PAPER

EFFECT OF ANTIOXIDANTS AND PACKING CONDITIONS ON STORAGE STABILITY OF CEREAL BAR FORTIFIED WITH HYDROLYZED COLLAGEN FROM SEABASS SKIN

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ABSTRACT

Effect of antioxidants (green tea powder (GT) and citric acid (CA)) and packing conditions on storage stability of cereal bar fortified with hydrolyzed collagen (HC) from seabass skin was studied up to 6 months of storage at 25°C in dark. Up to 3 months of storage, the addition of antioxidants impeded lipid oxidation, especially those cereal bars packed in polypropylene with normal heat seal (PP). Changes in moisture content, water activity, color, texture, PV, TBARS and formation of volatiles were effectively retarded when samples were packed under N_2 gas in laminated polyethylene/aluminium foil bag (LF) for 6 months of storage.

Keywords: hydrolyzed collagen, cereal bar, antioxidants, packing condition, physicochemical parameters

1. INTRODUCTION

The market for functional foods has been continuously growing (VICENTINI *et al*., 2016). Fish hydrolyzed collagen (HC), has been demonstrated to contain low-molecular-weight peptides with a wide range of bioactivities (GÓMEZ-GUILLÉN *et al*., 2011). Bioactivity and health enhancing potentials have led to the use of HC, especially from marine animal, for developing functional foods, cosmetics and pharmaceutical products (RUSTAD *et al*., 2011; ZHUANG *et al*., 2009). With increasing consumer demand for health promoting food, fish HC is of increasing interest as a bioactive ingredient because of its associated nutraceutical aspects (THIANSILAKUL *et al*., 2007). It can be fortified in drinks to enhance bioactivity such as antioxidant activity (CHUAYCHAN *et al*., 2016). However, fish HC addition can potentially bring about a fishy odor in the finished product, leading to rejection by consumers. Recently, bioactive HC powder without fishy odor was developed using enzymatic hydrolysis of seabass skin (BENJAKUL *et al*., 2017). Thus, it could be fortified at higher level imparting increased bioactivities.
Cereal bars are eaten regularly due to their nutritive values and ease of consumption. Several kinds of cereal bars have been produced to serve the growing functional food market (DEAN *et al*., 2007; TALENS *et al*., 2012). From our preliminary study, the relationship between health concerns of the consumers and purchase intention for cereal bar fortified with HC was investigated. The results indicated that HC powder can be used as supplement for the development of functional cereal bar. Focus group results suggested that production of cereal bars consisting of many grains with high levels of HC powder (5%) was feasible. In general, cereal bars have a high oil content, mainly from nuts and some grains, which are susceptible to oxidation during storage. Furthermore, HC powder is hygroscopic and absorbs water easily. These changes directly affect physical, chemical and sensory properties of the cereal bars fortified with HC powder during the extended storage.
Antioxidants have been employed in foods to prevent lipid oxidation, which can result in off-odor and toxicity in foods (ROSTAMZAD *et al*., 2011). Due to safety concern, synthetic antioxidants have been commonly replaced by natural antioxidants. Antioxidative compounds such as citric acid and green tea powder were reported to retard lipid oxidation in food products (ROSTAMZAD *et al*., 2011; LORENZO and MUNEKATA, 2016). Moreover, packaging materials and storage conditions are important factors governing the shelf-life of foods via lowering water migration as well as oxygen permeability (NILSUWAN *et al*., 2016; BAKKALBAŞı *et al*., 2012). Lipid oxidation can be inhibited by using a packaging material with good protection and barrier properties or by storing the products in atmospheres containing a low oxygen content (NILSUWAN *et al*., 2016; BAKKALBAŞı *et al*., 2012). The application of antioxidants along with appropriate packaging condition could be an effective means for the shelf-life extension of cereal bars fortified with HC powder. The objective of this study was to investigate the effect of some antioxidants and packaging conditions on quality changes of cereal bars fortified with HC powder during storage at 25°C.

2. MATERIALS AND METHODS

2.1. Enzymes/materials/chemicals

Alcalase (EC 3.4.21.62) (food grade) from *Bacillus licheniformis* and papain (E.C. 3.4.22.2) from papaya (*Carica papaya*) latex were gifted from Siam Victory Chemicals Co, Ltd. (Bangkok, Thailand). Citric acid was procured from Chemipan Corporation Co., Ltd.

(Bangkok, Thailand). Rolled oat meal (McGarrett, PK Trading (Thailand) Co., Ltd, Bangkok, Thailand), all dried fruits, nuts and grains (My choice, Central Food Retail Company Ltd, Nonthaburi, Thailand), green tea powder (Changtong Factory, Hat Yai, Songkhla, Thailand), strawberry flavour, corn syrup, honey and ingredients for preparing cereal bar were purchased from a local market in Hat Yai, Songkhla, Thailand. Ammonium thiocyanate, 2-thiobarbituric acid and 1,1,3,3-tetramethoxypropane were obtained from Sigma (St. Louis, MO, USA).

2.2. Production of hydrolyzed collagen (HC) from seabass skin

HC from seabass (*Lates calcarifer*) skin was prepared using two-step enzymatic hydrolysis process as described by BENJAKUL *et al*. (2017). Briefly, skins of seabass (*Lates calcarifer*) were washed and cut into small pieces (3.0 × 3.0 cm^2). The skins were then pretreated to remove non-collagenous proteins by soaking in 0.10 M NaOH with a skin/alkaline solution ratio of 1:10 (w/v) for 3 h. Alkali-treated skins were washed until neutral or faintly basic pH of wash water was obtained. The pretreated skins were subsequently immersed in 0.1 M citric acid with a skin/solution ratio of 1:10 (w/v) for 2 h. The swollen skins were washed until wash water became neutral or faintly acidic. The prepared skins were subjected to hydrolysis.

To the pretreated skins, deionized (DI) water was added at a ratio of 1:5 (w/v). The pH of mixture was adjusted to 7.0 using 1.0 M NaOH and 1.0 M HCl and incubated at 40°C for 15 min. Papain (3% of solid content) was added and the hydrolysis was conducted at 40°C for 3 h with continuous stirring. The reaction was terminated by heating at 90°C for 15 min. For the second step of hydrolysis, Alcalase (2% solid content of fish skin) was added into the mixture, in which pH was readjusted to 8. Hydrolysis was performed at 50°C for 2 h. After inactivation at 90°C for 15 min, the resulting HC was filtered through 2 layers of cheesecloth and 2 layers of fiber grass filter to remove the debris. The filtrate was then fed to a filter unit, which consisted of 4 filter cartridges (CRC-20-BP-5, C.C.K, Taiwan) and 2 carbon capsules (Block carbon treatton 20, Treatton, Taiwan) using a diaphragm pump (R/O-450, Treatton, Taiwan). Feed flow rate was 2 L/min.

The resulting filtrate was then concentrated to obtain 10% solid using an alcohol recovery evaporator (Euro Best Technology Co., Ltd, Pathumthani, Thailand) at 50°C. HC concentrate was subjected to drying using a spray-dryer (LabPlant SD-06 Basic, North Yorkshire, England) equipped with a spry-drying chamber and a two-liquid-nozzle spray nozzle (0.5mm in size). The air inlet temperature was set at 200°C and outlet temperature was controlled at 90°C. The obtained powder was packed in laminated polyethylene/aluminium foil bag and sealed under vacuum. HC powder was kept at 4°C until used.

2.3. Preparation of cereal bar fortified with hydrolyzed collagen (HC)

The formulation and preparation of cereal bar fortified with HC are shown in Fig.1. Briefly, all dry ingredients were mixed together in a dough mixer (KitchenAid casserole multifunctional 5k, KitchenAid, Benton Harbor, MC, USA) at a low speed for 3 min. Liquid ingredients were separately mixed and preheated at 75°C for 5 min. Liquid mixture was then added to the dry mixture. The mixture was subsequently stirred at a low speed for 3 min. The resulting sticky cereal mixture (35 g) was placed on wax paper, transferred and pressed into aluminium baking tray (2.0×5.0 cm^2) with the height of 1.5 cm. The cereal bar in aluminium tray was baked in an electric oven (Mamaru MR-1214, Mamaru (Thailand) Co., Ltd., Bangkok, Thailand) at 180°C for 10 min. After baking, the cereal bars were allowed to cool at room temperature for 1 h. The resulting cereal bars were referred

to as 'Con' cereal bar. For another portion of cereal bar mixture, green tea powder (1.0% of total weight) and citric acid (0.01% of total weight) were mixed with cereal bar mixture in the same manner. Bars were formed and baked as previously described. The resulting cereal bars were named as 'GT+CA' cereal bar.

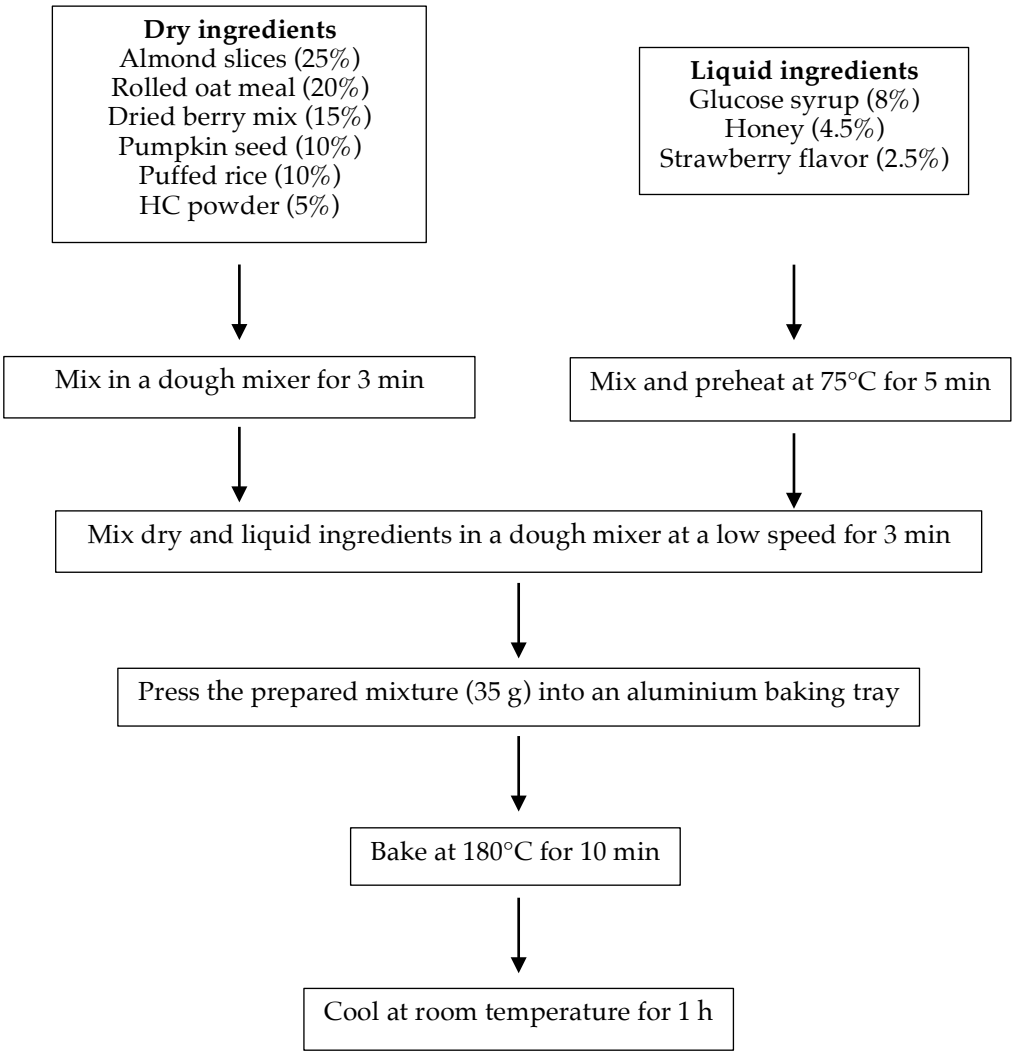

Figure 1. Diagram of preparation of cereal bar fortified with hydrolyzed collagen (HC).

2.4. Packing of cereal bars

Both 'Con' and 'GT+CA' cereal bars were packed under two different packing conditions. For the first group, the samples were placed in polypropylene (PP) pouch and heat sealed using an impulse sealer with a magnet Model ME-300HIM (S.N. MARK Ltd., Park, Nonthaburi, Thailand). The second group was placed in laminated polyethylene/aluminium foil bag with nitrogen gas flush (LF) (2.8-4.0% O_2) before being sealed using a Vacuum Sealer V-300 (FNB Machinery & Solutions, Bangkok, Thailand). PP bag (3.0×7.0 cm^2) had 89 μm thickness with a water vapor and oxygen permeabilities of 6.09 g.mm/day.m^2.mmHg (25°C) and 2.770 (mLO_2/day.pack), respectively. LF bag (3.0×7.0 cm^2) had 85 μm thickness with a water vapor and oxygen permeabilities of 5.76 g.mm/day.m^2.mmHg (25°C) and 0.002 (mL O_2/day.pack), respectively. The thickness of

packaging material was measured using a micrometer (Mitutoyo, Model ID-C112PM, Serial No. 00320, Mitutoyo Corp., Kawasaki-shi, Japan). Water vapor (WVP) and oxygen permeabilities were determined using ASTM method (SHIKU *et al*., 2004) and ambient oxygen ingress rate (AOIR) method (LARSEN *et al*., 2000), respectively. Oxygen content in packaging was investigated using Map-Pak Combi Gas Analyzer (AGC Instruments, Co Clare, Ireland).
The packaged samples were stored in the dark at the controlled temperature (25°C) for 6 months. At 0, 1, 2, 3, 4, 5 and 6 months of storage, the samples were taken for analyses.

2.5. Physicochemical analyses

2.5.1 Determination of moisture content and water activity

The samples were analyzed for moisture content using an oven method (AOAC, 2002). Water activity (a_w) was measured using a water activity meter (4TEV, Aqualab, Pullman, WA, USA).

2.5.2 Measurement of color parameters

The cereal bar was ground using a blender (Model MX-898N, Panasonic, Panasonic Sdn. Bhd., Kuala Lumpur, Malaysia) for 3 min. The color parameters of samples were then determined using a colorimeter (ColorFlex, Hunter Lab Reston, VA, USA). The color values were reported in the CIE system, including L^*, a^* and b^*, representing lightness, redness/greenness and yellowness/blueness, respectively. Total difference of color (ΔE^*) was calculated as described by TAKEUNGWONGTRAKUL *et al*. (2015).

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

where ΔL^*, $\Delta a^*$and $\Delta b^*$are the differences between the corresponding color parameter of the sample and that of day 0.

2.5.3 Measurement of textural properties

Hardness and crispiness of cereal bar were determined by a texture analyzer (Stable Micro Systems, Godalming, Surrey, UK) using a test speed of 1.0 mm/s with a load cell of 50 kg. A special pasta blade and plate (probe TA 47, 60 mm x 20 mm) were used to imitate the biting action of a tooth. The maximum force required to break cereal bar and the distance at break were calculated for each sample. Ten measurements were made for each sample.

2.5.4 Determination of lipid oxidation

2.5.4.1 Peroxide value (PV)

PV was determined in oil extracted from the cereal bar using the Bligh and Dyer method (Bligh and Dyer 1959). PV was determined using the ferric thiocyanate method (TAKEUNGWONGTRAKUL *et al*., 2015). A standard curve was prepared using cumene hydroperoxide with the concentration range of 0.5–2 ppm. PV was expressed as μg cumene hydroperoxide/g sample.

2.5.4.2 Thiobarbituric acid reactive substances (TBARS)

TBARS were determined using a distillation TBA method as described by KARNJANAPRATUM and BENJAKUL (2015b). Ten grams of sample, 97.5 mL of deionized water and 2.5 mL of 6 N HCl were transferred to a Kjeldahl flask. The mixture was heated and the distillate (200 mL) was collected. To determine TBARS, the distillate (0.2 mL) was added to 1 mL of TBAR solution (0.375% thiobarbituric acid, 15% TCA and 0.25M HCl) and heated in boiling water for 10 min. After cooling with running water and centrifugation at 5000xg for 10 min at room temperature, the absorbance of the pink solution was read at 532 nm. TBARS value was calculated from a standard curve of malondialdehyde (MDA) (0-10 mg/L) and expressed as μg MDA/g sample.

2.5.5 Volatile compounds

The volatile compounds in the cereal bar samples were determined at 0, 3 and 6 months of storage using solid-phase microextraction gas chromatography mass spectrometry (SPME GC–MS) following the method of TAKEUNGWONGTRAKUL and BENJAKUL (2017). Volatiles were expressed as the abundance (peak area).

2.6. Sensory evaluation

The packaged samples were taken for sensory evaluation at 0, 3 and 6 months. Sensory evaluation was performed by 50 untrained panelists. They were asked to evaluate for appearance, color, odor, flavor, taste, texture and overall likeness using a nine-point hedonic scale, in which a score of 1 = not like very much, 5 = neither like nor dislike and 9 = like extremely. The samples were labelled with random three-digit codes. Panelists were instructed to rinse their mouth with drinking water after each sample evaluation and the order of presentation of the samples was randomized (MEILGAARD *et al*., 2006). The samples with likeness score less than 7 (moderately like) were not used for further analyses.

2.7. Microbiological analysis

Total viable microbial count was enumerated by pour plating on Plate Count Agar (PCA, Difco Laboratories Inc., Detroit, MI, USA) at 37°C for 48 h. Yeast and mold counts were determined by pour plating using Potato Dextrose Agar (PDA, Laboratories Inc. Detroit, MI, USA) at 30°C for 72 h.

2.8. Statistical analysis

Completely randomized design was used. Experiments were run in triplicate using three lots of samples. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by the Duncan's multiple range tests (STEEL and TORRIE, 1980). Statistical analysis was performed using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1. Changes in moisture content and water activity during storage

Moisture content and water activity of HC fortified cereal bars without (Con) and with (GT+CA) addition of antioxidants, packed under different conditions during storage are shown in Fig. 1.

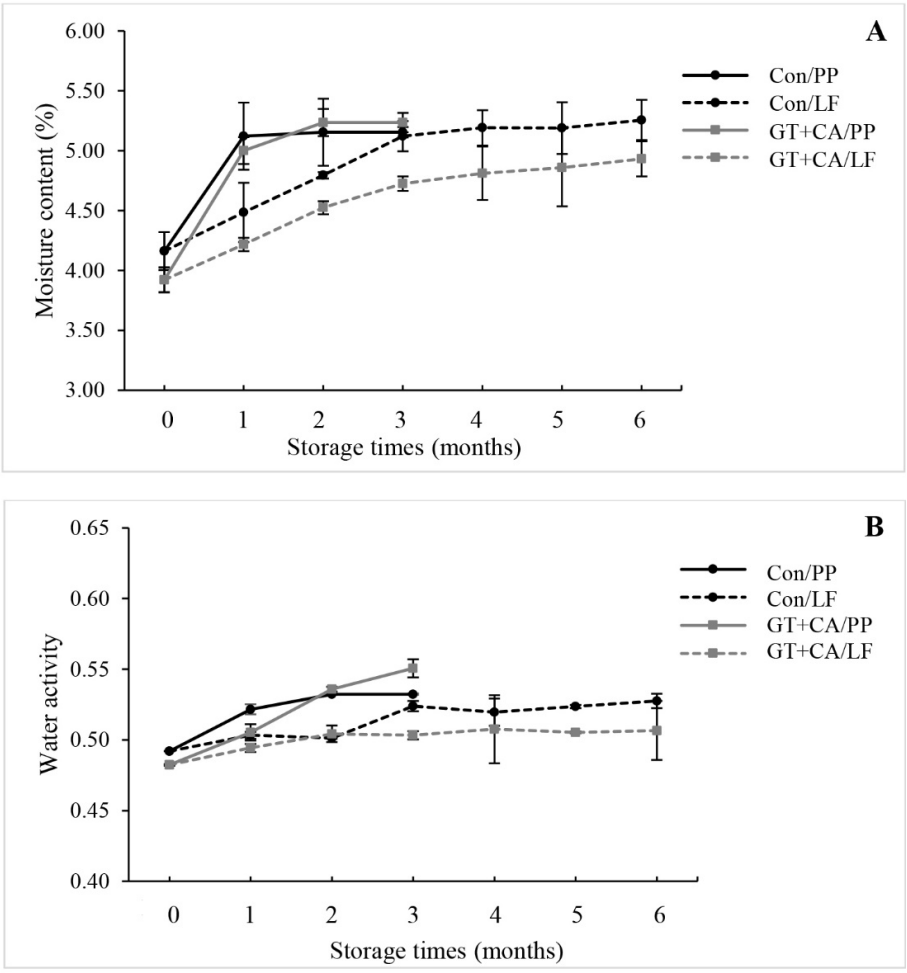

Figure 1. Moisture content (A) and water activity (B) of HC fortified cereal bars packed under different conditions during storage. Con; Cereal bar without antioxidants. GT+CA; Cereal bar with added antioxidants (1.0% Green tea powder, 0.01% Citric acid). PP; Polypropylene bag with normal heat seal. LF; Laminated polyethylene and aluminium foil bag with modified atmosphere pack (N_2 flush) before sealing. Bars represent the standard deviation (n=3).

Moisture contents of all samples during storage up to 6 months were in the range of 3.92-5.26% (w/w) (Fig. 1A). At Day 0, moisture contents of Con sample (4.16%) was significantly higher than that of GT+CA sample (3.92%) ($P<0.05$). Antioxidants (GT+CA) were added in the powder form, thereby lowering the moisture content of cereal bars. Sharp increase in moisture content was found for the samples packed in PP within the first month of storage ($P<0.05$), regardless of incorporation of antioxidants. Thereafter, the Con and GT+CA samples packed in PP bag had constant moisture content up to 3 months of storage ($P>0.05$). For the samples packed in LF bag, the moisture contents increased

linearly up to 3 months ($P<0.05$), irrespective of incorporation of antioxidant. However, no changes were found between 3-6 months of storage ($P>0.05$). It was noted that the Con sample had higher moisture content than GT+CA sample throughout the storage of 6 months. These results suggested that packaging played a profound role in preventing the migration of water into the cereal bars. LF bag showed the higher water vapor barrier property with a water vapor permeability of 5.67 g.mm/day.m^2.mmHg (25°C), compared with PP bag (6.09 g.mm/day.m^2.mmHg).

A similar result was observed for the change in water activity of cereal bars packed in different packing conditions during storage (Fig. 1B). At Day 0, the Con sample had higher water activity than GT+CA sample ($P<0.05$). Water activity of all samples continuously increased, especially those packed in PP bag, which showed the higher increasing rates, compared with those packed in LF bag, regardless of antioxidants used. Water activity of all samples during 6 months of storage was in the range of 0.48-0.53. Cereal bars with water activity lower than 0.6 are microbiologically safe under storage condition tested. FREITAS and MORETTI (2005) studied the stability of cereal bar with high protein using different packaging materials at room temperature. Increase in moisture content was observed during storage period tested and the best packaging with respect to water vapor permeability was the one containing aluminium (FREITAS and MORETTI, 2005). SENHOFA *et al*. (2015) found that the water activity of muesli stored in different packaging varied with packing materials and moisture permeability. In the present study, the packing condition had higher impact than the addition of antioxidants on moisture content and water activity change of cereal bars during storage.

3.2. Changes in color during storage

Color of HC fortified cereal bars without (Con) and with addition of antioxidants (GT+CA) packed under different packing conditions during 6 months of storage is presented in Table 1. For day 0, Con sample showed the lighter color as indicated by higher L^* values, compared to that of GT+CA sample ($P<0.05$). GT+CA sample was more greenish but less yellowish in color as indicated by the lower a^* and b^* values, respectively, compared to the Con sample ($P<0.05$). This could be related to green color of green tea powder used as antioxidant in the formulation, which contributed to the green color of product. Overall, the color of cereal bars was changed during storage, especially those packed in PP bag. The darker color with less yellowness as indicated by the decreased L^* and b^* values was observed for the Con sample packed in PP bag after storage for 3 months ($P<0.05$), compared to those found at Day 0. A similar result was obtained for the GT+CA sample packed in PP bag, in which less yellowness and redness indicated by lower a^* and b^* values were noted after 3 months of storage ($P<0.05$). On the other hand, a lower rate of change in color of samples during storage was observed when LF bag was used, particularly in combination of GT+CA addition. These results were in accordance with ΔE^* value. Increase in ΔE^* value at the higher rate was observed from the sample packed in PP bag, especially those of Con sample, during the storage. The addition of antioxidants (green tea and citric acid) could retard the discoloration of cereal bar during extended storage as evidenced by the retarded changes in lightness (L^* value) and yellowness (b^* value), compared with those of the Con sample. Moreover, lower O_2 permeability of LF bag with better water vapor barrier property could reduce chemical reactions, particularly lipid oxidation and Maillard reaction. Maillard reaction is predominant at room temperature in low-moisture products (a_w 0.5-0.7) with high protein content (BAPTISMA and CARVALHO, 2004). Lipid oxidation also resulted in propagation of the Maillard reaction (BECKER *et al*., 2009). Lipid oxidation occurred to a lower extent

when oxygen content in packaging was lowered and packaging material with low water vapor permeability was used (BAKKALBAŞı *et al*., 2012; NILSUWAN *et al*., 2016).

Table 1. Changes in color (L^*, a^*, b^* and ΔE^* values) of HC fortified cereal bars packed under different conditions during the storage.

Color	Storage time (month)	Con		GT+CA	
		PP	LF	PP	LF
L^*	0	67.94±0.96a		62.60±0.86a	
	1	65.56±0.70a	68.14±1.00a	61.83±1.54ab	61.82±1.43ab
	2	64.70±2.86a	66.91±1.03a	61.53±2.13ab	61.80±0.92ab
	3	61.23±2.65b	65.46±1.42a	61.33±3.50ab	60.52±2.21ab
	4	nd.	65.30±2.00a	nd.	60.10±3.72ab
	5	nd.	60.63±1.31b	nd.	60.10±1.39ab
	6	nd.	56.81±2.42c	nd.	58.28±0.89b
a^*	0	5.11±0.28abc		2.99±0.66a	
	1	5.73±0.68ab	6.28±1.34a	2.18±0.14bc	2.65±0.44ab
	2	4.28±1.33bc	5.42±1.14ab	2.16±0.64bc	2.52±0.21ab
	3	3.66±0.98c	5.17±0.23abc	1.46±0.55c	1.99±0.32bc
	4	nd.	5.13±0.56abc	nd.	1.68±0.21c
	5	nd.	4.31±0.40bc	nd.	1.60±0.33c
	6	nd.	4.31±0.39bc	nd.	1.55±0.47c
b^*	0	26.22±0.78a		22.29±0.46a	
	1	23.34±1.62bcd	24.97±0.37ab	22.57±0.74a	22.28±1.06a
	2	23.02±1.02bcd	24.84±0.41ab	20.67±0.29bc	22.14±0.61ab
	3	22.19±1.02cd	24.49±0.39ab	20.69±0.57bc	21.89±0.99ab
	4	nd.	23.43±1.98bc	nd.	21.33±0.64abc
	5	nd.	21.57±0.89cd	nd.	20.20±1.04cd
	6	nd.	21.22±1.54d	nd.	19.22±1.01d
ΔE^*	0				
	1	3.79±0.97a	1.72±1.14a	1.15±0.90a	0.85±0.86a
	2	4.63±2.18b	1.75±0.94a	2.11±1.28b	0.94±0.48b
	3	7.96±1.84c	3.02±0.61b	2.55±2.64b	2.34±1.49c
	4	nd.	3.84±1.61b	nd.	2.98±2.90c
	5	nd.	8.70±0.39c	nd.	3.54±0.85d
	6	nd.	12.23±1.65d	nd.	5.49±0.58e

Values and mean ± SD (n = 3). nd.; Not detected. Con; Cereal bar without antioxidants. GT+CA; Cereal bar with added antioxidants (1.0% Green tea powder, 0.01% Citric acid). PP; Polypropylene plastic with normal heat seal. LF; Laminated polyethylene and aluminium foil bag with modified atmosphere pack (N_2 flush) before sealing. Different lowercase letters for the same color attribute within the same column indicate significant difference ($P < 0.05$).

The generated lipid oxidation products, especially aldehydes and ketones, could be a carbonyl source for condensation with amines. As a consequence, browning discoloration via Maillard reaction could take place. Additionally, with increasing storage time,

Maillard reaction proceeded to higher extent. Thus, browning occurred to a higher degree, particularly for the Con sample packed in PP bag. Therefore, the addition of antioxidants and packing condition used played an important role in maintaining color of cereal bar during storage. The result indicated that LF bag showed higher preventive effect on color changes of cereal bar fortified with HC than PP bag during the extended storage.

3.3. Changes in hardness and crispiness during storage

Hardness and crispiness of cereal bars without (Con) and with addition of antioxidants (GT+CA) stored under different packing conditions during 6 months of storage are shown in Fig. 2.

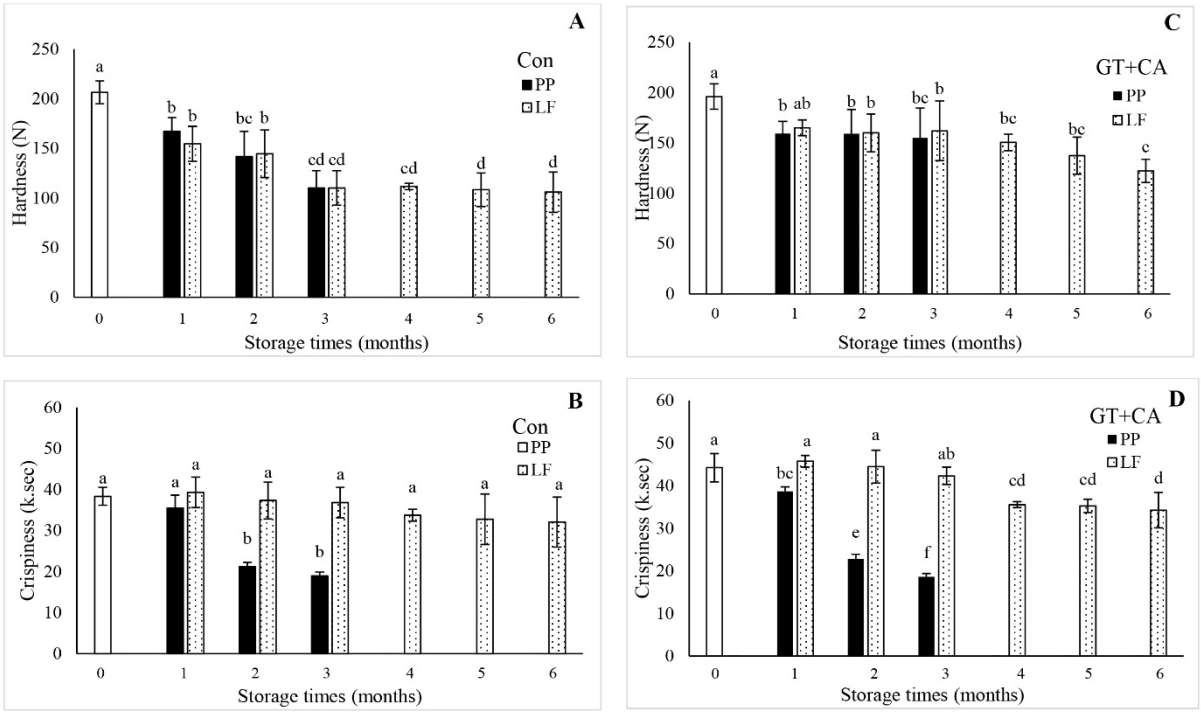

Figure 2. Hardness (A and C) and crispiness (B and D) of HC fortified cereal bars packed under different conditions during storage. Con; Cereal bar without antioxidants. GT+CA; Cereal bar with added antioxidants (1.0% Green tea powder, 0.01% Citric acid). PP; Polypropylene bag with normal heat seal. LF; Laminated polyethylene and aluminium foil bag with modified atmosphere pack (N_2 flush) before sealing. Bars represent the standard deviation (n=3).

The highest hardness (195.90-206.39 N) was observed for both Con (Fig. 2A) and GT+CA samples (Fig. 2C) at Day 0 of storage ($P<0.05$). Within the first 3 months of storage, hardness decreased for all samples packed in both PP bag and LF bag. There was no difference between cereal bars packed in PP bag and LF bag at the same storage time ($P>0.05$). After 3 months of storage, hardness of all samples packed in LF bag remained unchanged ($P>0.05$), excepted that of GT+CA samples after 6 months of storage, which had decreased hardness ($P<0.05$). This phenomenon was related to the slight increase in moisture content during storage (Fig. 1A). Increased moisture content more likely negatively affected the textural property of cereal bar. LF bag with better moisture barrier property packaging material prevented and reduced water vapor migration from environment to product, compared with PP bag. This was related with the lower crispiness of cereal bars packed in PP bag, compared to those packed in LF bag,

particularly within the first 3 months of storage (Fig. 2B and 2D). With extended storage, the samples packed in PP bag had marked decrease in crispiness ($P<0.05$). There was no change in crispiness for samples packed in LF bag throughout 6 months of storage ($P<0.05$), except those containing GT+CA, which had a slight decrease after 3 months of storage ($P<0.05$). Similar results were also reported for biscuits fortified with micro-encapsulated oil, in which high moisture content was related with lowered hardness (TAKEUNGWONGTRAKUL and BENJAKUL, 2017). The results indicated that packing condition had higher impact than added antioxidants on textural properties of cereal bars fortified with HC during storage of 6 months. Therefore, LF bag could be used for packing the cereal bars to maintain their textural property during storage.

3.4. Changes in peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) during storage

PV and TBARS of cereal bars without (Con) and with addition of antioxidants (GT+CA) stored under different packing conditions during 6 months of storage are depicted in Fig. 3. At day 0 of storage, GT+CA sample had lower PV than that of Con (Fig. 3A) ($P<0.05$). This result suggested that lipid oxidation could occur during cereal bar preparation and the addition of antioxidants, green tea powder and citric acid, could prevent lipid oxidation to some degree as indicated by the lower PV of GT+CA sample. PV of all samples notably increased within the first 2 months of storage ($P<0.05$) and remained unchanged during 2-3 months ($P>0.05$). After 3 months of storage, the highest PV was found for the Con packed in PP bag, compared with others ($P<0.05$). In general, PV of samples packed in PP bag was higher than that of samples packed in LF bag ($P<0.05$). Furthermore, the addition of antioxidants could suppress the formation of hydroperoxide. Nitrogen gas was purged into the sample packed in LF bag. This could also prevent oxidation in the sample in conjunction with antioxidants. In the low oxygen atmosphere, the oxidation of lipid occurs at negligible level. This result was in agreement with BAKKALBAŞı *et al*. (2012) who found that higher content of oxygen present in packaging increased lipid oxidation of walnuts during storage, especially at higher storage temperature. After 3 months of storage, sample packed in LF bag was further stored. It was found that the sharp decreases in PV were observed for both samples, Con and GT+CA samples. This was more likely caused by the decomposition of hydroperoxides to the secondary products (KARNJANAPRATUM and BENJAKUL, 2015a). The lower PV was observed for GT+CA sample, compared with those of Con sample ($P<0.05$). Addition of antioxidants in cereal bars had a preventive effect on lipid oxidation by retarding the radical chain reaction. Along with the nitrogen atmosphere, the oxidation of lipids in nuts or other ingredients used in formulation could be impeded more effectively.

Changes in TBARS of Con and GT+CA samples kept under different packing conditions during storage of 6 months are shown in Fig. 3B. The increase in TBARS indicated formation of secondary lipid oxidation products. Marked increases in TBARS were observed for all samples within the first month of storage ($P<0.05$). TBARS of Con sample packed in PP bag sharply decreased during 1-3 months, while GT+CA sample packed in PP bag showed gradual decrease after 2 months of storage ($P<0.05$). The decrease in TBARS with extended storage was probably due to the loss in those volatile secondary products (YARNPAKDEE *et al*., 2012). Green tea and citric acid added in cereal bars could prevent the formation of TBARS during storage of sample packed in PP bag, where oxygen was present to some extent. However, lower increase in TBARS value was observed for those packed in LF bag within the first month of storage ($P<0.05$), compared to those packed in PP bag. TBARS of samples packed in LF bag were slightly changed during 1-2 months of storage ($P<0.05$). During 2-3 months of storage, TBARS sharply

decreased in all samples ($P<0.05$). After 3 months of storage, drastic decrease in TBARS in sample packed in LF bag was observed. However, lower TBARS value was noticeable in the GT+CA sample, compared to Con sample. During 4-6 months of storage, TBARS value remained constant for both samples. Thus, the addition of antioxidants could prevent lipid oxidation of cereal bar during storage to some degree. Moreover, LF bag was able to effectively retard the early stages as well as the advanced stage of oxidation. As a result, the quality of cereal bar could be maintained during storage.

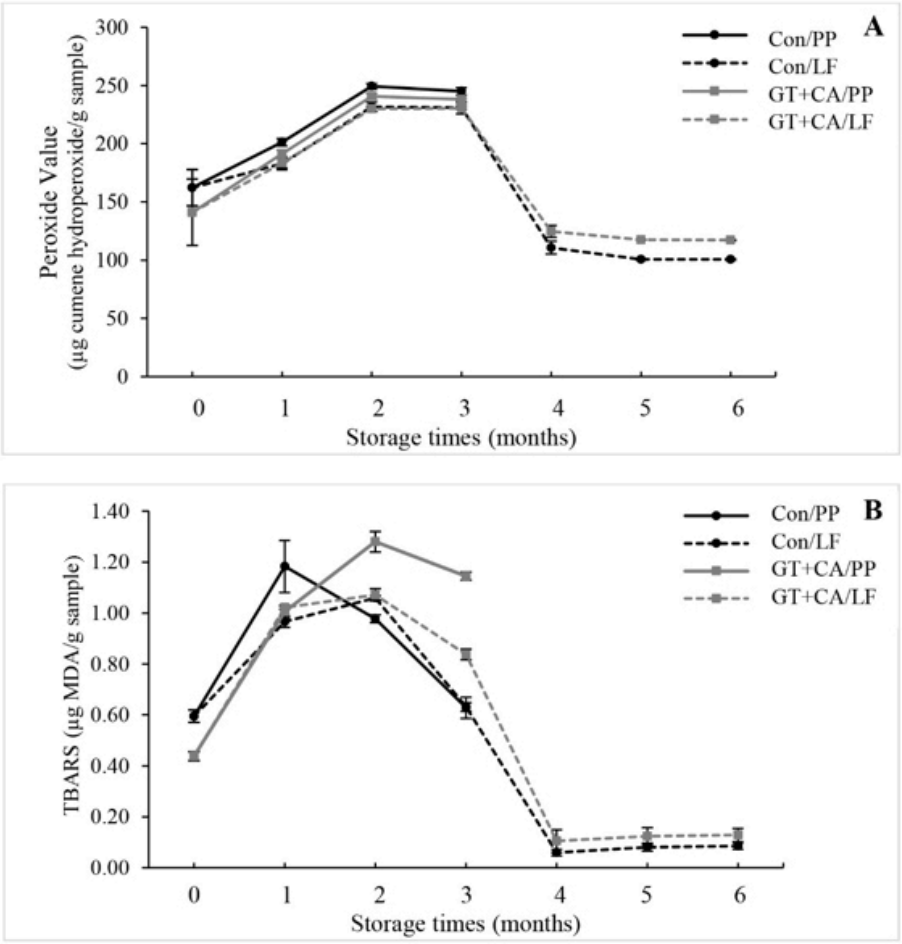

Figure 3. PV (A) and TBARS (B) values of HC fortified cereal bars packed under different conditions during storage. Con; Cereal bar without antioxidants. GT+CA; Cereal bar with added antioxidants (1.0% Green tea powder, 0.01% Citric acid). PP; Polypropylene bag with normal heat seal. LF; Laminated polyethylene and aluminium foil bag with modified atmosphere pack (N_2 flush) before sealing. Bars represent the standard deviation (n=3).

3.5. Volatile compounds

Volatile compounds in Con and GT+CA samples stored under different packing conditions at 0, 3 and 6 months of storage are displayed in Table 2. The types and abundance of volatile compounds detected in samples varied with antioxidants and packing conditions used as well as storage times. At day 0 of storage, 21 volatile compounds were identified for both samples, including 7 alcohols, 2 aldehydes, 3 ketones, 5 acids and 4 esters. It was noted that the abundance of total volatile compounds in GT+CA sample (308.83×10^6) was lower than that of Con sample (501.83×10^6). Some

volatiles were not detected in GT+CA sample, while they were found in Con sample. 1-Penten-3-ol was detected in GT+CA sample, indicating the presence of green odor/aroma in green tea (LEE *et al*., 2013). Most acids and ester volatile compounds were reported as fruity aroma (MOHAMED El HADI *et al*., 2013), which might be related to the mixed dried fruit and strawberry flavor used for preparing cereal bars. Several derivatives of volatiles can be formed by the oxidation of lipids. 1-Butanol 3-methyl-, 3-hexen-1-ol, 1,2-propanediol, 3-furanmethanol and benzyl alcohol were found in the Con sample with higher abundance, compared with those of GT+CA sample. Aldehydes and ketones are among the main contributors to flavor and their concentration was related to lipid oxidation (SAE-LEAW *et al*., 2016; TAKEUNGWONGTRAKUL and BENJAKUL, 2017). 2-Furan-carboxaldehyde, 5-hydroxymethylfurfural, 2-butanone, 3-hydroxy-, 2-propanone, 1-hydroxy-, ethanone, 1-(2-furanyl)-, butanoic acid ethyl ester, dipropylene glycol diacetate, 1,2-propanediol 1-acetate, 1,2-Propanediol 2-acetate and some acids were detected in the Con sample with higher abundance, compared with those of GT+CA sample. This result was in accordance with higher PV and TBARS of Con sample, compared to those of GT+CA sample at day 0 (Fig. 3). The results suggest that green tea and citric acid used in GT+CA sample could prevent lipid oxidation during cereal bar manufacture.

After 3 months of storage, marked increase in volatile compounds was observed for all samples packed in both PP and LF bags indicating that lipid oxidation took place in the cereal bars within 3 months of storage. Auto-oxidation could occur, especially in cereal, nuts and grains. Decomposition of lipid hydroperoxides is a complicated process and produces a multitude of constituents that may have biological effects and cause flavor deterioration in fat-containing foods (El-MAGOLI *et al*., 1982). This decomposition proceeds by homolytic cleavage of LO-OH to form alkoxy radicals LO·. These radicals undergo carbon-carbon cleavage to form breakdown products including aldehydes, ketones, alcohols, esters and furans (El-MAGOLI *et al*., 1982). New volatiles including 10 alcohols, 2 aldehydes, 1 ketone, 2 acids and 10 esters were identified for all samples tested. Notably, cereal bars packed in PP bag showed higher volatile abundance (706-921×10^6), compared to those packed in LF bag (419-505× 10^6). 1,2-propanediol, 3-hexen-1-ol and 1-hexanol were found as the major alcohols in all cereal bars. Hexanal and 2-furan-carboxaldehyde were dominant aldehydes, whereas 2-propanone, 1-hydroxy- was the predominant ketone. Aldehydes and ketones are known as the major contributors to the development of lipid oxidation off-odor and off-flavor (TAKEUNGWONGTRAKUL and BENJAKUL, 2017). Aliphatic alcohols such as 1-pentene-3-ol and 1-octen-3-ol contribute to off-flavor and they are produced by oxidative deterioration of food lipid (SAE-LEAW *et al*., 2016). Acetic acid was the major acid and butanoic acid ethyl ester was found as the high abundant ester in all samples, indicating the fruity flavor of cereal bars (MOHAMED EL HADI *et al*., 2013). Less volatile compounds were found for GT+CA sample, compared with the Con sample, regardless of packing condition used. This might be due to the influence of antioxidants used in GT+CA sample, which could prevent lipid oxidation. In addition, some new volatiles were detected only for those packed in PP bag such as 2-butanol and 2-penten-1-ol. This correlated well with the higher PV, compared with those packed in LF bag. The lowest abundance together with less types of volatiles was generally found for GT+CA sample packed in LF bag. Ethyl maltol, propanoic acid, propanoic acid ethyl ester, 2-hydroxypropyl propionate and 3-furancarboxylic acid methyl ester were found in all samples, excepted for GT+CA sample packed in LF bag. This was more likely owing to the higher barrier properties with lower oxygen content in LF bag as well as antioxidants added, which could prevent lipid oxidation during storage.

After 6 months of storage, some compounds were not found and new volatiles were generated. This was possibly due to the volatilization or decomposition of those aforementioned compounds. Simultaneously, new compounds were formed. The further

oxidation of lipids or some other reactions could change the abundance of volatile compounds during storage (ANDRÉS *et al*., 2004). Among alcohols, 2-butanol, 1-propanol,2-methyl-, 2-propanol, 1-methoxy-, 1-pentanol, 1-octanol, 2-propanol, 1,1'-oxybis-, benzene ethanol and maltol were not detected, while cyclohexanol was found as new volatile alcohol for both of Con and GT+CA samples packed in LF bag. 2-Propanone was generated as new ketone with disappearance of 2-butanone, 3-hydroxy- and 2(3H)-furanone, dihydro-. Marked increase in volatile compounds was observed for both Con and GT+CA samples with extended storage (6 month), compared with those of samples stored for 3 months. It was noted that GT+CA sample had lower abundance with less types of volatile compounds, compared to those of Con sample. The result indicated that lipid oxidation of GT+CA sample packed in LF bag could be reduced to some extent. Therefore, cereal bar fortified with HC with and without addition of antioxidants could be packed with LF bag to improve oxidative stability during 6 months of storage.

3.6. Sensory properties

Sensory properties of HC fortified cereal bar in the absence (Con) and the presence of antioxidants (GT+CA) stored under different packing conditions are shown in Fig. 4. At day 0, the likeness scores of all attributes tested, including appearance, color, odor, flavor, taste, texture and overall likeness for the Con and GT+CA samples were in the range of 7.5-8.2. Significant decrease in likeness score of both Con (Fig. 4A) and GT+CA samples (Fig. 4C) was observed for those packed in PP bag, especially after 3 months of storage ($P<0.05$). Likeness scores for all attributes of both samples stored in PP bag were lower than 7 (6.0-6.8) after 3 months of storage. There were no differences in likeness score of all attributes tested between both Con (Fig. 4B) and GT+CA samples (Fig. 4D) packed in LF bag during storage of 6 months ($P>0.05$). This result suggested that the packing condition had more impact than antioxidants on sensory properties during storage. The addition of antioxidants (GT+CA) had no marked influence on sensory qualities during storage. Con and GT+CA samples packed in PP were discarded at month 3 since likeness scores for all attributes tested were less than 7. Lower water vapor barrier property (6.09 g.mm/day.m^2.mmHg) with higher content of O_2 (18.0-18.8%) of PP bag could favor physical and chemical changes of cereal bar, whereas LF bag (2.8-4.0% O_2 content with water vapor permeability of 5.76 g.mm/day.m^2) showed superior property in keeping cereal bars. Water vapor from environment could migrate through packaging material and consequently increased the moisture content of product. This affected the appearance, color and texture of cereal bar. In addition, the high content of O_2 induced chemical change, especially lipid oxidation, during storage. Volatile compounds from lipid oxidation products were related to flavor deterioration known as rancidity (YARNPAKDEE *et al*., 2012). Moreover, aldehyde and ketone compounds from lipid oxidation were more likely involved in a yellowish discoloration via the Maillard reaction (YARNPAKDEE *et al*., 2012). Those reactions decreased the likeness score for appearance, odor, flavor and taste of cereal bars packed in PP bag. Similar results were reported for sensory property of stored muesli in different packaging (SENHOFA *et al*., 2015). Paper bag with low barrier property caused the greatest decrease of sensory quality during storage, compared with laminated low density polyethylene/aluminium foil container (SENHOFA *et al*., 2015). Also, LF bag was flushed with N_2 to replace air before sealing. This could help in retardation of lipid oxidation. Therefore, the packing conditions including packaging material and oxygen content, had direct impact on sensory properties of cereal bar fortified with HC during the storage. LF bag with nitrogen gas could improve the sensory quality of cereal bars during 6-month storage, regardless of antioxidant addition.

Table 2. Volatile compounds of HC fortified cereal bars packed under different conditions during storage.

Volatile compounds	Abundance (×10^6)							
	Month 0		Month 3				Month 6	
			PP		LF		LF	
	Con	GT+CA	Con	GT+CA	Con	GT+CA	Con	GT+CA
Alcohols								
2-Butanol	nd.	nd.	0.86	0.46	nd.	nd.	nd.	nd.
1-Propanol, 2-methyl-	nd.	nd.	1.99	1.69	1.53	1.31	nd.	nd.
2-Propanol, 1-methoxy-	nd.	nd.	0.42	0.36	0.35	0.28	nd.	nd.
1-Butanol	nd.	nd.	1.53	1.4	1.26	1.29	nd.	nd.
1-Penten-3-ol	1.29	nd.	2.71	2.69	nd.	nd.	nd.	nd.
1-Butanol, 3-methyl-	1.7	nd.	7.01	4.57	6.7	5.32	8.26	7.34
1-Pentanol	nd.	nd.	11.86	10.62	11.85	10.81	nd.	nd.
2-Penten-1-ol	nd.	nd.	1.59	1.4	nd.	nd.	5.88	6.14
1-Hexanol	3.98	3.1	31.97	31.09	29.49	23.48	207.7	117.33
3-Hexen-1-ol	90.1	41.21	89.01	65.77	64.47	72.34	303.91	295.12
Cyclohexanol	nd.	nd.	nd.	nd.	nd.	nd.	9.17	6.61
2,3-Butanediol	nd.	nd.	4.31	2.97	4.01	3.43	7.47	9.02
1-Octanol	nd.	nd.	0.32	0.69	0.31	nd.	nd.	nd.
1,2-Propanediol	258.83	167.47	342.48	255.17	175.83	111.76	71.61	94.19
3-Furanmethanol	2.8	2.28	4.98	4.56	3.23	5.87	46.86	55.63
2-Propanol, 1,1'-oxybis-	nd.	nd.	0.3	nd.	0.29	nd.	nd.	nd.
Benzyl alcohol	1.71	1.14	1.63	1.55	1.39	0.94	26.18	18.18
Benzeneethanol	nd.	nd.	0.21	nd.	0.2	nd.	nd.	nd.
Maltol	nd.	nd.	0.85	1.07	0.62	0.69	nd.	nd.
Ethyl maltol	nd.	nd.	1.19	1.16	1.09	nd.	21.73	21.79
Aldehydes								
2-Cyclopentenylethanal	nd.	nd.	nd.	nd.	nd.	nd.	234.1	51.68
Hexanal	nd.	nd.	9.61	9.3	6.66	2.87	23.07	9.74
2-furan-carboxaldehyde	16.17	5.37	6.72	5.82	4.83	4.32	65.28	62.25
Benzaldehyde	nd.	nd.	1.12	0.83	0.82	0.8	9.21	0.94

5-Hydroxymethylfurfural	2.52	nd.	0.19	nd.	nd.	nd.	nd.	nd.
Ketones								
2-Propanone	nd.	nd.	nd.	nd.	nd.	nd.	17.8	10.42
2-Butanone, 3-hydroxy-	3.89	nd.	1.9	1.9	1.88	1.37	nd.	nd.
2-Propanone, 1-hydroxy-	7.6	7.53	14.94	12.05	12.75	12.58	57.82	53.37
Ethanone, 1-(2-furanyl)-	0.84	nd.	1.61	1.32	1.25	1.22	17.16	9.45
2(3H)-Furanone, dihydro-	nd.	nd.	0.76	0.72	0.93	0.76	nd.	nd.
2-Butanone, 4-phenyl-	nd.	nd.	nd.	nd.	nd.	nd.	9.49	6.42
4H-Pyran-4-one, 3-hydroxy-2-methyl-	nd.	nd.	nd.	nd.	nd.	nd.	24.24	19.88
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	nd.	nd.	nd.	nd.	nd.	nd.	10.43	12.99
Acids								
Acetic acid	66.98	48.95	33.92	29.5	26.33	26.14	170.36	143.56
Propanoic acid	5.51	5.01	0.99	1.21	1.4	nd.	7.5	5.92
Butanoic acid	nd.	nd.	0.86	0.75	0.71	0.52	nd.	nd.
Pentanoic acid	0.41	nd.	0.56	0.55	0.41	0.36	7.19	5.58
Hexanoic acid	14.87	8.86	1.87	1.79	1.75	1.7	29.73	15.26
Hexanoic acid, 2-ethyl-	nd.	nd.	0.61	0.6	0.53	0.5	nd.	nd.
Butanoic acid, 2-methyl-	10.71	9.91	3.79	3.79	3.65	3.55	17.36	13.38
Esters								
Acetic acid ethyl ester	nd.	nd.	51.44	35.61	13.23	7.67	76.07	50.32
Propanoic acid, ethyl ester	nd.	nd.	1.48	0.75	0.46	nd.	nd.	nd.
Butanoic acid, ethyl ester	3.48	0.95	237.54	176.75	103.38	100.49	495.47	467.95
Butanoic acid, 2-methyl-, ethyl ester	nd.	nd.	12.01	7.81	5.15	4.77	48.12	47.71
Butanoic acid, 3-methyl-, ethyl ester	nd.	nd.	4.04	1.92	1.41	1.35	9.79	5.84
Hexanoic acid, ethyl ester	nd.	nd.	2.55	1.48	0.93	0.91	14.61	13.02
Butanoic acid, 2-ethyl-, methyl ester	nd.	nd.	nd.	nd.	nd.	nd.	25.15	22.09
Benzyl acetate	nd.	nd.	nd.	nd.	nd.	nd.	4.09	6.17
Methyl isobutyrate	nd.	nd.	2.09	1.72	1.64	1.28	nd.	nd.
Formic acid, octyl ester	nd.	nd.	nd.	nd.	nd.	nd.	7.82	5.43
Hexanoic acid, ethyl ester	nd.	nd.	2.55	1.48	0.93	0.91	13.02	14.61
Dipropylene glycol, diacetate	0.32	nd.	9.07	8.75	nd.	nd.	nd.	nd.
1,2-Propanediol, 1-acetate	4.57	3.78	5.8	5.55	5.15	5.04	33.97	nd.

2-Hydroxypropyl propionate	nd.	nd.	4.14	4.11	3.46	nd.	34.89	32.69
1,2-Propanediol, 2-acetate	3.55	3.27	2.79	2.64	2.54	2.27	11.31	11.25
Acetic acid, phenylmethyl ester	nd.	nd.	0.34	0.32	0.29	0.28	nd.	nd.
Ethane-1,1-diol dibutanoate	nd.	nd.	nd.	nd.	nd.	nd.	11.88	9.6
3-Furancarboxylic acid, methyl ester	nd.	nd.	0.51	0.44	0.22	nd.	9.64	7.4

nd.; Not detected. Con; Cereal bar without antioxidants. GT+CA; Cereal bar with added antioxidants (1.0% Green tea powder, 0.01% Citric acid). PP: Polypropylene plastic bag with normal heat seal. LF: Laminated polyethylene and aluminium foil bag with modified atmosphere pack (N_2 flush).

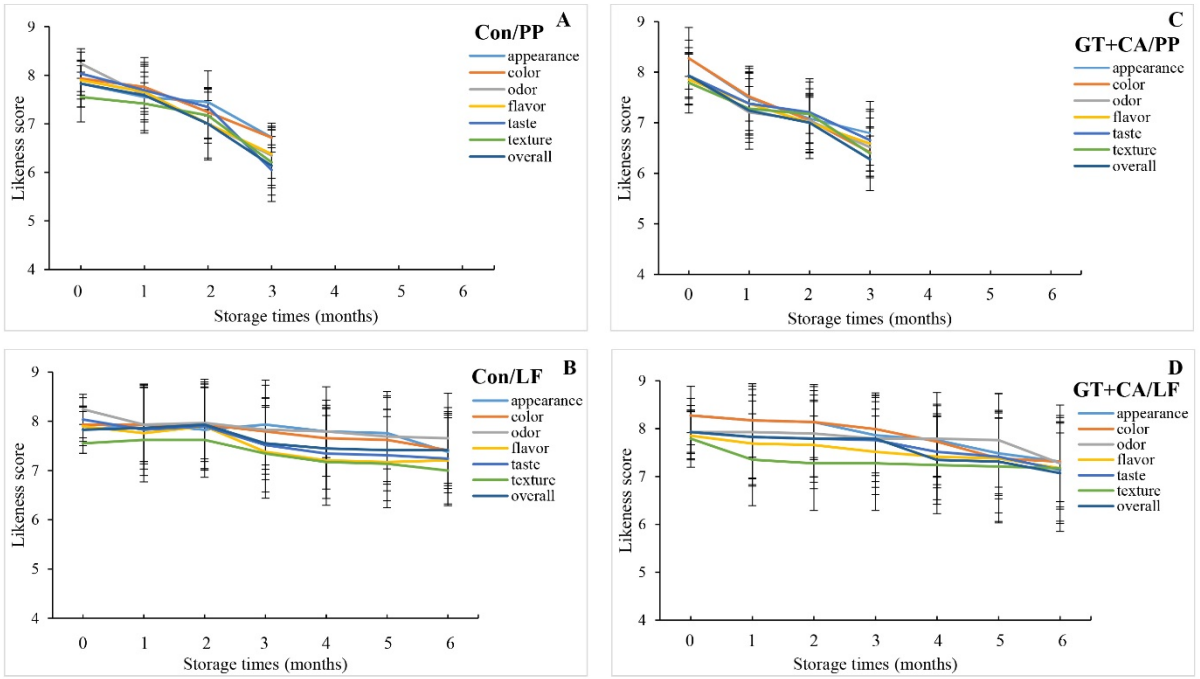

Figure 4. Sensory characteristics of HC fortified cereal bars packed under different conditions during storage. Con; Cereal bar without antioxidants. GT+CA; Cereal bar with added antioxidants (1% Green tea powder, 0.01% Citric acid). PP; Polypropylene plastic with normal heat seal. LF; Laminated polyethylene and aluminium foil bag with modified atmosphere pack (N_2 flush) before sealing. Bars represent the standard deviation (n=3).

3.7. Microbiological properties

Total viable count and yeast/mold counts of cereal bars stored under different packaging conditions were determined every month during storage of 6 months. Total viable counts for mesophilic aerobic microorganisms were less than 10^3 CFU/g sample for all samples tested during storage up to 6 months. On the other hand, yeast and mold counts were less than 10 CFU/g sample. The number of microorganisms was in accordance with the expected microbiological quality of processed foods, particularly cereal and cereal products (FDA, 2013). The result was in line with low water activity of the cereal bars, which was less than 0.6 during storage (Fig. 1B). This low water activity limits the microbial growth. SENHOFA *et al*. (2015) studied the storage stability of muesli in different types of packaging materials. Mesophilic aerobic bacteria, yeast and mold growth in muesli samples during storage was influenced by the presence of air and its diffusion through packaging material (SENHOFA *et al*., 2015). These results indicated that cereal bars with and without addition of antioxidants in all packaging conditions used were handled properly with appropriate storage condition, thus providing the product with microbiological safety and quality for consumers throughout the storage of 6 months.

4. CONCLUSIONS

Addition of antioxidants and packing conditions had profound impact on changes in quality and sensory properties of cereal bars fortified with HC during 6 months of storage at 25°C. Addition of green tea powder and citric acid as antioxidants could prevent some physicochemical changes and retard lipid oxidation to some degree, especially those cereal bars packed in PP bag. Based on sensory acceptance, cereal bars packed in LF bag in the presence of N_2 provided the better stability with lower lipid oxidation, compared with those packed in PP bag, regardless of antioxidant addition. Therefore, LF bag along with N_2 gas can be effectively used for packing cereal bars with improved storage stability.

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