similarly, the degradation of lignin can be achieved by using laccase enzyme (CAMARERO *et al.*, 2007). With the aim of recovering these important polymers, an enzymatic study was carried out with single or mixed hydrolytic enzymes in order to obtain a high quality lignocellulosic material.

2. MATERIAL AND METHODS

2.1. Enzymes

The commercial enzyme preparations of Pectinase from *Aspergillus niger* (30 kU/mL), Hemicellulase from *Aspergillus niger* (20 kU/g), Xylanase from *Bacillus subtilis* (20 kU/g), Laccase from white rot fungi (10 kU/mL) were purchased from from Creative Enzymes (Shirley, NY 11967, USA).

2.2. Pre-treatment of tomato stalks

Tomato stalks were obtained from Finagricola Company (Battipaglia, Italy). Mechanical and thermal pre-treatments were conducted: the stalks were first milled to 4mm chips and then suspended in 10 volumes of water and sterilized in autoclave at 121°C for 30 minutes. After that, the samples were centrifuged at 10000×*g* for 10 min at 20°C; the pellet of pre-treated chips was stored at 4°C until the enzymatic treatments.

2.3. Enzymatic Treatments

Test tube-experiments were conducted on thermally and mechanically pre-treated chips. In particular, 2 g of pre-treated chips were suspended in 20 mL of sodium acetate buffer (50 mM, pH 5) and stirred at 150 rpm in orbital shaker (Forma Scientific) at 50°C with or without enzymes (control). The enzyme-substrate ratio was 1:20 (w/w or v/w). Single enzymes in sequence at 24 h intervals or mixed enzymes together were added to the reaction mixture and the hydrolytic activity was monitored at increased incubation time. Aliquots of the incubation solutions were taken for subsequent determinations at fixed time intervals (3, 6, 9, 24, 30, 72 h). In order to stop the enzymatic reaction, the samples were boiled in water bath for 10 min and then centrifuged at 10000×*g* for 5 min. Both pellet and supernatant from control and enzymatically processed chips were used for the further chemical determinations and image analyses.

2.4. Chemical characterization and image analyses

The total carbohydrate release in the incubation solution was calculated by Dubois method applied on the supernatant of digested samples, and was expressed as mg of glucose equivalent/g of sample (DUBOIS *et al.*, 1956). The pellets resulting from the same centrifugation were lyophilized for the further determination of cellulose, hemicellulose and lignin contents by Van Soest Method (VAN SOEST, 1963).

The same lyophilized samples were coated with gold particles and the microstructure was examined by means of Scanning Electron Microscopy (SEM) (LEO EVO 40 SEM, Zeiss, Germany) with a 20 kV acceleration voltage and a magnification of 250 and 500×.

3. RESULTS AND DISCUSSION

The thermally and mechanically pre-treated samples were used for the extraction of the fibers following enzymatic method. The selected enzymes were pectinase, hemicellulase, xylanase and laccase for the hydrolysis of water insoluble pectins, hemicellulose and lignin (DEMUNER *et al.*, 2011). Preliminary results showed the efficacy of mixed enzymes in cutting down pectins, hemicelluloses and xylanes in shorter soluble fragments, which can be removed with the liquid phase. The highest total sugar release in the incubation solution was recorded at the maximum incubation time for all analyzed enzymes, although, mixed enzymes showed a higher positive effect on the polysaccharide extraction (Fig. 1).

Prolonging the soaking of chips with enzymes might improve the solubilisation of cell wall components. However, in a large scale setup very long incubation time would lead to inferior product quality and energy inefficiency (BABBAR *et al.*, 2016). An advantage of enzyme assisted extraction is the requirement of less amount of solvents for the extraction of desired polysaccharides, making the process greener (NADAR *et al.*, 2018). The selection of appropriate enzyme and its concentration depends on the composition of plant cell walls (GHOSH and BISWAS, 2015). The percent enzyme loading will primarily depend on the cost of enzymes used and the quality of the biomolecules extracted (JIANG *et al.*, 2010). During the crude pectic extraction from agro-industrial residues, Babbar *et al.* examined the effect of different extraction strategies on the composition of both neutral and acidic pectic polysaccharides. In this study, the milled chips treated with enzymes showed an increase in the percentage of cellulose and a reduction of hemicellulose and lignin content compared to the control sample (Table 1).

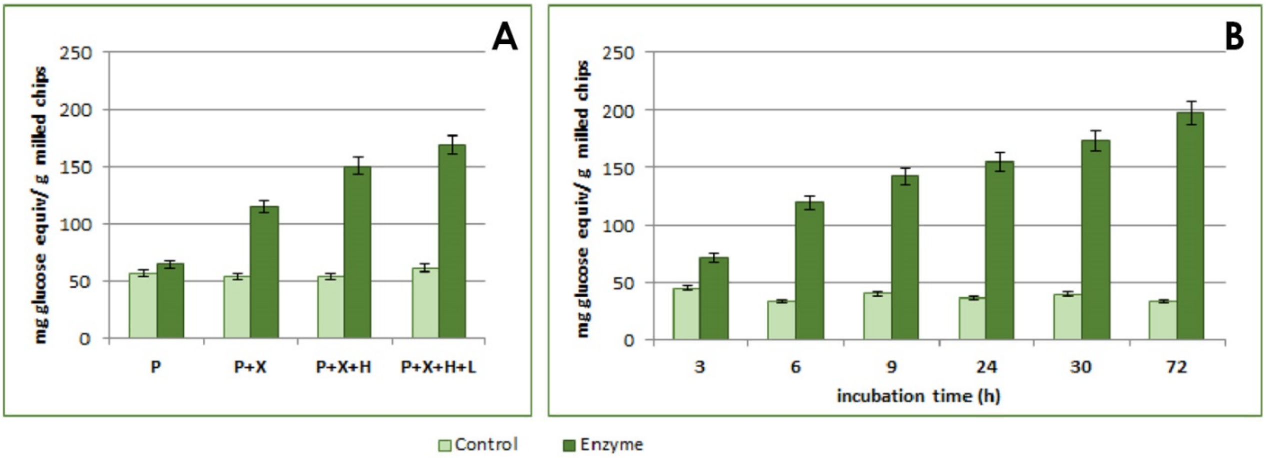

Figure 1. Total sugar release in the incubation solution of digestion mixture performed with single enzymes in sequence added at 24 h intervals (A) and mixed enzymes (B).
P, pectinase; X, xylanase; H, hemicellulase; L, laccase.

Table 1. Polymers content of treated milled chips.

Treated milled chips	Hemicellulose (%)	Cellulose (%)	Lignin (%)
Control	20.4±0.85	26.53±0.50	11.9±0.12
Mixed Enzymes	17.8±0.42	32.85±1.0	10.35±0.07

Values are expressed as Mean±SD (n=3).

The morphology of the tomato stalks, control and enzymatically processed chips was observed by Scanning Electron Microscopy (SEM) (Fig. 2). In the stalks samples the fibrous component was covered with hemicellulose coating material, which gave the sample a rough appearance similar to a bark (Fig. 2-A). Moreover, the lumen of the xylem vessels was clearly visible in cross section (Fig. 2-B). The enzymatic treatment of milled chips allowed to remove the hemicellulose component from the surface of the lignocellulosic structures well visible in Fig. 2-C, leading to the release of clean fibers (Fig. 2-D).
Mixed enzymes are seen to have a positive effect on the polysaccharide extraction. An advantage of enzyme assisted extraction is the requirement of less amount of solvents for the extraction of desired polysaccharides, making the process greener (SHAMRAJA *et al.*, 2018).

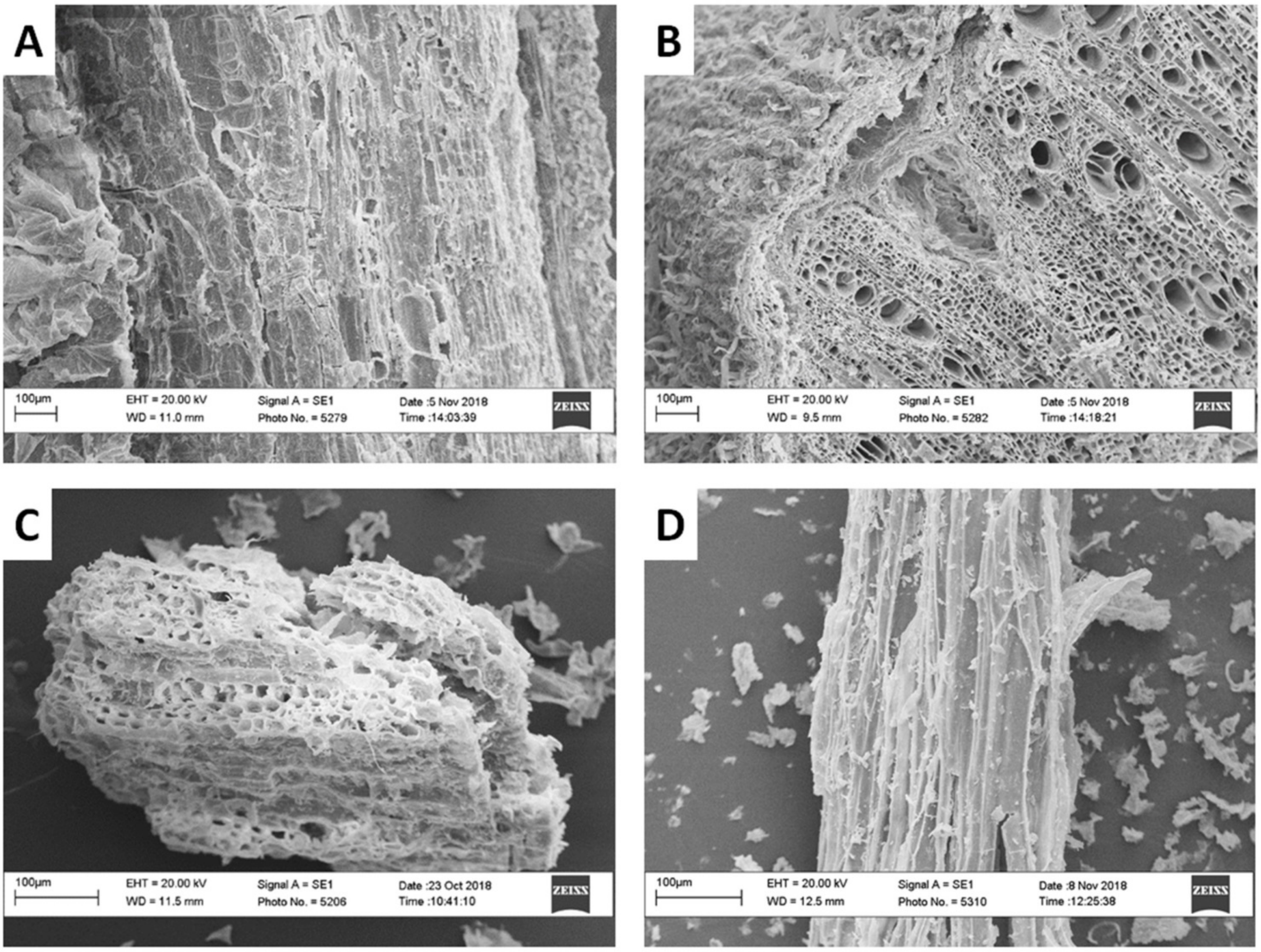

Figure 2. SEM images of tomato chips [longitudinal (A) & transverse (B) sections, (250x)] and treated milled powder [control (C) & enzymatically processed (D), (500x)].

4. CONCLUSIONS

The obtained results demonstrated that tested enzymes can be applied in the tomato stalks pulp processing, to improve lignocellulosic material, which can be used as starting material in different industrial sectors. The advantages of applying an enzymatic treatment for the recovery of plant waste consist in the use of environmentally friendly processes, in energy saving and, finally, in cost reduction.

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CARRANGEENAN CRYOPROTECTANT OF FROZEN COCONUT MEAT

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ABSTRACT

The objective of this study was to investigate the impact of the co-presence of texturizing agents and k-carrageenan on protecting coconut meat against freeze-thaw damage. k-carrageenan with calcium chloride or citric acid showed good textural, drip loss and browning protection. Calcium chloride produced greater enhancement of textural firmness in the blanched than in the unblanched coconut. However, the drip loss and browning of the blanched coconut were more highly developed. Citric acid had an adverse impact on the firmness and browning of the blanched coconut while beneficially reducing the drip loss. Calcium chloride likely enhances the cryoprotectability of carrageenan in fresh and blanched coconut meat.

Keywords: carrageenan, calcium, citric acid, freeze, thaw, coconut

1. INTRODUCTION

Freezing is an excellent preservation method; however, it is also detrimental due to physical disruption it causing to the cells or cell components of plant tissues. After frozen and thawed, fruit and vegetables have a soggy texture as the cell membrane and wall break down and the cellular liquid separates from the matrices (JUL, 1984). Drip loss and other quality deterioration are developed (SRIWIMON and BOONSUPTHIP, 2011). Several attempts have been made to reduce the susceptibility of fruit and vegetable tissues to freezing deterioration (BOONSUPTHIP and LEE, 2003; BOONSUPTHIP *et al.*, 2009). Hydrocolloids are a key hydro-dynamically active ingredient that binds and controls water to reduce the damage caused by ice crystals (ZEYNALI *et al.*, 2019).

Generally, κ-carrageenan is used to improve cryo-protectability, texture firmness, emulsion stability, and sliceability and juiciness (SOUKOULIS, 2008; ZHANG *et al.*, 2018). In fact, the κ-carrageenan gel network is subjected to freezing and freezing and thawing damages (KOZŁOWICZ, 2013) and consequently, it cannot protect a variety of frozen food products. However, there are proven benefits. For example, κ-carrageenan cryo-protected chicken myofibrillar protein (actin and myosin) from denaturation when stored at -25°C for 3 months (KOVAČEVIĆ *et al.*, 2009). Pre-conditioning using κ-carrageenan was beneficial to frozen pre-cut carrot (MAITY *et al.*, 2011), melons (RESENDE and Cal, 2002), and a tapioca starch model (AGUDELO *et al.*, 2014). The presence of κ-carrageenan was a crucial factor for the cryoprotection of ice cream (SOUKOULIS *et al.*, 2008).

A mixture of cryoprotectants evidently improved the quality of frozen food such as blends of κ-carrageenan and xanthan gum to improve the texture of frozen/thawed mashed potatoes by retarding starch retrogradation, increasing the water holding capacity and enhancing consumer acceptance (ALVAREZ *et al.*, 2009). κ-Carrageenan and whey protein isolate retarded ice recrystallization (CHUN *et al.*, 2012). κ-Carrageenan, potassium ion and locus bean gum helped to improve its freezing-thawing stability (LUNDIN, and HERMANSSON, 1995). Other advantageous blends include carrageenan-pectin and xanthan gum-guar gum for cooked carrot, potatoes and turnips (DOWNEY, 2002); xanthan-carrageenan-sodium caseinate and guar-pectin-whey protein concentrate for cooked potatoes (DOWNEY, 2003); and sodium casienate-xanthan gum for mashed potatoes (FERNANDEZ *et al.*, 2009). In addition, a mixture of hydrocolloids having small molecules can also be useful such as pectin-sugar-$CaCl_2$ for the prevention of freezing losses and phyto-chemical deterioration of strawberries (RENO *et al.*, 2011). The presence of other substances, pH and ionic strength can affect the molecular behavior of κ-carrageenan. As calcium chloride and citric acid are commonly used to pre-condition fruit and vegetables prior to freezing, κ-carrageenan functionality as a cryoprotectant could be affected by these substances.

The objective of this study was to evaluate κ-carrageenan cryoprotectibility in frozen coconut meat when calcium chloride or citric acid was present. Coconut meat has soft tissue that is easily altered by a texturizing agent and damaged by freeze-thaw cycles. The carrageenan gel-network was selected as it is subjected to freezing. A loss of water holding capacity is its weak point. The co-presence of texturizing agent was expected to enhance the cryo-protectability of carrageenan. The conformational change of κ-carrageenan by these substances was expected to influence ice crystallization and recrystallization characteristics in a way that would strengthen textural firmness and reduce drip loss and discoloration of frozen/thawed coconut meat. As blanching causes tissue softening, its impact on hydrocolloid ability was also observed.

2. MATERIAL AND METHODS

2.1. Sample preparation and pre-treatment

Young aromatic coconut fruit (Rachaburi province, Thailand, harvested at 200-210 days after blooming) had a meat layer about 3-4 mm thick. The white meat was sliced into pieces (5 x 5 cm) and stored in an ice bath prior to use. Samples were dipped in a calcium chloride ($CaCl_2$) solution and left for 10 min at room temperature, and in a 1% carrageenan solution and left for 10 min. Individual slices of coconut were stored in sealed plastic bags before being frozen and stored overnight at -20°C. A freeze-thaw cycle was performed by freezing at -20°C for 14 h, thawing at room temperature for 2 h, freezing at -20°C for 6 h and thawing at room temperature for 2 h. Ten freeze-thaw cycles were applied.

2.2. Texture measurement

A slice of coconut was placed on the flat platform of a texture analyzer (TA.XT plus, Stable Micro Systems Ltd., Godalming, UK) with a 49 N load cell. A cylindrical Perspex probe (6 mm diameter, 35 mm length) was used to puncture through the coconut tissue from the outer endosperm toward the inner endosperm, penetrating to 80% of the total fruit width. The crosshead speed was set at 2 mm/s. The textural parameter measured on the resulting force-distance curves was used to describe the firmness or maximum resistance to penetration. The mean values of 10 replicates, expressed in g force, were reported.

2.3. Drip loss measurement

Samples were weighed before freezing (m_1), and after undergoing 10 freeze-thaw cycles and then laid over absorbent papers at room temperature until a constant weight was reached (m_2). The drip loss (DL) was calculated using equation (1):

$$DL = [(m_1 - m_2) / m_1] \times 100 \tag{1}$$

2.4. Color measurement

Color measurements (Hunter L*, a* and b* values) were performed on the surface of the samples using a Hunter Lab DP 9000 (Hunter Associates Laboratory Inc., Reston, Virginia. The browning index (BI) was calculated using equation (2):

$$BI = [100(x-0.31)]/0.17 \tag{2}$$

$$x = (a^*+1.75L^*)/(5.645L^*+a^*-3.012b^*) \tag{3}$$

2.5. Statistical analysis

The experiment was arranged using a completely randomized design with three replications. Analysis of variance (ANOVA, 95% significance level) was carried out using the statistical analytical software package SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). Differences between means were assessed using Duncan's multiple range test with differences considered significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Textural hardness

The hardness of fresh coconut meat (Raw) was largely reduced by 10 freeze-thaw cycles (None) (Fig. 1a). κ-Carrageenan had satisfactory textural protection in the presence of calcium chloride and citric acid. Calcium chloride (0.5% and 1%) was more effective than citric acid (0.5% and 1%), especially at high concentration. Calcium ions are commonly used to protect plant tissue by increasing the cell wall strength and tissue firmness. In the presence of calcium (gel-strengthener) ions, κ-carrageenan gels by electrostatic interaction among the negatively charged sulfate groups of κ-carrageenan and the positively charged ions (CHUN *et al.*, 2012). This gel likely strengthened the coconut meat texture. Citric acid softens plant tissue due to pH reduction (ZIVANOVIC *et al.*, 2003). The softened tissue possibly freed ions of potassium, calcium, magnesium, manganese, sodium and cobalt, which are trivially available in coconut meat (SOLANGI and ZAFAR, 2011), to support the electrostatic gel of κ-carrageenan, and to strengthen the coconut texture to better withstand freeze-thaw damage.

Once the coconut meat had been blanched for a short-time prior to treatment with all the solutions, the texture of the blanched coconut became slightly softer than that of the fresh (None) coconut. Calcium chloride produced greater enhancement of textural hardness for the blanched than for the unblanched coconut. Blanching softens plant tissue by heat; however, it also strengthens by way of catalysis of pectin and divalent ion linkages (SANJUÁN and HERNANDO, 2005). In addition, tissue modification by blanching was reported to enhance the diffusion of small compounds (sugar, calcium ions) into plant tissue, and to support textural protection impact (NERI and PITTIA, 2014). However, the softening impact of citric acid was potentially enhanced by blanching.

3.2. Drip loss

κ-Carrageenan decreased the drip loss in the presence of calcium chloride and citric acid (Fig. 1b).

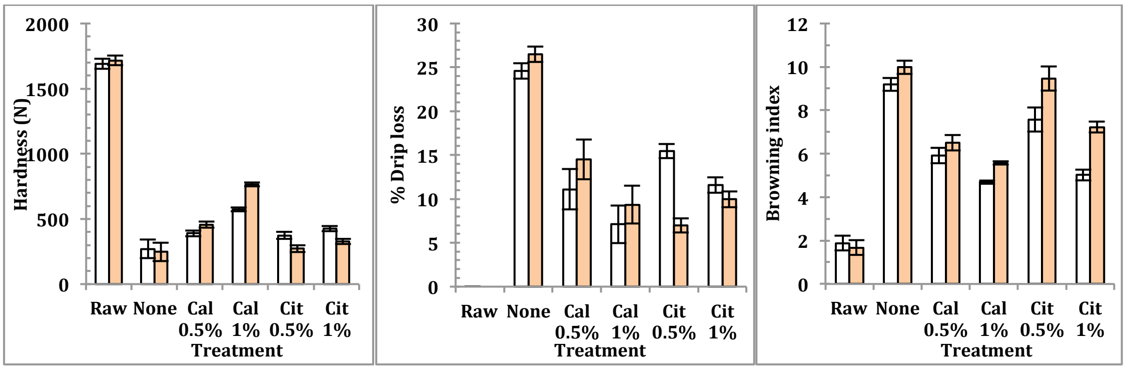


Figure 1. Effect of texturizing agents (calcium chloride and citric acid) on carrageenan cryoprotectability of coconut quality: (a) hardness, (b) %drip loss and (c) browning index. The coconut was fresh (☐ Fresh) and blanched (▨Blanched) before treatments.

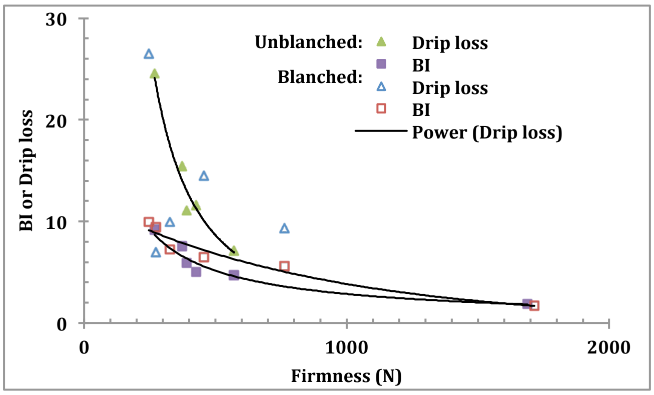
Calcium chloride was more effective than citric acid for the unblanched samples. As expected, improvement in drip loss was associated with the better coconut texture integrity. Interestingly, the impact of citric acid on drip loss was significantly improved by blanching. Such softened tissues by a combination of citric acid and blanching possibly interacted well with the κ-carrageenan to allow greater water holding capacity.

3.3. Browning index

κ-Carrageenan inhibited browning of the unblanched and blanched coconut meat samples in the presence of calcium chloride and citric acid (Fig. 1c). Calcium chloride had a stronger impact than citric acid, especially at high concentration. Better protection of color was potentially associated with that of texture and drip loss. Citric acid is generally applied to inhibit polyphenoloxidase (PPO) and browning (Hussein *et al.*, 2014). However, combinations of κ-carrageenan and calcium chloride produced better browning protection. Browning of the blanched coconut was more highly developed than those of the unblanched samples. This blanching condition was possibly too mild; leading to stimulation of enzymatic browning instead.

3.4. Relationship

Interestingly, firmness of the unblanched coconut was associated with its drip loss and browning index (BI) (Fig. 2). However, the firmness of the blanched coconut was only related to its browning index suggesting that the mechanism of κ-carrageenan in combination with either calcium chloride or citric acid to protect unblanched coconut meat against freeze-thaw damage was different from that applying to the blanched coconut.



Relationship	Equation	R^2
Unblanched		
Firmness-Drip loss	$y = 253576x^{-1.655}$	0.9545
Firmness-BI	$y = 999.04x^{-0.848}$	0.9644
Blanched		
Firmness-Drip loss	No correlation	
Firmness-BI	$y = 12.107e^{-0.001x}$	0.9756

y = Firmness, x = Drip loss or BI

Figure 2. Firmness relationships with drip loss and browning index (BI) of unblanched and blanched coconut meats after 10 freeze-thaw cycles.

4. CONCLUSION

Calcium chloride can enhance the cryoprotectability of κ-carrageenan in fresh and blanched coconut meat. Calcium chloride was more effective than citric acid, especially at high concentrations. Once the coconut meat had been blanched for a short time period prior to treatment with all the solutions, the texture of the blanched coconut became slightly softer than that of fresh coconut. Interestingly, the firmness of the unblanched coconut was associated with its drip loss and browning index. However, the firmness of the blanched coconut was only related to its browning index. The calcium chloride-κ-carrageenan mixture could be a good choice for the cryoprotection of frozen plants. Firming the texture of plant tissue prior to freezing is potentially beneficial. Citric acid, if needed to be used with κ-carrageenan, offered lower benefits than calcium chloride. However, for blanched plants, citric acid is likely to better reduce drip loss. Other combination of texturizing agents and hydrocolloids would be interesting for further exploration.

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ENZYMATIC MODIFICATION OF PROTEINS AND PEPTIDES FROM OILSEEDS BY-PRODUCTS

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ABSTRACT

Nowadays, the worldwide attention on waste reduction is increasing. In this view, the development of new processes is interesting for food industries that aim to reuse wastes both as a food product with added value or for other uses such as edible packaging. Modification of proteins and peptides by transglutaminase represents a suitable way to manipulate polymeric matrices in order to change its physico-chemical and textural properties. For this purpose, whole proteins from de-fatted flour and/or peptides residual from the enzymatic oil extraction from walnut and pumpkin seeds were incubated in the presence of microbial transglutaminase and monitored through SDS-PAGE.

Keywords: walnuts, pumpkin seeds, oilseeds, transglutaminase enzyme, protein, peptide

1. INTRODUCTION

The growing worldwide attention to waste reduction leads food companies to focus on the recovery of by-products. Among this, the development of new processes is interesting and useful for large food industries that aim to reuse wastes both as a food product with added value or for other uses such as edible packaging (TAHA and IBRAHIM, 2002; BURGOS *et al.*, 2016). Modification of proteins and peptides by enzymes such as Transglutaminase (TG) has been the subject of numerous studies by scientists for many years. TG is an enzyme capable of catalyzing the formation of covalent crosslinks between peptide-bound glutamynil residue and ε-amino group of lysine residues in proteins and peptides (ANDO *et al.*, 1989; NONAKA *et al.*, 1989). In the absence of amine substrate in the reaction system, water becomes the acyl acceptor and the γ-carboaximide groups of glutamine residues are deaminated, becoming glutamic acid residues.

This work focuses the attention on the recovery of waste deriving from different processes applied in oil extraction. Today, the extractions with organic solvents are the most effective and used methods to obtain oil from oilseeds. However, enzymatic treatments aiming to improve oil extractability from oilseeds represent for companies a new and environmentally friendly way to produce oil (ROSENTHAL *et al.*, 2001). However, regardless of whether the oil is chemically or enzymatically extracted, a large amount of waste is produced from the process, consisting essentially of proteins, peptides and fibers. This material can be considered an excellent one start point to re-use by-products as a new source of polymers for developing of edible packaging.

The aim of this study was to assess the capability of the TG to promote the formation of networks in different kind of proteins derived from oilseed by-products, such as de-fatted flour and proteolysed by-product from enzymatic oil extraction processing, in view of their using as a cross-linking protein component in edible films. For this purpose, whole proteins from de-fatted flour and/or peptides residual from the enzymatic oil extraction from walnut and pumpkin seeds were incubated in the presence of microbial TG and monitored through SDS-PAGE.

2. MATERIAL AND METHODS

2.1. Enzymes

Transglutaminase (ACTIVA WM, 92 U/g, produced by Ajinomoto) was provided by Prodotti Gianni S.r.l., MI, Italy. Viscozyme was obtained from Sigma-Aldrich, St. Louis, USA. The commercial Papain (100 kU/mL) was purchased from Creative Enzymes (Shirley, NY 11967, USA).

2.2. De-fatting of flours

Deshelled walnuts and pumpkin seeds, from local market, were ground to a fine powder by using a coffee grinder at high speed. Then, the powder was diluted 1:10 (w/v) with N-hexane and stirred at 150 rpm in orbital shaker (Forma Scientific) for 60 min at 37°C. The samples were then centrifuged at 15000×g for 10 min at 20°C; the supernatant was discarded and the pellet was allowed to dry overnight. The protein content was determined using the Bradford assay (BRADFORD, 1976).

2.3. Enzymatic treatments

2.3.1 Papain assay

Walnuts and pumpkin seeds were ground to a fine powder by using a coffee grinder at high speed, then were subjected to papain treatment to simulate the enzyme-assisted oil extraction process in aqueous phase. In particular, 5 g of powder of each sample were dispersed in distilled water at 1:3 powder-to-water ratio and 7 mg/mL of papain were added. The reaction mixture was incubated at 50°C and 150 rpm in an orbital shaker (Forma Scientific) for 16 h, and after that was centrifuged at 4000×g for 30 min. The water phase was carefully collected by using a Pasteur pipette and then boiled for 2 min to stop the enzymatic reaction. A control was performed without boiling. All the samples were stored at -20°C until the SDS-PAGE analysis.

2.3.2 Viscozyme assay

Defatted flours obtained from walnuts and pumpkin seeds were disperded in distilled water at concentration of 10 mg/mL in a total volume of 5 mL; then 40 μL of Viscozyme were added and the reaction mixtures were stirred at 150 rpm in orbital shaker (Forma Scientific) for 4 hours at 37°C.

2.3.3 Transglutaminase assay

The transglutaminase assay was performed with whole proteins from de-fatted flour, treated and not with Viscozyme, and peptides residual from the enzymatic oil extraction, boiled or not, from walnut and pumpkin seeds. Especially, 500 μg of each sample were incubated with 10 mU of mTG in a final volume of 60 μL and stirred at 150 rpm in orbital shaker (Forma Scientific) for 2 or 16 hours at 37°C.

2.4. SDS-PAGE

The SDS-PAGE was performed according to LAEMMLI (1970) using 15% resolving and 5% stacking gels. After electrophoretic running the gels were stained with Coomassie brilliant blue G-250 and destained in a 10% Methanol/10% Acetic Acid solution. Finally, the image of the gels was acquired by using a digital camera.

3. RESULTS AND DISCUSSION

Oilseeds by-products were produced from ground walnut and pumpkin seeds at lab scale with two different methods simulating the oil extraction processes: organic solvent extraction was performed by de-fatting with hexane, giving rise to de-fatted flours; while enzyme assisted extraction was achieved by papain treatment in aqueous phase in order to obtain the oil outcrop and the release of proteolysed proteins and peptides in the water phase. The two different wastes (de-fatted flours and protein hydrolysates) were further subjected to mTG enzymatic treatment to add value to oilseeds by-products. A basic flow chart of processing step used for preparing the samples is shown in Fig. 1.

In particular, the de-fatted flour was also pre-treated with Viscozyme to obtain better solubilisation of proteins. On the other side, the aqueous waste was collected and boiled to stop the enzyme reaction. Then, whole proteins from de-fatted flour and peptides residual

from the enzymatic oil extraction from walnut and pumpkin seeds were incubated in the presence of mTG and the cross-linking activity was monitored through SDS-PAGE.
Both walnut and pumpkin seed proteins and peptides resulted able to be crosslinked by transglutaminase. In particular, electrophoresis showed that proteins and peptides have formed the networks and the high molecular-weight bands are increased both after 2 and 16 hours of incubation with mTG (Fig. 2).

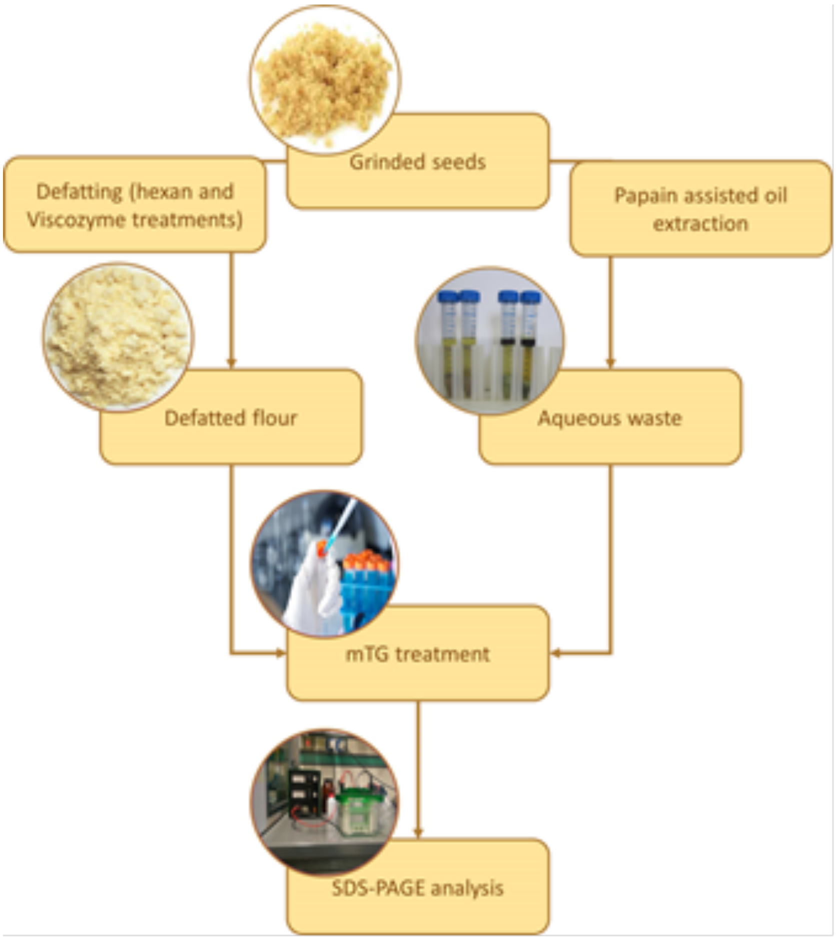

Figure 1 - Flow chart of experimental design.

The pre-treatment with Viscozyme of seed flours allows a better solubilization of proteins and, in fact, the electrophoretic profiles are richer in bands (Figs. 2A, B).
Pumpkin peptide samples showed an abnormal migration: this is due to a problem of coagulation of the sample when subjected to the necessary thermal treatment before loading the gel (Fig. 2C). Further experiments are needed to avoid this artifact.

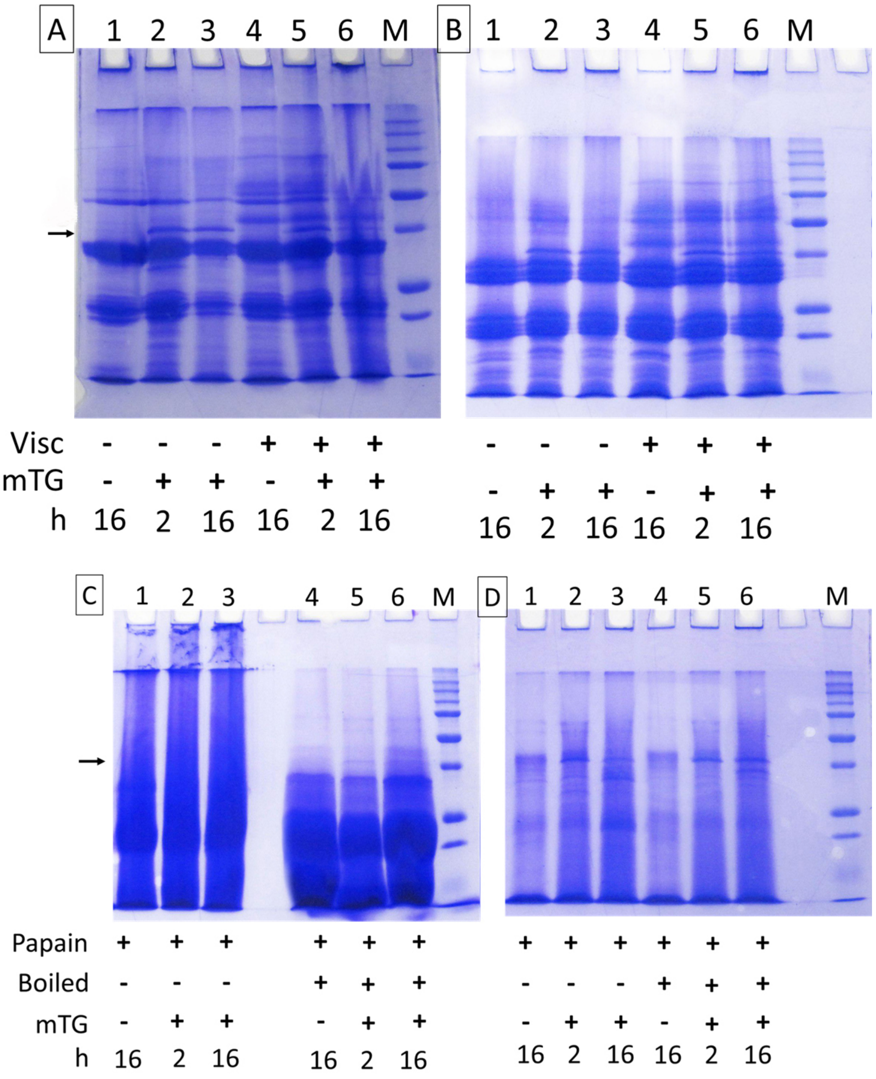

Figure 2. SDS-PAGE profile of proteins (A-B) and peptides (C-D) from pumpkin (A and C) and walnut (B and D) samples treated with mTG in different conditions. h, hours of mTG incubation. Black arrow indicate the mTG band. M, Precision Plus Protein™.All Blue Prestained Protein Standards (Bio-Rad).

4. CONCLUSIONS

These preliminary results suggest that the oilseeds by-products could be a new opportunity for design edible film and coatings in combination with other hydrocolloids of carbohydrate origin such as pectin.

Microbial TG allow to promote the formation of networks in different kind of proteins derived from oilseeds by-product, such as de-fatted flour and proteolyzed by-product from enzymatic oil extraction processing. So, these advantages are a starting point for further investigations for the recovery and re-use of waste from oilseeds by-products.

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