



Effects of Cooking Temperature in Repetitive Cooking-Chilling Cycles on Resistant Starch Content and Quality Characteristics of Fish Crackers

Nor, M. Z. M.¹, Talib, R. A.^{1,4*}, Noranizan, M. A.², Chin, N. L.¹ and Hashim, K.³

¹Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

²Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

³Malaysian Nuclear Agency, Bangi, 43000 Kajang, Selangor, Malaysia

⁴Halal Products Research Institute, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

This work describes the effects of different cooking temperatures in repetitive cooking-chilling (RCC) process on resistant starch (RS) content in fish crackers prepared in a ratio of 1:1 fish to sago starch formulation. In this work, three sets of four RCC cycles were performed on fish crackers, in which each set was cooked at fixed temperatures of 100, 115 and 121°C, respectively. The chilling temperature was fixed at 4°C in all cases. Subjecting the fish crackers to a higher cooking temperature for up to 4 cycles of RCC can increase the RS content. However, quality degradation was observed in the characteristics of the fish crackers. During the first RCC cycle, cooking at a higher temperature had caused the crackers to crack and burst. Besides, defragmentation to the shape of the fish cracker gels was also observed during the first RCC cycle, coupled with softer texture and high moisture content. When the products were subjected to frying, their linear expansion decreased, the texture became harder and the colour turned darker. This work demonstrated that the application of higher cooking temperature up to 4 RCC cycles was able to enhance the RS content in the fish crackers, but it was less able to attain the product's perfect shape. On the contrary, fish crackers that were exposed to lower cooking temperatures contained lower RS but with less shape damage.

Keywords: Resistant starch, fish cracker, cooking, chilling, temperature

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E-mail addresses:

zuhair@upm.edu.my (Nor, M. Z. M.),

rosnita@upm.edu.my (Talib, R. A.),

noraadzahan@upm.edu.my (Noranizan, M. A.),

chinnl@upm.edu.my (Chin, N. L.),

kbhashim@nuclearmalaysia.gov.my (Hashim, K.)

*Corresponding Author

INTRODUCTION

Fish crackers or “keropok” are well-known amongst Malaysians as one of the most popular snacks. Fish crackers consist of dough formed from a mixture of starch, fish flesh and water, which is then shaped and boiled to gelatinize the starch, cut into thin slices

and dried before marketed. It is commonly fried before being served. Resistant starch (RS) is a type of starch that passes through small intestine without being digested, similar to dietary fibre. It is reported that the content of RS can be improved in fish cracker production via a repetitive cooking-chilling (RCC) process. However, there are some minor drawbacks of the quality characteristics of chilled and fried products that have been reported (Nor *et al.*, 2014). The repeated cooking-chilling cycles at the cooking temperature of 100°C and chilling of 4°C increased the extent of starch gelatinization for each of the successive cooking cycles and promoted retrogradation upon cooling. This promoted the formation of resistant starch type 3. Resistant starch type 3 (RS3) is a resistant starch formed from retrograded starch during food processing. Other types of RS include resistant starch type 1 (RS1; i.e. physically inaccessible to digestive enzymes found in whole or partly ground cereal grains, seeds and legumes), resistant starch type 2 (RS2; i.e. a natural crystalline resistant starch granules found in unripe banana and some legumes), and resistant starch type 4 (RS4; i.e. a chemically modified resistant starch). In another study, Nor *et al.* (2012) reported that fish crackers formulated from sago starch had the highest RS content in the dried samples as compared to tapioca and wheat starches due to higher amylose content. There is a possibility to improve the RCC by increasing its cooking temperature to above 100°C in order to obtain better RS yield in the treated products. This approach is based on the previous studies which reported that high temperature treatment via autoclaving and pressure cooking produces better RS yield in the food processed. The RS yield from wheat starch increased progressively as the autoclaving temperature was increased from about 2.5% at 100°C to about 9% at 134°C (Berry, 1986). Parchure and Kulkarni (1997) further reported that a 10-minute pressure cooking of native rice and amaranth produced RS content which was 1.7 times higher compared to those boiled for 15 minutes. Meanwhile, the repetition of heat treatment and cooling at different heating temperatures was undertaken by Sievert and Pomeranz (1989) who reported that by 20 repetitions of autoclaving (121°C, 134°C and 148°C for 1 hour) and cooling (4°C for overnight) of amylo maize VII starch, the RS level was raised from 20% to over 40%. Considering the potential of producing fish crackers with high RS content via higher cooking temperature in the RCC treatment, the objective of this study was therefore to investigate the effects of different cooking temperatures in the RCC treatment on the RS content and other quality characteristics of fish crackers.

MATERIALS AND METHODS

Materials

Fresh sardines (*Sardina pilchardus*) were purchased from a wet market in Serdang, Malaysia, while salt, sugar and monosodium glutamate (MSG) were purchased from a grocery store. Sago starch (Metroxylon sago) was purchased from Songiing Holding Sdn. Bhd. in Sibul, Malaysia, whereas filtered ice water was obtained from the laboratory.

Preparation of the Fish Crackers

Fig. 1 illustrates the fish cracker preparation methods using a modified version of the procedure previously described by Siaw *et al.* (1985). The fish cracker formulation is as follows: 1:1

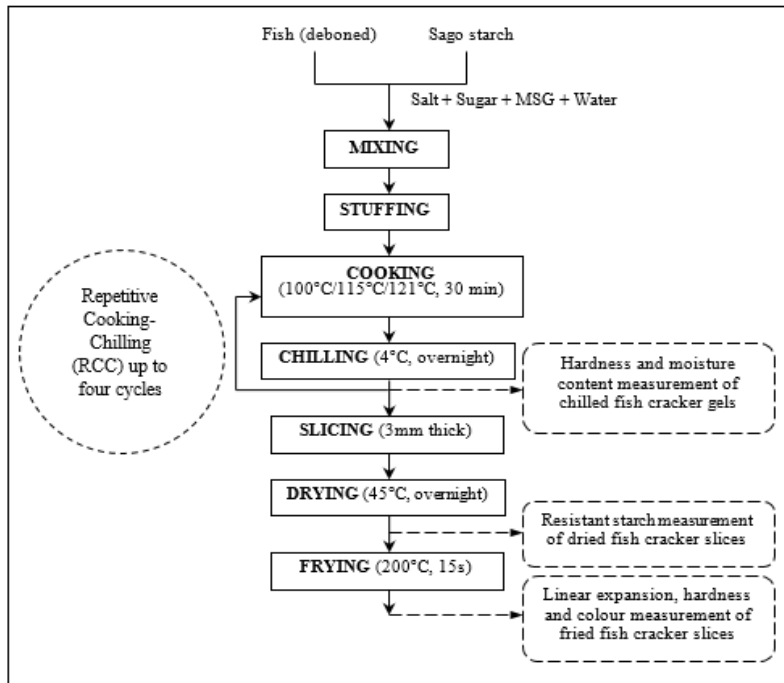


Fig. 1: Application of different cooking temperatures in the RCC cycles in fish cracker production

fish to sago starch ratio (1kg: 1kg), 2% salt, 1% sugar, 0.1% monosodium glutamate (MSG) and 30% ice water (% weight based on the total weight of starch and wet fish). Firstly, the fresh sardines were manually beheaded, degutted and deboned using a knife to obtain fresh flesh after the fish had been transported from the local wet market to the laboratory. The fish flesh was ground using a meat grinder (National, Japan) before being transferred into a silent cutter (Khin Shang Hoo Iron Works, Taiwan) and mix it with salt, sugar and MSG. Then, ice water and sago starch were added until the mixture formed homogenous dough. This mixing process took approximately 20 minutes. Meanwhile, a sausage stuffer (F-Dick, Germany) was used to stuff the dough-like mixture into circular cellulose casings (2.5 cm diameter and 12 cm length). Both ends of the stuffed casings were tied with cotton strings, and the stuffed rods were cooked for 30 minutes at fixed temperatures of 100°C, 115°C and 121°C, respectively (Kyaw *et al.*, 2001). After cooking, the cooked gels were immediately immersed in ice water to prevent shrinkage and to facilitate separation from the casings. The gels were then chilled overnight in a cold room at 4°C to complete one cooking-chilling cycle. The cooking-chilling cycle was repeated up to four times. The chilled gels were manually cut into 3 mm slices, which were later oven dried at 45°C overnight until the final moisture content was around 8–10%. The dried crackers (already considered a half finished product) were deep fried in palm oil at 200°C for 15 seconds until they fully expanded. These fried crackers were considered as the final products. Samples were taken after chilling (in gel form), after drying (in dried slice form) and after frying (in expanded cracker form) to obtain measurements. The protein contents for the single cooking-chilling fish crackers with similar formulation of tapioca, wheat and sago starches were found to be 15.6%, 24.9% and 15.7%, respectively, with a standard fat content of 0.4% (Yu, 1991).

Measurement of Resistant Starch in the Dried Fish Crackers

Protein removal was performed prior to the RS analysis (Goñi *et al.*, 1996), while the Resistant Starch Assay Analysis (Megazyme International Ireland Ltd. Co., Ireland) was used for the RS content determination. The method included the incubation with α -amylase (37°C, 16 hours) to hydrolyse the digestible starch, the solubilisation of precipitate using 2M KOH, the incubation with amyloglucosidase (50°C, 30 minutes) and the quantification of glucose using glucose oxidase/oxidase reagent.

Measurement of Instrumental Hardness and Moisture Content in the Chilled Fish Crackers

The chilled gels were maintained at room temperature (32°C) for 2 hours prior to the analysis (Cheow *et al.*, 2004) and cut into cylinders (25 mm diameter and 25 mm length). The hardness test was performed using a texture analyser (Stable Micro System, TA.XT.Plus, UK). The samples were placed on a heavy-duty platform and deformation was applied onto the sample using a compression probe (P/36R) 35 mm in diameter at a constant speed of 2 mm/s. The compression tests were set at a distance of 50% deformation. Force-deformation data were recorded to determine the textural characteristics of the samples. The hardness force was obtained from the absolute value of the peak height and the hardness measurements were performed in quadruplicate.

The moisture content was determined using the oven drying method (AOAC, 2000). The chilled gels were divided into two sections corresponding to the centre and periphery of the cylindrical rods and each section was used to determine the moisture content of the gel. The centre portion was 15 mm in diameter. A balance of the centre portion was used to determine the peripheral portion and three samples were used for each measurement.

Measurement of Linear Expansion, Hardness and Colour in Fried Fish Crackers

The linear expansion percentage of the fried fish cracker sample was determined according to Yu *et al.* (1981). Three parallel lines were drawn on the dried fish crackers using a marker. The length of each line was measured before and after frying. The calculation for the linear expansion is as follows:

$$\% \text{ linear expansion} = \quad \times 100 \quad [1]$$

The textural analysis of the fried fish cracker sample was performed using a texture analyser (Stable Micro System, TA-XT2i, United Kingdom). The samples were placed on a covered hollow cylindrical base (25 mm inner diameter, 1.5 mm thickness, stainless steel). Deformation was applied using a 5 mm spherical compression probe (P/0.25S) at a constant speed of 3 mm/s until the sample cracked. The maximum force of break was used as an indicator of the sample's hardness. The average value was calculated from 25 measurements. The colour of the fried samples was determined using a colour reader (Konica Minolta, CR-10, Japan). Ten measurements were used to determine lightness (L^*), redness (a^*) and yellowness (b^*), and the average value was recorded for each sample.

Observation of the Physical Condition after Cooking

Observations were carried out on the physical condition of the chilled fish cracker gels subjected to a single cycle of repetitive cooking-chilling (RCC). Each sample was classified according to the attributes listed in the Appendix.

Data Analysis

All the tests were conducted in triplicates, and a statistical analysis was performed using Microsoft Excel 2007 (Vista Edition, Microsoft Corporation, USA) and Statistical Analysis System (SAS) software (Version 9.2, SAS Institute, Inc., USA). A significance level of $\alpha = 0.05$ was used throughout the analysis, and p-values larger than α ($p > 0.05$) indicated that the difference between the treatments was not significant. When a significant difference was found from the ANOVA results, the treatments were compared using the t-test.

RESULTS AND DISCUSSION*Effects of Different Cooking Temperatures in RCC on the Resistant Starch Content of Dried Fish Crackers*

The results of one to four RCC cycles on the RS, which yielded dried fish crackers under different cooking temperatures are shown in Table 1. The one-way ANOVA test performed showed that the RCC treatment indicated significant differences ($p < 0.05$) in resistant starch formation in each cycle for the samples at different cooking temperatures. Similar trends also reported by Nor *et al.* (2014) for different formulation samples cooked at 100°C. The repeated cooking and chilling processes facilitate further gelatinization and retrogradation of the fish crackers, thus promoting the formation of RS.

TABLE 1: Average RS values in the dried sago starch based-fish crackers for each cooking-chilling cycle at different cooking temperatures

Temperature	Resistant starch in each cycle (% dwb)			
	1	2	3	4
100°C	3.07±0.02 ^{a1}	3.44±0.05 ^{b1}	3.68±0.11 ^{bc1}	3.81±0.12 ^{c1}
115°C	3.04±0.07 ^{a1}	3.56±0.26 ^{b1}	3.74±0.20 ^{bc1}	3.99±0.11 ^{c23}
121°C	3.36±0.10 ^{a2}	3.79±0.20 ^{b1}	3.99±0.23 ^{bc1}	4.23±0.20 ^{c3}

*Mean of triplicate determinations ± standard deviation

**Means within a row with different letters are significantly different ($p < 0.05$)

***Means within a column with different numbers are significantly different ($p < 0.05$)

When the cooking temperatures were compared, the ranking of the RS content in the dried samples exposed to the RCC treatment is as follows: 121°C > 115°C > 100°C. Sago starch-formulated fish cracker cooked at 121°C up to four cycles of RCC exhibited the highest RS yield, which was 4.23% (dwb). This value is higher as compared to the RS content in selected commercial fish crackers available in the Malaysian market ranging from 0.91% to 1.77% (dwb) (Nor *et al.*, 2012).

The comparison between different cooking temperatures showed that higher temperatures would produce appreciably higher RS. This is in agreement with Shin-Kyung *et al.* (1997) who reported that the heating of corn starches at 121°C provided higher yield of RS than it was at 100°C. In addition, Berry (1986) reported that the yield of RS from wheat starch increased progressively as the autoclaving temperature increased, i.e. from about 2.5% at 100°C to about 9% at 134°C.

Sievert and Pomeranz (1989) reported that although the differences were small, amylo maize VII starch autoclaved at 134°C had a higher RS content as compared to the same samples autoclaved at 121°C after completing four autoclaving-cooling cycles. Nevertheless, it was also reported that at a higher autoclave temperature (148°C), the amount of RS obtained was lowered. However, this finding was not further discussed.

The increase in RS as the cooking temperature increased might be due to more amylose leached out from the starch granules. Mohd Adzahan *et al.* (2010) reported that approximately 96% of amylose leached out at 120°C compared with approximately 88% amylose being leached out at 100°C when solubilised sago starch was heated for 1 hour. In addition, higher cooking temperature also caused thermal degradation of amylopectin into shorter amylopectin chains that could be attributed to the formation of RS since the entanglement of retrograded amylopectin molecules might reduce enzyme susceptibility (Eerlingen & Delcour, 1995). Jiranuntakul *et al.* (2012) observed thermal degradation of amylopectin molecules when subjected to heat-moisture treatment at 120°C and 140°C as compared to that at 100°C in waxy starches. The degradation of amylopectin is indicated by smaller molecules in the starch chain distribution. However, this is not shown in the current work.

Since the current study only used a small scale autoclave which has safety limit of 121°C, the treatment at a higher autoclaving temperature could not be performed. Despite this limitation, the application of higher temperature would lead to a higher processing cost, so limiting the autoclaving temperature at 121°C would be the best choice. The effects on other quality characteristics are also a major concern.

Effects of Different Cooking Temperatures in RCC on the Hardness and Moisture Content of Chilled Fish Cracker Gels

Fig.2 displays the effects of the repetitive cooking-chilling (RCC) treatment on the hardness of chilled fish cracker gels at different cooking temperatures. There were increases in the hardness values from cycle one to cycle two when the samples were cooked at 115°C and 121°C. However, the values decreased in the subsequent cycles, leaving the hardness values after completing the four cooking-chilling cycles to be lesser than the values in the first cycle. However, the values are still higher than the samples that were cooked-chilled for four cycles at 100°C. In overall, the samples cooked at the temperatures higher than 100°C produced higher hardness values. Kyaw *et al.* (2001) reported an increase in the gel strength of the fish cracker gels made from wheat starch when cooked under the cooking temperatures ranging from 100°C to 121°C. They proposed that this finding might be due to the swelling and water uptake of starch during gelatinization upon heating. In contrast, as the fish cracker gels were made based on tapioca starch, they found a decrease in the strength of the fish cracker gels,

although being cooked in the same range of the cooking temperatures. This could possibly be due to the population of fragmented starch granules that increased as the cooking temperature increased. However, their study only focused on a single cooking cycle.

The moisture contents in the centre and periphery parts of each sample treated with RCC at different cooking temperatures are displayed in Fig.3 (a) and Fig.3 (b), respectively. The RCC treatment caused a decrease in the moisture content for each sample from cycle one to cycle four. It was observed that the samples cooked at the temperatures higher than 100°C had lower moisture content (centre and periphery), which was probably due to their compact structure that supports the findings of hardness values in Fig.2.

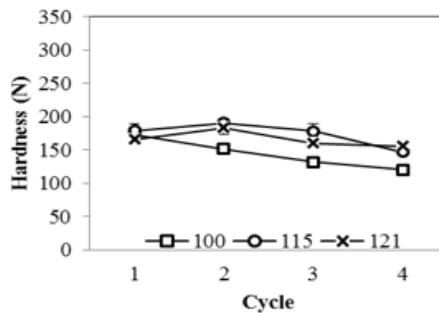


Fig.2: Hardness values of chilled sago starch based-fish cracker gels when subjected to cooking-chilling repetitions at different cooking temperatures

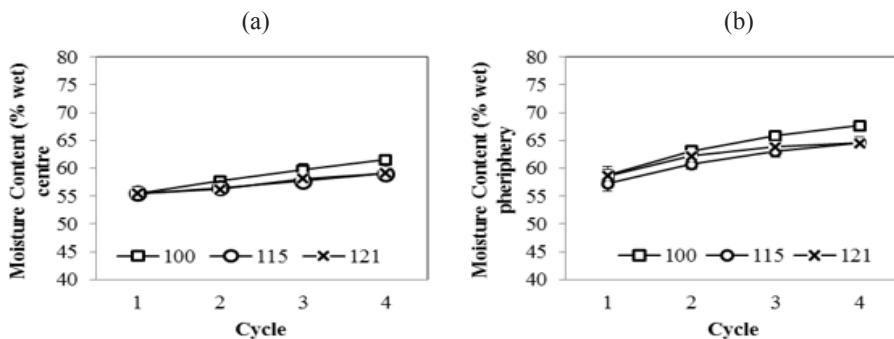


Fig.3: Moisture content values—centre (a) and periphery (b) of the chilled sago starch-based fish crackers when subjected to repetitive cooking-chilling cycles at different cooking temperatures

Effects of Different Cooking Temperatures in RCC on the Linear Expansion, Hardness and Colour of Fried Fish Crackers

Table 2 shows the linear expansion values of the fried fish crackers treated with RCC at different cooking temperatures. Similar to the findings of Nor *et al.* (2013), the RCC treatment caused a decrease in the linear expansion of the treated fish crackers. At higher cooking temperatures (115°C and 121°C), the samples became less expanded after frying. This could probably be due to the extreme effects of the high temperature treatment that caused more damages to the starch granules. Kyaw *et al.* (2001) reported that the cooking of fish crackers made of tapioca starch at a temperature above 108°C would cause a reduced expansion due to the water being released into the fused fish and starch structure.

TABLE 2: Average linear expansion values in the fried sago starch based-fish crackers for each cooking-chilling cycle at different cooking temperatures

Temperature	Linear expansion in each cycle (%)			
	1	2	3	4
100°C	77.60±2.95 ^{a1}	71.88±1.32 ^{b1}	66.34±4.49 ^{c1}	59.45±1.91 ^{d1}
115°C	73.10±5.77 ^{a1}	67.10±8.21 ^{ab12}	61.91±8.59 ^{ab12}	59.79±3.90 ^{b1}
121°C	73.79±1.51 ^{a1}	61.04±3.27 ^{b2}	54.58±2.66 ^{c2}	48.73±1.41 ^{d2}

*Mean of triplicate determinations ± standard deviation

**Means within a row with different letters are significantly different (p<0.05)

***Means within a column with different numbers are significantly different (p<0.05)

The most likely factor affecting the reduction in the linear expansion is the formation of resistant starch in the fish crackers. The treated samples had less expandability due to the strong hydrogen bonding involved in the RS formation. Furthermore, extensive heating in excess water through repetitions of the cooking-chilling process had caused the maximal swelling and the rupture of the starch granules which resulted in a reduced amount of expansion.

Fig.4 displays a comparison of the hardness values of fried sago-starch-based fish crackers treated at different cooking temperatures of RCC. Repetition of cooking-chilling cycles caused an increase in the hardness value of each sample. The samples cooked at higher cooking temperatures tended to become harder after frying. As hardness and expansion are inversely proportional (Yu & Low, 1992), the same trend was also observed by comparing the LE values in Table 3 with the hardness values shown in Fig.4. Sago starch fish crackers treated with four cycles of RCC at 121°C demonstrated the lowest linear expansion value (48.79%) although they had the highest hardness value (21.1 N). Hardness has often been related to the crispiness in crispy foods. Low hardness shows high crispiness score and this meets consumers' preferences in the selection of fish crackers (Huda *et al.*, 2009).

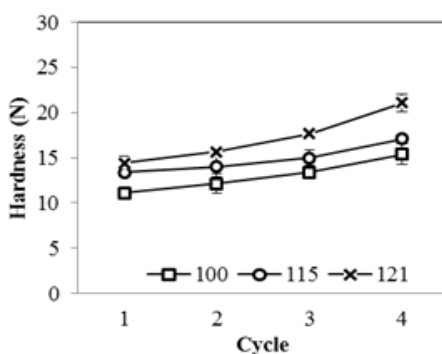


Fig.4: Hardness values of the fried sago starch based-fish crackers when subjected to cooking-chilling repetitions at different cooking temperatures

Fig.5 (a), (b) and (c) show the comparison of colour values for the fried sago starch fish crackers treated by the RCC at different cooking temperatures. RCC caused a reduction in L^* for all the samples from the first to the fourth cycle, but no significant changes were observed for a^* and b^* . It was also observed that higher cooking temperatures produced fish crackers with lower L^* and higher a^* . All the changes in the colour of the products were mainly associated with the expansion ability of the samples (Altan *et al.*, 2008). Darker fish crackers were obtained from less expandable products which could be related to their compact structure. To date, there has been no report on assessing consumers' preferences for specific colour values of fish crackers although some researchers have observed that lighter coloured fish crackers are generally preferred (Huda *et al.*, 2010).

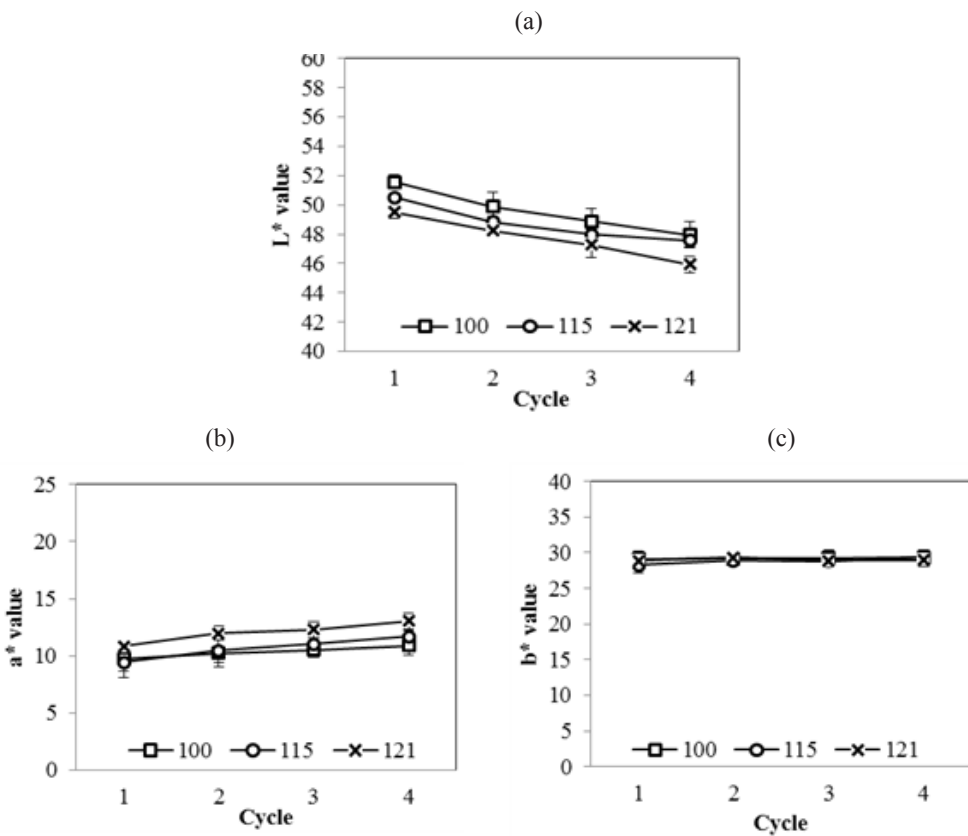


Fig.5: (a), (b) and (c). Colour values of the fried sago starch-based fish crackers when subjected to cooking-chilling repetitions at different cooking temperatures

Effects of Different Cooking Temperatures in RCC on the Physical Conditions of the Chilled Fish Cracker Gels

Table 3 shows the percentage of different physical conditions of the chilled fish cracker gels after being subjected to the first cycle of the RCC treatment. The percentage was calculated based on the number of samples identified in each attribute over the total samples cooked

in the same batch. From the observation made, it could be seen that the chilled fish cracker gels cooked at 100°C were almost in perfect condition after the treatment compared to those cooked above 100°C. This finding is important because gels in a perfect condition will facilitate further processes.

Fish crackers cooked at 100°C were observed to have almost a perfect shape. Meanwhile, higher cooking temperatures that require higher pressure above the atmospheric pressure during cooking may cause physical damage to the gels. Fish cracker gels cooked at 115°C (10 psi) produced ~77% perfect condition gels, whereas only 36% of the samples were in the perfect condition after being subjected to cooking at 121°C (15 psi). It is believed that any further increase of the cooking temperature may cause more damages to the shape of the samples. Kyaw *et al.* (2001) studied the effects of pressure cooking on the microstructure and expansion of the fish crackers cooked up to 121°C. They also highlighted the gradual increases of the tapioca and wheat starch swelling power with an increase in the cooking temperature before the granules were hydrated with simultaneous loss of their polarization crosses and burst. In this study, the observation was done only during the first RCC cycle because the gels were initially formed at this stage, while the subsequent cycles had lesser effects on the shape formation of the gels.

TABLE 3 : Conditions of the chilled fish cracker gels after the first repetitive cooking-chilling cycles

Attribute	Physical condition of the gels after the first RCC cycle (%)		
	100°C	115°C	121°C
Perfect	98.3	77.0	36.1
Minor Crack	0.0	0.00	27.7
Major Crack	0.0	7.5	14.1
Minor Burst	1.7	7.5	12.4
Major Burst	0.0	4.0	4.8
Fragmented	0.0	4.0	4.9

CONCLUSION

From the experiment, it is apparent that as the cooking temperature increases, the RS content in the fish crackers also increases. The cooking temperature at 121°C with the repetition of four cycles resulted in the highest RS content. However, it could be seen that there were some defects on the physical characteristics of the fish crackers such as cracks and burst. Despite these, the process of cooking at high temperature with the repetition of four cycles is still meaningful to further promote the nutritional aspects of the fish cracker, apart from highlighting the fact that fish crackers are processed products that contain high protein, high carbohydrate and high calcium.

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APPENDIX

List of physical condition attributes and descriptions of the chilled fish cracker gels after single RCC

No.	Attribute	Description	Illustration	No.	Attribute	Description	Illustration
1	Perfect	Sample was in perfect shape after cooking and chilling without any obvious deformation		4	Minor burst	Sample which burst less than 50% of the whole body	
2	Minor crack	Sample was found to have cracks not more than 50% of the whole body		5	Major burst	Sample which burst more than 50% of the whole body	
3	Major crack	Sample was found to have a crack more than 50% of the whole body		6	Fragment	Sample was found to be fragmented at some parts/whole part of the body	