Research Article
Effect of Addition of Resistant Starch on Oxidative Stability of Fried Fish Crackers as Influenced by Storage Temperatures and Packaging Materials

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Abstract: The aim of the present work was to investigate the effect of the addition of resistant starch (unmodified potato starch) on physicochemical properties and lipid stability of fried fish crackers packed in two types of packaging. Fish crackers added with one part of resistant starch were prepared, packed into two types of packaging with four different layers of packaging material; (i) polyethylene terephthalate-polyethylene-aluminium-linear low density polyethylene and (ii) oriented polypropylene-polyethylene-metallized polyethylene terephthalate-linear low density polyethylene and stored at 25, 40 and 60°C for 12 weeks. The linear expansion and oil absorption of the fried fish cracker were $58.00 \pm 3.46\%$ and $12.60 \pm 1.34\%$ respectively. Physical analyses showed an increase in moisture contents (from 2.75-3.47% to 4.08-4.54%), water activities (0.297 to $0.436a_w$) and a* and b* values (5.27 to 9.14% and 21.09 to 25.27%, respectively), while a decrease in L* value (from 63 to 58%), hardness (from 2.110 to 1.117 kg) and crispiness (from 12.46 to 8.18 kg/sec) throughout 12 weeks of storage at all temperatures tested. The lipid yield of the crackers increased during the storage time and the concentrations of conjugated dienes and thiobarbituric acid reactive substances showed a gradual increase and decrease, respectively. These results showed that the fried fish crackers in the storage study had undergone lipid oxidation where physical and chemical deterioration were observed and measured. In conclusion, the addition of one part of resistant starch in crackers has given positive effect on the stability of the resulting fried fish crackers.

Keywords: Conjugated dienes, fried fish crackers, lipid oxidation, physico-chemical analyses, thiobarbituricacis reactive substances, unmodified potato starch

INTRODUCTION

The development of healthier fish crackers designate beneficial method taking into account the selective conduct of modern consumers and their request for food that not only delicious but is also healthy and nutritious (Fuentes-Zaragoza *et al.*, 2010). To make the fish crackers healthier, the starch in fish crackers is converted into resistant starch (Nor *et al.*, 2014). The texture of the fried product can be enhanced and the dough machinability can be improved by the addition of starches in fried snacks. Due to the brittleness of the films that will produced during cooking and drying, high amylose contents will provide a strong texture as with cereals and increase crispiness to fried product. Oil absorption can also be controlled by high-amylose starches (Mason, 2009).

Deep-fried crispy snacks from tapioca, banana, rice and seafood (such as fish crackers, prawn crackers, squid crackers and fish chips) are popular in Malaysia. These snacks are rich in carbohydrate, protein and fat. Seafood products are indeed a good source of protein but lack fiber. The addition of fibers to seafood products will not only improve their functionality, but also create new functional foods with health benefits (Borderias *et al.*, 2005).

The sum of starch and starch degradation products that not absorbed and stay in the small intestine of fit individuals is defined as Resistant Starch (RS) (Eerlingen and Delcour, 1995). It has the benefits of dietary fibers, which affect the digestive tract, blood cholesterol level, microbial flora and the glycemic index as well as help in the restraint of diabetes (Nor *et al.*, 2014). Attention has been aroused in non-digestible starch fractions when some starch is not completely digested or absorbed in the small intestine and their capability to conduct functions alike dietary fiber in the large intestine (Cummings and Englyst,

1991; Asp, 1994). However, RS does not greatly affect the sensory quality of food unlike traditional sources of fiber (Fuentes-Zaragoza *et al.*, 2010).

Starch is essential in colonic physiology and can give protective effects against colorectal cancer as agreed by nutritionists (Cassidy *et al.*, 1994; Silvi *et al.*, 1999). RS that arrives at the colon where it provides benefits, including the growth of favorable bacteria is not digested by pancreatic amylases in the small intestine (Thompson, 2000). Starches are classified into three types: RS1, RS2 and RS3 (Englyst *et al.*, 1992). RS1 is starch that cannot be digested in the small intestine by the food matrix. RS2 is granular starch that not swollen. RS3 is gelatinized starch that retrogrades to become enzyme-resistant. In later years, chemically modified (e.g., cross linked) starch (RS4) is introduced (Thompson, 2000).

Potato (*Solanum tuberosum*) is the world's first non-grain food commodity, ranks third behind rice (*Oryza sativa*) and wheat (*Triticum aestivum*) in world food production. (Camire *et al.*, 2009). Potatoes are vegetable that consumed directly and can act as raw material for food production such as snack, pre-formed meals, potato derivatives, starch and starch derivatives (Alvani *et al.*, 2011). Potato starch has been used widely in a variety of food systems and is special amongst other commercial starches (e.g., cereal types) (Yusuph *et al.*, 2003). This specialty is due to the large granule size, relatively long amylose and amylopectin chains, purity, presence of phosphate ester groups on amylopectin and, ability to form thick visco-elastic gels upon heating and subsequent cooling (Vasanthan *et al.*, 1999; Alvani *et al.*, 2011). Potato starch is classified as RS2 (Englyst *et al.*, 1992) as less than 10% (w/w) of the starch is digested within 20 min by α-amylase *in vitro* (Oates, 1997).

Several chemical changes take place involving the internal food constituents and the external environmental factors during the processing and storage of fried foods. These changes will decrease the shelf-life of food and food deterioration can happen. The major chemical changes are related with the enzymatic reactions, oxidative reactions, particularly lipid oxidation and non-enzymatic browning (Singh and Cadwallader, 2004). In foods, lipids are the least stable macro-constituents and greatly prone to oxidation. The development of oxidative rancidity depends on the degree of unsaturation is the result from oxidation. The food becomes unsuitable for consume and is refused by consumers when oxidation happens. In rancid foods, the development of off-flavor is clearly detected and the production of free radicals during the autocatalytic process leads to other unwanted reactions, e.g., change in color, loss of vitamins and proteins degradation (Singh, 1999). Numerous oxidized products of rancidity are considered to be unhealthy besides the production of off-flavors (Eskin and Przybylski, 2001).

Flavor degradations of snack that developed from oxidation of oils and savory flavoring components are being preserved by packaging materials by:

- Stopping the entry of environmental oxygen into the packaging
- Blocking components of environmental light
- Slowing migration of volatile flavorings out of the packaging (Dunn, 2001).

In the absence of light, an oxidation reaction is take place at slower rate. Brown-colored glassine paper or brown pigmented plastic layers in co-extruded films are primarily used as light-barrier packaging, which blocked about 80% of ambient light. After that, metallized films are being introduced and can block about 99+% of light (Dunn, 2001). In the present work, two types of packaging with four different layers of packaging material were used:

- Polyethylene terephthalate-polyethylene-aluminium-linear low density polyethylene (PET-PE-ALU-LLDPE)
- Oriented polypropylene-polyethylene-metallized polyethylene terephthalate-linear low density polyethylene (OPP-PE-MPET-LLDPE).

The aim of the present work was therefore to investigate the effect of the addition of resistant starch (unmodified potato starch) on physicochemical properties, lipid stability and protein characteristics of fried fish crackers packed in two types of packaging, stored for 12 weeks at 25, 40 and 60°C.

MATERIALS AND METHODS

Materials: Round scad fish (*Decapterusruselli*) was purchased from a wet market in Selangor, Malaysia andtransported in ice box to the processing laboratory, Faculty of Food Science and Technology, Universiti Putra Malaysia, for immediate processing. Tapioca starch, sago starch, salt, sugar, monosodium glutamate, sodium bicarbonate and palm oil were purchased from a local food market. The unmodified potato starch was purchased from Bob's Red Mill (UK). The analytical-grade chloroform and iso-octane were purchased from Fisher (USA). The analytical-grade methanol was purchased from Merck (Germany) and thiobarbituric acid reactive substances kits from Cell Biolabs (USA).

Methods:
Preparation of crackers: The preparation of resistant starch fried fish crackers was performed following the recipe prescribed by the Department of Fisheries, Malaysia, with a slight modification. First, fish meat, tapioca flour, sago flour, rice bran flour, salt, sugar, monosodium glutamate, sodium bicarbonate and iced water were mixed thoroughly using a mixer until homogenized dough was formed. Next, the dough was molded into rectangular portions ≈2.5 cm and boiled for 30 min. Then, the boiled dough was drained and chilled overnight (4°C) before sliced into thin layers (≈2 mm).

The layers were then cabinet-dried at 40°C for 2 h. Next, the dried slices were deep fried in palm cooking oil at 180-200°C for 30 sec using an electric fryer.

Determination of linear expansion: The linear expansion of the fried fish crackers was determined based on the method by Yu (1991). The dried fish crackers was marked with three to five lines across and each line was measured before and after frying in oil at 180-200°C (Kyaw *et al.*, 2001; Cheow *et al.*, 2004) using Eq. (1):

$$\text{Linear expansion (LE\%)} =$$
$$\frac{\text{Length after puffing - length before puffing}}{\text{Length before puffing}} \times 100 \qquad (1)$$

Determination of oil absorption: The oil absorption of fried fish crackers was measured according to the method proposed by Mohamed *et al.* (1988) using Eq. (2):

$$\text{Oil absorption (OA\%)} =$$
$$\frac{\text{W of moisture after frying - W of moisture before frying}}{\text{Weight of moisture before frying}} \times 100 \qquad (2)$$

The effect of storage temperatures and periods: Fried fish crackers were packed into the commercially obtained PET-PE-ALU-LLDPE (packaging A) and OPP-PE-MPET-LLDPE (packaging B) each weighing ≈18 g. Packed samples were then separately incubated at 25, 40 and 60°C (Shel Lab, USA). Samples were removed for analyses at 1, 2, 3, 4, 6, 8, 10 and 12 weeks intervals.

Determination of moisture content: The moisture content of fried fish crackers was determined using the oven-drying method (AOAC International, 2007).

Determination of water activity: The water activity of fried fish crackers was determined using the Water Activity Analyzer (AquaLab, USA).

Determination of color: The color (lightness, redness and yellowness; L*, a* and b*) of fried fish crackers was measured using Ultra Scan PRO (Hunter Lab, Japan).

Determination of hardness and crispiness: The hardness (N/cm^2) and crispiness ($N/cm^2/s$) of fried fish crackers were measured by a penetration test using the Texture Analyzer (TA-XT2 Stable Micro System, UK). The conditions of the texture analyzer were as follows: Pre-test speed = 1.0 mm/sec; post-test speed = 5.0 mm/sec; test speed = 2.0 mm/sec; distance = strain, 100%; time = 5.0 sec; trigger type = auto; trigger force = 10 g.

Extraction of lipids: Fried fish crackers were mixed with chloroform:methanol (2:1 v/v) in 1:5 (w/v) ratio,

flushed with argon, left for 30 min, then centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was collected, the extraction was repeated and the two extracts were pooled. The meal of fried fish crackers was dried for 2-3 h at room temperature and used for protein analyses. Following extraction, the supernatant was evaporated at 40°C using a rotary evaporator (Heidolph, Germany) and the extracted lipid of fried fish crackers was weighed to determine the lipid recovery based on initial sample weight. Dried lipid extracts were flushed with argon, sealed and stored at -18°C for further analyses.

Determination of conjugated dienes: The conjugated dienes in the lipid extracts of fried fish crackers were determined by a modification of AOCS standard method (Th 1a-64). Firstly, 30 µL oil was diluted in 10 mL iso-octane. Absorbance at 234 nm was measured against iso-octane blank using quartz cells. Concentrations of conjugated dienes (mM) were calculated using the Beer's Law Eq. (3) with a molar extinction coefficient of 29500 for iso-octane:

$$A = \varepsilon b c, \qquad (3)$$

where,

A   = Absorbance value
b   = Thickness of cuvette (1 cm)
ε = 2950 $M^{-1}cm^{-1}$
c   = Concentration (mM)

Determination of thiobarbituric acid reactive substances: OxiSelect™ TBARS Assay Kit (MDA Quantitation) was used for the determination of thiobarbituric acid reactive substances (TBARS) and prepared according to the manufacturer's instruction. Firstly, 100 µL unknown samples or MDA standards was added to separate microcentrifuge tubes. Next, 100 µL SDS Lysis Solution was added to both the unknown samples and the MDA standards. The solution was mixed thoroughly. The samples were incubated for 5 min at room temperature. Next, 250 µL TBA reagent was added to each sample and standard. Each tube was incubated at 95°C for 45-60 min. The tubes were then cooled at room temperature in an ice bath for 5 min. Then, the tubes were centrifuged at 3000 rpm for 15 min. The supernatant was removed for further analysis. 200 µL of the MDA standards and samples were transferred to a 96 well microplate compatible with a spectrophotometric plate reader. The concentration of the final reaction product was measured by the spectrophotometric method at 532 nm.

Statistical analysis: Statistical analyses were performed using Minitab statistical software (Minitab 16, Minitab Inc., Pennsylvania, USA). The Analysis of Variance (ANOVA) was performed to compare the

means of data. All determinations were done in duplicate and presented as average. Tukey Test was applied to compare the average mean values. The confidence limits used in the present work were based on a level of 95% ($p < 0.05$).

RESULTS AND DISCUSSION

Linear expansion and oil absorption: The linear expansion for fried fish crackers with RS was $58.00 \pm 3.46\%$[b] while the oil absorption was $12.60 \pm 1.34\%$[c]. The linear expansion of fried fish crackers with RS was significantly lower than the control ($75.67 \pm 5.86\%$[a]) but slightly (yet insignificantly) higher than the fried fish crackers with rice bran ($53.33 \pm 2.08\%$[b]). The oil absorption of fried fish crackers with RS was significantly lower than the control ($27.86 \pm 0.80\%$[a]) and with rice bran ($18.56 \pm 0.39\%$[b]).

Fish crackers expand into porous snack product during frying and the degree of expansion is measured as linear expansion (Ramesh *et al.*, 2018). Moelyanto (1992) stated that the fish crackers quality depend on several factors such as the fish content and the linear expansion. The linear expansion of fish crackers is influenced by processing parameters such as the proportion of flour to fish, various kinds of flour, homogeneous dough, boiling time, the thickness of slices, the moisture content of dry slices and the cooking oil temperature. Yu (1991) reported that the ideal percentage of linear expansion of fish crackers as 77%. Expandability is important because consumers prefer fish crackers with the highest linear expansion (Nurul *et al.*, 2009).

Studies by Mohamed *et al.* (1989) showed that the linear expansion, oil absorption and the crispiness of crackers correlated with the total amylopectin content in the flour used. The oil absorption of the fried crackers is higher in the sample with higher linear expansion and lower in the sample with lower linear expansion. As shown in the results, as the linear expansion of the fried fish crackers increased, more air cells were formed and more oil was trapped and consequently oil absorption too increased. But apparently, the oil absorption of fried fish crackers with RS was significantly lower than with rice bran which might be due to the lower content of total amylopectin.

Moisture contents of fried fish crackers with resistant starch: Figure 1 shows the moisture contents of fried fish crackers over 12 weeks of storage at three different temperatures (25, 40 and 60°C). The moisture content of fried fish crackers with RS increased significantly ($p < 0.05$) from 3.30 to 4.47%, 3.47 to 4.41% and 3.01 to 4.54% over 12 weeks storage at 25°C, 40°C and 60°C respectively in packaging A. Meanwhile, in packaging B, the moisture content of
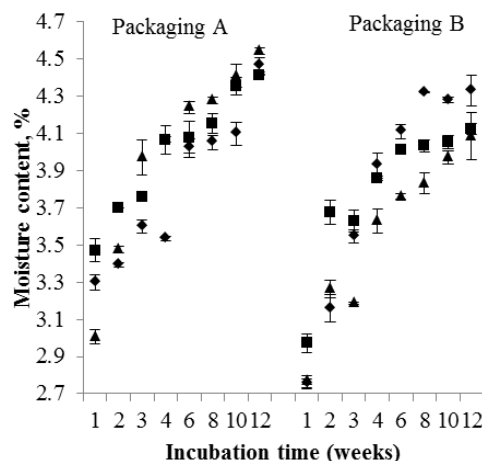

Fig. 1: Moisture contents (%) between packaging A (PET-PE-ALU-LLDPE) and packaging B (OPP-PE-MPET-LLDPE) over 12 weeks of storage at three different temperatures ---◆--- 25°C ---■--- 40°C ---▲--- 60°C

fried fish crackers with RS increased significantly ($p < 0.05$) from 2.75 to 4.33%, 2.97 to 4.13% and 2.77 to 4.08% over 12 weeks storage at 25°C, 40°C and 60°C respectively.

Foods that are dried to very low moisture content (<2-3%) may become susceptible to oxidation (Labuza and Dugan Jr., 1971) and other physical changes or instabilities brought about by the water loss, water gain, or migration of water in the food. The change in moisture alone may cause the product to become unacceptable, though frequently it also leads to other problems such as microbial or chemical degradation. Moisture transfer occurs in foods due to gradients in chemical potential, which is directly related to the food's water activity (a_w) (Singh and Anderson, 2004).

Water activities of fried fish crackers with resistant starch: Figure 2 shows the a_w of fried fish crackers over 12 weeks of storage at three different temperatures. The a_w of fried fish crackers with RS stored at 25°C increased significantly ($p < 0.05$) from 0.312 to 0.406 a_w in packaging A and from 0.316 to 0.426 a_w in packaging B. At 40°C, the a_w increased significantly from 0.309 to 0.436 a_w in packaging A and from 0.297 to 0.433 a_w in packaging B. At 60°C, the a_w increased significantly from 0.297 to 0.431 a_w in packaging A and from 0.297 to 0.409 a_w in packaging B.

Water activity appreciably influences the oxidative stability of oil/fat-containing low-moisture foods, particularly powder foods. According to food stability map (Labuza and Dugan Jr., 1971), lipid oxidation rate is lowest between 0.2 and 0.3 a_w for most dry foods, where water exists in monolayer at which water is bound tightly to the food surface and thus cannot act as an aqueous phase and is not available for reactants and catalysts. Lipid oxidation rate increases appreciably when a_w is greater than 0.4 or lower than 0.2. In fact, the oxidative stability of low-moisture foods does not
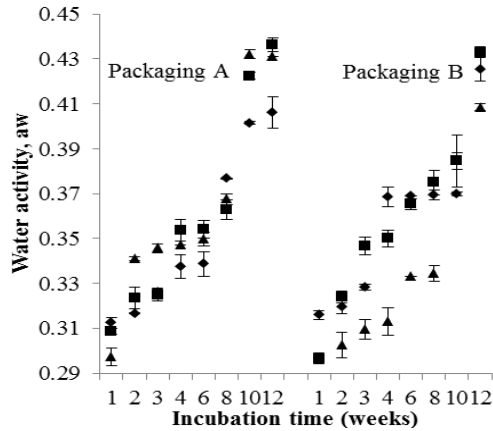
Fig. 2: Water activities (a$_w$) between packaging A (PET-PE-ALU-LLDPE) and packaging B (OPP-PE-MPET-LLDPE) over 12 weeks of storage at three different temperatures --◆-- 25°C —■— 40°C ---▲--- 60°C
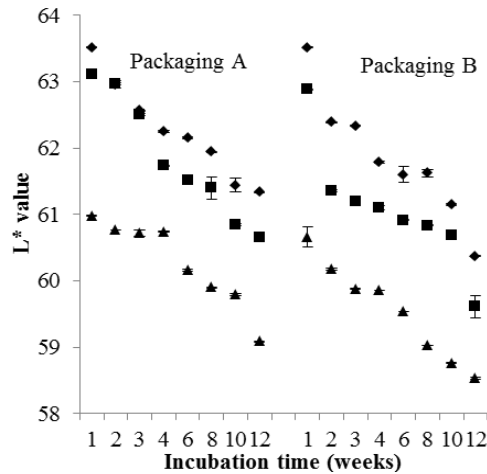

Fig. 3: L* between packaging A (PET-PE-ALU-LLDPE) and packaging B (OPP-PE-MPET-LLDPE) over 12 weeks of storage at three different temperatures --◆-- 25°C —■— 40°C ---▲--- 60°C

Fig. 4: a* between packaging A (PET-PE-ALU-LLDPE) and packaging B (OPP-PE-MPET-LLDPE) over 12 weeks of storage at three different temperatures --◆-- 25°C —■— 40°C ---▲--- 60°C

Fig. 5: b* between packaging A (PET-PE-ALU-LLDPE) and packaging B (OPP-PE-MPET-LLDPE) over 12 weeks of storage at three different temperatures --◆-- 25°C —■— 40°C ---▲--- 60°C

always follow the food stability map, rather, it is more product-related (Jensen and Risbo, 2007; Raitio *et al.*, 2011).

Colors of fried fish crackers with resistant starch: As shown in Fig. 3, the lightness (L*) values of fried fish crackers with RS ranged from 58.53 to 63.52. The lightness of fried fish crackers with RS decreased significantly (p<0.05) as the storage time increased at all temperatures in both packaging A and B. The color of fried fish crackers with RS was the darkest at 60°C, in both packaging. The redness (a*) values of fried fish crackers with RS as shown in Fig. 4 ranged from 5.27 to 9.14. The values increased significantly (p<0.05) throughout the 12 weeks of storage. The redness of the samples stored at 60°C increased from 6.21 to 9.14 in packaging A and from 6.24 to 8.99 in packaging B. The
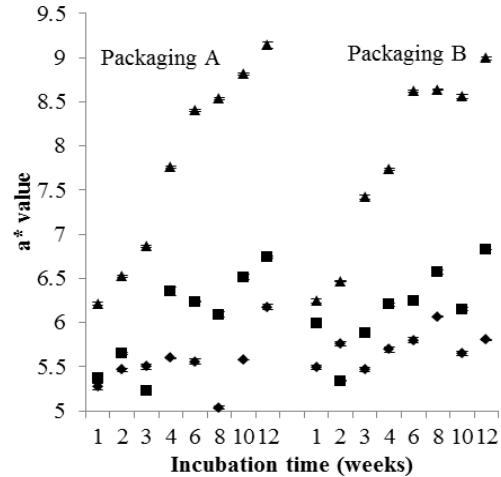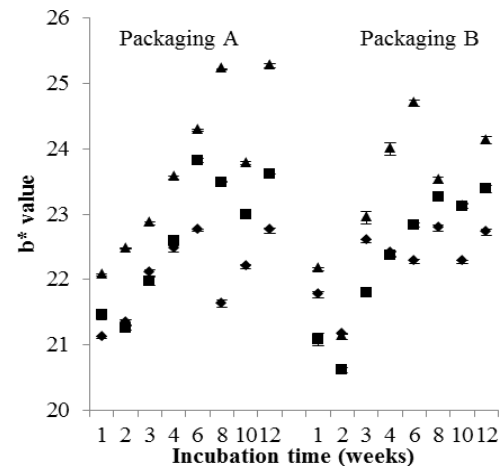
yellowness (b*) values of fried fish crackers with RS as shown in Fig. 5 also significantly increased (p<0.05) and ranged from 21 to 25. The yellowness at 60°C was also the highest. It has been demonstrated before that as the lightness decreased, the redness and yellowness increased (Maneerote *et al.*, 2009).

There are many factors contributing to the color of fried crackers, including the ratio of fish to starch in the formulation, the type of starch and additives used, the cracker thickness, the species of fish used and the presence of sugar and the Maillard reaction (Huda *et al.*, 2010). Non-enzymatic browning (Maillard browning) is a major cause of quality change and degradation of nutritional content in many foods. This type of browning occurs due to the interaction between reducing sugars and amino acids. These reactions result
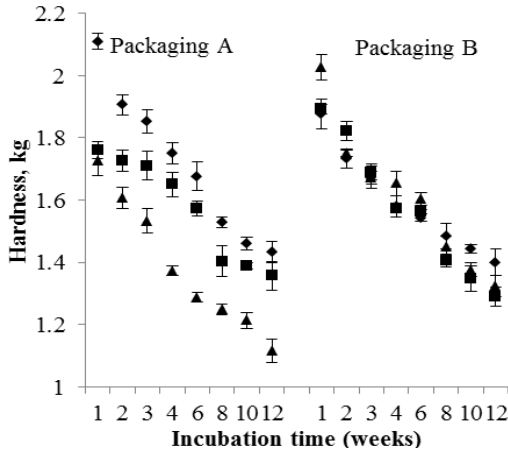
Fig. 6: Hardness between packaging A (PET-PE-ALU-LLDPE) and packaging B (OPP-PE-MPET-LLDPE) over 12 weeks of storage at three different temperatures ---♦--- 25°C ───■─── 40°C ----▲---- 60°C
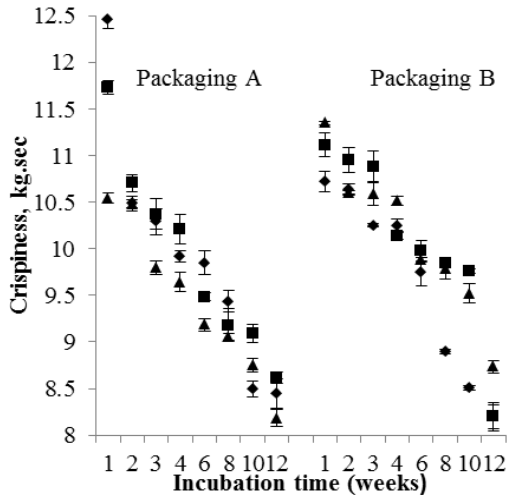

Fig. 7: Crispiness between packaging A (PET-PE-ALU-LLDPE) and packaging B (OPP-PE-MPET-LLDPE) over 12 weeks of storage at three different temperatures ---♦--- 25°C ───■─── 40°C ----▲---- 60°C

in the loss of protein solubility, darkening of light-colored dried products and the development of bitter flavors (Singh and Cadwallader, 2004). Non-enzymatic browning involves a reaction that forms an unstable Schiff's base and further transformation through the Amadori rearrangement. The reactions continue further through the Strecker degradation and polymerization reactions to form volatiles and dark pigments. This causes a browning of the color and sometimes changes in texture of the food product (Singh and Anderson, 2004).

Hardness and crispiness of fried fish crackers with resistant starch: Texture is one of the factors determining the eating quality of foods, especially for crackers and cookies. The results of texture analyses

(i.e., hardness and crispiness) of fried fish crackers with RS are shown in Fig. 6. The hardness ranged from 2.110 to 1.117 kg and decreased significantly ($p < 0.05$) throughout the storage. The hardness of fried fish crackers in packaging A and B were slightly similar. The hardness of fried fish crackers with RS was higher than control and with rice bran. Hardness of crackers is inversely correlated to its expansion and amylopectin content (Mohamed *et al.*, 1989). If a high amylopectin is present, the cracker would be hard (crunchy) because expansion is low and less water is being trapped (Noranizan, 2002). Therefore, some amylose should be present to give adequate resistance to breakage and an acceptable texture (Teixeira *et al.*, 2000).

The quality of fish crackers is also judged from their crispiness, which can be determined mechanically (as in the present work) or through sensory evaluation. Crispiness is synonymous to freshness. The crispiness of fried fish crackers with RS significantly ($p < 0.05$) decreased during the storage period. As shown in Fig. 7, the crispiness of fried fish crackers with RS ranged from 12.46 to 8.18 kg/sec. Dry food products such as crackers are expected and preferred to be crisp. However, it can lose their desired crispiness during storage or upon opening of the package, or if they are stored in a high humidity environment where they will absorb water (lowering Tg) and undergo glass transition to become tough and soggy (Singh and Anderson, 2004).

Lipid extractability of fried fish crackers with resistant starch: The lipid yield of fried fish crackers with RS stored at 25, 40 and 60°C as shown in Fig. 8 ranged from 16.06 to 22.67%. The extraction of oil for fried fish crackers with RS did not differ much than that of the control (12.95-22.08%) and with rice bran (19.07-23.75%). Lipid extractability at three different
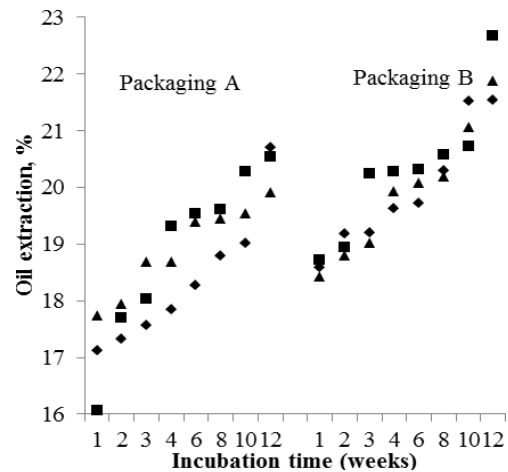

Fig. 8: Lipid yields (%) between packaging A (PET-PE-ALU-LLDPE) and packaging B (OPP-PE-MPET-LLDPE) over 12 weeks of storage at three different temperatures ---♦--- 25°C ───■─── 40°C ----▲---- 60°C
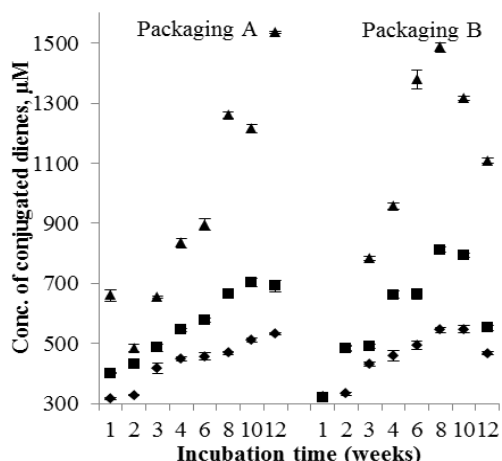
Fig. 9: Conjugated diene concentrations (µM) between packaging A (PET-PE-ALU-LLDPE) and packaging B (OPP-PE-MPET-LLDPE) over 12 weeks of storage at three different temperatures

Fig. 10: Thiobarbituric acid reactive substances concentrations (µM/µL) between packaging A (PET-PE-ALU-LLDPE) and packaging B (OPP-PE-MPET-LLDPE) over 12 weeks of storage at three different temperatures

temperatures increased albeit insignificantly (p>0.05) in both packaging A and B. The addition of starch could decrease the oil content in fried products and the extent of the decrease might be correlated to the amylose content (Ahamed *et al*., 1997). During frying, starch granules with higher amylose content could release more amylose and subsequently provide better film barrier, which could preferably inhibit the oil from penetrating into the food material (Zhang *et al*., 2014). Lipid extractability was also reduced during storage, indicating that oxidized lipids may have been irreversibly bound to proteins or starch (Hwang and Winkler-Moser, 2016).

Conjugated dienes of fried fish crackers with resistant starch: In oxidative stability and shelf-life study, Conjugated Dienes (CDs) value measures the concentration of primary oxidation product formed during lipid oxidation. Figure 9 shows the CDs of fried fish crackers with RS over 12 weeks of storage at three different temperatures. The CDs were found to increase from week one to week twelve of storage at 25°C (315.03-521.75 µM), 40°C (401.58-691.86 µM) and 60°C (659.44-1536.72 µM) in packaging A and decreased after week ten in packaging B. The concentration of CDs significantly increased (p<0.05) at 60°C in both packaging A and B.

The formation of hydroperoxides generated from unsaturated fatty acids such as linoleic and linolenic acids is usually accompanied by the generation of CDs due to the rearrangement of the double bonds, which absorb ultraviolet (UV) light at 230-235 nm (Shahidi and Zhong, 2005). Positive correlations between the sensory and CDs data have been reported in several foods, such as salami (Larrauri *et al*., 2013) and peanuts (Nepote *et al*., 2009; Riveros *et al*., 2010, 2013). The
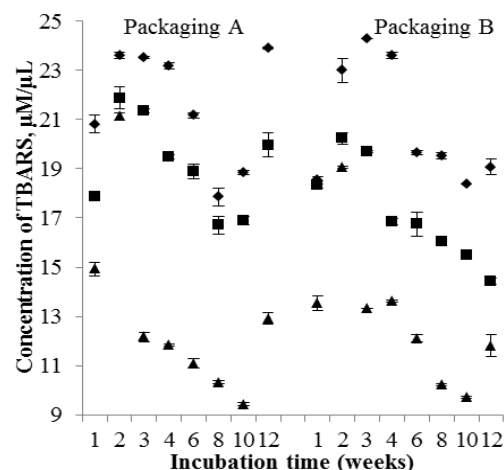
primary lipid oxidation products of the auto-oxidation cascade are tasteless and odorless fatty acid hydroperoxides (LOOH) and if a polyunsaturated fatty acid is oxidized, each peroxide group is accompanied by a CD. The amount of lipid hydroperoxides usually increases at the beginning of an oxidation cascade but decreases later as hydroperoxide breakdown to secondary oxidation products becomes higher than the rate of formation (Gardner, 1983).

Thiobarbituric acid reactive substances of fried fish crackers with resistant starch: Secondary lipid oxidation is measured based on the TBARS value which is an index of Malonaldehyde (MDA) concentration. MDA is one of the main end products of lipid oxidation (Piccolo *et al*., 2014). The formation of TBARS during storage of fried fish crackers with RS is shown in Fig. 10. The TBARS values measured were same as fried fish crackers with rice bran (9-24 µM/µL) but lower than control (15-28 µM/µL). The low TBARS value of the fried fish crackers indicates that the secondary oxidation did not accelerate during storage.

There were no significant changes in the TBARS values during the 6 weeks storage at 25°C in both packaging A and B indicating the stability of the prototypes against oxidation which might probably due to low a_w of the product (Belitz *et al*., 2009). Meanwhile, at 60°C, the TBARS values decreased significantly (p<0.05) from week two until week ten in packaging A. The TBARS values were found to increase from week one to week two of storage at 25, 40 and 60°C, then decreased in the following week until week ten. After that, the concentration of TBARS increased significantly (p<0.05) until week twelve. Generally, TBARS results have correlated well with

sensory analyses, supporting the involvement of TBARS in processes that generate off-flavors and odors detected by consumers (Schaich, 2016). TBARS was found to be satisfactory in assessing the quality of fats and oils such as lard, cooking fat and soybean, sunflower and rapeseed oils, in the early stages of rancidity (Pokorny *et al.*, 1985). Previously, it was proposed that the presence of interfering substances such as protein might affect the TBARS measurement (Yang and Boyle, 2016).

CONCLUSION

Addition of RS affected the physical properties and lipid oxidation of fried fish crackers such as linear expansion, oil absorption, moisture content, water activity, color, hardness, crispiness as well as conjugated dienes and TBARS. The fried fish crackers with RS were found to have lower linear expansion and oil absorption as compared to the control. The present work showed increasing trend for moisture content, water activity, redness (a*) and yellowness (b*) while decreasing trend in hardness, crispiness and lightness (L*) in fried fish crackers during storage study. The concentration of conjugated dienes was slightly higher while TBARS slightly lower than control. Therefore, it can be concluded that the addition of one part of RS and packing in the right material packaging could maintain the quality of fried fish crackers physically and chemically.

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CONFLICT OF INTEREST

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