

BUKTI KORESPONDENSI

Judul Artikel : Effect of Frozen Storage Condition on the Physicochemical and Sensory Properties of Cassava Sticks

Jurnal : Food Research
Volume 7, Supplementary 1, Februari 2023

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No.	Perihal	Tanggal
1.	Bukti konfirmasi penerimaan submit artikel, artikel yang di-submit dan revisi artikel yang harus dilakukan	5 Februari 2022
2.	Bukti konfirmasi submit revisi artikel pertama	8 Februari 2022
3.	Bukti konfirmasi penerimaan submit revisi artikel	9 Februari 2022
4.	Bukti konfirmasi review dan hasil review dari 2 Reviewer	10 Maret 2022
5.	Bukti konfirmasi submit revisi artikel kedua, respon kepada reviewer dan artikel yang di-resubmit	20 Maret 2022
6.	Bukti konfirmasi revisi artikel yang harus dilakukan	23 Maret 2022
7.	Bukti konfirmasi submit revisi artikel ketiga	24 Maret 2022
8.	Bukti konfirmasi revisi artikel yang harus dilakukan	26 Maret 2022
9.	Bukti konfirmasi submit revisi artikel keempat dan balasannya	28 Maret 2022
10.	Bukti konfirmasi artikel diterima dan acceptance letter	18 Oktober 2022
11.	Bukti konfirmasi galley proof artikel	4 Februari 2023
12.	Bukti konfirmasi submit revisi galley proof artikel dan galley proof artikel revisi yang di-resubmit	7 Februari 2023
13.	Bukti konfirmasi galley proof hasil revisi, persetujuan galley proof dan balasannya	7 dan 8 Februari 2023

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2.

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(8 Februari 2022)

Effects of frozen storage duration on the physicochemical and sensory properties of cassava sticks

Abstract

In this study, the cassava stick was stored at -20°C for three months to investigate its effects on the physicochemical and sensory properties of cassava sticks. The effects of frozen storage duration were monitored every month with three replications. A randomized block design with a single factor was used as the experimental design. According to the results, storing cassava sticks under frozen significantly increased ($P<0.05$) the oil absorption and had no effect ($P>0.05$) on the moisture content. A significant alteration ($P<0.05$) in texture was observed through the increase of cassava stick hardness from 1.11 to 2.54 N. Frozen storage duration also influenced ($P<0.05$) the lightness and yellowness, but not the redness ($P>0.05$) of cassava sticks. Fat oxidation also occurred during storage, marked by a significant increase ($P<0.05$) of free fatty acid (0.06 to 0.14%) and peroxide value (0 to 34.53 mg peroxide/kg lipid) on three months of frozen storage. Thus, this study concludes that the frozen storage duration affected the physical and chemical properties of cassava sticks. Moreover, cassava sticks stored frozen for three months were acceptable for panelists with neither like nor dislike (4.30) average acceptance and had no significant difference ($P>0.05$) with other samples.

Keywords: Cassava, Cassava stick, Frozen Storage duration

1. Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most consumed crops in the world, especially in Sub-Saharan Africa (SSA) and in developing countries of Asia such as Cambodia, Vietnam, and Laos (Benesi *et al.*, 2004; International Atomic Energy Agency, 2018). Cassava is considered as the substitute for rice as the primary carbohydrate source and can be consumed in various ways (fried, boiled, or steamed). Besides

23 carbohydrates, cassava is also rich in calcium (50 mg/100 g), phosphorus (40 mg/100 g), vitamin C (25
24 mg/100g) and contains a significant amount of thiamine, riboflavin, and nicotinic acid (International
25 Atomic Energy Agency, 2018). In Indonesia, cassava is generally processed into traditional food such as
26 tiwul, gatot, gaplek, gethuk, tapai, and cassava chips. Furthermore, it can also be processed to a modern
27 food such as cassava stick.

28 Cassava stick has similar characteristics to French fries due to its shoestring-like shape, golden
29 brown color, crunchy exterior, and fluffy interior. Those characteristics are obtained through several
30 processes such as blanching, drying, frying, and each step affects the quality of the final product (Lamberg
31 *et al.*, 1990). Despite those processes, other processes are also required to extend shelf life since cassava
32 sticks are classified as perishable food. Among other processes that might be employed for long-term
33 preservation (high pressure, infrared irradiation, pulsed electric field, and ultrasound), freezing or frozen
34 storage is still one of the most used methods (Rahman & Velez-Ruiz, 2007). Generally, frozen storage
35 temperature is 0°F (-18°C) and even colder depending on the type of the food (WFLO Commodity Storage
36 Manual, 2008).

37 Several studies stated that storing food (fruit, meat, and French fries) under freezing temperatures
38 affects the physical and chemical properties (Celli *et al.*, 2016; Medic *et al.*, 2018; Sattar *et al.*, 2015).
39 However, based on our knowledge, there is no study reported about the effect of frozen storage on the
40 cassava sticks properties. Therefore, this study aimed to investigate the physical and chemical changes on
41 cassava sticks during frozen storage. In addition, the sensory evaluation of cassava sticks during frozen
42 storage was also studied.

43 **2. Materials and methods**

44 **2.1. Materials**

45 Cassava tubers (*Manihot esculenta* Crantz) used in this study were yellow cassava (Malang 2) and
46 purchased from a local market in Malang, Indonesia. The cooking oil was purchased from a market in
47 Surabaya, Indonesia. All chemicals and other reagents used in this study were analytical grades.

48 2.2. Preparation of cassava stick

49 Cassava tubers were washed two times using tap water to remove physical contaminations. The
50 cleaned cassavas were then peeled, cut into strips of 4 x 1 x 1 cm, and soaked in 0.1% (w/v) CaCl₂ for 15
51 min to increase the crispiness of the cassava stick. Next, the soaked cassava sticks were steamed in a
52 steamer (98°C, 15 min) and cooled to room temperature before being pre-fried (170°C, 30 s) in cassava
53 sticks : cooking oil ratio of 1:12 (w/v) using a deep fryer (Fritel Professional 35 SICO, Belgium). Later, the
54 pre-fried cassava sticks were vacuum packed in a polypropylene plastic bag using a vacuum sealer
55 (Maksindo DZ300, Indonesia) and frozen at -20°C for different storage duration (0, 1, 2, 3 months) in a
56 chest freezer (Modena MD 45, Italy). The frozen cassava sticks were then thawed in a water bath (15 min)
57 and fried for 2 min at the same condition as the pre-frying process. At the end of frying, the fryer basket
58 was immediately shaken for 10 seconds and sticks were cooled to room temperature before analyzing the
59 physicochemical properties.

60 2.3. Moisture content

61 All pre-fried and fried cassava stick samples were minced before immediately analyzing the
62 moisture content. The moisture content was determined using a drying oven (Binder ED 53, Germany)
63 with the thermogravimetric method (AOAC, 2006) for 3-5 hours at 105 ± 2 °C.

64 2.4. Oil absorption analysis

65 The oil absorption analysis was determined according to the method by Mohammed *et al.* (1988).
66 Approximately 5 g of minced pre-fried and fried cassava sticks were oven-dried at 105°C for 3-5 hours and
67 weighed until a stable weight of the sample was obtained. The oil absorption was calculated using the
68 following equation:

69
$$\text{Oil absorption (\%)} = \frac{W_2 - W_1}{W_1} \times 100\%$$

70 where W_1 is the weight of pre-fried cassava stick (g) and W_2 is the weight of fried cassava stick (g).

71 2.5. *Hardness*

72 Hardness as the selected texture characteristics were determined using a TA-XT plus (Stable Micro
73 System, UK) equipped with a three-point bend rig using compression test mode with the following
74 conditions: pre-test speed= 2 mm/s, test speed= 0.5 mm/s, post-test speed= 10 mm/s, and distance= 10
75 mm. The hardness of the samples was expressed as Newton and determined as the maximum force
76 required to compress the sample.

77 2.6. *Free fatty acid analysis*

78 The free fatty acid of cassava sticks was analyzed using a method by Sudarmadji *et al.* (1984).
79 Approximately 28.2 g samples were minced before adding 50 mL of neutralized alcohol and 2 mL
80 phenolphthalein. The mixture was then titrated with 0.1 N NaOH until a stable pink color occurred for 30
81 s. The percentage of free fatty acid was expressed as the percentage of palmitic acid and calculated using
82 the following equation:

83
$$\text{Palmitic acid (\%)} = \frac{V_{\text{NaOH}} \times N_{\text{NaOH}} \times Mr}{W \times 1000} \times 100\%$$

84 where V_{NaOH} is the titre of NaOH (mL), N_{NaOH} is the concentration of NaOH (N), Mr is the molecular
85 weight of palmitic acid (256.42 g/mol), and W is the sample weight (g).

86 2.7. *Peroxide value*

87 An iodometric titration method was used to analyze the peroxide value as the primary oxidation
88 product (Sudarmadji *et al.*, 1984). Briefly, 5 g sample was mixed with 30 mL acetic acid – chloroform (3:2)
89 and 0.5 mL saturated aqueous potassium iodide solution was added. Later, the mixture was shaken
90 vigorously for 1 minute before 30 mL distilled water was added. The mixture was titrated with 0.1 M
91 $\text{Na}_2\text{S}_2\text{O}_3$ until the yellow color had almost disappeared. Next, 0.5 mL of 1% (w/v) starch indicator solution

92 was added and the titration continued until the blue color disappeared. The peroxide value was calculated
93 using the following formula:

$$94 \quad \text{Peroxide value (meq peroxide/kg oil)} = \frac{V_{\text{thio}} \times N_{\text{thio}} \times 1000}{W}$$

95 where V_{thio} is the titre of $\text{Na}_2\text{S}_2\text{O}_3$ (mL), N_{thio} is the concentration of $\text{Na}_2\text{S}_2\text{O}_3$, and W is the sample
96 weight (g).

97 2.8. Color

98 Cassava stick color was identified using a color reader (Color Reader Minolta, CR-10). The L^* value
99 (0 = blackness, 100 = whiteness), a^* value (+ = redness, - = greenness), and b^* value (+ = yellowness, - =
100 blueness) of each sample were observed.

101 2.9. Sensory evaluation

102 In this study, the Hedonic Scale Scoring (preferred test) was used to measure the level of preference
103 and acceptance of the product by consumers (Kusuma *et al.*, 2017). The sensory evaluation was evaluated
104 by 100 untrained panelists. The panelists were students of the Faculty of Agricultural Technology, Widya
105 Mandala Catholic University Surabaya. The sensory attributes tested were color, hardness, crispiness,
106 aroma, and taste. A scoring system of 1-7 points was used to represent the sensory characteristics of each
107 sample. On this scale, 1 was defined as dislike extremely, 2 was defined as dislike moderately, 3 was
108 defined as dislike slightly, 4 was defined as neither like nor dislike, 5 was defined as like slightly, 6 was
109 defined as like moderately, and 7 was defined as like extremely.

110 2.10. Statistical analysis

111 All experiments were analyzed at least three times in triplicate and represented as mean values
112 \pm SD. Statistical analyses were performed using SPSS for Windows (version 19.0, SPSS Inc., USA) and
113 compared with Duncan Multiple Range Test (DMRT) at $P < 0.05$.

114 3. Results and discussion

115 3.1. Moisture content and oil absorption

116 The moisture content of fried cassava sticks is shown in Table 1. Based on the result, different frozen
117 storage durations have no significant difference ($P>0.05$) on the moisture content of cassava sticks, even
118 though there was a downward trend in the moisture content of frozen cassava sticks for 2 and 3 months.
119 Similar results were reported by Medic *et al* (2018), where frozen storage duration had no significant on
120 the moisture content of pork loin and belly rib. However, several researchers also stated that freezing
121 significantly ($P<0.05$) influenced the moisture content of the fried potato (Adedeji & Ngadi, 2017), lamb
122 (Coombs *et al*, 2017), and pork ham (Medic *et al.*, 2018). According to Fennema (1985), water in food
123 converts to ice crystals during freezing at -18°C and easily removed from food when the food is dried or
124 fried; so that the moisture content decreases.

125 Oil absorption analysis was conducted to measure the ability of cassava sticks to absorb oil during
126 frying by using a modifying thermogravimetric method (Mohamed *et al.*, 1988). Table 1 showed that
127 frozen storage duration significantly increased ($P<0,05$) the oil absorption of cassava sticks. Cassava stick
128 stored frozen for 2 months has the highest oil absorption (58.45%) but has no significant difference on
129 cassava stick stored frozen for 3 months (57.84%). In Adedeji & Ngadi (2017) study, oil absorption also
130 showed a significant increase in fried potato that was stored frozen. However, they also stated that there
131 was no interaction between storage duration and freezing method, which contradicts our study.

132 According to Adedeji *et al.* (2009), moisture loss during frying affects the amount of oil absorbed in
133 fried food. This statement is in accordance with Fellows (1990) statement, where there is an oil transfer
134 into the product to replace evaporated water during frying. In addition, moisture evaporation from fried
135 food during frying damages the cellular structure of plant tissues, resulting in an increase in porosity which
136 contributes to oil absorption (Mellema, 2003; Dana & Saguy, 2006; Rimac-Brncic *et al.*, 2004). On the other
137 hand, during frozen storage, all water in food converts to ice crystal and causes volume expansion which
138 is characterized by the damage to cell membranes during ice crystal formation (Fennema, 1985; Celli *et*

139 *al.*, 2016). This volume expansion causes the water in cassava sticks to be easier to evaporate during frying
140 and increases oil absorption.

141 3.2. Hardness

142 In this study, hardness was chosen as the analyzed texture attribute to evaluate the impact of frozen
143 storage duration on the texture of cassava sticks. According to Szczesniak (2002), hardness is the force
144 required to change the shape of material due to its resistance to resist deformation. The effect of frozen
145 storage duration on the hardness of cassava sticks is presented in Figure 1. As shown in that Figure, frozen
146 storage duration significantly affected ($P < 0.05$) the hardness of the cassava sticks. Cassava stick that
147 stored frozen for 3 months has the highest force (2.54) among other samples (0.63 – 1.41). Our results
148 were in accordance with the results of Adedeji & Ngadi (2017). In that study, they found a marginal
149 increase in the hardness of fried potatoes as the frozen storage duration increased. Therefore, the
150 increase of fried cassava sticks' hardness might be due to the retrogradation of cassava starch during
151 frozen storage. Our statement was supported by a similar study by Yu *et al.* (2010), where the hardness
152 of cooked rice increased continually with the amylopectin retrogradation during frozen storage. So that,
153 based on those findings, it indicates that amylopectin retrogradation contributed to the hardness increase
154 during storage on the high starch food products.

155 3.3. Free fatty acid and peroxide value

156 This study assessed the percentage of free fatty acid and peroxide values to monitor the fat
157 oxidation in cassava sticks during frozen storage. The increase of free fatty acid and peroxide value
158 indicates the rancidity of food products that produce off-flavors (Maity *et al.*, 2012) and might reduce
159 consumers' preferences. The free fatty acid of cassava stick was expressed as the percentage of palmitic
160 acid since the cooking oil used to fry was palm oil. Meanwhile, the peroxide value as the primary oxidation
161 product of fat oxidation was expressed as meq peroxide/ kg fat. The percentage of free fatty acid and

162 peroxide value of fried cassava sticks is shown in Figure 2. A significant increased ($P<0.05$) of free fatty
163 acid was recorded during 2 months of frozen storage (0.13 %) and had no significant difference ($P>0.05$)
164 with cassava sticks stored frozen for 3 months (0.14%). On the other hand, the peroxide value of cassava
165 stick was also significantly increased ($P<0.05$) as the frozen storage duration increased (0 – 34.53 meq
166 peroxide/ kg fat). Similar results were also reported by some studies where frozen storage duration
167 increased the free fatty acid and peroxide value of potato strips (Kizito *et al.*, 2017), pork meat (Medic *et*
168 *al.*, 2018), and ready-to-fry vegetable snacks (Maity *et al.*, 2012).

169 According to Crosa *et al.* (2014), free fatty acids are formed through the nucleophilic attack at
170 triacylglycerol's ester bond and have been associated with the undesirable odors and taste of food
171 products. Furthermore, the formation of hydroperoxides during storage generally occurred during the
172 early stage of oxidation (Fennema, 1985) and later decomposed to other secondary oxidation products
173 such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (Kizito *et al.*, 2017; Medic *et al.*, 2018).
174 Another factor that might increase the formation of hydroperoxides was the contact of cassava stick with
175 oxygen before and after storage; since oxygen is one of the reactants that caused fat oxidation (Giri &
176 Mangaraj, 2012).

177 3.4. Color properties

178 Color is generally considered an important attribute that significantly affects the physical
179 properties of food, the perception of consumers, and determines the nutritional quality of food products
180 (Patras *et al.*, 2011; Hutchings, 2002). In this study, the color of cassava sticks is shown in Table 2 and was
181 measured using Hunter's L^* , a^* , and b^* color attributes. The L^* value expressed the degree of lightness,
182 where the highest value was measured in the cassava stick stored frozen for 3 months (75.20). However,
183 the results were not significantly different ($P>0.05$) with cassava stick that was not stored frozen (71.55)
184 and significantly different ($P<0.05$) with other samples (66.52 – 67.98). The different results were shown
185 on the a^* value, which expressed the degree of redness ($+a^*$) and greenness ($-a^*$) of cassava sticks. All

186 samples had no significant difference ($P>0.05$) on the degree of redness (2.94 – 4.62). Meanwhile, the b^*
187 value expressed the degree of yellowness ($+b^*$) and blueness ($-b^*$). The results show that the b^* values of
188 cassava stick (25.77) that stored frozen for 2 months was significantly lower ($P<0.05$) than other samples
189 (30.79 – 32.08).

190 Our results showed that the color of cassava sticks was greatly affected by the frozen storage
191 duration. Research by Oner & Wall (2012) also found that frozen storage significantly influenced ($P<0.05$)
192 the color of French fries. The color change of fried cassava sticks during frozen storage duration might be
193 due to the surface moisture desiccation (Maity *et al.*, 2012). The amount of moisture loss during the frying
194 process also influence the color of fried cassava stick since moisture loss is associated with crust formation
195 and accelerates the Maillard browning (Sahin, 2000). The Maillard browning occurs due to the reaction
196 between amino acid and reducing sugar during frying and as the result, increases the coloration of yellow,
197 red, and brown color in fried food (Pedreschi *et al.*, 2005). Another factor that might influence the crust
198 color of cassava sticks was the heat transfer rate in the cassava sticks during frying (Maity *et al.*, 2012).

199 3.5. Sensory evaluation

200 The sensory evaluation results of cassava sticks stored frozen in different storage durations are
201 shown as a spider plot in Figure 3. According to the results, the highest score for the color attribute was
202 the 0-month storage cassava sticks (5.60) and significantly difference ($P<0.05$) with other samples (3.20 –
203 4.96). The decrease in panelists' color preference was due to the color changes in cassava sticks that tend
204 to be darker and brownly than the regular cassava sticks. Meanwhile, on the hardness attribute, the
205 panelists' preference significantly decreased ($P<0.05$) as the frozen storage duration increased (5.30 –
206 4.20). This result was in accordance with our objective analysis on hardness, where frozen storage
207 duration significantly increased the hardness of cassava sticks due to the starch retrogradation. The next
208 sensory attribute tested was the crispiness and the results were quite varied. Cassava sticks stored frozen
209 for 0 months (4.88) had no significant difference ($P>0.05$) with 2 months stored cassava stick (4.66) but

210 showed significant difference ($P < 0.05$) with cassava sticks stored frozen for 1 month (3.86) and 3 months
211 (3.68). This result indicated that panelists perceived that frozen storage duration influenced the crispiness
212 of fried cassava sticks. Furthermore, the frozen storage duration significantly influenced ($P < 0.05$) the
213 panelists' preferences on the aroma of cassava sticks. The aroma of cassava sticks tended to be different
214 for each treatment and might be influenced by the formation of hydroperoxides and free fatty acids. The
215 last sensory attribute evaluated was the taste of cassava sticks. According to the result, the frozen storage
216 duration had no significant difference ($P > 0.05$) on the panelists' perception of the taste of cassava sticks
217 (4.16 – 4.88). Overall, the average score of panelists' preference showed that frozen storage duration did
218 not significantly affect ($P > 0.05$) panelists' acceptance of the cassava stick.

219 **4. Conclusion**

220 Frozen storage can be used to extend the shelf life of food products. However, the use of frozen
221 storage on cassava sticks affects its physical and chemical properties. Different frozen storage duration
222 influenced the oil absorption of cassava sticks, though the moisture content showed no significant change.
223 The effect of frozen storage duration was also significant on the texture of cassava sticks, especially on
224 hardness. Another studied quality characteristic, such as cassava stick surface color, was substantially
225 declined during the frozen storage. The free fatty acid and peroxide value of cassava sticks was gradually
226 increased with elongation of frozen storage duration. According to the sensory evaluation results, frozen
227 storage duration influenced all of the sensory attributes besides the taste of cassava sticks. Cassava sticks
228 stored frozen for 3 months were still acceptable by the panelists and the acceptance was defined as
229 neither like nor dislike.

230 **Conflict of interest**

231 The authors declare no conflict of interest.

232 **Acknowledgments**

233 This study was financially supported by the Lembaga Penelitian dan Pengabdian Masyarakat (LPPM)
234 Widya Mandala Surabaya Catholic University through PPPG Research Grants 2015/2016.
235

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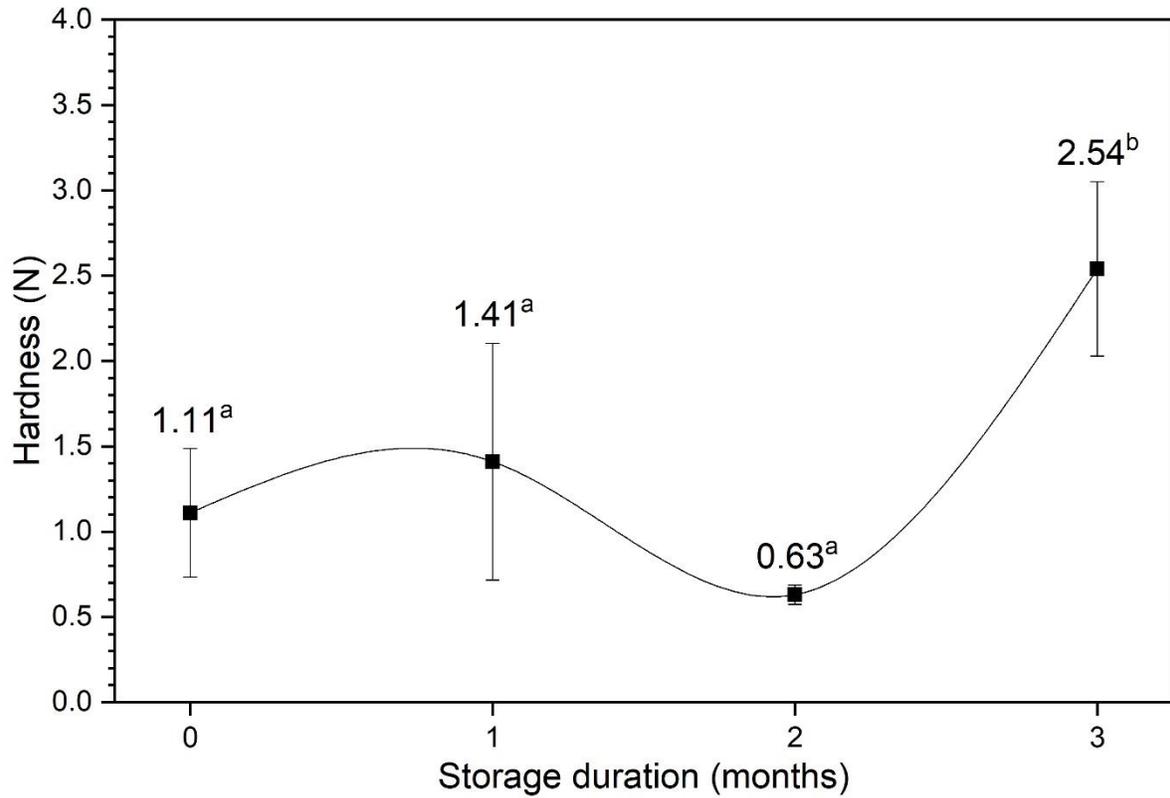
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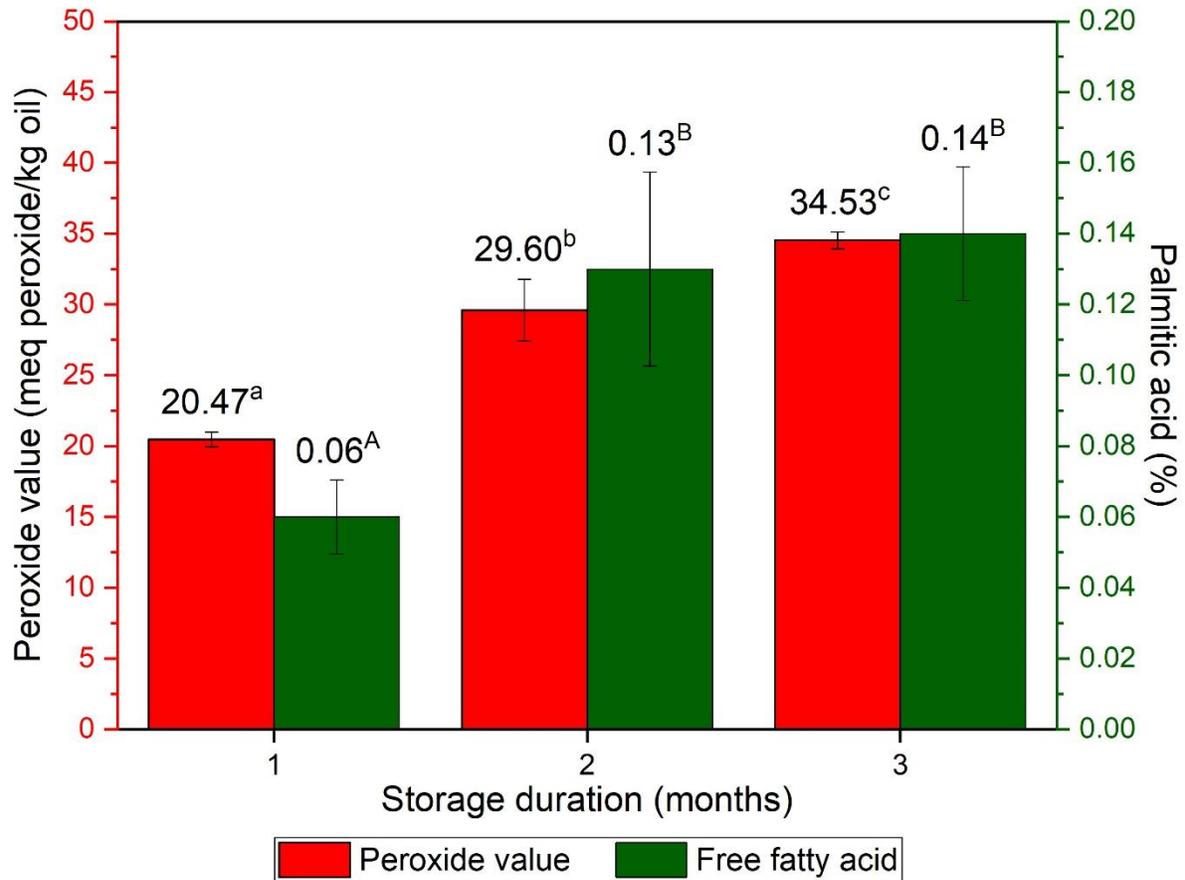
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318 Figure 1. Hardness of cassava stick stored frozen in different storage duration. Means \pm standard
319 deviation (n = 3 for each group) in the same column followed by the different letter are significantly
320 different (P<0.05). The statistical significance was evaluated by one-way ANOVA followed by DMRT test

321 ($\alpha = 0.05$).

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324

325 Figure 2. Free fatty acid and peroxide value of cassava stick stored frozen in different storage duration.

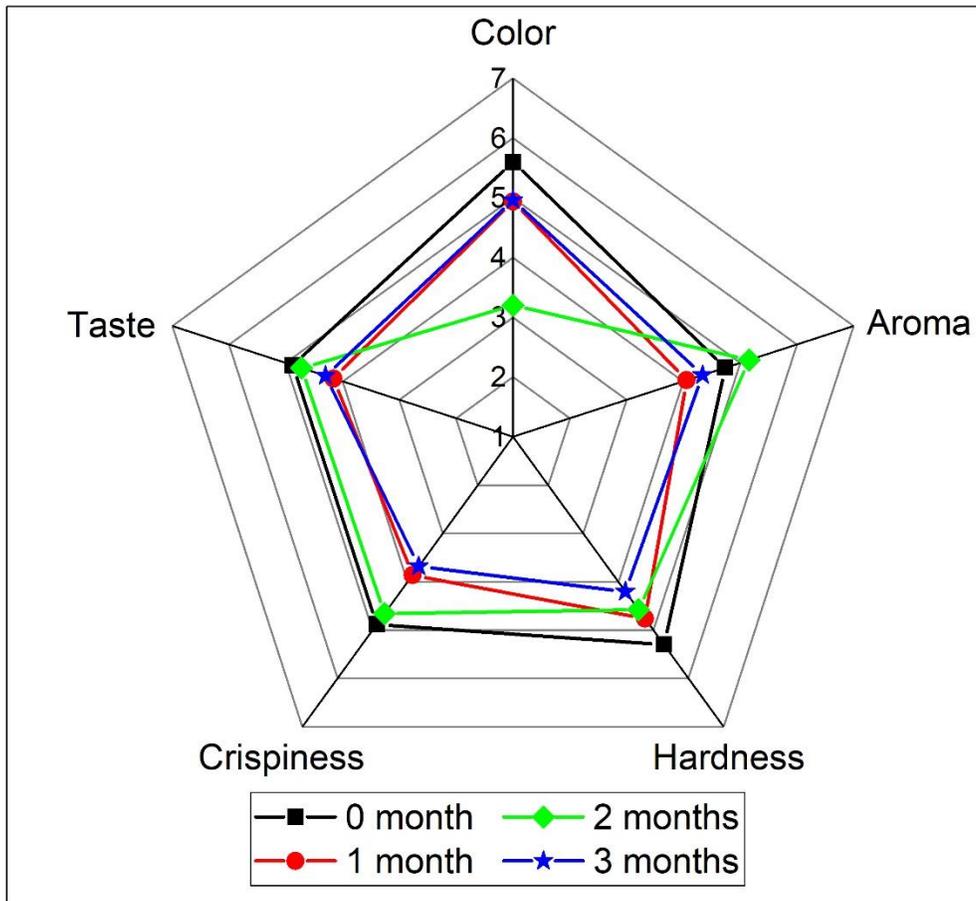
326 Means \pm standard deviation (n = 3 for each group) in the same column followed by the different letter

327 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA

328

followed by DMRT test ($\alpha = 0.05$).

329



330

331 Figure 3. Sensory evaluation of cassava stick stored frozen in different storage duration. Means \pm
 332 standard deviation (n = 100 for each group) in the same column followed by the different letter are
 333 significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA followed by
 334 DMRT test ($\alpha = 0.05$).

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338

339 **List of Tables**

340 Table 1. Moisture content and oil absorption of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Moisture content (%)	Oil absorption (%)
0	45.13 ± 7.46 ^a	20.87 ± 15.91 ^a
1	48.83 ± 3.27 ^a	25.66 ± 9.51 ^a
2	28.68 ± 7.95 ^a	58.45 ± 2.47 ^b
3	27.75 ± 10.13 ^a	57.94 ± 24.71 ^b

341 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter
342 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA
343 followed by DMRT test ($\alpha = 0.05$).

344

345

Table 2. Color of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Color		
	L*	a*	b*
0	71.55 ± 4.08 ^{ab}	2.95 ± 0.78 ^a	32.08 ± 3.02 ^b
1	67.98 ± 2.78 ^a	4.62 ± 1.93 ^a	30.79 ± 1.87 ^b
2	66.52 ± 1.15 ^a	3.21 ± 0.37 ^a	25.77 ± 0.98 ^a
3	75.20 ± 1.80 ^b	4.01 ± 0.30 ^a	31.40 ± 1.66 ^b

346 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter

347 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA

348 followed by DMRT test ($\alpha = 0.05$).

349

350

1 **Effects of frozen storage duration on the physicochemical and sensory properties of cassava sticks**

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10

11 **Abstract**

12 In this study, the cassava stick was stored at -20°C for three months to investigate its effects on the
13 physicochemical and sensory properties of cassava sticks. The effects of frozen storage duration were
14 monitored every month with three replications. A randomized block design with a single factor was used
15 as the experimental design. According to the results, storing cassava sticks under frozen significantly
16 increased ($P<0.05$) the oil absorption and had no effect ($P>0.05$) on the moisture content. A significant
17 alteration ($P<0.05$) in texture was observed through the increase of cassava stick hardness from 1.11 to
18 2.54 N. Frozen storage duration also influenced ($P<0.05$) the lightness and yellowness, but not the redness
19 ($P>0.05$) of cassava sticks. Fat oxidation also occurred during storage, marked by a significant increase
20 ($P<0.05$) of free fatty acid (0.06 to 0.14%) and peroxide value (0 to 34.53 mg peroxide/kg lipid) on three
21 months of frozen storage. Thus, this study concludes that the frozen storage duration affected the physical

22 and chemical properties of cassava sticks. Moreover, cassava sticks stored frozen for three months were
23 acceptable for panelists with neither like nor dislike (4.30) average acceptance, and had no significant
24 difference ($P>0.05$) with other samples.

25 **Keywords:** Cassava, Cassava stick, Frozen Storage duration

26 1. Introduction

27 Cassava (*Manihot esculenta* Crantz) is one of the most consumed crops in the world, especially in
28 Sub-Saharan Africa (SSA) and in developing countries of Asia such as Cambodia, Vietnam, and Laos (Benesi
29 *et al.*, 2004; International Atomic Energy Agency, 2018). Cassava is considered as the substitute for rice as
30 the primary carbohydrate source and can be consumed in various ways (fried, boiled, or steamed). Besides
31 carbohydrates, cassava is also rich in calcium (50 mg/100 g), phosphorus (40 mg/100 g), vitamin C (25
32 mg/100g) and contains a significant amount of thiamine, riboflavin, and nicotinic acid (International
33 Atomic Energy Agency, 2018). In Indonesia, cassava is generally processed into traditional food such as
34 tiwul, gatot, gapek, gethuk, tapai, and cassava chips. Furthermore, it can also be processed to a modern
35 food such as cassava stick.

36 Cassava stick has similar characteristics to French fries due to its shoestring-like shape, golden
37 brown color, crunchy exterior, and fluffy interior. Those characteristics are obtained through several
38 processes such as blanching, drying, frying, and each step affects the quality of the final product (Lamberg
39 *et al.*, 1990). Despite those processes, other processes are also required to extend shelf life since cassava
40 sticks are classified as perishable food. Among other processes that might be employed for long-term
41 preservation (high pressure, infrared irradiation, pulsed electric field, and ultrasound), freezing or frozen
42 storage is still one of the most used methods (Rahman & Velez-Ruiz, 2007). Generally, frozen storage
43 temperature is 0°F (-18°C) and even colder depending on the type of the food (WFLO Commodity Storage
44 Manual, 2008).

45 Several studies stated that storing food (fruit, meat, and French fries) under freezing temperatures
46 affects the physical and chemical properties (Celli *et al.*, 2016; Medic *et al.*, 2018; Sattar *et al.*, 2015).
47 However, based on our knowledge, there is no study reported about the effect of frozen storage on the
48 cassava sticks properties. Therefore, this study aimed to investigate the physical and chemical changes on
49 cassava sticks during frozen storage. In addition, the sensory evaluation of cassava sticks during frozen
50 storage was also studied.

51 **2. Materials and methods**

52 *2.1. Materials*

53 Cassava tubers (*Manihot esculenta* Crantz) used in this study were yellow cassava (Malang 2) and
54 purchased from a local market in Malang, Indonesia. The cooking oil was purchased from a market in
55 Surabaya, Indonesia. All chemicals and other reagents used in this study were analytical grades.

56 *2.2. Preparation of cassava stick*

57 Cassava tubers were washed two times using tap water to remove physical contaminations. The
58 cleaned cassavas were then peeled, cut into strips of 4 x 1 x 1 cm, and soaked in 0.1% (w/v) CaCl₂ for 15
59 min to increase the crispiness of the cassava stick. Next, the soaked cassava sticks were steamed in a
60 steamer (98°C, 15 min) and cooled to room temperature before being pre-fried (170°C, 30 s) in cassava
61 sticks : cooking oil ratio of 1:12 (w/v) using a deep fryer (Fritel Professional 35 SICO, Belgium). Later, the
62 pre-fried cassava sticks were vacuum packed in a polypropylene plastic bag using a vacuum sealer
63 (Maksindo DZ300, Indonesia) and frozen at -20°C for different storage duration (0, 1, 2, 3 months) in a
64 chest freezer (Modena MD 45, Italy). The frozen cassava sticks were then thawed in a water bath (15 min)
65 and fried for 2 min at the same condition as the pre-frying process. At the end of frying, the fryer basket
66 was immediately shaken for 10 seconds and sticks were cooled to room temperature before analyzing the
67 physicochemical properties.

68 *2.3. Moisture content*

69 All pre-fried and fried cassava stick samples were minced before immediately analyzing the
70 moisture content. The moisture content was determined using a drying oven (Binder ED 53, Germany)
71 with the thermogravimetric method (AOAC, 2006) for 3-5 hours at 105 ± 2 °C.

72 2.4. Oil absorption analysis

73 The oil absorption analysis was determined according to the method by Mohammed *et al.* (1988).
74 Approximately 5 g of minced pre-fried and fried cassava sticks were oven-dried at 105°C for 3-5 hours and
75 weighed until a stable weight of the sample was obtained. The oil absorption was calculated using the
76 following equation:

$$77 \text{ Oil absorption (\%)} = \frac{W_2 - W_1}{W_1} \times 100\%$$

78 where W_1 is the weight of pre-fried cassava stick (g) and W_2 is the weight of fried cassava stick (g).

79 2.5. Hardness

80 Hardness as the selected texture characteristics were determined using a TA-XT plus (Stable Micro
81 System, UK) equipped with a three-point bend rig using compression test mode with the following
82 conditions: pre-test speed= 2 mm/s, test speed= 0.5 mm/s, post-test speed= 10 mm/s, and distance= 10
83 mm. The hardness of the samples was expressed as Newton and determined as the maximum force
84 required to compress the sample.

85 2.6. Free fatty acid analysis

86 The free fatty acid of cassava sticks was analyzed using a method by Sudarmadji *et al.* (1984).
87 Approximately 28.2 g samples were minced before adding 50 mL of neutralized alcohol and 2 mL
88 phenolphthalein. The mixture was then titrated with 0.1 N NaOH until a stable pink color occurred for 30
89 s. The percentage of free fatty acid was expressed as the percentage of palmitic acid and calculated using
90 the following equation:

$$91 \text{ Palmitic acid (\%)} = \frac{V_{\text{NaOH}} \times N_{\text{NaOH}} \times Mr}{W \times 1000} \times 100\%$$

92 where V_{NaOH} is the titre of NaOH (mL), N_{NaOH} is the concentration of NaOH (N), M_r is the molecular
93 weight of palmitic acid (256.42 g/mol), and W is the sample weight (g).

94 2.7. Peroxide value

95 An iodometric titration method was used to analyze the peroxide value as the primary oxidation
96 product (Sudarmadji *et al.*, 1984). Briefly, 5 g sample was mixed with 30 mL acetic acid – chloroform (3:2)
97 and 0.5 mL saturated aqueous potassium iodide solution was added. Later, the mixture was shaken
98 vigorously for 1 minute before 30 mL distilled water was added. The mixture was titrated with 0.1 M
99 $\text{Na}_2\text{S}_2\text{O}_3$ until the yellow color had almost disappeared. Next, 0.5 mL of 1% (w/v) starch indicator solution
100 was added and the titration continued until the blue color disappeared. The peroxide value was calculated
101 using the following formula:

$$102 \quad \text{Peroxide value (meq peroxide/kg oil)} = \frac{V_{\text{thio}} \times N_{\text{thio}} \times 1000}{W}$$

103 where V_{thio} is the titre of $\text{Na}_2\text{S}_2\text{O}_3$ (mL), N_{thio} is the concentration of $\text{Na}_2\text{S}_2\text{O}_3$, and W is the sample
104 weight (g).

105 2.8. Color

106 Cassava stick color was identified using a color reader (Color Reader Minolta, CR-10). The L^* value
107 (0 = blackness, 100 = whiteness), a^* value (+ = redness, - = greenness), and b^* value (+ = yellowness, - =
108 blueness) of each sample were observed.

109 2.9. Sensory evaluation

110 In this study, the Hedonic Scale Scoring (preferred test) was used to measure the level of preference
111 and acceptance of the product by consumers (Kusuma *et al.*, 2017). The sensory evaluation was evaluated
112 by 100 untrained panelists. The panelists were students of the Faculty of Agricultural Technology, Widya
113 Mandala Catholic University Surabaya. The sensory attributes tested were color, hardness, crispiness,
114 aroma, and taste. A scoring system of 1-7 points was used to represent the sensory characteristics of each
115 sample. On this scale, 1 was defined as dislike extremely, 2 was defined as dislike moderately, 3 was

116 defined as dislike slightly, 4 was defined as neither like nor dislike, 5 was defined as like slightly, 6 was
117 defined as like moderately, and 7 was defined as like extremely.

118 2.10. Statistical analysis

119 All experiments were analyzed at least three times in triplicate and represented as mean values
120 \pm SD. Statistical analyses were performed using SPSS for Windows (version 19.0, SPSS Inc., USA) and
121 compared with Duncan Multiple Range Test (DMRT) at $P < 0.05$.

122 3. Results and discussion

123 3.1. Moisture content and oil absorption

124 The moisture content of fried cassava sticks is shown in Table 1. Based on the result, different frozen
125 storage durations have no significant difference ($P > 0.05$) on the moisture content of cassava sticks, even
126 though there was a downward trend in the moisture content of frozen cassava sticks for 2 and 3 months.
127 Similar results were reported by Medic *et al* (2018), where frozen storage duration had no significant on
128 the moisture content of pork loin and belly rib. However, several researchers also stated that freezing
129 significantly ($P < 0.05$) influenced the moisture content of the fried potato (Adedeji & Ngadi, 2017), lamb
130 (Coombs *et al*, 2017), and pork ham (Medic *et al.*, 2018). According to Fennema (1985), water in food
131 converts to ice crystals during freezing at -18°C and easily removed from food when the food is dried or
132 fried; so that the moisture content decreases.

133 Oil absorption analysis was conducted to measure the ability of cassava sticks to absorb oil during
134 frying by using a modifying thermogravimetric method (Mohamed *et al.*, 1988). Table 1 showed that
135 frozen storage duration significantly increased ($P < 0.05$) the oil absorption of cassava sticks. Cassava stick
136 stored frozen for 2 months has the highest oil absorption (58.45%) but has no significant difference on
137 cassava stick stored frozen for 3 months (57.84%). In Adedeji & Ngadi (2017) study, oil absorption also
138 showed a significant increase in fried potato that was stored frozen. However, they also stated that there
139 was no interaction between storage duration and freezing method, which contradicts our study.

140 According to Adedeji *et al.* (2009), moisture loss during frying affects the amount of oil absorbed in
141 fried food. This statement is in accordance with Fellows (1990) statement, where there is an oil transfer
142 into the product to replace evaporated water during frying. In addition, moisture evaporation from fried
143 food during frying damages the cellular structure of plant tissues, resulting in an increase in porosity which
144 contributes to oil absorption (Mellema, 2003; Dana & Saguy, 2006; Rimac-Brncic *et al.*, 2004). On the other
145 hand, during frozen storage, all water in food converts to ice crystal and causes volume expansion which
146 is characterized by the damage to cell membranes during ice crystal formation (Fennema, 1985; Celli *et*
147 *al.*, 2016). This volume expansion causes the water in cassava sticks to be easier to evaporate during frying
148 and increases oil absorption.

149 3.2. Hardness

150 In this study, hardness was chosen as the analyzed texture attribute to evaluate the impact of frozen
151 storage duration on the texture of cassava sticks. According to Szczesniak (2002), hardness is the force
152 required to change the shape of material due to its resistance to resist deformation. The effect of frozen
153 storage duration on the hardness of cassava sticks is presented in Figure 1. As shown in that Figure, frozen
154 storage duration significantly affected ($P < 0.05$) the hardness of the cassava sticks. Cassava stick that
155 stored frozen for 3 months has the highest force (2.54) among other samples (0.63 – 1.41). Our results
156 were in accordance with the results of Adedeji & Ngadi (2017). In that study, they found a marginal
157 increase in the hardness of fried potatoes as the frozen storage duration increased. Therefore, the
158 increase of fried cassava sticks' hardness might be due to the retrogradation of cassava starch during
159 frozen storage. Our statement was supported by a similar study by Yu *et al.* (2010), where the hardness
160 of cooked rice increased continually with the amylopectin retrogradation during frozen storage. So that,
161 based on those findings, it indicates that amylopectin retrogradation contributed to the hardness increase
162 during storage on the high starch food products.

163 3.3. Free fatty acid and peroxide value

164 This study assessed the percentage of free fatty acid and peroxide values to monitor the fat
165 oxidation in cassava sticks during frozen storage. The increase of free fatty acid and peroxide value
166 indicates the rancidity of food products that produce off-flavors (Maity *et al.*, 2012) and might reduce
167 consumers' preferences. The free fatty acid of cassava stick was expressed as the percentage of palmitic
168 acid since the cooking oil used to fry was palm oil. Meanwhile, the peroxide value as the primary oxidation
169 product of fat oxidation was expressed as meq peroxide/ kg fat. The percentage of free fatty acid and
170 peroxide value of fried cassava sticks is shown in Figure 2. A significant increased ($P<0.05$) of free fatty
171 acid was recorded during 2 months of frozen storage (0.13 %) and had no significant difference ($P>0.05$)
172 with cassava sticks stored frozen for 3 months (0.14%). On the other hand, the peroxide value of cassava
173 stick was also significantly increased ($P<0.05$) as the frozen storage duration increased (0 – 34.53 meq
174 peroxide/ kg fat). Similar results were also reported by some studies where frozen storage duration
175 increased the free fatty acid and peroxide value of potato strips (Kizito *et al.*, 2017), pork meat (Medic *et*
176 *al.*, 2018), and ready-to-fry vegetable snacks (Maity *et al.*, 2012).

177 According to Crosa *et al.* (2014), free fatty acids are formed through the nucleophilic attack at
178 triacylglycerol's ester bond and have been associated with the undesirable odors and taste of food
179 products. Furthermore, the formation of hydroperoxides during storage generally occurred during the
180 early stage of oxidation (Fennema, 1985) and later decomposed to other secondary oxidation products
181 such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (Kizito *et al.*, 2017; Medic *et al.*, 2018).
182 Another factor that might increase the formation of hydroperoxides was the contact of cassava stick with
183 oxygen before and after storage; since oxygen is one of the reactants that caused fat oxidation (Giri &
184 Mangaraj, 2012).

185 3.4. Color properties

186 Color is generally considered an important attribute that significantly affects the physical
187 properties of food, the perception of consumers, and determines the nutritional quality of food products

188 (Patras *et al.*, 2011; Hutchings, 2002). In this study, the color of cassava sticks is shown in Table 2 and was
189 measured using Hunter's L*, a*, and b* color attributes. The L* value expressed the degree of lightness,
190 where the highest value was measured in the cassava stick stored frozen for 3 months (75.20). However,
191 the results were not significantly different ($P>0.05$) with cassava stick that was not stored frozen (71.55)
192 and significantly different ($P<0.05$) with other samples (66.52 – 67.98). The different results were shown
193 on the a* value, which expressed the degree of redness (+a*) and greenness (-a*) of cassava sticks. All
194 samples had no significant difference ($P>0.05$) on the degree of redness (2.94 – 4.62). Meanwhile, the b*
195 value expressed the degree of yellowness (+b*) and blueness (-b*). The results show that the b* values of
196 cassava stick (25.77) that stored frozen for 2 months was significantly lower ($P<0.05$) than other samples
197 (30.79 – 32.08).

198 Our results showed that the color of cassava sticks was greatly affected by the frozen storage
199 duration. Research by Oner & Wall (2012) also found that frozen storage significantly influenced ($P<0.05$)
200 the color of French fries. The color change of fried cassava sticks during frozen storage duration might be
201 due to the surface moisture desiccation (Maity *et al.*, 2012). The amount of moisture loss during the frying
202 process also influence the color of fried cassava stick since moisture loss is associated with crust formation
203 and accelerates the Maillard browning (Sahin, 2000). The Maillard browning occurs due to the reaction
204 between amino acid and reducing sugar during frying and as the result, increases the coloration of yellow,
205 red, and brown color in fried food (Pedreschi *et al.*, 2005). Another factor that might influence the crust
206 color of cassava sticks was the heat transfer rate in the cassava sticks during frying (Maity *et al.*, 2012).

207 3.5. Sensory evaluation

208 The sensory evaluation results of cassava sticks stored frozen in different storage durations are
209 shown as a spider plot in Figure 3. According to the results, the highest score for the color attribute was
210 the 0-month storage cassava sticks (5.60) and significantly difference ($P<0.05$) with other samples (3.20 –
211 4.96). The decrease in panelists' color preference was due to the color changes in cassava sticks that tend

212 to be darker and brownly than the regular cassava sticks. Meanwhile, on the hardness attribute, the
213 panelists' preference significantly decreased ($P < 0.05$) as the frozen storage duration increased (5.30 –
214 4.20). This result was in accordance with our objective analysis on hardness, where frozen storage
215 duration significantly increased the hardness of cassava sticks due to the starch retrogradation. The next
216 sensory attribute tested was the crispiness and the results were quite varied. Cassava sticks stored frozen
217 for 0 months (4.88) had no significant difference ($P > 0.05$) with 2 months stored cassava stick (4.66) but
218 showed significant difference ($P < 0.05$) with cassava sticks stored frozen for 1 month (3.86) and 3 months
219 (3.68). This result indicated that panelists perceived that frozen storage duration influenced the crispiness
220 of fried cassava sticks. Furthermore, the frozen storage duration significantly influenced ($P < 0.05$) the
221 panelists' preferences on the aroma of cassava sticks. The aroma of cassava sticks tended to be different
222 for each treatment and might be influenced by the formation of hydroperoxides and free fatty acids. The
223 last sensory attribute evaluated was the taste of cassava sticks. According to the result, the frozen storage
224 duration had no significant difference ($P > 0.05$) on the panelists' perception of the taste of cassava sticks
225 (4.16 – 4.88). Overall, the average score of panelists' preference showed that frozen storage duration did
226 not significantly affect ($P > 0.05$) panelists' acceptance of the cassava stick.

227 **4. Conclusion**

228 Frozen storage can be used to extend the shelf life of food products. However, the use of frozen
229 storage on cassava sticks affects its physical and chemical properties. Different frozen storage duration
230 influenced the oil absorption of cassava sticks, though the moisture content showed no significant change.
231 The effect of frozen storage duration was also significant on the texture of cassava sticks, especially on
232 hardness. Another studied quality characteristic, such as cassava stick surface color, was substantially
233 declined during the frozen storage. The free fatty acid and peroxide value of cassava sticks was gradually
234 increased with elongation of frozen storage duration. According to the sensory evaluation results, frozen
235 storage duration influenced all of the sensory attributes besides the taste of cassava sticks. Cassava sticks

236 stored frozen for 3 months were still acceptable by the panelists and the acceptance was defined as
237 neither like nor dislike.

238 **Conflict of interest**

239 The authors declare no conflict of interest.

240 **Acknowledgments**

241 This study was financially supported by the Lembaga Penelitian dan Pengabdian Masyarakat (LPPM)
242 Widya Mandala Surabaya Catholic University through PPPG Research Grants 2015/2016.

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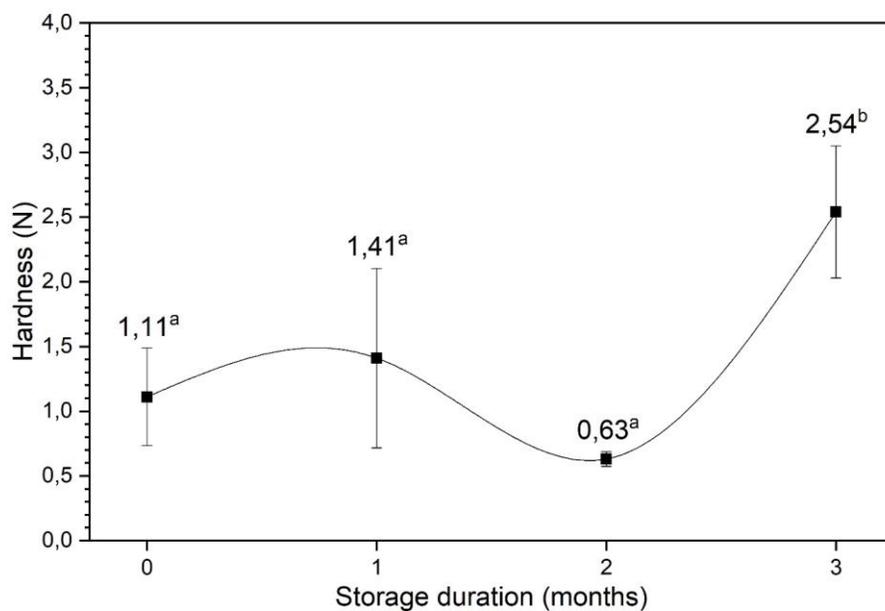
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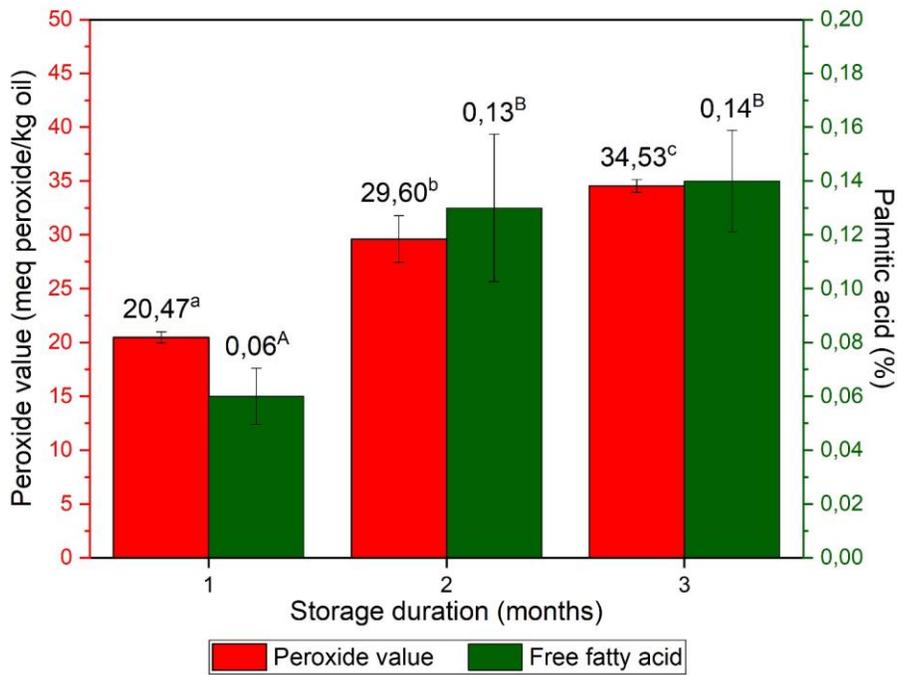
322 **List of Figures**



323
324 Figure 1. Hardness of cassava stick stored frozen in different storage duration. Means \pm standard
325 deviation ($n = 3$ for each group) in the same column followed by the different letter are significantly
326 different ($P < 0.05$). The statistical significance was evaluated by one-way ANOVA followed by DMRT test
327 ($\alpha = 0.05$).

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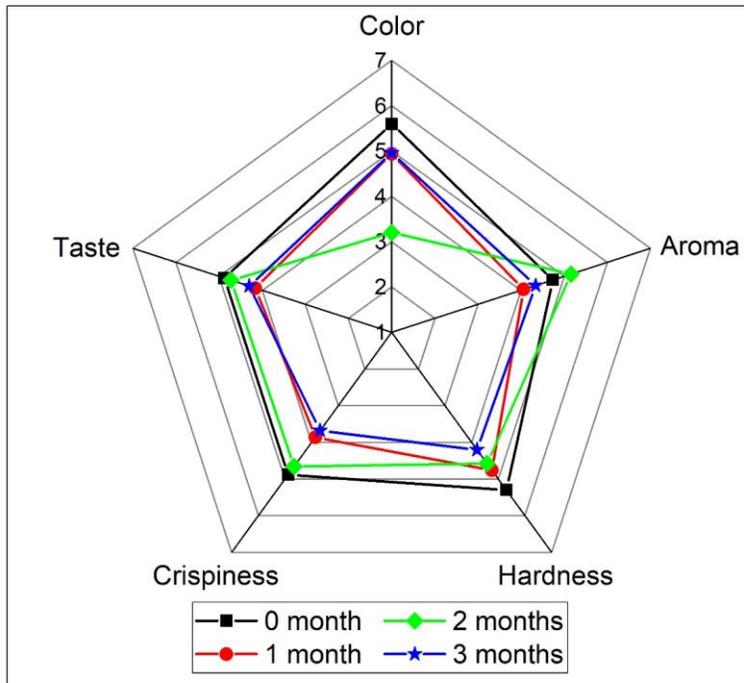
331 Figure 2. Free fatty acid and peroxide value of cassava stick stored frozen in different storage duration.

332 Means \pm standard deviation (n = 3 for each group) in the same column followed by the different letter

333 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA

334 followed by DMRT test ($\alpha = 0.05$).

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336

337 Figure 3. Sensory evaluation of cassava stick stored frozen in different storage duration. Means \pm
 338 standard deviation (n = 100 for each group) in the same column followed by the different letter are
 339 significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA followed by
 340 DMRT test ($\alpha = 0.05$).

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344

345 **List of Tables**

346 Table 1. Moisture content and oil absorption of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Moisture content (%)	Oil absorption (%)
0	45,13 ± 7,46 ^a	20,87 ± 15,91 ^a
1	48,83 ± 3,27 ^a	25,66 ± 9,51 ^a
2	28,68 ± 7,95 ^a	58,45 ± 2,47 ^b
3	27,75 ± 10,13 ^a	57,94 ± 24,71 ^b

347 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter
 348 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA
 349 followed by DMRT test (α = 0.05).

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350 **Table 2.** Color of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Color		
	L*	a*	b*
0	71,55 ± 4,08 ^{ab}	2,95 ± 0,78 ^a	32,08 ± 3,02 ^b
1	67,98 ± 2,78 ^a	4,62 ± 1,93 ^a	30,79 ± 1,87 ^b
2	66,52 ± 1,15 ^a	3,21 ± 0,37 ^a	25,77 ± 0,98 ^a
3	75,20 ± 1,80 ^b	4,01 ± 0,30 ^a	31,40 ± 1,66 ^b

351 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter
 352 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA
 353 followed by DMRT test (α = 0.05).

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22 oxidation also occurred during storage, marked by a significant increase ($P < 0.05$) of free fatty acid (0.06
23 to 0.14%) and peroxide value (0 to 34.53 mg peroxide/kg lipid) on three months of frozen storage. Thus,
24 this study concludes that frozen storage duration affected the physical and chemical properties of cassava
25 sticks. Moreover, cassava sticks stored frozen for three months were acceptable for panelists with neither
26 like nor dislike (4.30) average acceptance, and had no significant difference ($P > 0.05$) with other samples.

27 **Keywords:** Cassava, Cassava stick, Frozen storage duration

28 1. Introduction

29 Cassava (*Manihot esculenta* Crantz) is one of the most consumed crops in the world, especially in
30 Sub-Saharan Africa (SSA) and in developing countries of Asia such as Cambodia, Vietnam, and Laos (Benesi
31 *et al.*, 2004; International Atomic Energy Agency, 2018). Cassava is considered as the substitute for rice as
32 the primary carbohydrate source and can be consumed in various ways (fried, boiled, or steamed). Besides
33 carbohydrates, cassava is also rich in calcium (50 mg/100 g), phosphorus (40 mg/100 g), vitamin C (25
34 mg/100g) and contains a significant amount of thiamine, riboflavin, and nicotinic acid (International
35 Atomic Energy Agency, 2018). In Indonesia, cassava is generally processed into traditional food such as
36 *tiwul*, *gatot*, *gaplek*, *gethuk*, *tapai*, and cassava chips. Furthermore, it can also be processed into a modern
37 food such as cassava stick.

38 Cassava stick has similar characteristics to French fries due to its shoestring-like shape, golden
39 brown color, crunchy exterior, and fluffy interior. Those characteristics are obtained through several
40 processes such as blanching, drying, frying, and each step affects the quality of the final product (Lamberg
41 *et al.*, 1990). Other processes are also required to extend shelf life since cassava sticks are classified as
42 perishable food. Among other processes that might be employed for long-term preservation (high
43 pressure, infrared irradiation, pulsed electric field, and ultrasound), freezing or frozen storage is still one

44 of the most used methods (Rahman and Velez-Ruiz, 2007). Generally, frozen storage temperature is 0°F
45 (-18°C) and even colder depending on the type of the food (WFLO Commodity Storage Manual, 2008).

46 Several studies stated that storing food (fruit, meat, and French fries) under freezing temperatures
47 affects the physical and chemical properties (Celli *et al.*, 2016; Medic *et al.*, 2018; Sattar *et al.*, 2015).
48 However, based on our knowledge, there is no study reported about the effect of frozen storage on the
49 cassava sticks properties. Therefore, this study aimed to investigate the physical and chemical changes on
50 cassava sticks during frozen storage. In addition, the sensory evaluation of cassava sticks during frozen
51 storage was also studied.

52 **2. Materials and methods**

53 *2.1. Materials*

54 Cassava tubers (*Manihot esculenta* Crantz) used in this study were yellow cassava (Malang 2),
55 purchased from a local market in Malang, Indonesia. The cooking oil was purchased from a market in
56 Surabaya, Indonesia. All chemicals and other reagents used in this study were analytical grades.

57 *2.2. Preparation of cassava stick*

58 Cassava tubers were washed two times using tap water to remove physical contaminations. The
59 cleaned cassava tubers were then peeled, cut into strips of 4 x 1 x 1 cm, and soaked in 0.1% (w/v) CaCl₂
60 for 15 min to increase the crispiness of the cassava stick. Next, the soaked cassava sticks were steamed in
61 a steamer (98°C, 15 min) and cooled to room temperature before being pre-fried (170°C, 30 s) in cassava
62 sticks : cooking oil ratio of 1:12 (w/v) using a deep fryer (Fritel Professional 35 SICO, Belgium). Later, the
63 pre-fried cassava sticks were vacuum packed in a polypropylene plastic bag using a vacuum sealer
64 (Maksindo DZ300, Indonesia) and frozen at -20°C for different storage duration (0, 1, 2, 3 months) in a
65 chest freezer (Modena MD 45, Italy). The frozen cassava sticks were then thawed in a water bath (15 min)
66 and fried for 2 min at the same condition as the pre-frying process. At the end of frying, the fryer basket

67 was immediately shaken for 10 seconds and sticks were cooled to room temperature before analyzing the
68 physicochemical properties.

69 2.3. *Moisture content*

70 All fried cassava stick samples were minced before immediately analyzing the moisture content.
71 The moisture content was determined using a drying oven (Binder ED 53, Germany) with the
72 thermogravimetric method (AOAC, 2006) for 3-5 hours at 105 ± 2 °C.

73 2.4. *Oil absorption analysis*

74 The oil absorption analysis was determined according to the method by Mohammed *et al.* (1988).
75 Approximately 5 g of minced pre-fried and fried cassava sticks were oven-dried at 105°C for 3-5 hours and
76 weighed until a stable weight of the sample was obtained. The oil absorption was calculated using the
77 following equation:

$$78 \text{ Oil absorption (\%)} = \frac{W_2 - W_1}{W_1} \times 100\%$$

79 where W_1 is the weight of pre-fried cassava stick (g) and W_2 is the weight of fried cassava stick (g).

80 2.5. *Hardness*

81 Hardness as the selected texture characteristics were determined using a TA-XT plus (Stable Micro
82 System, UK) equipped with a three-point bend rig using compression test mode with the following
83 conditions: pre-test speed= 2 mm/s, test speed= 0.5 mm/s, post-test speed= 10 mm/s, and distance= 10
84 mm. The hardness of fried cassava stick was expressed as Newton and determined as the maximum force
85 required to compress the sample.

86 2.6. *Free fatty acid analysis*

87 The free fatty acid of cassava sticks was analyzed using a method by Sudarmadji *et al.* (1984).
88 Approximately 28.2 g of fried cassava sticks were minced before adding 50 mL of neutralized alcohol and
89 2 mL phenolphthalein. The mixture was then titrated with 0.1 N NaOH until a stable pink color occurred

90 for 30 s. The percentage of free fatty acid was expressed as the percentage of palmitic acid and calculated
91 using the following equation:

$$92 \quad \text{Palmitic acid (\%)} = \frac{V_{\text{NaOH}} \times N_{\text{NaOH}} \times \text{Mr}}{W \times 1000} \times 100\%$$

93 where V_{NaOH} is the titre of NaOH (mL), N_{NaOH} is the concentration of NaOH (N), Mr is the molecular
94 weight of palmitic acid (256.42 g/mol), and W is the sample weight (g).

95 2.7. Peroxide value

96 An iodometric titration method was used to analyze the peroxide value as the primary oxidation
97 product (Sudarmadji *et al.*, 1984). Briefly, 5 g of fried cassava sticks were mixed with 30 mL acetic acid –
98 chloroform (3:2) and 0.5 mL saturated aqueous potassium iodide solution was added. Later, the mixture
99 was shaken vigorously for 1 minute before 30 mL distilled water was added. The mixture was titrated with
100 0.1 M $\text{Na}_2\text{S}_2\text{O}_3$ until the yellow color had almost disappeared. Next, 0.5 mL of 1% (w/v) starch indicator
101 solution was added and the titration continued until the blue color disappeared. The peroxide value was
102 calculated using the following formula:

$$103 \quad \text{Peroxide value (meq peroxide/kg oil)} = \frac{V_{\text{thio}} \times N_{\text{thio}} \times 1000}{W}$$

104 where V_{thio} is the titre of $\text{Na}_2\text{S}_2\text{O}_3$ (mL), N_{thio} is the concentration of $\text{Na}_2\text{S}_2\text{O}_3$, and W is the sample
105 weight (g).

106 2.8. Color

107 The color of fried cassava stick was identified using a color reader (Color Reader Minolta, CR-10).
108 The L^* value (0 = blackness, 100 = whiteness), a^* value (+ = redness, - = greenness), and b^* value (+ =
109 yellowness, - = blueness) of each sample were observed.

110 2.9. Sensory evaluation

111 In this study, the Hedonic Scale Scoring (preferred test) was used to measure the level of preference
112 and acceptance of the product by consumers (Kusuma *et al.*, 2017). The sensory evaluation was evaluated

113 by 100 untrained panelists. The panelists were students of the Faculty of Agricultural Technology, Widya
114 Mandala Catholic University Surabaya. The sensory attributes tested were color, hardness, crispiness,
115 aroma, and taste. A scoring system of 1-7 points was used to represent the sensory characteristics of each
116 sample. On this scale, 1 was defined as dislike extremely, 2 was defined as dislike moderately, 3 was
117 defined as dislike slightly, 4 was defined as neither like nor dislike, 5 was defined as like slightly, 6 was
118 defined as like moderately, and 7 was defined as like extremely.

119 2.10. Statistical analysis

120 All experiments were analyzed at least three times in triplicate and represented as mean values
121 \pm SD. Statistical analyses were performed using SPSS for Windows (version 19.0, SPSS Inc., USA) and
122 compared with Duncan Multiple Range Test (DMRT) at $P < 0.05$.

123 3. Results and discussion

124 3.1. Moisture content and oil absorption

125 The moisture content of fried cassava sticks is shown in Table 1. Based on the result, different frozen
126 storage durations have no significant difference ($P > 0.05$) on the moisture content of cassava sticks, even
127 though there was a downward trend in the moisture content of frozen cassava sticks for 2 and 3 months.
128 Similar results were reported by Medic *et al.* (2018), where frozen storage duration had no significant on
129 the moisture content of pork loin and belly rib. However, several researchers also stated that freezing
130 significantly ($P < 0.05$) influenced the moisture content of the fried potato (Adedeji and Ngadi, 2017), lamb
131 (Coombs *et al.*, 2017), and pork ham (Medic *et al.*, 2018). According to Fennema (1985), water in food
132 converts to ice crystals during freezing at -18°C and easily removed from food when the food is dried or
133 fried; so that the moisture content decreases.

134 Oil absorption analysis was conducted to measure the ability of cassava sticks to absorb oil during
135 frying by using a modifying thermogravimetric method (Mohamed *et al.*, 1988). Table 1 showed that
136 frozen storage duration significantly increased ($P < 0.05$) the oil absorption of cassava sticks. Cassava stick

137 stored frozen for 2 months has the highest oil absorption (58.45%) but has no significant difference with
138 cassava stick stored frozen for 3 months (57.84%). In Adedeji's and Ngadi's (2017) study, oil absorption
139 also showed a significant increase in fried potato that was stored frozen. However, they also stated that
140 there was no interaction between storage duration and freezing method, which contradicts our study.

141 According to Adedeji *et al.* (2009), moisture loss during frying affects the amount of oil absorbed in
142 fried food. This statement is in accordance with Fellows (1990) statement, where there is an oil transfer
143 into the product to replace evaporated water during frying. In addition, moisture evaporation from fried
144 food during frying damages the cellular structure of plant tissues, resulting in an increase in porosity which
145 contributes to oil absorption (Mellema, 2003; Dana and Saguy, 2006; Rimac-Brcic *et al.*, 2004). On the
146 other hand, during frozen storage, all water in food converts to ice crystal and causes volume expansion
147 which is characterized by the damage to cell membranes during ice crystal formation (Fennema, 1985;
148 Celli *et al.*, 2016). This volume expansion causes the water in cassava sticks to be easier to evaporate
149 during frying and increases oil absorption.

150 3.2. Hardness

151 In this study, hardness was chosen as the analyzed texture attribute to evaluate the impact of frozen
152 storage duration on the texture of cassava sticks. According to Szczesniak (2002), hardness is the force
153 required to change the shape of material due to its resistance to resist deformation. The effect of frozen
154 storage duration on the hardness of cassava sticks is presented in Figure 1. As shown in that figure, frozen
155 storage duration significantly affected ($P < 0.05$) the hardness of the cassava sticks. Cassava stick that was
156 stored frozen for 3 months has the highest force (2.54) among other samples (0.63 – 1.41). Our results
157 were in accordance with the results of Adedeji and Ngadi (2017). In that study, they found a marginal
158 increase in the hardness of fried potatoes as the frozen storage duration increased. Therefore, the
159 increase of fried cassava sticks' hardness might be due to the retrogradation of cassava starch during
160 frozen storage. Our statement was supported by a similar study by Yu *et al.* (2010), where the hardness

161 of cooked rice increased continually with the amylopectin retrogradation during frozen storage. Based on
162 those findings, amylopectin retrogradation contributed to the hardness increase during storage on the
163 high starch food products.

164 3.3. Free fatty acid and peroxide value

165 This study assessed the percentage of free fatty acid and peroxide values to monitor the fat
166 oxidation in cassava sticks during frozen storage. The increase of free fatty acid and peroxide value
167 indicates the rancidity of food products that produce off-flavors (Maity *et al.*, 2012) and might reduce
168 consumers' preferences. The free fatty acid of cassava stick was expressed as the percentage of palmitic
169 acid since the cooking oil used to fry was palm oil. Meanwhile, the peroxide value as the primary oxidation
170 product of fat oxidation was expressed as meq peroxide/kg fat. The percentage of free fatty acid and
171 peroxide value of fried cassava sticks is shown in Figure 2. A significant increased ($P < 0.05$) of free fatty
172 acid was recorded during 2 months of frozen storage (0.13 %) and had no significant difference ($P > 0.05$)
173 with cassava sticks stored frozen for 3 months (0.14%). On the other hand, the peroxide value of cassava
174 stick was also significantly increased ($P < 0.05$) as the frozen storage duration increased (0 – 34.53 meq
175 peroxide/ kg fat). Similar results were also reported by some studies where frozen storage duration
176 increased the free fatty acid and peroxide value of potato strips (Kizito *et al.*, 2017), pork meat (Medic *et al.*
177 *et al.*, 2018), and ready-to-fry vegetable snacks (Maity *et al.*, 2012).

178 According to Crosa *et al.* (2014), free fatty acids are formed through the nucleophilic attack at
179 triacylglycerol's ester bond and have been associated with the undesirable odors and taste of food
180 products. Furthermore, the formation of hydroperoxides during storage generally occurred during the
181 early stage of oxidation (Fennema, 1985) and later decomposed to other secondary oxidation products
182 such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (Kizito *et al.*, 2017; Medic *et al.*, 2018).
183 Another factor that might increase the formation of hydroperoxides was the contact of cassava stick with

184 oxygen before and after storage; since oxygen is one of the reactants that caused fat oxidation (Giri and
185 Mangaraj, 2012).

186 3.4. Color properties

187 Color is generally considered an important attribute that significantly affects the physical
188 properties of food, the perception of consumers, and determines the nutritional quality of food products
189 (Patras *et al.*, 2011; Hutchings, 2002). In this study, the color of cassava sticks is shown in Table 2 and was
190 measured using Hunter's L*, a*, and b* color attributes. The L* value expressed the degree of lightness,
191 where the highest value was measured in the cassava stick stored frozen for 3 months (75.20). However,
192 the results were not significantly different ($P>0.05$) with cassava stick that was not stored frozen (71.55)
193 and significantly different ($P<0.05$) with other samples (66.52 – 67.98). The different results were shown
194 on the a* value, which expressed the degree of redness (+a*) and greenness (-a*) of cassava sticks. All
195 samples had no significant difference ($P>0.05$) on the degree of redness (2.94 – 4.62). Meanwhile, the b*
196 value expressed the degree of yellowness (+b*) and blueness (-b*). The results show that the b* values of
197 cassava stick (25.77) that stored frozen for 2 months was significantly lower ($P<0.05$) than other samples
198 (30.79 – 32.08).

199 Research by Oner and Wall (2012) also found that frozen storage significantly influenced
200 ($P<0.05$) the color of French fries. The color change of fried cassava sticks during frozen storage duration
201 might be due to the surface moisture desiccation (Maity *et al.*, 2012). The amount of moisture loss during
202 the frying process also influence the color of fried cassava stick since moisture loss is associated with crust
203 formation and accelerates the Maillard browning (Sahin, 2000). The Maillard browning occurs due to the
204 reaction between amino acid and reducing sugar during frying and as the result, increases the coloration
205 of yellow, red, and brown color in fried food (Pedreschi *et al.*, 2005). Another factor that might influence
206 the crust color of cassava sticks was the heat transfer rate in the cassava sticks during frying (Maity *et al.*,
207 2012).

208 3.5. Sensory evaluation

209 The sensory evaluation results of cassava sticks stored frozen in different storage durations are
210 shown as a spider plot in Figure 3. According to the results, the highest score for the color attribute was
211 the 0-month storage cassava sticks (5.60) and significantly difference ($P < 0.05$) with other samples (3.20 –
212 4.96). The decrease in panelists' color preference was due to the color changes in cassava sticks that tend
213 to be darker and brownish than the regular cassava sticks. Meanwhile, on the hardness attribute, the
214 panelists' preference significantly decreased ($P < 0.05$) as the frozen storage duration increased (5.30 –
215 4.20). This result was in accordance with our objective analysis on hardness, where frozen storage
216 duration significantly increased the hardness of cassava sticks due to the starch retrogradation. The next
217 sensory attribute tested was the crispiness and the results were quite varied. Cassava sticks stored frozen
218 for 0 months (4.88) had no significant difference ($P > 0.05$) with 2 months stored cassava stick (4.66) but
219 showed significant difference ($P < 0.05$) with cassava sticks stored frozen for 1 month (3.86) and 3 months
220 (3.68). This result indicated that panelists perceived that frozen storage duration influenced the crispiness
221 of fried cassava sticks. Furthermore, the frozen storage duration significantly influenced ($P < 0.05$) the
222 panelists' preferences on the aroma of cassava sticks. The aroma of cassava sticks tended to be different
223 for each treatment and might be influenced by the formation of hydroperoxides and free fatty acids. The
224 last sensory attribute evaluated was the taste of cassava sticks. According to the result, the frozen storage
225 duration had no significant difference ($P > 0.05$) on the panelists' perception of the taste of cassava sticks
226 (4.16 – 4.88). Overall, the average score of panelists' preference showed that frozen storage duration did
227 not significantly affect ($P > 0.05$) panelists' acceptance of the cassava stick.

228 4. Conclusion

229 Frozen storage can be used to extend the shelf life of food products. However, the use of frozen
230 storage on cassava sticks affects its physical and chemical properties. Different frozen storage duration
231 influenced the oil absorption of cassava sticks, though the moisture content showed no significant change.

232 The effect of frozen storage duration was also significant on the texture of cassava sticks, especially on
233 hardness. Another studied quality characteristic, such as cassava stick surface color, was substantially
234 declined during the frozen storage. The free fatty acid and peroxide value of cassava sticks was gradually
235 increased with elongation of frozen storage duration. According to the sensory evaluation results, frozen
236 storage duration influenced all of the sensory attributes besides the taste of cassava sticks. Cassava sticks
237 stored frozen for 3 months were still acceptable by the panelists and the acceptance was defined as
238 neither like nor dislike.

239 **Conflict of interest**

240 The authors declare no conflict of interest.

241 **Acknowledgments**

242 This study was financially supported by the Lembaga Penelitian dan Pengabdian Masyarakat (LPPM)
243 Widya Mandala Surabaya Catholic University through PPPG Research Grants 2015/2016.

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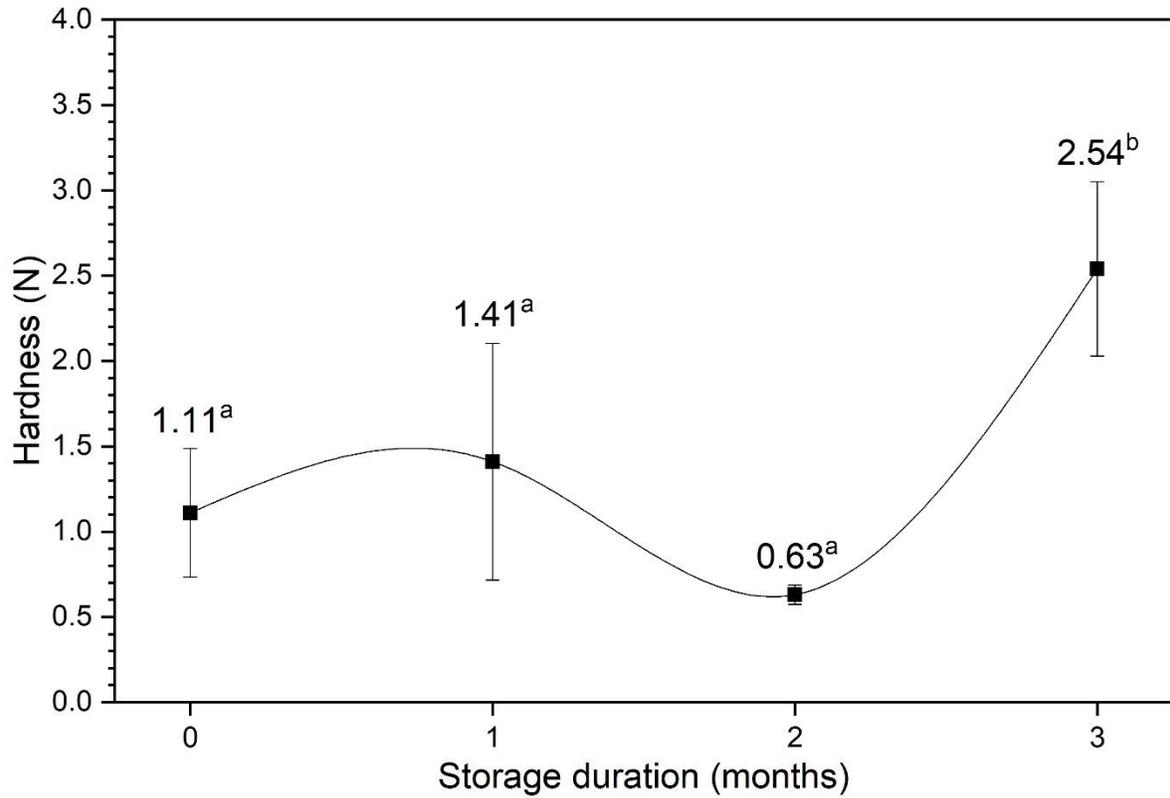
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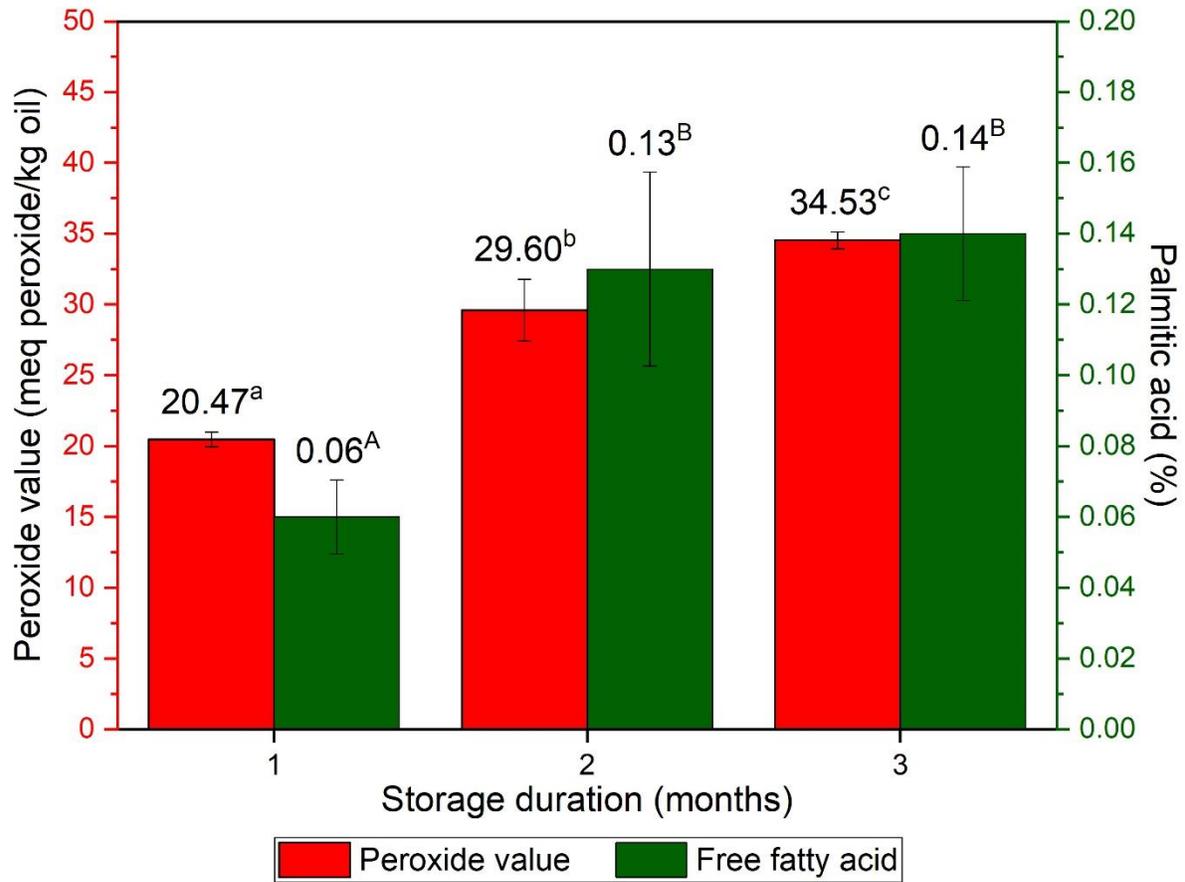
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318 Figure 1. Hardness of cassava stick stored frozen in different storage duration. Means \pm standard
319 deviation (n = 3 for each group) in the same column followed by the different letter are significantly
320 different (P<0.05). The statistical significance was evaluated by one-way ANOVA followed by DMRT test

321 ($\alpha = 0.05$).

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324

325 Figure 2. Free fatty acid and peroxide value of cassava stick stored frozen in different storage duration.

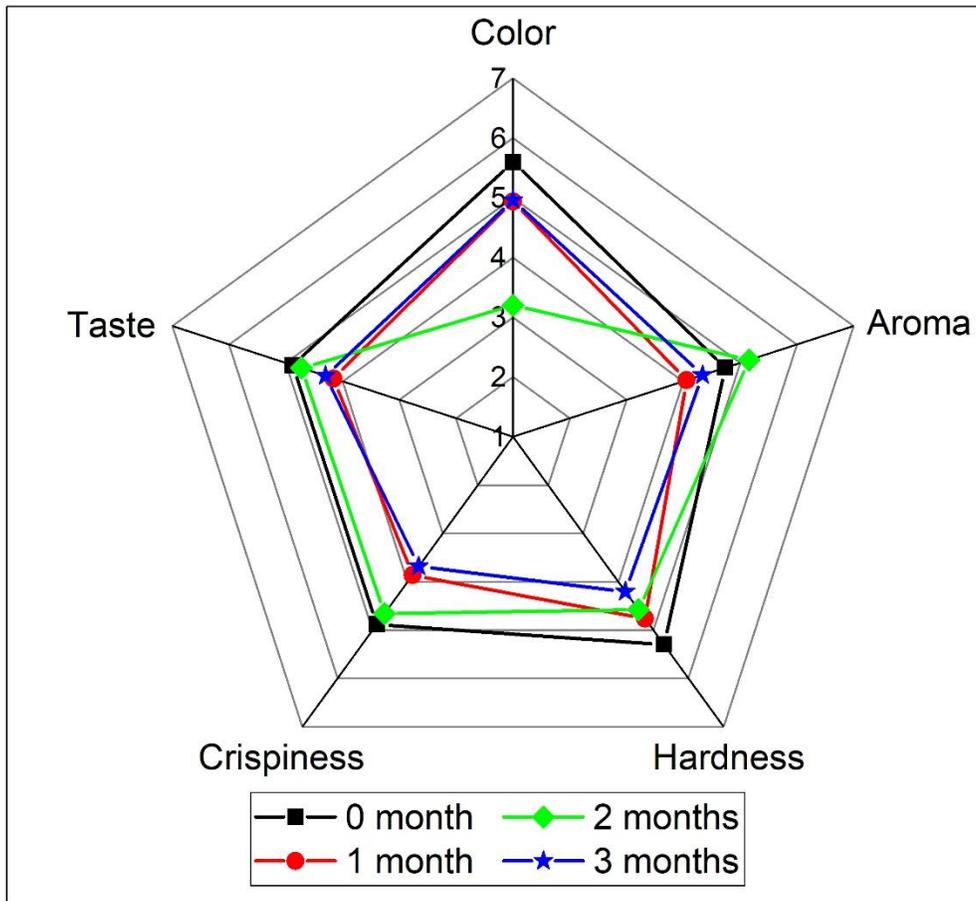
326 Means \pm standard deviation (n = 3 for each group) in the same column followed by the different letter

327 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA

328

followed by DMRT test ($\alpha = 0.05$).

329



330

331 Figure 3. Sensory evaluation of cassava stick stored frozen in different storage duration. Means \pm
 332 standard deviation (n = 100 for each group) in the same column followed by the different letter are
 333 significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA followed by
 334 DMRT test ($\alpha = 0.05$).

335

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337

338

339 **List of Tables**

340 Table 1. Moisture content and oil absorption of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Moisture content (%)	Oil absorption (%)
0	45.13 ± 7.46 ^a	20.87 ± 15.91 ^a
1	48.83 ± 3.27 ^a	25.66 ± 9.51 ^a
2	28.68 ± 7.95 ^a	58.45 ± 2.47 ^b
3	27.75 ± 10.13 ^a	57.94 ± 24.71 ^b

341 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter
 342 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA
 343 followed by DMRT test (α = 0.05).

344 **Table 2.** Color of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Color		
	L*	a*	b*
0	71.55 ± 4.08 ^{ab}	2.95 ± 0.78 ^a	32.08 ± 3.02 ^b
1	67.98 ± 2.78 ^a	4.62 ± 1.93 ^a	30.79 ± 1.87 ^b
2	66.52 ± 1.15 ^a	3.21 ± 0.37 ^a	25.77 ± 0.98 ^a
3	75.20 ± 1.80 ^b	4.01 ± 0.30 ^a	31.40 ± 1.66 ^b

345 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter
 346 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA
 347 followed by DMRT test (α = 0.05).

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Effects of frozen storage duration on the physicochemical and sensory properties of cassava sticks

Abstract

In this study, the cassava stick was stored at -20°C for three months to investigate its effects on the physicochemical and sensory properties of cassava sticks. The effects of frozen storage duration were monitored every month with three replications. A randomized block design with a single factor was used as the experimental design. According to the results, storing cassava sticks under frozen significantly increased ($P<0.05$) the oil absorption and had no effect ($P>0.05$) on the moisture content. A significant alteration ($P<0.05$) in texture was observed through the increase of cassava stick hardness from 1.11 to 2.54 N. Frozen storage duration also influenced ($P<0.05$) the lightness and yellowness, but not the redness ($P>0.05$) of cassava sticks. Fat oxidation also occurred during storage, marked by a significant increase ($P<0.05$) of free fatty acid (0.06 to 0.14%) and peroxide value (0 to 34.53 mg peroxide/kg lipid) on three months of frozen storage. Thus, this study concludes that the frozen storage duration affected the physical and chemical properties of cassava sticks. Moreover, cassava sticks stored frozen for three months were acceptable for panelists with neither like nor dislike (4.30) average acceptance and had no significant difference ($P>0.05$) with other samples.

Keywords: Cassava, Cassava stick, Frozen Storage duration

1. Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most consumed crops in the world, especially in Sub-Saharan Africa (SSA) and in developing countries of Asia such as Cambodia, Vietnam, and Laos (Benesi *et al.*, 2004; International Atomic Energy Agency, 2018). Cassava is considered as the substitute for rice as the primary carbohydrate source and can be consumed in various ways (fried, boiled, or steamed). Besides

23 carbohydrates, cassava is also rich in calcium (50 mg/100 g), phosphorus (40 mg/100 g), vitamin C (25
24 mg/100g) and contains a significant amount of thiamine, riboflavin, and nicotinic acid (International
25 Atomic Energy Agency, 2018). In Indonesia, cassava is generally processed into traditional food such as
26 tiwul, gatot, gaplek, gethuk, tapai, and cassava chips. Furthermore, it can also be processed to a modern
27 food such as cassava stick.

28 Cassava stick has similar characteristics to French fries due to its shoestring-like shape, golden
29 brown color, crunchy exterior, and fluffy interior. Those characteristics are obtained through several
30 processes such as blanching, drying, frying, and each step affects the quality of the final product (Lamberg
31 *et al.*, 1990). Despite those processes, other processes are also required to extend shelf life since cassava
32 sticks are classified as perishable food. Among other processes that might be employed for long-term
33 preservation (high pressure, infrared irradiation, pulsed electric field, and ultrasound), freezing or frozen
34 storage is still one of the most used methods (Rahman & Velez-Ruiz, 2007). Generally, frozen storage
35 temperature is 0°F (-18°C) and even colder depending on the type of the food (WFLO Commodity Storage
36 Manual, 2008).

37 Several studies stated that storing food (fruit, meat, and French fries) under freezing temperatures
38 affects the physical and chemical properties (Celli *et al.*, 2016; Medic *et al.*, 2018; Sattar *et al.*, 2015).
39 However, based on our knowledge, there is no study reported about the effect of frozen storage on the
40 cassava sticks properties. Therefore, this study aimed to investigate the physical and chemical changes on
41 cassava sticks during frozen storage. In addition, the sensory evaluation of cassava sticks during frozen
42 storage was also studied.

43 **2. Materials and methods**

44 **2.1. Materials**

45 Cassava tubers (*Manihot esculenta* Crantz) used in this study were yellow cassava (Malang 2) and
46 purchased from a local market in Malang, Indonesia. The cooking oil was purchased from a market in
47 Surabaya, Indonesia. All chemicals and other reagents used in this study were analytical grades.

48 2.2. *Preparation of cassava stick*

49 Cassava tubers were washed two times using tap water to remove physical contaminations. The
50 cleaned cassavas were then peeled, cut into strips of 4 x 1 x 1 cm, and soaked in 0.1% (w/v) CaCl₂ for 15
51 min to increase the crispiness of the cassava stick. Next, the soaked cassava sticks were steamed in a
52 steamer (98°C, 15 min) and cooled to room temperature before being pre-fried (170°C, 30 s) in cassava
53 sticks : cooking oil ratio of 1:12 (w/v) using a deep fryer (Fritel Professional 35 SICO, Belgium). Later, the
54 pre-fried cassava sticks were vacuum packed in a polypropylene plastic bag using a vacuum sealer
55 (Maksindo DZ300, Indonesia) and frozen at -20°C for different storage duration (0, 1, 2, 3 months) in a
56 chest freezer (Modena MD 45, Italy). The frozen cassava sticks were then thawed in a water bath (15 min)
57 and fried for 2 min at the same condition as the pre-frying process. At the end of frying, the fryer basket
58 was immediately shaken for 10 seconds and sticks were cooled to room temperature before analyzing the
59 physicochemical properties.

60 2.3. *Moisture content*

61 All pre-fried and fried cassava stick samples were minced before immediately analyzing the
62 moisture content. The moisture content was determined using a drying oven (Binder ED 53, Germany)
63 with the thermogravimetric method (AOAC, 2006) for 3-5 hours at 105 ± 2 °C.

64 2.4. *Oil absorption analysis*

65 The oil absorption analysis was determined according to the method by Mohammed *et al.* (1988).
66 Approximately 5 g of minced pre-fried and fried cassava sticks were oven-dried at 105°C for 3-5 hours and
67 weighed until a stable weight of the sample was obtained. The oil absorption was calculated using the
68 following equation:

69
$$\text{Oil absorption (\%)} = \frac{W_2 - W_1}{W_1} \times 100\%$$

70 where W_1 is the weight of pre-fried cassava stick (g) and W_2 is the weight of fried cassava stick (g).

71 2.5. *Hardness*

72 Hardness as the selected texture characteristics were determined using a TA-XT plus (Stable Micro
73 System, UK) equipped with a three-point bend rig using compression test mode with the following
74 conditions: pre-test speed= 2 mm/s, test speed= 0.5 mm/s, post-test speed= 10 mm/s, and distance= 10
75 mm. The hardness of the samples was expressed as Newton and determined as the maximum force
76 required to compress the sample.

77 2.6. *Free fatty acid analysis*

78 The free fatty acid of cassava sticks was analyzed using a method by Sudarmadji *et al.* (1984).
79 Approximately 28.2 g samples were minced before adding 50 mL of neutralized alcohol and 2 mL
80 phenolphthalein. The mixture was then titrated with 0.1 N NaOH until a stable pink color occurred for 30
81 s. The percentage of free fatty acid was expressed as the percentage of palmitic acid and calculated using
82 the following equation:

83
$$\text{Palmitic acid (\%)} = \frac{V_{\text{NaOH}} \times N_{\text{NaOH}} \times Mr}{W \times 1000} \times 100\%$$

84 where V_{NaOH} is the titre of NaOH (mL), N_{NaOH} is the concentration of NaOH (N), Mr is the molecular
85 weight of palmitic acid (256.42 g/mol), and W is the sample weight (g).

86 2.7. *Peroxide value*

87 An iodometric titration method was used to analyze the peroxide value as the primary oxidation
88 product (Sudarmadji *et al.*, 1984). Briefly, 5 g sample was mixed with 30 mL acetic acid – chloroform (3:2)
89 and 0.5 mL saturated aqueous potassium iodide solution was added. Later, the mixture was shaken
90 vigorously for 1 minute before 30 mL distilled water was added. The mixture was titrated with 0.1 M
91 $\text{Na}_2\text{S}_2\text{O}_3$ until the yellow color had almost disappeared. Next, 0.5 mL of 1% (w/v) starch indicator solution

92 was added and the titration continued until the blue color disappeared. The peroxide value was calculated
93 using the following formula:

94
$$\text{Peroxide value (meq peroxide/kg oil)} = \frac{V_{\text{thio}} \times N_{\text{thio}} \times 1000}{W}$$

95 where V_{thio} is the titre of $\text{Na}_2\text{S}_2\text{O}_3$ (mL), N_{thio} is the concentration of $\text{Na}_2\text{S}_2\text{O}_3$, and W is the sample
96 weight (g).

97 2.8. Color

98 Cassava stick color was identified using a color reader (Color Reader Minolta, CR-10). The L^* value
99 (0 = blackness, 100 = whiteness), a^* value (+ = redness, - = greenness), and b^* value (+ = yellowness, - =
100 blueness) of each sample were observed.

101 2.9. Sensory evaluation

102 In this study, the Hedonic Scale Scoring (preferred test) was used to measure the level of preference
103 and acceptance of the product by consumers (Kusuma *et al.*, 2017). The sensory evaluation was evaluated
104 by 100 untrained panelists. The panelists were students of the Faculty of Agricultural Technology, Widya
105 Mandala Catholic University Surabaya. The sensory attributes tested were color, hardness, crispiness,
106 aroma, and taste. A scoring system of 1-7 points was used to represent the sensory characteristics of each
107 sample. On this scale, 1 was defined as dislike extremely, 2 was defined as dislike moderately, 3 was
108 defined as dislike slightly, 4 was defined as neither like nor dislike, 5 was defined as like slightly, 6 was
109 defined as like moderately, and 7 was defined as like extremely.

110 2.10. Statistical analysis

111 All experiments were analyzed at least three times in triplicate and represented as mean values
112 \pm SD. Statistical analyses were performed using SPSS for Windows (version 19.0, SPSS Inc., USA) and
113 compared with Duncan Multiple Range Test (DMRT) at $P < 0.05$.

114 3. Results and discussion

115 3.1. Moisture content and oil absorption

116 The moisture content of fried cassava sticks is shown in Table 1. Based on the result, different frozen
117 storage durations have no significant difference ($P>0.05$) on the moisture content of cassava sticks, even
118 though there was a downward trend in the moisture content of frozen cassava sticks for 2 and 3 months.
119 Similar results were reported by Medic *et al* (2018), where frozen storage duration had no significant on
120 the moisture content of pork loin and belly rib. However, several researchers also stated that freezing
121 significantly ($P<0.05$) influenced the moisture content of the fried potato (Adedeji & Ngadi, 2017), lamb
122 (Coombs *et al*, 2017), and pork ham (Medic *et al.*, 2018). According to Fennema (1985), water in food
123 converts to ice crystals during freezing at -18°C and easily removed from food when the food is dried or
124 fried; so that the moisture content decreases.

125 Oil absorption analysis was conducted to measure the ability of cassava sticks to absorb oil during
126 frying by using a modifying thermogravimetric method (Mohamed *et al.*, 1988). Table 1 showed that
127 frozen storage duration significantly increased ($P<0,05$) the oil absorption of cassava sticks. Cassava stick
128 stored frozen for 2 months has the highest oil absorption (58.45%) but has no significant difference on
129 cassava stick stored frozen for 3 months (57.84%). In Adedeji & Ngadi (2017) study, oil absorption also
130 showed a significant increase in fried potato that was stored frozen. However, they also stated that there
131 was no interaction between storage duration and freezing method, which contradicts our study.

132 According to Adedeji *et al.* (2009), moisture loss during frying affects the amount of oil absorbed in
133 fried food. This statement is in accordance with Fellows (1990) statement, where there is an oil transfer
134 into the product to replace evaporated water during frying. In addition, moisture evaporation from fried
135 food during frying damages the cellular structure of plant tissues, resulting in an increase in porosity which
136 contributes to oil absorption (Mellema, 2003; Dana & Saguy, 2006; Rimac-Brncic *et al.*, 2004). On the other
137 hand, during frozen storage, all water in food converts to ice crystal and causes volume expansion which
138 is characterized by the damage to cell membranes during ice crystal formation (Fennema, 1985; Celli *et*

139 *al.*, 2016). This volume expansion causes the water in cassava sticks to be easier to evaporate during frying
140 and increases oil absorption.

141 3.2. *Hardness*

142 In this study, hardness was chosen as the analyzed texture attribute to evaluate the impact of frozen
143 storage duration on the texture of cassava sticks. According to Szczesniak (2002), hardness is the force
144 required to change the shape of material due to its resistance to resist deformation. The effect of frozen
145 storage duration on the hardness of cassava sticks is presented in Figure 1. As shown in that Figure, frozen
146 storage duration significantly affected ($P < 0.05$) the hardness of the cassava sticks. Cassava stick that
147 stored frozen for 3 months has the highest force (2.54) among other samples (0.63 – 1.41). Our results
148 were in accordance with the results of Adedeji & Ngadi (2017). In that study, they found a marginal
149 increase in the hardness of fried potatoes as the frozen storage duration increased. Therefore, the
150 increase of fried cassava sticks' hardness might be due to the retrogradation of cassava starch during
151 frozen storage. Our statement was supported by a similar study by Yu *et al.* (2010), where the hardness
152 of cooked rice increased continually with the amylopectin retrogradation during frozen storage. So that,
153 based on those findings, it indicates that amylopectin retrogradation contributed to the hardness increase
154 during storage on the high starch food products.

155 3.3. *Free fatty acid and peroxide value*

156 This study assessed the percentage of free fatty acid and peroxide values to monitor the fat
157 oxidation in cassava sticks during frozen storage. The increase of free fatty acid and peroxide value
158 indicates the rancidity of food products that produce off-flavors (Maity *et al.*, 2012) and might reduce
159 consumers' preferences. The free fatty acid of cassava stick was expressed as the percentage of palmitic
160 acid since the cooking oil used to fry was palm oil. Meanwhile, the peroxide value as the primary oxidation
161 product of fat oxidation was expressed as meq peroxide/ kg fat. The percentage of free fatty acid and

162 peroxide value of fried cassava sticks is shown in Figure 2. A significant increased ($P<0.05$) of free fatty
163 acid was recorded during 2 months of frozen storage (0.13 %) and had no significant difference ($P>0.05$)
164 with cassava sticks stored frozen for 3 months (0.14%). On the other hand, the peroxide value of cassava
165 stick was also significantly increased ($P<0.05$) as the frozen storage duration increased (0 – 34.53 meq
166 peroxide/ kg fat). Similar results were also reported by some studies where frozen storage duration
167 increased the free fatty acid and peroxide value of potato strips (Kizito *et al.*, 2017), pork meat (Medic *et*
168 *al.*, 2018), and ready-to-fry vegetable snacks (Maity *et al.*, 2012).

169 According to Crosa *et al.* (2014), free fatty acids are formed through the nucleophilic attack at
170 triacylglycerol's ester bond and have been associated with the undesirable odors and taste of food
171 products. Furthermore, the formation of hydroperoxides during storage generally occurred during the
172 early stage of oxidation (Fennema, 1985) and later decomposed to other secondary oxidation products
173 such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (Kizito *et al.*, 2017; Medic *et al.*, 2018).
174 Another factor that might increase the formation of hydroperoxides was the contact of cassava stick with
175 oxygen before and after storage; since oxygen is one of the reactants that caused fat oxidation (Giri &
176 Mangaraj, 2012).

177 3.4. Color properties

178 Color is generally considered an important attribute that significantly affects the physical
179 properties of food, the perception of consumers, and determines the nutritional quality of food products
180 (Patras *et al.*, 2011; Hutchings, 2002). In this study, the color of cassava sticks is shown in Table 2 and was
181 measured using Hunter's L^* , a^* , and b^* color attributes. The L^* value expressed the degree of lightness,
182 where the highest value was measured in the cassava stick stored frozen for 3 months (75.20). However,
183 the results were not significantly different ($P>0.05$) with cassava stick that was not stored frozen (71.55)
184 and significantly different ($P<0.05$) with other samples (66.52 – 67.98). The different results were shown
185 on the a^* value, which expressed the degree of redness ($+a^*$) and greenness ($-a^*$) of cassava sticks. All

186 samples had no significant difference ($P>0.05$) on the degree of redness (2.94 – 4.62). Meanwhile, the b^*
187 value expressed the degree of yellowness ($+b^*$) and blueness ($-b^*$). The results show that the b^* values of
188 cassava stick (25.77) that stored frozen for 2 months was significantly lower ($P<0.05$) than other samples
189 (30.79 – 32.08).

190 Our results showed that the color of cassava sticks was greatly affected by the frozen storage
191 duration. Research by Oner & Wall (2012) also found that frozen storage significantly influenced ($P<0.05$)
192 the color of French fries. The color change of fried cassava sticks during frozen storage duration might be
193 due to the surface moisture desiccation (Maity *et al.*, 2012). The amount of moisture loss during the frying
194 process also influence the color of fried cassava stick since moisture loss is associated with crust formation
195 and accelerates the Maillard browning (Sahin, 2000). The Maillard browning occurs due to the reaction
196 between amino acid and reducing sugar during frying and as the result, increases the coloration of yellow,
197 red, and brown color in fried food (Pedreschi *et al.*, 2005). Another factor that might influence the crust
198 color of cassava sticks was the heat transfer rate in the cassava sticks during frying (Maity *et al.*, 2012).

199 3.5. Sensory evaluation

200 The sensory evaluation results of cassava sticks stored frozen in different storage durations are
201 shown as a spider plot in Figure 3. According to the results, the highest score for the color attribute was
202 the 0-month storage cassava sticks (5.60) and significantly difference ($P<0.05$) with other samples (3.20 –
203 4.96). The decrease in panelists' color preference was due to the color changes in cassava sticks that tend
204 to be darker and brownly than the regular cassava sticks. Meanwhile, on the hardness attribute, the
205 panelists' preference significantly decreased ($P<0.05$) as the frozen storage duration increased (5.30 –
206 4.20). This result was in accordance with our objective analysis on hardness, where frozen storage
207 duration significantly increased the hardness of cassava sticks due to the starch retrogradation. The next
208 sensory attribute tested was the crispiness and the results were quite varied. Cassava sticks stored frozen
209 for 0 months (4.88) had no significant difference ($P>0.05$) with 2 months stored cassava stick (4.66) but

210 showed significant difference ($P < 0.05$) with cassava sticks stored frozen for 1 month (3.86) and 3 months
211 (3.68). This result indicated that panelists perceived that frozen storage duration influenced the crispiness
212 of fried cassava sticks. Furthermore, the frozen storage duration significantly influenced ($P < 0.05$) the
213 panelists' preferences on the aroma of cassava sticks. The aroma of cassava sticks tended to be different
214 for each treatment and might be influenced by the formation of hydroperoxides and free fatty acids. The
215 last sensory attribute evaluated was the taste of cassava sticks. According to the result, the frozen storage
216 duration had no significant difference ($P > 0.05$) on the panelists' perception of the taste of cassava sticks
217 (4.16 – 4.88). Overall, the average score of panelists' preference showed that frozen storage duration did
218 not significantly affect ($P > 0.05$) panelists' acceptance of the cassava stick.

219 **4. Conclusion**

220 Frozen storage can be used to extend the shelf life of food products. However, the use of frozen
221 storage on cassava sticks affects its physical and chemical properties. Different frozen storage duration
222 influenced the oil absorption of cassava sticks, though the moisture content showed no significant change.
223 The effect of frozen storage duration was also significant on the texture of cassava sticks, especially on
224 hardness. Another studied quality characteristic, such as cassava stick surface color, was substantially
225 declined during the frozen storage. The free fatty acid and peroxide value of cassava sticks was gradually
226 increased with elongation of frozen storage duration. According to the sensory evaluation results, frozen
227 storage duration influenced all of the sensory attributes besides the taste of cassava sticks. Cassava sticks
228 stored frozen for 3 months were still acceptable by the panelists and the acceptance was defined as
229 neither like nor dislike.

230 **Conflict of interest**

231 The authors declare no conflict of interest.

232 **Acknowledgments**

233 This study was financially supported by the Lembaga Penelitian dan Pengabdian Masyarakat (LPPM)
234 Widya Mandala Surabaya Catholic University through PPPG Research Grants 2015/2016.

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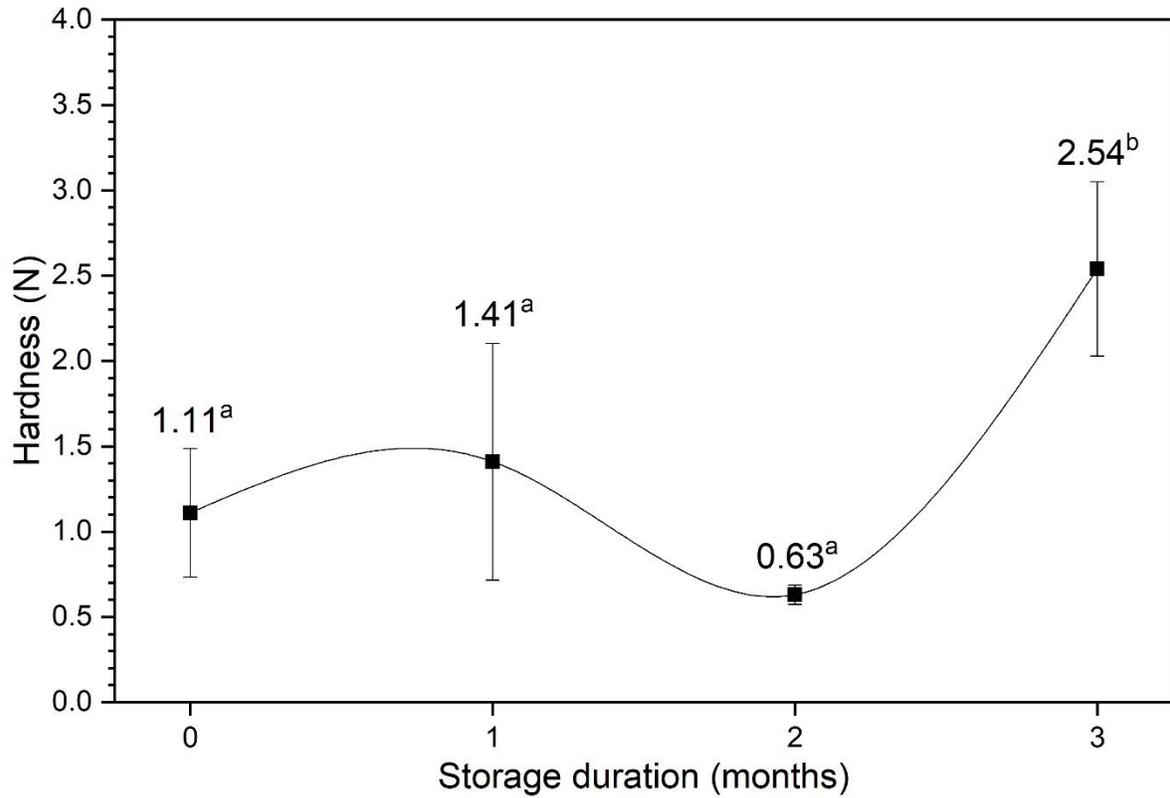
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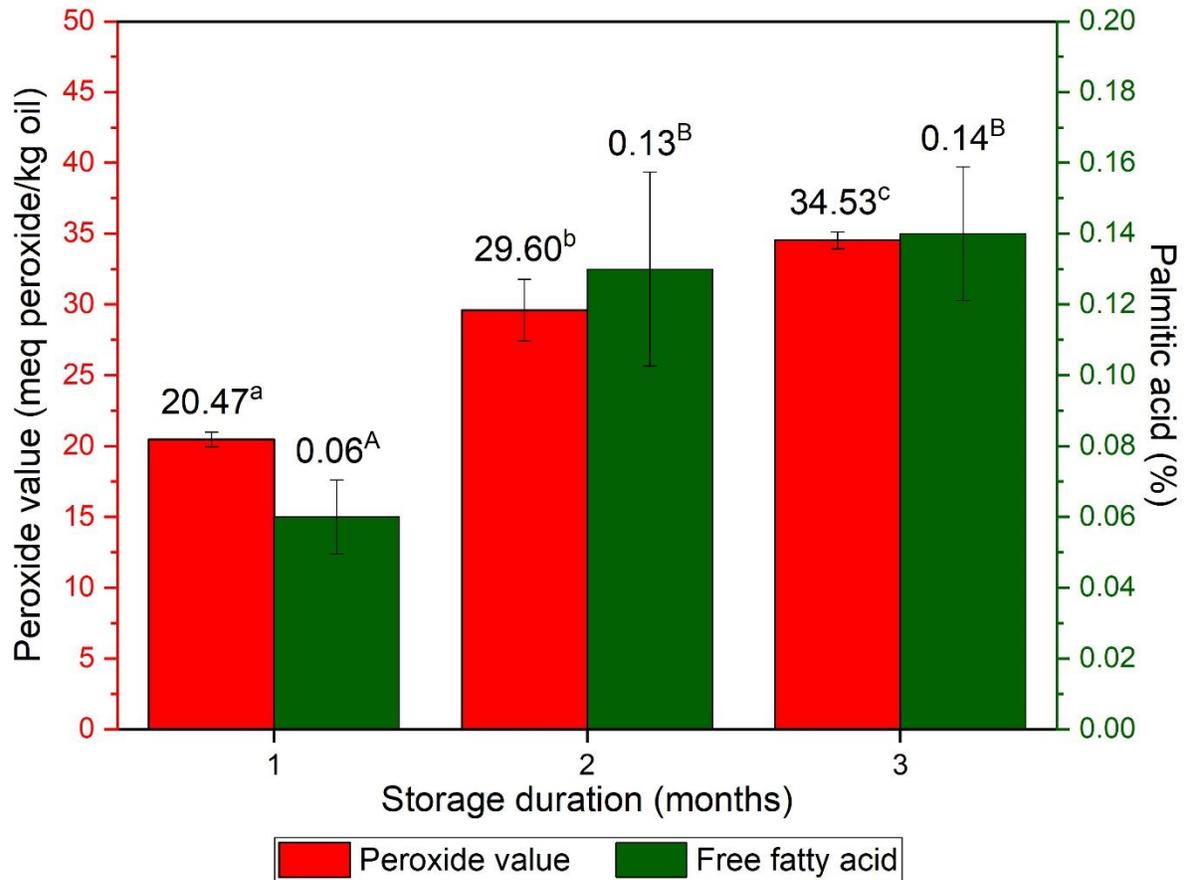


317

318 Figure 1. Hardness of cassava stick stored frozen in different storage duration. Means \pm standard
319 deviation (n = 3 for each group) in the same column followed by the different letter are significantly
320 different (P<0.05). The statistical significance was evaluated by one-way ANOVA followed by DMRT test
321 ($\alpha = 0.05$).

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324

325 Figure 2. Free fatty acid and peroxide value of cassava stick stored frozen in different storage duration.

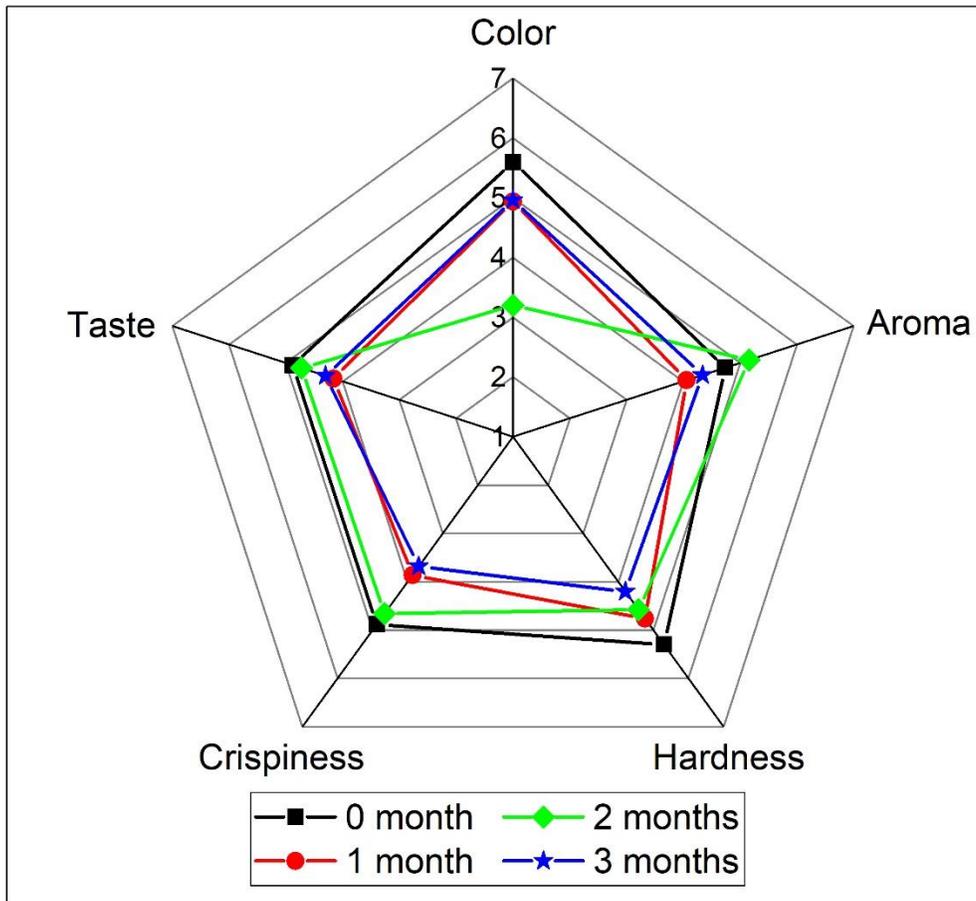
326 Means \pm standard deviation (n = 3 for each group) in the same column followed by the different letter

327 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA

328

followed by DMRT test ($\alpha = 0.05$).

329



330

331 Figure 3. Sensory evaluation of cassava stick stored frozen in different storage duration. Means \pm
 332 standard deviation (n = 100 for each group) in the same column followed by the different letter are
 333 significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA followed by
 334 DMRT test ($\alpha = 0.05$).

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338

339 **List of Tables**

340 Table 1. Moisture content and oil absorption of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Moisture content (%)	Oil absorption (%)
0	45.13 ± 7.46 ^a	20.87 ± 15.91 ^a
1	48.83 ± 3.27 ^a	25.66 ± 9.51 ^a
2	28.68 ± 7.95 ^a	58.45 ± 2.47 ^b
3	27.75 ± 10.13 ^a	57.94 ± 24.71 ^b

341 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter

342 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA

343 followed by DMRT test ($\alpha = 0.05$).

344

345

Table 2. Color of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Color		
	L*	a*	b*
0	71.55 ± 4.08 ^{ab}	2.95 ± 0.78 ^a	32.08 ± 3.02 ^b
1	67.98 ± 2.78 ^a	4.62 ± 1.93 ^a	30.79 ± 1.87 ^b
2	66.52 ± 1.15 ^a	3.21 ± 0.37 ^a	25.77 ± 0.98 ^a
3	75.20 ± 1.80 ^b	4.01 ± 0.30 ^a	31.40 ± 1.66 ^b

346 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter

347 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA

348 followed by DMRT test ($\alpha = 0.05$).

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1 **Effects of frozen storage duration on the physicochemical and sensory properties of cassava sticks**

2

3 **Abstract**

4 In this study, the cassava stick was stored at -20°C for three months to investigate its effects on the
5 physicochemical and sensory properties of cassava sticks. The effects of frozen storage duration were
6 monitored every month with three replications. A randomized block design with a single factor was used
7 as the experimental design. According to the results, storing cassava sticks under frozen significantly
8 increased ($P < 0.05$) the oil absorption and had no effect ($P > 0.05$) on the moisture content. A significant
9 alteration ($P < 0.05$) in texture was observed through the increase of cassava stick hardness from 1.11 to
10 2.54 N. Frozen storage duration also influenced ($P < 0.05$) the lightness and yellowness, but not the redness
11 ($P > 0.05$) of cassava sticks. Fat oxidation also occurred during storage, marked by a significant increase
12 ($P < 0.05$) of free fatty acid (0.06 to 0.14%) and peroxide value (0 to 34.53 mg peroxide/kg lipid) on three
13 months of frozen storage. Thus, this study concludes that ~~the~~ frozen storage duration affected the physical
14 and chemical properties of cassava sticks. Moreover, cassava sticks stored frozen for three months were
15 acceptable for panelists with neither like nor dislike (4.30) average acceptance, and had no significant
16 difference ($P > 0.05$) with other samples.

17 **Keywords:** Cassava, Cassava stick, Frozen Storage duration

18 **1. Introduction**

19 Cassava (*Manihot esculenta* Crantz) is one of the most consumed crops in the world, especially in
20 Sub-Saharan Africa (SSA) and in developing countries of Asia such as Cambodia, Vietnam, and Laos (Benesi
21 *et al.*, 2004; International Atomic Energy Agency, 2018). Cassava is considered as the substitute for rice as
22 the primary carbohydrate source and can be consumed in various ways (fried, boiled, or steamed). Besides

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23 carbohydrates, cassava is also rich in calcium (50 mg/100 g), phosphorus (40 mg/100 g), vitamin C (25
24 mg/100_g) and contains a significant amount of thiamine, riboflavin, and nicotinic acid (International
25 Atomic Energy Agency, 2018). In Indonesia, cassava is generally processed into traditional food such as
26 *tiwul, gatot, gaplek, gethuk, tapai,* and cassava chips. Furthermore, it can also be processed *in*to a modern
27 food such as cassava stick.

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28 Cassava stick has similar characteristics to French fries due to its shoestring-like shape, golden
29 brown color, crunchy exterior, and fluffy interior. Those characteristics are obtained through several
30 processes such as blanching, drying, frying, and each step affects the quality of the final product (Lamberg
31 *et al.*, 1990). ~~Despite those processes,~~ other processes are also required to extend shelf life since cassava
32 sticks are classified as perishable food. Among other processes that might be employed for long-term
33 preservation (high pressure, infrared irradiation, pulsed electric field, and ultrasound), freezing or frozen
34 storage is still one of the most used methods (Rahman & Velez-Ruiz, 2007). Generally, frozen storage
35 temperature is 0°F (-18°C) and even colder depending on the type of the food (WFLO Commodity Storage
36 Manual, 2008).

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37 Several studies stated that storing food (fruit, meat, and French fries) under freezing temperatures
38 affects the physical and chemical properties (Celli *et al.*, 2016; Medic *et al.*, 2018; Sattar *et al.*, 2015).
39 However, based on our knowledge, there is no study reported about the effect of frozen storage on the
40 cassava sticks properties. Therefore, this study aimed to investigate the physical and chemical changes on
41 cassava sticks during frozen storage. In addition, the sensory evaluation of cassava sticks during frozen
42 storage was also studied.

43 2. Materials and methods

44 2.1. Materials

45 Cassava tubers (*Manihot esculenta* Crantz) used in this study were yellow cassava (Malang 2) ~~and~~
46 purchased from a local market in Malang, Indonesia. The cooking oil was purchased from a market in
47 Surabaya, Indonesia. All chemicals and other reagents used in this study were analytical grades.

48 2.2. Preparation of cassava stick

49 Cassava tubers were washed two times using tap water to remove physical contaminations. The
50 cleaned cassavas were then peeled, cut into strips of 4 x 1 x 1 cm, and soaked in 0.1% (w/v) CaCl₂ for 15
51 min to increase the crispiness of the cassava stick. Next, the soaked cassava sticks were steamed in a
52 steamer (98°C, 15 min) and cooled to room temperature before being pre-fried (170°C, 30 s) in cassava
53 sticks : cooking oil ratio of 1:12 (w/v) using a deep fryer (Fritel Professional 35 SICO, Belgium). Later, the
54 pre-fried cassava sticks were vacuum packed in a polypropylene plastic bag using a vacuum sealer
55 (Maksindo DZ300, Indonesia) and frozen at -20°C for different storage duration (0, 1, 2, 3 months) in a
56 chest freezer (Modena MD 45, Italy). The frozen cassava sticks were then thawed in a water bath (15 min)
57 and fried for 2 min at the same condition as the pre-frying process. At the end of frying, the fryer basket
58 was immediately shaken for 10 seconds and sticks were cooled to room temperature before analyzing the
59 physicochemical properties.

60 2.3. Moisture content

61 All pre-fried and fried cassava stick samples were minced before immediately analyzing the
62 moisture content. The moisture content was determined using a drying oven (Binder ED 53, Germany)
63 with the thermogravimetric method (AOAC, 2006) for 3-5 hours at 105 ± 2 °C.

64 2.4. Oil absorption analysis

65 The oil absorption analysis was determined according to the method by Mohammed *et al.* (1988).
66 Approximately 5 g of minced pre-fried and fried cassava sticks were oven-dried at 105°C for 3-5 hours and
67 weighed until a stable weight of the sample was obtained. The oil absorption was calculated using the
68 following equation:

69
$$\text{Oil absorption (\%)} = \frac{W_2 - W_1}{W_1} \times 100\%$$

70 where W_1 is the weight of pre-fried cassava stick (g) and W_2 is the weight of fried cassava stick (g).

71 **2.5. Hardness**

72 Hardness as the selected texture characteristics were determined using a TA-XT plus (Stable Micro
73 System, UK) equipped with a three-point bend rig using compression test mode with the following
74 conditions: pre-test speed= 2 mm/s, test speed= 0.5 mm/s, post-test speed= 10 mm/s, and distance= 10
75 mm. The hardness of the samples was expressed as Newton and determined as the maximum force
76 required to compress the sample.

77 **2.6. Free fatty acid analysis**

78 The free fatty acid of cassava sticks was analyzed using a method by Sudarmadji *et al.* (1984).
79 Approximately 28.2 g samples were minced before adding 50 mL of neutralized alcohol and 2 mL
80 phenolphthalein. The mixture was then titrated with 0.1 N NaOH until a stable pink color occurred for 30
81 s. The percentage of free fatty acid was expressed as the percentage of palmitic acid and calculated using
82 the following equation:

83
$$\text{Palmitic acid (\%)} = \frac{V_{\text{NaOH}} \times N_{\text{NaOH}} \times Mr}{W \times 1000} \times 100\%$$

84 where V_{NaOH} is the titre of NaOH (mL), N_{NaOH} is the concentration of NaOH (N), Mr is the molecular
85 weight of palmitic acid (256.42 g/mol), and W is the sample weight (g).

86 **2.7. Peroxide value**

87 An iodometric titration method was used to analyze the peroxide value as the primary oxidation
88 product (Sudarmadji *et al.*, 1984). Briefly, 5 g sample was mixed with 30 mL acetic acid – chloroform (3:2)
89 and 0.5 mL saturated aqueous potassium iodide solution was added. Later, the mixture was shaken
90 vigorously for 1 minute before 30 mL distilled water was added. The mixture was titrated with 0.1 M
91 $\text{Na}_2\text{S}_2\text{O}_3$ until the yellow color had almost disappeared. Next, 0.5 mL of 1% (w/v) starch indicator solution

92 was added and the titration continued until the blue color disappeared. The peroxide value was calculated
93 using the following formula:

94
$$\text{Peroxide value (meq peroxide/kg oil)} = \frac{V_{\text{thio}} \times N_{\text{thio}} \times 1000}{W}$$

95 where V_{thio} is the titre of $\text{Na}_2\text{S}_2\text{O}_3$ (mL), N_{thio} is the concentration of $\text{Na}_2\text{S}_2\text{O}_3$, and W is the sample
96 weight (g).

97 2.8. Color

98 Cassava stick color was identified using a color reader (Color Reader Minolta, CR-10). The L^* value
99 (0 = blackness, 100 = whiteness), a^* value (+ = redness, - = greenness), and b^* value (+ = yellowness, - =
100 blueness) of each sample were observed.

101 2.9. Sensory evaluation

102 In this study, the Hedonic Scale Scoring (preferred test) was used to measure the level of preference
103 and acceptance of the product by consumers (Kusuma *et al.*, 2017). The sensory evaluation was evaluated
104 by 100 untrained panelists. The panelists were students of the Faculty of Agricultural Technology, Widya
105 Mandala Catholic University Surabaya. The sensory attributes tested were color, hardness, crispiness,
106 aroma, and taste. A scoring system of 1-7 points was used to represent the sensory characteristics of each
107 sample. On this scale, 1 was defined as dislike extremely, 2 was defined as dislike moderately, 3 was
108 defined as dislike slightly, 4 was defined as neither like nor dislike, 5 was defined as like slightly, 6 was
109 defined as like moderately, and 7 was defined as like extremely.

110 2.10. Statistical analysis

111 All experiments were analyzed at least three times in triplicate and represented as mean values
112 \pm SD. Statistical analyses were performed using SPSS for Windows (version 19.0, SPSS Inc., USA) and
113 compared with Duncan Multiple Range Test (DMRT) at $P < 0.05$.

114 3. Results and discussion

115 3.1. Moisture content and oil absorption

116 The moisture content of fried cassava sticks is shown in Table 1. Based on the result, different frozen
117 storage durations have no significant difference ($P>0.05$) on the moisture content of cassava sticks, even
118 though there was a downward trend in the moisture content of frozen cassava sticks for 2 and 3 months.
119 Similar results were reported by Medic *et al.* (2018), where frozen storage duration had no significant on
120 the moisture content of pork loin and belly rib. However, several researchers also stated that freezing
121 significantly ($P<0.05$) influenced the moisture content of the fried potato (Adedeji & Ngadi, 2017), lamb
122 (Coombs *et al.*, 2017), and pork ham (Medic *et al.*, 2018). According to Fennema (1985), water in food
123 converts to ice crystals during freezing at -18°C and easily removed from food when the food is dried or
124 fried; so that the moisture content decreases.

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125 Oil absorption analysis was conducted to measure the ability of cassava sticks to absorb oil during
126 frying by using a modifying thermogravimetric method (Mohamed *et al.*, 1988). Table 1 showed that
127 frozen storage duration significantly increased ($P<0,05$) the oil absorption of cassava sticks. Cassava stick
128 stored frozen for 2 months has the highest oil absorption (58.45%) but has no significant difference on
129 cassava stick stored frozen for 3 months (57.84%). In Adedeji's & Ngadi's (2017) study, oil absorption also
130 showed a significant increase in fried potato that was stored frozen. However, they also stated that there
131 was no interaction between storage duration and freezing method, which contradicts our study.

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132 According to Adedeji *et al.* (2009), moisture loss during frying affects the amount of oil absorbed in
133 fried food. This statement is in accordance with Fellows (1990) statement, where there is an oil transfer
134 into the product to replace evaporated water during frying. In addition, moisture evaporation from fried
135 food during frying damages the cellular structure of plant tissues, resulting in an increase in porosity which
136 contributes to oil absorption (Mellema, 2003; Dana & Saguy, 2006; Rimac-Brcncic *et al.*, 2004). On the other
137 hand, during frozen storage, all water in food converts to ice crystal and causes volume expansion which
138 is characterized by the damage to cell membranes during ice crystal formation (Fennema, 1985; Celli *et*

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139 *al.*, 2016). This volume expansion causes the water in cassava sticks to be easier to evaporate during frying
140 and increases oil absorption.

141 3.2. Hardness

142 In this study, hardness was chosen as the analyzed texture attribute to evaluate the impact of frozen
143 storage duration on the texture of cassava sticks. According to Szczesniak (2002), hardness is the force
144 required to change the shape of material due to its resistance to resist deformation. The effect of frozen
145 storage duration on the hardness of cassava sticks is presented in Figure 1. As shown in that ~~f~~Figure, frozen
146 storage duration significantly affected ($P < 0.05$) the hardness of the cassava sticks. Cassava stick that
147 stored frozen for 3 months has the highest force (2.54) among other samples (0.63 – 1.41). Our results
148 were in accordance with the results of Adedeji & Ngadi (2017). In that study, they found a marginal
149 increase in the hardness of fried potatoes as the frozen storage duration increased. Therefore, the
150 increase of fried cassava sticks' hardness might be due to the retrogradation of cassava starch during
151 frozen storage. Our statement was supported by a similar study by Yu *et al.* (2010), where the hardness
152 of cooked rice increased continually with the amylopectin retrogradation during frozen storage. ~~So that,~~
153 based on those findings, ~~it indicates that~~ amylopectin retrogradation contributed to the hardness increase
154 during storage on the high starch food products.

155 3.3. Free fatty acid and peroxide value

156 This study assessed the percentage of free fatty acid and peroxide values to monitor the fat
157 oxidation in cassava sticks during frozen storage. The increase of free fatty acid and peroxide value
158 indicates the rancidity of food products that produce off-flavors (Maity *et al.*, 2012) and might reduce
159 consumers' preferences. The free fatty acid of cassava stick was expressed as the percentage of palmitic
160 acid since the cooking oil used to fry was palm oil. Meanwhile, the peroxide value as the primary oxidation
161 product of fat oxidation was expressed as meq peroxide/-kg fat. The percentage of free fatty acid and

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162 peroxide value of fried cassava sticks is shown in Figure 2. A significant increased ($P<0.05$) of free fatty
163 acid was recorded during 2 months of frozen storage (0.13 %) and had no significant difference ($P>0.05$)
164 with cassava sticks stored frozen for 3 months (0.14%). On the other hand, the peroxide value of cassava
165 stick was also significantly increased ($P<0.05$) as the frozen storage duration increased (0 – 34.53 meq
166 peroxide/-kg fat). Similar results were also reported by some studies where frozen storage duration
167 increased the free fatty acid and peroxide value of potato strips (Kizito *et al.*, 2017), pork meat (Medic *et*
168 *al.*, 2018), and ready-to-fry vegetable snacks (Maity *et al.*, 2012).

169 According to Crosa *et al.* (2014), free fatty acids are formed through the nucleophilic attack at
170 triacylglycerol's ester bond and have been associated with the undesirable odors and taste of food
171 products. Furthermore, the formation of hydroperoxides during storage generally occurred during the
172 early stage of oxidation (Fennema, 1985) and later decomposed to other secondary oxidation products
173 such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (Kizito *et al.*, 2017; Medic *et al.*, 2018).
174 Another factor that might increase the formation of hydroperoxides was the contact of cassava stick with
175 oxygen before and after storage; since oxygen is one of the reactants that caused fat oxidation (Giri &
176 Mangaraj, 2012).

177 3.4. Color properties

178 Color is generally considered an important attribute that significantly affects the physical
179 properties of food, the perception of consumers, and determines the nutritional quality of food products
180 (Patras *et al.*, 2011; Hutchings, 2002). In this study, the color of cassava sticks is shown in Table 2 and was
181 measured using Hunter's L^* , a^* , and b^* color attributes. The L^* value expressed the degree of lightness,
182 where the highest value was measured in the cassava stick stored frozen for 3 months (75.20). However,
183 the results were not significantly different ($P>0.05$) with cassava stick that was not stored frozen (71.55)
184 and significantly different ($P<0.05$) with other samples (66.52 – 67.98). The different results were shown
185 on the a^* value, which expressed the degree of redness ($+a^*$) and greenness ($-a^*$) of cassava sticks. All

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186 samples had no significant difference ($P>0.05$) on the degree of redness (2.94 – 4.62). Meanwhile, the b^*
187 value expressed the degree of yellowness ($+b^*$) and blueness ($-b^*$). The results show that the b^* values of
188 cassava stick (25.77) that stored frozen for 2 months was significantly lower ($P<0.05$) than other samples
189 (30.79 – 32.08).

190 Our results showed that the color of cassava sticks was greatly affected by the frozen storage
191 duration. Research by Oner & Wall (2012) also found that frozen storage significantly influenced ($P<0.05$)
192 the color of French fries. The color change of fried cassava sticks during frozen storage duration might be
193 due to the surface moisture desiccation (Maity *et al.*, 2012). The amount of moisture loss during the frying
194 process also influence the color of fried cassava stick since moisture loss is associated with crust formation
195 and accelerates the Maillard browning (Sahin, 2000). The Maillard browning occurs due to the reaction
196 between amino acid and reducing sugar during frying and as the result, increases the coloration of yellow,
197 red, and brown color in fried food (Pedreschi *et al.*, 2005). Another factor that might influence the crust
198 color of cassava sticks was the heat transfer rate in the cassava sticks during frying (Maity *et al.*, 2012).

199 3.5. Sensory evaluation

200 The sensory evaluation results of cassava sticks stored frozen in different storage durations are
201 shown as a spider plot in Figure 3. According to the results, the highest score for the color attribute was
202 the 0-month storage cassava sticks (5.60) and significantly difference ($P<0.05$) with other samples (3.20 –
203 4.96). The decrease in panelists' color preference was due to the color changes in cassava sticks that tend
204 to be darker and brownly than the regular cassava sticks. Meanwhile, on the hardness attribute, the
205 panelists' preference significantly decreased ($P<0.05$) as the frozen storage duration increased (5.30 –
206 4.20). This result was in accordance with our objective analysis on hardness, where frozen storage
207 duration significantly increased the hardness of cassava sticks due to the starch retrogradation. The next
208 sensory attribute tested was the crispiness and the results were quite varied. Cassava sticks stored frozen
209 for 0 months (4.88) had no significant difference ($P>0.05$) with 2 months stored cassava stick (4.66) but

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210 showed significant difference ($P < 0.05$) with cassava sticks stored frozen for 1 month (3.86) and 3 months
211 (3.68). This result indicated that panelists perceived that frozen storage duration influenced the crispiness
212 of fried cassava sticks. Furthermore, the frozen storage duration significantly influenced ($P < 0.05$) the
213 panelists' preferences on the aroma of cassava sticks. The aroma of cassava sticks tended to be different
214 for each treatment and might be influenced by the formation of hydroperoxides and free fatty acids. The
215 last sensory attribute evaluated was the taste of cassava sticks. According to the result, the frozen storage
216 duration had no significant difference ($P > 0.05$) on the panelists' perception of the taste of cassava sticks
217 (4.16 – 4.88). Overall, the average score of panelists' preference showed that frozen storage duration did
218 not significantly affect ($P > 0.05$) panelists' acceptance of the cassava stick.

219 **4. Conclusion**

220 Frozen storage can be used to extend the shelf life of food products. However, the use of frozen
221 storage on cassava sticks affects its physical and chemical properties. Different frozen storage duration
222 influenced the oil absorption of cassava sticks, though the moisture content showed no significant change.
223 The effect of frozen storage duration was also significant on the texture of cassava sticks, especially on
224 hardness. Another studied quality characteristic, such as cassava stick surface color, was substantially
225 declined during the frozen storage. The free fatty acid and peroxide value of cassava sticks was gradually
226 increased with elongation of frozen storage duration. According to the sensory evaluation results, frozen
227 storage duration influenced all of the sensory attributes besides the taste of cassava sticks. Cassava sticks
228 stored frozen for 3 months were still acceptable by the panelists and the acceptance was defined as
229 neither like nor dislike.

230 **Conflict of interest**

231 The authors declare no conflict of interest.

232 **Acknowledgments**

233 This study was financially supported by the Lembaga Penelitian dan Pengabdian Masyarakat (LPPM)

234 Widya Mandala Surabaya Catholic University through PPPG Research Grants 2015/2016.

235

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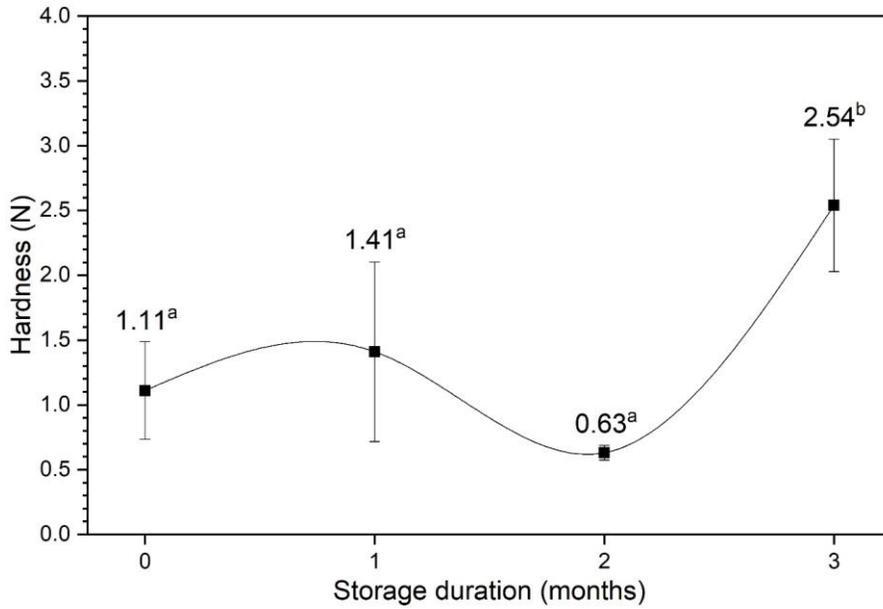
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316 List of Figures

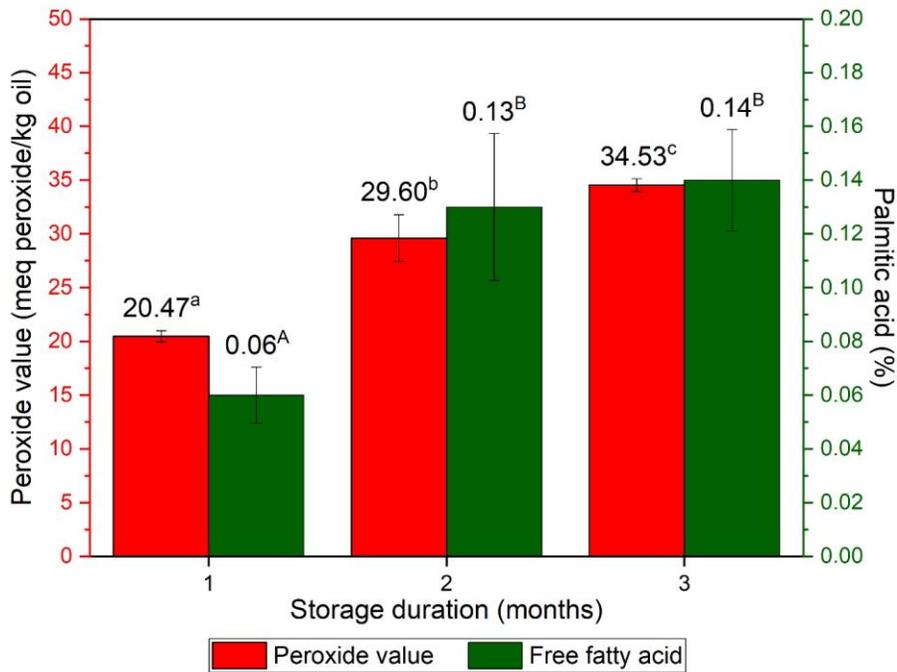


317

318 Figure 1. Hardness of cassava stick stored frozen in different storage duration. Means \pm standard
319 deviation ($n = 3$ for each group) in the same column followed by the different letter are significantly
320 different ($P < 0.05$). The statistical significance was evaluated by one-way ANOVA followed by DMRT test
321 ($\alpha = 0.05$).

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323



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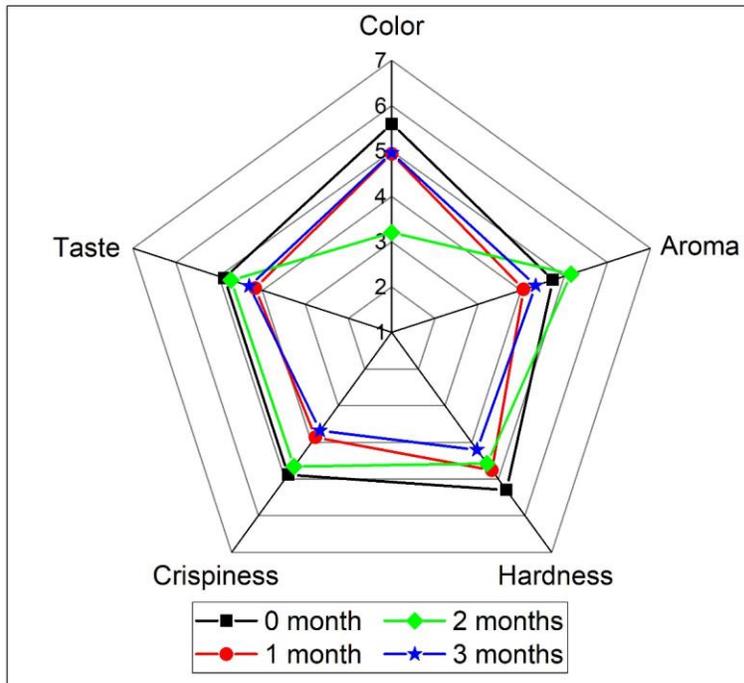
325 Figure 2. Free fatty acid and peroxide value of cassava stick stored frozen in different storage duration.

326 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter

327 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA

328 followed by DMRT test ($\alpha = 0.05$).

329



330

331 Figure 3. Sensory evaluation of cassava stick stored frozen in different storage duration. Means \pm
 332 standard deviation (n = 100 for each group) in the same column followed by the different letter are
 333 significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA followed by
 334 DMRT test ($\alpha = 0.05$).

335

336

337

338

339 **List of Tables**

340 Table 1. Moisture content and oil absorption of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Moisture content (%)	Oil absorption (%)
0	45.13 ± 7.46 ^a	20.87 ± 15.91 ^a
1	48.83 ± 3.27 ^a	25.66 ± 9.51 ^a
2	28.68 ± 7.95 ^a	58.45 ± 2.47 ^b
3	27.75 ± 10.13 ^a	57.94 ± 24.71 ^b

341 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter

342 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA

343 followed by DMRT test ($\alpha = 0.05$).

344

345

Table 2. Color of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Color		
	L*	a*	b*
0	71.55 ± 4.08 ^{ab}	2.95 ± 0.78 ^a	32.08 ± 3.02 ^b
1	67.98 ± 2.78 ^a	4.62 ± 1.93 ^a	30.79 ± 1.87 ^b
2	66.52 ± 1.15 ^a	3.21 ± 0.37 ^a	25.77 ± 0.98 ^a
3	75.20 ± 1.80 ^b	4.01 ± 0.30 ^a	31.40 ± 1.66 ^b

346 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter

347 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA

348 followed by DMRT test ($\alpha = 0.05$).

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I have revised the manuscript according to the comments of reviewers. Please find it in the attached file. I also attached my response to the reviewer's comments in the evaluation forms.

Best regards,
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22 oxidation also occurred during storage, marked by a significant increase ($P < 0.05$) of free fatty acid (0.06
23 to 0.14%) and peroxide value (0 to 34.53 mg peroxide/kg lipid) on three months of frozen storage. Thus,
24 this study concludes that frozen storage duration affected the physical and chemical properties of cassava
25 sticks. Moreover, cassava sticks stored frozen for three months were acceptable for panelists with neither
26 like nor dislike (4.30) average acceptance, and had no significant difference ($P > 0.05$) with other samples.

27 **Keywords:** Cassava, Cassava stick, Frozen storage duration

28 1. Introduction

29 Cassava (*Manihot esculenta* Crantz) is one of the most consumed crops in the world, especially in
30 Sub-Saharan Africa (SSA) and in developing countries of Asia such as Cambodia, Vietnam, and Laos (Benesi
31 *et al.*, 2004; International Atomic Energy Agency, 2018). Cassava is considered as the substitute for rice as
32 the primary carbohydrate source and can be consumed in various ways (fried, boiled, or steamed). Besides
33 carbohydrates, cassava is also rich in calcium (50 mg/100 g), phosphorus (40 mg/100 g), vitamin C (25
34 mg/100g) and contains a significant amount of thiamine, riboflavin, and nicotinic acid (International
35 Atomic Energy Agency, 2018). In Indonesia, cassava is generally processed into traditional food such as
36 *tiwul*, *gatot*, *gaplek*, *gethuk*, *tapai*, and cassava chips. Furthermore, it can also be processed into a modern
37 food such as cassava stick.

38 Cassava stick has similar characteristics to French fries due to its shoestring-like shape, golden
39 brown color, crunchy exterior, and fluffy interior. Those characteristics are obtained through several
40 processes such as blanching, drying, frying, and each step affects the quality of the final product (Lamberg
41 *et al.*, 1990). Other processes are also required to extend shelf life since cassava sticks are classified as
42 perishable food. Among other processes that might be employed for long-term preservation (high
43 pressure, infrared irradiation, pulsed electric field, and ultrasound), freezing or frozen storage is still one

44 of the most used methods (Rahman and Velez-Ruiz, 2007). Generally, frozen storage temperature is 0°F
45 (-18°C) and even colder depending on the type of the food (WFLO Commodity Storage Manual, 2008).

46 Several studies stated that storing food (fruit, meat, and French fries) under freezing temperatures
47 affects the physical and chemical properties (Celli *et al.*, 2016; Medic *et al.*, 2018; Sattar *et al.*, 2015).
48 However, based on our knowledge, there is no study reported about the effect of frozen storage on the
49 cassava sticks properties. Therefore, this study aimed to investigate the physical and chemical changes on
50 cassava sticks during frozen storage. In addition, the sensory evaluation of cassava sticks during frozen
51 storage was also studied.

52 **2. Materials and methods**

53 *2.1. Materials*

54 Cassava tubers (*Manihot esculenta* Crantz) used in this study were yellow cassava (Malang 2),
55 purchased from a local market in Malang, Indonesia. The cooking oil was purchased from a market in
56 Surabaya, Indonesia. All chemicals and other reagents used in this study were analytical grades.

57 *2.2. Preparation of cassava stick*

58 Cassava tubers were washed two times using tap water to remove physical contaminations. The
59 cleaned cassava tubers were then peeled, cut into strips of 4 x 1 x 1 cm, and soaked in 0.1% (w/v) CaCl₂
60 for 15 min to increase the crispiness of the cassava stick. Next, the soaked cassava sticks were steamed in
61 a steamer (98°C, 15 min) and cooled to room temperature before being pre-fried (170°C, 30 s) in cassava
62 sticks : cooking oil ratio of 1:12 (w/v) using a deep fryer (Fritel Professional 35 SICO, Belgium). Later, the
63 pre-fried cassava sticks were vacuum packed in a polypropylene plastic bag using a vacuum sealer
64 (Maksindo DZ300, Indonesia) and frozen at -20°C for different storage duration (0, 1, 2, 3 months) in a
65 chest freezer (Modena MD 45, Italy). The frozen cassava sticks were then thawed in a water bath (15 min)
66 and fried for 2 min at the same condition as the pre-frying process. At the end of frying, the fryer basket

67 was immediately shaken for 10 seconds and sticks were cooled to room temperature before analyzing the
68 physicochemical properties.

69 2.3. *Moisture content*

70 All fried cassava stick samples were minced before immediately analyzing the moisture content.
71 The moisture content was determined using a drying oven (Binder ED 53, Germany) with the
72 thermogravimetric method (AOAC, 2006) for 3-5 hours at 105 ± 2 °C.

73 2.4. *Oil absorption analysis*

74 The oil absorption analysis was determined according to the method by Mohammed *et al.* (1988).
75 Approximately 5 g of minced pre-fried and fried cassava sticks were oven-dried at 105°C for 3-5 hours and
76 weighed until a stable weight of the sample was obtained. The oil absorption was calculated using the
77 following equation:

$$78 \text{ Oil absorption (\%)} = \frac{W_2 - W_1}{W_1} \times 100\%$$

79 where W_1 is the weight of pre-fried cassava stick (g) and W_2 is the weight of fried cassava stick (g).

80 2.5. *Hardness*

81 Hardness as the selected texture characteristics were determined using a TA-XT plus (Stable Micro
82 System, UK) equipped with a three-point bend rig using compression test mode with the following
83 conditions: pre-test speed= 2 mm/s, test speed= 0.5 mm/s, post-test speed= 10 mm/s, and distance= 10
84 mm. The hardness of fried cassava stick was expressed as Newton and determined as the maximum force
85 required to compress the sample.

86 2.6. *Free fatty acid analysis*

87 The free fatty acid of cassava sticks was analyzed using a method by Sudarmadji *et al.* (1984).
88 Approximately 28.2 g of fried cassava sticks were minced before adding 50 mL of neutralized alcohol and
89 2 mL phenolphthalein. The mixture was then titrated with 0.1 N NaOH until a stable pink color occurred

90 for 30 s. The percentage of free fatty acid was expressed as the percentage of palmitic acid and calculated
91 using the following equation:

$$92 \quad \text{Palmitic acid (\%)} = \frac{V_{\text{NaOH}} \times N_{\text{NaOH}} \times \text{Mr}}{W \times 1000} \times 100\%$$

93 where V_{NaOH} is the titre of NaOH (mL), N_{NaOH} is the concentration of NaOH (N), Mr is the molecular
94 weight of palmitic acid (256.42 g/mol), and W is the sample weight (g).

95 2.7. Peroxide value

96 An iodometric titration method was used to analyze the peroxide value as the primary oxidation
97 product (Sudarmadji *et al.*, 1984). Briefly, 5 g of fried cassava sticks were mixed with 30 mL acetic acid –
98 chloroform (3:2) and 0.5 mL saturated aqueous potassium iodide solution was added. Later, the mixture
99 was shaken vigorously for 1 minute before 30 mL distilled water was added. The mixture was titrated with
100 0.1 M $\text{Na}_2\text{S}_2\text{O}_3$ until the yellow color had almost disappeared. Next, 0.5 mL of 1% (w/v) starch indicator
101 solution was added and the titration continued until the blue color disappeared. The peroxide value was
102 calculated using the following formula:

$$103 \quad \text{Peroxide value (meq peroxide/kg oil)} = \frac{V_{\text{thio}} \times N_{\text{thio}} \times 1000}{W}$$

104 where V_{thio} is the titre of $\text{Na}_2\text{S}_2\text{O}_3$ (mL), N_{thio} is the concentration of $\text{Na}_2\text{S}_2\text{O}_3$, and W is the sample
105 weight (g).

106 2.8. Color

107 The color of fried cassava stick was identified using a color reader (Color Reader Minolta, CR-10).
108 The L^* value (0 = blackness, 100 = whiteness), a^* value (+ = redness, - = greenness), and b^* value (+ =
109 yellowness, - = blueness) of each sample were observed.

110 2.9. Sensory evaluation

111 In this study, the Hedonic Scale Scoring (preferred test) was used to measure the level of preference
112 and acceptance of the product by consumers (Kusuma *et al.*, 2017). The sensory evaluation was evaluated

113 by 100 untrained panelists. The panelists were students of the Faculty of Agricultural Technology, Widya
114 Mandala Catholic University Surabaya. The sensory attributes tested were color, hardness, crispiness,
115 aroma, and taste. A scoring system of 1-7 points was used to represent the sensory characteristics of each
116 sample. On this scale, 1 was defined as dislike extremely, 2 was defined as dislike moderately, 3 was
117 defined as dislike slightly, 4 was defined as neither like nor dislike, 5 was defined as like slightly, 6 was
118 defined as like moderately, and 7 was defined as like extremely.

119 2.10. Statistical analysis

120 All experiments were analyzed at least three times in triplicate and represented as mean values
121 \pm SD. Statistical analyses were performed using SPSS for Windows (version 19.0, SPSS Inc., USA) and
122 compared with Duncan Multiple Range Test (DMRT) at $P < 0.05$.

123 3. Results and discussion

124 3.1. Moisture content and oil absorption

125 The moisture content of fried cassava sticks is shown in Table 1. Based on the result, different frozen
126 storage durations have no significant difference ($P > 0.05$) on the moisture content of cassava sticks, even
127 though there was a downward trend in the moisture content of frozen cassava sticks for 2 and 3 months.
128 Similar results were reported by Medic *et al.* (2018), where frozen storage duration had no significant on
129 the moisture content of pork loin and belly rib. However, several researchers also stated that freezing
130 significantly ($P < 0.05$) influenced the moisture content of the fried potato (Adedeji and Ngadi, 2017), lamb
131 (Coombs *et al.*, 2017), and pork ham (Medic *et al.*, 2018). According to Fennema (1985), water in food
132 converts to ice crystals during freezing at -18°C and easily removed from food when the food is dried or
133 fried; so that the moisture content decreases.

134 Oil absorption analysis was conducted to measure the ability of cassava sticks to absorb oil during
135 frying by using a modifying thermogravimetric method (Mohamed *et al.*, 1988). Table 1 showed that
136 frozen storage duration significantly increased ($P < 0.05$) the oil absorption of cassava sticks. Cassava stick

137 stored frozen for 2 months has the highest oil absorption (58.45%) but has no significant difference with
138 cassava stick stored frozen for 3 months (57.84%). In Adedeji's and Ngadi's (2017) study, oil absorption
139 also showed a significant increase in fried potato that was stored frozen. However, they also stated that
140 there was no interaction between storage duration and freezing method, which contradicts our study.

141 According to Adedeji *et al.* (2009), moisture loss during frying affects the amount of oil absorbed in
142 fried food. This statement is in accordance with Fellows (1990) statement, where there is an oil transfer
143 into the product to replace evaporated water during frying. In addition, moisture evaporation from fried
144 food during frying damages the cellular structure of plant tissues, resulting in an increase in porosity which
145 contributes to oil absorption (Mellema, 2003; Dana and Saguy, 2006; Rimac-Brcic *et al.*, 2004). On the
146 other hand, during frozen storage, all water in food converts to ice crystal and causes volume expansion
147 which is characterized by the damage to cell membranes during ice crystal formation (Fennema, 1985;
148 Celli *et al.*, 2016). This volume expansion causes the water in cassava sticks to be easier to evaporate
149 during frying and increases oil absorption.

150 3.2. Hardness

151 In this study, hardness was chosen as the analyzed texture attribute to evaluate the impact of frozen
152 storage duration on the texture of cassava sticks. According to Szczesniak (2002), hardness is the force
153 required to change the shape of material due to its resistance to resist deformation. The effect of frozen
154 storage duration on the hardness of cassava sticks is presented in Figure 1. As shown in that figure, frozen
155 storage duration significantly affected ($P < 0.05$) the hardness of the cassava sticks. Cassava stick that was
156 stored frozen for 3 months has the highest force (2.54) among other samples (0.63 – 1.41). Our results
157 were in accordance with the results of Adedeji and Ngadi (2017). In that study, they found a marginal
158 increase in the hardness of fried potatoes as the frozen storage duration increased. Therefore, the
159 increase of fried cassava sticks' hardness might be due to the retrogradation of cassava starch during
160 frozen storage. Our statement was supported by a similar study by Yu *et al.* (2010), where the hardness

161 of cooked rice increased continually with the amylopectin retrogradation during frozen storage. Based on
162 those findings, amylopectin retrogradation contributed to the hardness increase during storage on the
163 high starch food products.

164 3.3. Free fatty acid and peroxide value

165 This study assessed the percentage of free fatty acid and peroxide values to monitor the fat
166 oxidation in cassava sticks during frozen storage. The increase of free fatty acid and peroxide value
167 indicates the rancidity of food products that produce off-flavors (Maity *et al.*, 2012) and might reduce
168 consumers' preferences. The free fatty acid of cassava stick was expressed as the percentage of palmitic
169 acid since the cooking oil used to fry was palm oil. Meanwhile, the peroxide value as the primary oxidation
170 product of fat oxidation was expressed as meq peroxide/kg fat. The percentage of free fatty acid and
171 peroxide value of fried cassava sticks is shown in Figure 2. A significant increased ($P<0.05$) of free fatty
172 acid was recorded during 2 months of frozen storage (0.13 %) and had no significant difference ($P>0.05$)
173 with cassava sticks stored frozen for 3 months (0.14%). On the other hand, the peroxide value of cassava
174 stick was also significantly increased ($P<0.05$) as the frozen storage duration increased (0 – 34.53 meq
175 peroxide/ kg fat). Similar results were also reported by some studies where frozen storage duration
176 increased the free fatty acid and peroxide value of potato strips (Kizito *et al.*, 2017), pork meat (Medic *et*
177 *al.*, 2018), and ready-to-fry vegetable snacks (Maity *et al.*, 2012).

178 According to Crosa *et al.* (2014), free fatty acids are formed through the nucleophilic attack at
179 triacylglycerol's ester bond and have been associated with the undesirable odors and taste of food
180 products. Furthermore, the formation of hydroperoxides during storage generally occurred during the
181 early stage of oxidation (Fennema, 1985) and later decomposed to other secondary oxidation products
182 such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (Kizito *et al.*, 2017; Medic *et al.*, 2018).
183 Another factor that might increase the formation of hydroperoxides was the contact of cassava stick with

184 oxygen before and after storage; since oxygen is one of the reactants that caused fat oxidation (Giri and
185 Mangaraj, 2012).

186 3.4. Color properties

187 Color is generally considered an important attribute that significantly affects the physical
188 properties of food, the perception of consumers, and determines the nutritional quality of food products
189 (Patras *et al.*, 2011; Hutchings, 2002). In this study, the color of cassava sticks is shown in Table 2 and was
190 measured using Hunter's L*, a*, and b* color attributes. The L* value expressed the degree of lightness,
191 where the highest value was measured in the cassava stick stored frozen for 3 months (75.20). However,
192 the results were not significantly different ($P>0.05$) with cassava stick that was not stored frozen (71.55)
193 and significantly different ($P<0.05$) with other samples (66.52 – 67.98). The different results were shown
194 on the a* value, which expressed the degree of redness (+a*) and greenness (-a*) of cassava sticks. All
195 samples had no significant difference ($P>0.05$) on the degree of redness (2.94 – 4.62). Meanwhile, the b*
196 value expressed the degree of yellowness (+b*) and blueness (-b*). The results show that the b* values of
197 cassava stick (25.77) that stored frozen for 2 months was significantly lower ($P<0.05$) than other samples
198 (30.79 – 32.08).

199 Research by Oner and Wall (2012) also found that frozen storage significantly influenced
200 ($P<0.05$) the color of French fries. The color change of fried cassava sticks during frozen storage duration
201 might be due to the surface moisture desiccation (Maity *et al.*, 2012). The amount of moisture loss during
202 the frying process also influence the color of fried cassava stick since moisture loss is associated with crust
203 formation and accelerates the Maillard browning (Sahin, 2000). The Maillard browning occurs due to the
204 reaction between amino acid and reducing sugar during frying and as the result, increases the coloration
205 of yellow, red, and brown color in fried food (Pedreschi *et al.*, 2005). Another factor that might influence
206 the crust color of cassava sticks was the heat transfer rate in the cassava sticks during frying (Maity *et al.*,
207 2012).

208 3.5. Sensory evaluation

209 The sensory evaluation results of cassava sticks stored frozen in different storage durations are
210 shown as a spider plot in Figure 3. According to the results, the highest score for the color attribute was
211 the 0-month storage cassava sticks (5.60) and significantly difference ($P<0.05$) with other samples (3.20 –
212 4.96). The decrease in panelists' color preference was due to the color changes in cassava sticks that tend
213 to be darker and brownish than the regular cassava sticks. Meanwhile, on the hardness attribute, the
214 panelists' preference significantly decreased ($P<0.05$) as the frozen storage duration increased (5.30 –
215 4.20). This result was in accordance with our objective analysis on hardness, where frozen storage
216 duration significantly increased the hardness of cassava sticks due to the starch retrogradation. The next
217 sensory attribute tested was the crispiness and the results were quite varied. Cassava sticks stored frozen
218 for 0 months (4.88) had no significant difference ($P>0.05$) with 2 months stored cassava stick (4.66) but
219 showed significant difference ($P<0.05$) with cassava sticks stored frozen for 1 month (3.86) and 3 months
220 (3.68). This result indicated that panelists perceived that frozen storage duration influenced the crispiness
221 of fried cassava sticks. Furthermore, the frozen storage duration significantly influenced ($P<0.05$) the
222 panelists' preferences on the aroma of cassava sticks. The aroma of cassava sticks tended to be different
223 for each treatment and might be influenced by the formation of hydroperoxides and free fatty acids. The
224 last sensory attribute evaluated was the taste of cassava sticks. According to the result, the frozen storage
225 duration had no significant difference ($P>0.05$) on the panelists' perception of the taste of cassava sticks
226 (4.16 – 4.88). Overall, the average score of panelists' preference showed that frozen storage duration did
227 not significantly affect ($P>0.05$) panelists' acceptance of the cassava stick.

228 4. Conclusion

229 Frozen storage can be used to extend the shelf life of food products. However, the use of frozen
230 storage on cassava sticks affects its physical and chemical properties. Different frozen storage duration
231 influenced the oil absorption of cassava sticks, though the moisture content showed no significant change.

232 The effect of frozen storage duration was also significant on the texture of cassava sticks, especially on
233 hardness. Another studied quality characteristic, such as cassava stick surface color, was substantially
234 declined during the frozen storage. The free fatty acid and peroxide value of cassava sticks was gradually
235 increased with elongation of frozen storage duration. According to the sensory evaluation results, frozen
236 storage duration influenced all of the sensory attributes besides the taste of cassava sticks. Cassava sticks
237 stored frozen for 3 months were still acceptable by the panelists and the acceptance was defined as
238 neither like nor dislike.

239 **Conflict of interest**

240 The authors declare no conflict of interest.

241 **Acknowledgments**

242 This study was financially supported by the Lembaga Penelitian dan Pengabdian Masyarakat (LPPM)
243 Widya Mandala Surabaya Catholic University through PPPG Research Grants 2015/2016.

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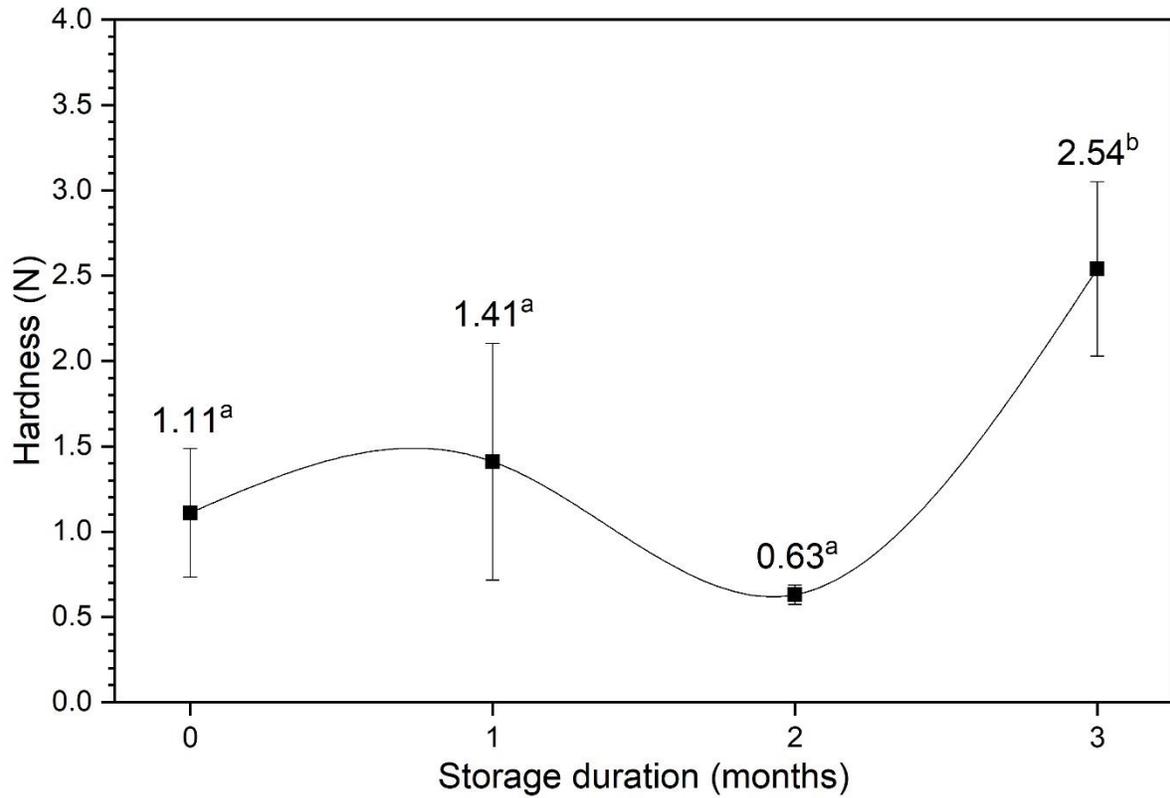
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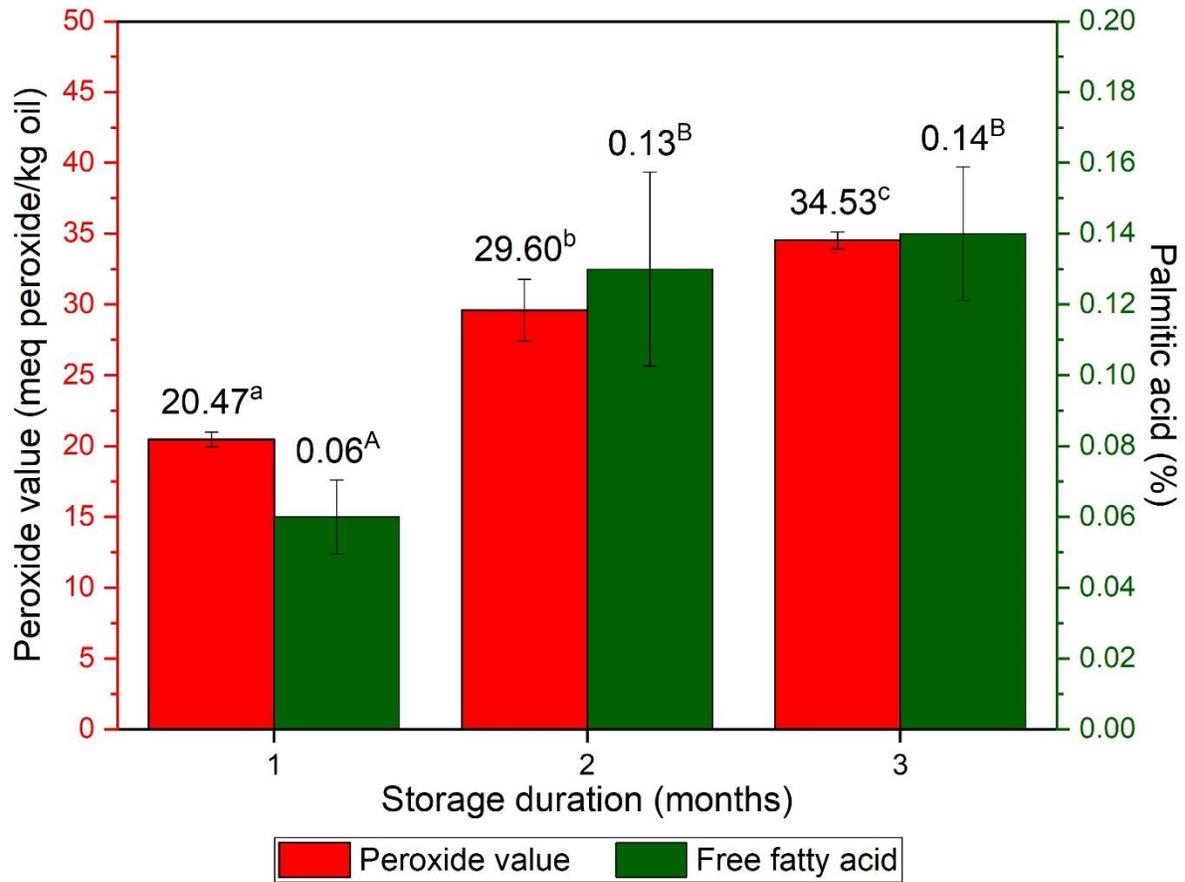
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318 Figure 1. Hardness of cassava stick stored frozen in different storage duration. Means \pm standard
319 deviation (n = 3 for each group) in the same column followed by the different letter are significantly
320 different (P<0.05). The statistical significance was evaluated by one-way ANOVA followed by DMRT test

321 ($\alpha = 0.05$).

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325 Figure 2. Free fatty acid and peroxide value of cassava stick stored frozen in different storage duration.

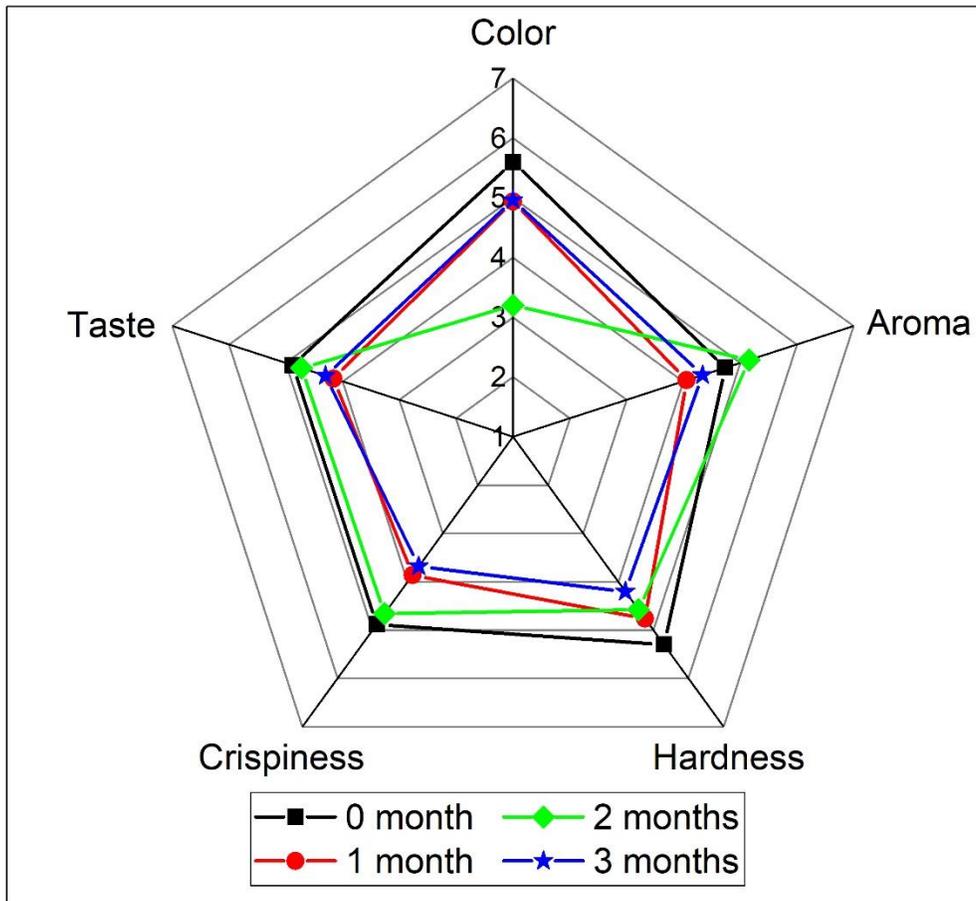
326 Means \pm standard deviation (n = 3 for each group) in the same column followed by the different letter

327 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA

328

followed by DMRT test ($\alpha = 0.05$).

329



330

331 Figure 3. Sensory evaluation of cassava stick stored frozen in different storage duration. Means \pm
 332 standard deviation (n = 100 for each group) in the same column followed by the different letter are
 333 significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA followed by
 334 DMRT test ($\alpha = 0.05$).

335

336

337

338

339 **List of Tables**

340 Table 1. Moisture content and oil absorption of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Moisture content (%)	Oil absorption (%)
0	45.13 ± 7.46 ^a	20.87 ± 15.91 ^a
1	48.83 ± 3.27 ^a	25.66 ± 9.51 ^a
2	28.68 ± 7.95 ^a	58.45 ± 2.47 ^b
3	27.75 ± 10.13 ^a	57.94 ± 24.71 ^b

341 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter
 342 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA
 343 followed by DMRT test (α = 0.05).

344 **Table 2.** Color of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Color		
	L*	a*	b*
0	71.55 ± 4.08 ^{ab}	2.95 ± 0.78 ^a	32.08 ± 3.02 ^b
1	67.98 ± 2.78 ^a	4.62 ± 1.93 ^a	30.79 ± 1.87 ^b
2	66.52 ± 1.15 ^a	3.21 ± 0.37 ^a	25.77 ± 0.98 ^a
3	75.20 ± 1.80 ^b	4.01 ± 0.30 ^a	31.40 ± 1.66 ^b

345 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter
 346 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA
 347 followed by DMRT test (α = 0.05).

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Bukti konfirmasi revisi artikel yang harus dilakukan

(23 Maret 2022)

Manuscript ID: FR-IFC-005 - chat x +

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Dear Dr. Chatarina Yayuk Trisnawati,

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1 **Effects of frozen storage duration on the physicochemical and sensory properties of cassava sticks**

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10

11 **Abstract**

12 Cassava sticks is a processed food made from cassava that has similar characteristics to French fries.
13 However, cassava sticks are also classified as perishable food, so it is required to employ other process
14 that might increases its preservation, such as frozen storage. In this study, the cassava stick was stored at
15 -20°C for three months to investigate its effects on the physicochemical and sensory properties of cassava
16 sticks. The effects of frozen storage duration were monitored every month with three replications. A
17 randomized block design with a single factor was used as the experimental design. According to the
18 results, storing cassava sticks under frozen condition significantly increased ($P<0.05$) the oil absorption
19 and had no effect ($P>0.05$) on the moisture content. A significant alteration ($P<0.05$) in texture was
20 observed through the increase of cassava stick hardness from 1.11 to 2.54 N. Frozen storage duration also
21 influenced ($P<0.05$) the lightness and yellowness, but not the redness ($P>0.05$) of cassava sticks. Fat

22 oxidation also occurred during storage, marked by a significant increase ($P < 0.05$) of free fatty acid (0.06
23 to 0.14%) and peroxide value (0 to 34.53 mg peroxide/kg lipid) on three months of frozen storage. Thus,
24 this study concludes that frozen storage duration affected the physical and chemical properties of cassava
25 sticks. Moreover, cassava sticks stored frozen for three months were acceptable for panelists with neither
26 like nor dislike (4.30) average acceptance, and had no significant difference ($P > 0.05$) with other samples.

27 **Keywords:** Cassava, Cassava stick, Frozen storage duration

28 1. Introduction

29 Cassava (*Manihot esculenta* Crantz) is one of the most consumed crops in the world, especially in
30 Sub-Saharan Africa (SSA) and in developing countries of Asia such as Cambodia, Vietnam, and Laos (Benesi
31 *et al.*, 2004; International Atomic Energy Agency, 2018). Cassava is considered as the substitute for rice as
32 the primary carbohydrate source and can be consumed in various ways (fried, boiled, or steamed). Besides
33 carbohydrates, cassava is also rich in calcium (50 mg/100 g), phosphorus (40 mg/100 g), vitamin C (25
34 mg/100g) and contains a significant amount of thiamine, riboflavin, and nicotinic acid (International
35 Atomic Energy Agency, 2018). In Indonesia, cassava is generally processed into traditional food such as
36 *tiwul*, *gatot*, *gapek*, *gethuk*, *tapai*, and cassava chips. Furthermore, it can also be processed into a modern
37 food such as cassava stick.

38 Cassava stick has similar characteristics to French fries due to its shoestring-like shape, golden
39 brown color, crunchy exterior, and fluffy interior. Those characteristics are obtained through several
40 processes such as blanching, drying, frying, and each step affects the quality of the final product (Lamberg
41 *et al.*, 1990). Other processes are also required to extend shelf life since cassava sticks are classified as
42 perishable food. Among other processes that might be employed for long-term preservation (high
43 pressure, infrared irradiation, pulsed electric field, and ultrasound), freezing or frozen storage is still one

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44 of the most used methods (Rahman and Velez-Ruiz, 2007). Generally, frozen storage temperature is 0°F
45 (-18°C) and even colder depending on the type of the food (WFLO Commodity Storage Manual, 2008).

46 Several studies stated that storing food (fruit, meat, and French fries) under freezing temperatures
47 affects the physical and chemical properties (Celli *et al.*, 2016; Medic *et al.*, 2018; Sattar *et al.*, 2015).
48 However, based on our knowledge, there is no study reported about the effect of frozen storage on the
49 cassava sticks properties. Therefore, this study aimed to investigate the physical and chemical changes on
50 cassava sticks during frozen storage. In addition, the sensory evaluation of cassava sticks during frozen
51 storage was also studied.

52 2. Materials and methods

53 2.1. Materials

54 Cassava tubers (*Manihot esculenta* Crantz) used in this study were yellow cassava (Malang 2),
55 purchased from a local market in Malang, Indonesia. The cooking oil was purchased from a market in
56 Surabaya, Indonesia. All chemicals and other reagents used in this study were analytical grades.

57 2.2. Preparation of cassava stick

58 Cassava tubers were washed two times using tap water to remove physical contaminations. The
59 cleaned cassava tubers were then peeled, cut into strips of 4 x 1 x 1 cm, and soaked in 0.1% (w/v) CaCl₂
60 for 15 min to increase the crispiness of the cassava stick. Next, the soaked cassava sticks were steamed in
61 a steamer (98°C, 15 min) and cooled to room temperature before being pre-fried (170°C, 30 s) in cassava
62 sticks : cooking oil ratio of 1:12 (w/v) using a deep fryer (Fritel Professional 35 SICO, Belgium). Later, the
63 pre-fried cassava sticks were vacuum packed in a polypropylene plastic bag using a vacuum sealer
64 (Maksindo DZ300, Indonesia) and frozen at -20°C for different storage duration (0, 1, 2, 3 months) in a
65 chest freezer (Modena MD 45, Italy). The frozen cassava sticks were then thawed in a water bath (15 min)
66 and fried for 2 min at the same condition as the pre-frying process. At the end of frying, the fryer basket

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67 was immediately shaken for 10 seconds and sticks were cooled to room temperature before analyzing the
68 physicochemical properties.

69 2.3. *Moisture content*

70 All fried cassava stick samples were minced before immediately analyzing the moisture content.
71 The moisture content was determined using a drying oven (Binder ED 53, Germany) with the
72 thermogravimetric method (AOAC, 2006) for 3-5 hours at 105 ± 2 °C.

73 2.4. *Oil absorption analysis*

74 The oil absorption analysis was determined according to the method by Mohammed *et al.* (1988).
75 Approximately 5 g of minced pre-fried and fried cassava sticks were oven-dried at 105°C for 3-5 hours and
76 weighed until a stable weight of the sample was obtained. The oil absorption was calculated using the
77 following equation:

$$78 \text{ Oil absorption (\%)} = \frac{W_2 - W_1}{W_1} \times 100\%$$

79 where W_1 is the weight of pre-fried cassava stick (g) and W_2 is the weight of fried cassava stick (g).

80 2.5. *Hardness*

81 Hardness as the selected texture characteristics were determined using a TA-XT plus (Stable Micro
82 System, UK) equipped with a three-point bend rig using compression test mode with the following
83 conditions: pre-test speed= 2 mm/s, test speed= 0.5 mm/s, post-test speed= 10 mm/s, and distance= 10
84 mm. The hardness of fried cassava stick was expressed as Newton and determined as the maximum force
85 required to compress the sample.

86 2.6. *Free fatty acid analysis*

87 The free fatty acid of cassava sticks was analyzed using a method by Sudarmadji *et al.* (1984).
88 Approximately 28.2 g of fried cassava sticks were minced before adding 50 mL of neutralized alcohol and
89 2 mL phenolphthalein. The mixture was then titrated with 0.1 N NaOH until a stable pink color occurred

90 for 30 s. The percentage of free fatty acid was expressed as the percentage of palmitic acid and calculated
91 using the following equation:

$$92 \quad \text{Palmitic acid (\%)} = \frac{V_{\text{NaOH}} \times N_{\text{NaOH}} \times \text{Mr}}{W \times 1000} \times 100\%$$

93 where V_{NaOH} is the titre of NaOH (mL), N_{NaOH} is the concentration of NaOH (N), Mr is the molecular
94 weight of palmitic acid (256.42 g/mol), and W is the sample weight (g).

95 2.7. Peroxide value

96 An iodometric titration method was used to analyze the peroxide value as the primary oxidation
97 product (Sudarmadji *et al.*, 1984). Briefly, 5 g of fried cassava sticks were mixed with 30 mL acetic acid –
98 chloroform (3:2) and 0.5 mL saturated aqueous potassium iodide solution was added. Later, the mixture
99 was shaken vigorously for 1 minute before 30 mL distilled water was added. The mixture was titrated with
100 0.1 M $\text{Na}_2\text{S}_2\text{O}_3$ until the yellow color had almost disappeared. Next, 0.5 mL of 1% (w/v) starch indicator
101 solution was added and the titration continued until the blue color disappeared. The peroxide value was
102 calculated using the following formula:

$$103 \quad \text{Peroxide value (meq peroxide/kg oil)} = \frac{V_{\text{thio}} \times N_{\text{thio}} \times 1000}{W}$$

104 where V_{thio} is the titre of $\text{Na}_2\text{S}_2\text{O}_3$ (mL), N_{thio} is the concentration of $\text{Na}_2\text{S}_2\text{O}_3$, and W is the sample
105 weight (g).

106 2.8. Color

107 The color of fried cassava stick was identified using a color reader (Color Reader Minolta, CR-10).
108 The L^* value (0 = blackness, 100 = whiteness), a^* value (+ = redness, - = greenness), and b^* value (+ =
109 yellowness, - = blueness) of each sample were observed.

110 2.9. Sensory evaluation

111 In this study, the Hedonic Scale Scoring (preferred test) was used to measure the level of preference
112 and acceptance of the product by consumers (Kusuma *et al.*, 2017). The sensory evaluation was evaluated

113 by 100 untrained panelists. The panelists were students of the Faculty of Agricultural Technology, Widya
114 Mandala Catholic University Surabaya. The sensory attributes tested were color, hardness, crispiness,
115 aroma, and taste. A scoring system of 1-7 points was used to represent the sensory characteristics of each
116 sample. On this scale, 1 was defined as dislike extremely, 2 was defined as dislike moderately, 3 was
117 defined as dislike slightly, 4 was defined as neither like nor dislike, 5 was defined as like slightly, 6 was
118 defined as like moderately, and 7 was defined as like extremely.

119 2.10. *Statistical analysis*

120 All experiments were analyzed at least three times in triplicate and represented as mean values
121 \pm SD. Statistical analyses were performed using SPSS for Windows (version 19.0, SPSS Inc., USA) and
122 compared with Duncan Multiple Range Test (DMRT) at $P < 0.05$.

123 3. Results and discussion

124 3.1. *Moisture content and oil absorption*

125 The moisture content of fried cassava sticks is shown in Table 1. Based on the result, different frozen
126 storage durations have no significant difference ($P > 0.05$) on the moisture content of cassava sticks, even
127 though there was a downward trend in the moisture content of frozen cassava sticks for 2 and 3 months.
128 Similar results were reported by Medic *et al.* (2018), where frozen storage duration had no significant on
129 the moisture content of pork loin and belly rib. However, several researchers also stated that freezing
130 significantly ($P < 0.05$) influenced the moisture content of the fried potato (Adedeji and Ngadi, 2017), lamb
131 (Coombs *et al.*, 2017), and pork ham (Medic *et al.*, 2018). According to Fennema (1985), water in food
132 converts to ice crystals during freezing at -18°C and easily removed from food when the food is dried or
133 fried; so that the moisture content decreases.

134 Oil absorption analysis was conducted to measure the ability of cassava sticks to absorb oil during
135 frying by using a modifying thermogravimetric method (Mohamed *et al.*, 1988). Table 1 showed that
136 frozen storage duration significantly increased ($P < 0,05$) the oil absorption of cassava sticks. Cassava stick

137 stored frozen for 2 months has the highest oil absorption (58.45%) but has no significant difference with
138 cassava stick stored frozen for 3 months (57.84%). In Adedeji's and Ngadi's (2017) study, oil absorption
139 also showed a significant increase in fried potato that was stored frozen. However, they also stated that
140 there was no interaction between storage duration and freezing method, which contradicts our study.

141 According to Adedeji *et al.* (2009), moisture loss during frying affects the amount of oil absorbed in
142 fried food. This statement is in accordance with Fellows (1990) statement, where there is an oil transfer
143 into the product to replace evaporated water during frying. In addition, moisture evaporation from fried
144 food during frying damages the cellular structure of plant tissues, resulting in an increase in porosity which
145 contributes to oil absorption (Mellema, 2003; Dana and Saguy, 2006; Rimac-Brcic *et al.*, 2004). On the
146 other hand, during frozen storage, all water in food converts to ice crystal and causes volume expansion
147 which is characterized by the damage to cell membranes during ice crystal formation (Fennema, 1985;
148 Celli *et al.*, 2016). This volume expansion causes the water in cassava sticks to be easier to evaporate
149 during frying and increases oil absorption.

150 3.2. **Hardness**

151 In this study, hardness was chosen as the analyzed texture attribute to evaluate the impact of frozen
152 storage duration on the texture of cassava sticks. According to Szczesniak (2002), hardness is the force
153 required to change the shape of material due to its resistance to resist deformation. The effect of frozen
154 storage duration on the hardness of cassava sticks is presented in Figure 1. As shown in that figure, frozen
155 storage duration significantly affected ($P < 0.05$) the hardness of the cassava sticks. Cassava stick that was
156 stored frozen for 3 months has the highest force (2.54) among other samples (0.63 – 1.41). Our results
157 were in accordance with the results of Adedeji and Ngadi (2017). In that study, they found a marginal
158 increase in the hardness of fried potatoes as the frozen storage duration increased. Therefore, the
159 increase of fried cassava sticks' hardness might be due to the retrogradation of cassava starch during
160 frozen storage. Our statement was supported by a similar study by Yu *et al.* (2010), where the hardness

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161 of cooked rice increased continually with the amylopectin retrogradation during frozen storage. Based on
162 those findings, amylopectin retrogradation contributed to the hardness increase during storage on the
163 high starch food products.

164 3.3. Free fatty acid and peroxide value

165 This study assessed the percentage of free fatty acid and peroxide values to monitor the fat
166 oxidation in cassava sticks during frozen storage. The increase of free fatty acid and peroxide value
167 indicates the rancidity of food products that produce off-flavors (Maity *et al.*, 2012) and might reduce
168 consumers' preferences. The free fatty acid of cassava stick was expressed as the percentage of palmitic
169 acid since the cooking oil used to fry was palm oil. Meanwhile, the peroxide value as the primary oxidation
170 product of fat oxidation was expressed as meq peroxide/kg fat. The percentage of free fatty acid and
171 peroxide value of fried cassava sticks is shown in Figure 2. A significant increased ($P<0.05$) of free fatty
172 acid was recorded during 2 months of frozen storage (0.13 %) and had no significant difference ($P>0.05$)
173 with cassava sticks stored frozen for 3 months (0.14%). On the other hand, the peroxide value of cassava
174 stick was also significantly increased ($P<0.05$) as the frozen storage duration increased (0 – 34.53 meq
175 peroxide/ kg fat). Similar results were also reported by some studies where frozen storage duration
176 increased the free fatty acid and peroxide value of potato strips (Kizito *et al.*, 2017), pork meat (Medic *et*
177 *al.*, 2018), and ready-to-fry vegetable snacks (Maity *et al.*, 2012).

178 According to Crosa *et al.* (2014), free fatty acids are formed through the nucleophilic attack at
179 triacylglycerol's ester bond and have been associated with the undesirable odors and taste of food
180 products. Furthermore, the formation of hydroperoxides during storage generally occurred during the
181 early stage of oxidation (Fennema, 1985) and later decomposed to other secondary oxidation products
182 such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (Kizito *et al.*, 2017; Medic *et al.*, 2018).
183 Another factor that might increase the formation of hydroperoxides was the contact of cassava stick with

184 oxygen before and after storage; since oxygen is one of the reactants that caused fat oxidation (Giri and
185 Mangaraj, 2012).

186 3.4. Color properties

187 Color is generally considered an important attribute that significantly affects the physical
188 properties of food, the perception of consumers, and determines the nutritional quality of food products
189 (Patras *et al.*, 2011; Hutchings, 2002). In this study, the color of cassava sticks is shown in Table 2 and was
190 measured using Hunter's L*, a*, and b* color attributes. The L* value expressed the degree of lightness,
191 where the highest value was measured in the cassava stick stored frozen for 3 months (75.20). However,
192 the results were not significantly different ($P>0.05$) with cassava stick that was not stored frozen (71.55)
193 and significantly different ($P<0.05$) with other samples (66.52 – 67.98). The different results were shown
194 on the a* value, which expressed the degree of redness (+a*) and greenness (-a*) of cassava sticks. All
195 samples had no significant difference ($P>0.05$) on the degree of redness (2.94 – 4.62). Meanwhile, the b*
196 value expressed the degree of yellowness (+b*) and blueness (-b*). The results show that the b* values of
197 cassava stick (25.77) that stored frozen for 2 months was significantly lower ($P<0.05$) than other samples
198 (30.79 – 32.08).

199 Research by Oner and Wall (2012) also found that frozen storage significantly influenced
200 ($P<0.05$) the color of French fries. The color change of fried cassava sticks during frozen storage duration
201 might be due to the surface moisture desiccation (Maity *et al.*, 2012). The amount of moisture loss during
202 the frying process also influence the color of fried cassava stick since moisture loss is associated with crust
203 formation and accelerates the Maillard browning (Sahin, 2000). The Maillard browning occurs due to the
204 reaction between amino acid and reducing sugar during frying and as the result, increases the coloration
205 of yellow, red, and brown color in fried food (Pedreschi *et al.*, 2005). Another factor that might influence
206 the crust color of cassava sticks was the heat transfer rate in the cassava sticks during frying (Maity *et al.*,
207 2012).

208 3.5. Sensory evaluation

209 The sensory evaluation results of cassava sticks stored frozen in different storage durations are
210 shown as a spider plot in Figure 3. According to the results, the highest score for the color attribute was
211 the 0-month storage cassava sticks (5.60) and significantly difference ($P<0.05$) with other samples (3.20 –
212 4.96). The decrease in panelists' color preference was due to the color changes in cassava sticks that tend
213 to be darker and brownish than the regular cassava sticks. Meanwhile, on the hardness attribute, the
214 panelists' preference significantly decreased ($P<0.05$) as the frozen storage duration increased (5.30 –
215 4.20). This result was in accordance with our objective analysis on hardness, where frozen storage
216 duration significantly increased the hardness of cassava sticks due to the starch retrogradation. The next
217 sensory attribute tested was the crispiness and the results were quite varied. Cassava sticks stored frozen
218 for 0 months (4.88) had no significant difference ($P>0.05$) with 2 months stored cassava stick (4.66) but
219 showed significant difference ($P<0.05$) with cassava sticks stored frozen for 1 month (3.86) and 3 months
220 (3.68). This result indicated that panelists perceived that frozen storage duration influenced the crispiness
221 of fried cassava sticks. Furthermore, the frozen storage duration significantly influenced ($P<0.05$) the
222 panelists' preferences on the aroma of cassava sticks. The aroma of cassava sticks tended to be different
223 for each treatment and might be influenced by the formation of hydroperoxides and free fatty acids. The
224 last sensory attribute evaluated was the taste of cassava sticks. According to the result, the frozen storage
225 duration had no significant difference ($P>0.05$) on the panelists' perception of the taste of cassava sticks
226 (4.16 – 4.88). Overall, the average score of panelists' preference showed that frozen storage duration did
227 not significantly affect ($P>0.05$) panelists' acceptance of the cassava stick.

228 4. Conclusion

229 Frozen storage can be used to extend the shelf life of food products. However, the use of frozen
230 storage on cassava sticks affects its physical and chemical properties. Different frozen storage duration
231 influenced the oil absorption of cassava sticks, though the moisture content showed no significant change.

232 The effect of frozen storage duration was also significant on the texture of cassava sticks, especially on
233 hardness. Another studied quality characteristic, such as cassava stick surface color, was substantially
234 declined during the frozen storage. The free fatty acid and peroxide value of cassava sticks was gradually
235 increased with elongation of frozen storage duration. According to the sensory evaluation results, frozen
236 storage duration influenced all of the sensory attributes besides the taste of cassava sticks. Cassava sticks
237 stored frozen for 3 months were still acceptable by the panelists and the acceptance was defined as
238 neither like nor dislike.

239 **Conflict of interest**

240 The authors declare no conflict of interest.

241 **Acknowledgments**

242 This study was financially supported by the Lembaga Penelitian dan Pengabdian Masyarakat (LPPM)
243 Widya Mandala Surabaya Catholic University through PPPG Research Grants 2015/2016.

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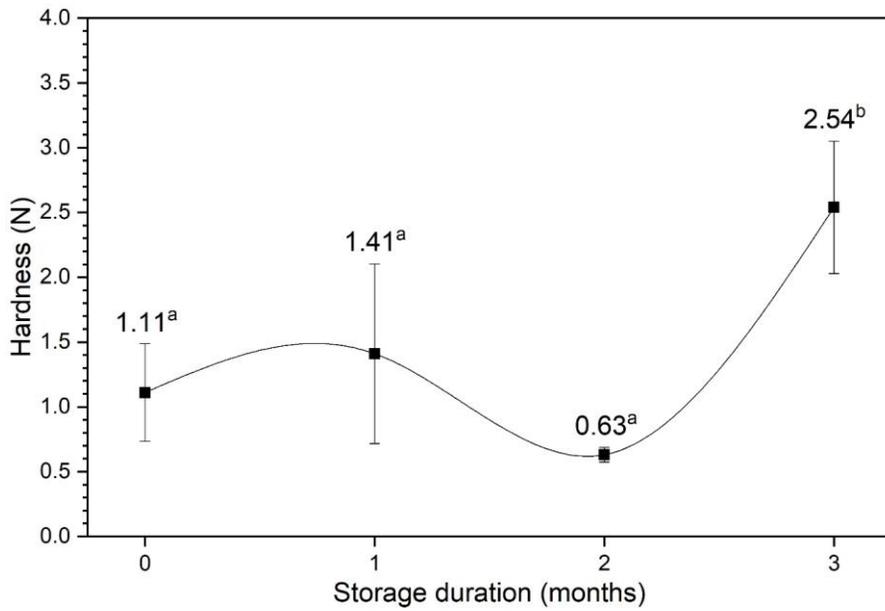
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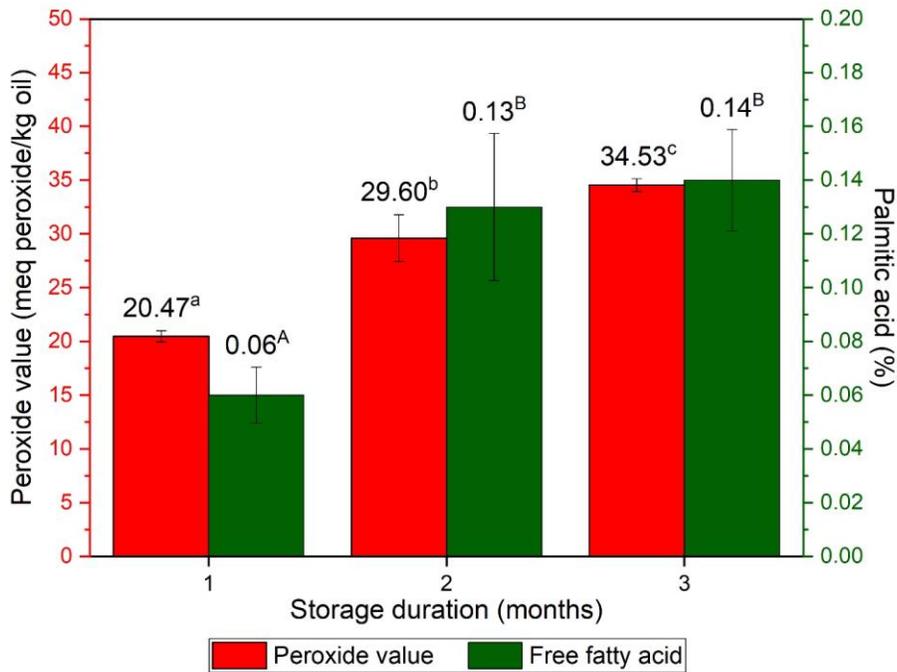


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318 Figure 1. Hardness of cassava stick stored frozen in different storage duration. Means \pm standard
319 deviation ($n = 3$ for each group) in the same column followed by the different letter are significantly
320 different ($P < 0.05$). The statistical significance was evaluated by one-way ANOVA followed by DMRT test
321 ($\alpha = 0.05$).

322

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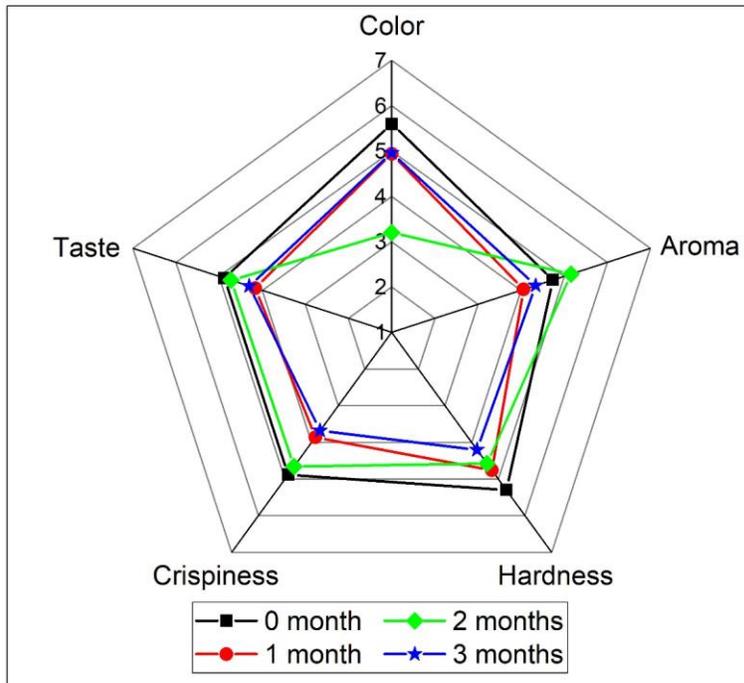
325 Figure 2. Free fatty acid and peroxide value of cassava stick stored frozen in different storage duration.

326 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter

327 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA

328 followed by DMRT test ($\alpha = 0.05$).

329



330

331 Figure 3. Sensory evaluation of cassava stick stored frozen in different storage duration. Means \pm
 332 standard deviation (n = 100 for each group) in the same column followed by the different letter are
 333 significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA followed by
 334 DMRT test ($\alpha = 0.05$).

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339 **List of Tables**

340 Table 1. Moisture content and oil absorption of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Moisture content (%)	Oil absorption (%)
0	45.13 ± 7.46 ^a	20.87 ± 15.91 ^a
1	48.83 ± 3.27 ^a	25.66 ± 9.51 ^a
2	28.68 ± 7.95 ^a	58.45 ± 2.47 ^b
3	27.75 ± 10.13 ^a	57.94 ± 24.71 ^b

341 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter
 342 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA
 343 followed by DMRT test (α = 0.05).

344 **Table 2. Color** of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Color		
	L*	a*	b*
0	71.55 ± 4.08 ^{ab}	2.95 ± 0.78 ^a	32.08 ± 3.02 ^b
1	67.98 ± 2.78 ^a	4.62 ± 1.93 ^a	30.79 ± 1.87 ^b
2	66.52 ± 1.15 ^a	3.21 ± 0.37 ^a	25.77 ± 0.98 ^a
3	75.20 ± 1.80 ^b	4.01 ± 0.30 ^a	31.40 ± 1.66 ^b

345 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter
 346 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA
 347 followed by DMRT test (α = 0.05).

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A screenshot of a Gmail inbox in a web browser. The browser's address bar shows the URL 'mail.google.com/mail/u/0/#search/foodresearch.my%40outlook.com/FMfcgzGmthprxhkjXKkZhnCKgbMFmxF'. The Gmail interface includes a left sidebar with navigation options like 'Compose', 'Inbox', 'Starred', 'Snoozed', 'Sent', 'Drafts', and 'More'. The main content area displays an email from 'Chatarina Yayuk Trisnawati, STP., MP.' dated 'Mar 24, 2022, 10:30 PM'. The email body contains a greeting, a request to find an attached revised manuscript, and a sign-off. Below the text is a section for 'One attachment • Scanned by Gmail' with a thumbnail of a document titled 'FR-IFC-005_Rev...'. At the bottom of the browser window, the Windows taskbar is visible, showing the search bar, system tray with temperature and time, and various application icons.

22 oxidation also occurred during storage, marked by a significant increase ($P < 0.05$) of free fatty acid (0.06
23 to 0.14%) and peroxide value (0 to 34.53 mg peroxide/kg lipid) on three months of frozen storage. Thus,
24 this study concludes that frozen storage duration affected the physical and chemical properties of cassava
25 sticks. Moreover, cassava sticks stored frozen for three months were acceptable for panelists with neither
26 like nor dislike (4.30) average acceptance, and had no significant difference ($P > 0.05$) with other samples.

27 **Keywords:** Cassava, Cassava stick, Frozen storage duration

28 1. Introduction

29 Cassava (*Manihot esculenta* Crantz) is one of the most consumed crops in the world, especially in
30 Sub-Saharan Africa (SSA) and in developing countries of Asia such as Cambodia, Vietnam, and Laos (Benesi
31 *et al.*, 2004; International Atomic Energy Agency, 2018). Cassava is considered as the substitute for rice as
32 the primary carbohydrate source and can be consumed in various ways (fried, boiled, or steamed). Besides
33 carbohydrates, cassava is also rich in calcium (50 mg/100 g), phosphorus (40 mg/100 g), vitamin C (25
34 mg/100g) and contains a significant amount of thiamine, riboflavin, and nicotinic acid (International
35 Atomic Energy Agency, 2018). In Indonesia, cassava is generally processed into traditional food such as
36 *tiwul*, *gatot*, *gaplek*, *gethuk*, *tapai*, and cassava chips. Furthermore, it can also be processed into a modern
37 food such as cassava stick.

38 Cassava stick has similar characteristics to French fries due to its shoestring-like shape, golden
39 brown colour, crunchy exterior, and fluffy interior. Those characteristics are obtained through several
40 processes such as blanching, drying, frying, and each step affects the quality of the final product (Lamberg
41 *et al.*, 1990). Other processes are also required to extend shelf life since cassava sticks are classified as
42 perishable food. Among other processes that might be employed for long-term preservation (high
43 pressure, infrared irradiation, pulsed electric field, and ultrasound), freezing or frozen storage is still one

44 of the most used methods (Rahman and Velez-Ruiz, 2007). Generally, frozen storage temperature is 0°F
45 (-18°C) and even colder depending on the type of the food (WFLO Commodity Storage Manual, 2008).

46 Several studies showed that storing food (fruit, meat, and French fries) under freezing temperatures
47 affects the physical and chemical properties (Sattar *et al.*, 2015; Celli *et al.*, 2016; Medic *et al.*, 2018).
48 However, so far there is no study reported about the effect of frozen storage on the cassava sticks
49 properties. Therefore, this study aimed to investigate the physical and chemical changes on cassava sticks
50 during frozen storage. In addition, the sensory evaluation of cassava sticks during frozen storage was also
51 studied.

52 **2. Materials and methods**

53 *2.1. Materials*

54 Cassava tubers (*Manihot esculenta* Crantz) used in this study were yellow cassava (Malang 2),
55 purchased from a local market in Malang, Indonesia. The cooking oil was purchased from a market in
56 Surabaya, Indonesia. All chemicals and other reagents used in this study were analytical grades.

57 *2.2. Preparation of cassava stick*

58 Cassava tubers were washed two times using tap water to remove physical contaminations. The
59 cleaned cassava tubers were then peeled, cut into strips of 4 x 1 x 1 cm, and soaked in 0.1% (w/v) CaCl₂
60 for 15 min to increase the crispiness of the cassava stick. Next, the soaked cassava sticks were steamed in
61 a steamer (98°C, 15 min) and cooled to room temperature before being pre-fried (170°C, 30 s) in cassava
62 sticks : cooking oil ratio of 1:12 (w/v) using a deep fryer (Fritel Professional 35 SICO, Belgium). Later, the
63 pre-fried cassava sticks were vacuum packed in a polypropylene plastic bag using a vacuum sealer
64 (Maksindo DZ300, Indonesia) and frozen at -20°C for different storage duration (0, 1, 2, 3 months) in a
65 chest freezer (Modena MD 45, Italy). The frozen cassava sticks were then thawed in a water bath (15 min)
66 and fried for 2 min at the same condition as the pre-frying process. At the end of frying, the fryer basket

67 was immediately shaken for 10 seconds and sticks were cooled to room temperature before analyzing the
68 physicochemical properties.

69 2.3. *Moisture content*

70 All fried cassava stick samples were minced before immediately analyzing the moisture content.
71 The moisture content was determined using a drying oven (Binder ED 53, Germany) with the
72 thermogravimetric method (AOAC, 2006) for 3-5 hours at 105 ± 2 °C.

73 2.4. *Oil absorption analysis*

74 The oil absorption analysis was determined according to the method by Mohammed *et al.* (1988).
75 Approximately 5 g of minced pre-fried and fried cassava sticks were oven-dried at 105°C for 3-5 hours and
76 weighed until a stable weight of the sample was obtained. The oil absorption was calculated using the
77 following equation:

$$78 \text{ Oil absorption (\%)} = \frac{W_2 - W_1}{W_1} \times 100\%$$

79 where W_1 is the weight of pre-fried cassava stick (g) and W_2 is the weight of fried cassava stick (g).

80 2.5. *Hardness*

81 Hardness as the selected texture characteristics were determined using a TA-XT plus (Stable Micro
82 System, UK) equipped with a three-point bend rig using compression test mode with the following
83 conditions: pre-test speed= 2 mm/s, test speed= 0.5 mm/s, post-test speed= 10 mm/s, and distance= 10
84 mm. The hardness of fried cassava stick was expressed as Newton and determined as the maximum force
85 required to compress the sample.

86 2.6. *Free fatty acid analysis*

87 The free fatty acid of cassava sticks was analyzed using a method by Sudarmadji *et al.* (1984).
88 Approximately 28.2 g of fried cassava sticks were minced before adding 50 mL of neutralized alcohol and
89 2 mL phenolphthalein. The mixture was then titrated with 0.1 N NaOH until a stable pink colour occurred

90 for 30 s. The percentage of free fatty acid was expressed as the percentage of palmitic acid and calculated
91 using the following equation:

$$92 \quad \text{Palmitic acid (\%)} = \frac{V_{\text{NaOH}} \times N_{\text{NaOH}} \times \text{Mr}}{W \times 1000} \times 100\%$$

93 where V_{NaOH} is the titre of NaOH (mL), N_{NaOH} is the concentration of NaOH (N), Mr is the molecular
94 weight of palmitic acid (256.42 g/mol), and W is the sample weight (g).

95 2.7. Peroxide value

96 An iodometric titration method was used to analyze the peroxide value as the primary oxidation
97 product (Sudarmadji *et al.*, 1984). Briefly, 5 g of fried cassava sticks were mixed with 30 mL acetic acid –
98 chloroform (3:2) and 0.5 mL saturated aqueous potassium iodide solution was added. Later, the mixture
99 was shaken vigorously for 1 minute before 30 mL distilled water was added. The mixture was titrated with
100 0.1 M $\text{Na}_2\text{S}_2\text{O}_3$ until the yellow colour had almost disappeared. Next, 0.5 mL of 1% (w/v) starch indicator
101 solution was added and the titration continued until the blue colour disappeared. The peroxide value was
102 calculated using the following formula:

$$103 \quad \text{Peroxide value (meq peroxide/kg oil)} = \frac{V_{\text{thio}} \times N_{\text{thio}} \times 1000}{W}$$

104 where V_{thio} is the titre of $\text{Na}_2\text{S}_2\text{O}_3$ (mL), N_{thio} is the concentration of $\text{Na}_2\text{S}_2\text{O}_3$, and W is the sample
105 weight (g).

106 2.8. Colour

107 The colour of fried cassava stick was identified using a colour reader (Colour Reader Minolta, CR-
108 10). The L^* value (0 = blackness, 100 = whiteness), a^* value (+ = redness, - = greenness), and b^* value (+ =
109 yellowness, - = blueness) of each sample were observed.

110 2.9. Sensory evaluation

111 In this study, the Hedonic Scale Scoring (preferred test) was used to measure the level of preference
112 and acceptance of the product by consumers (Kusuma *et al.*, 2017). The sensory evaluation was evaluated

113 by 100 untrained panelists. The panelists were students of the Faculty of Agricultural Technology, Widya
114 Mandala Catholic University Surabaya. The sensory attributes tested were colour, hardness, crispiness,
115 aroma, and taste. A scoring system of 1-7 points was used to represent the sensory characteristics of each
116 sample. On this scale, 1 was defined as dislike extremely, 2 was defined as dislike moderately, 3 was
117 defined as dislike slightly, 4 was defined as neither like nor dislike, 5 was defined as like slightly, 6 was
118 defined as like moderately, and 7 was defined as like extremely.

119 2.10. Statistical analysis

120 All experiments were analyzed at least three times in triplicate and represented as mean values
121 \pm SD. Statistical analyses were performed using SPSS for Windows (version 19.0, SPSS Inc., USA) and
122 compared with Duncan Multiple Range Test (DMRT) at $P < 0.05$.

123 3. Results and discussion

124 3.1. Moisture content and oil absorption

125 The moisture content of fried cassava sticks is shown in Table 1. Based on the result, different frozen
126 storage durations have no significant difference ($P > 0.05$) on the moisture content of cassava sticks, even
127 though there was a downward trend in the moisture content of frozen cassava sticks for 2 and 3 months.
128 Similar results were reported by Medic *et al.* (2018), where frozen storage duration had no significant on
129 the moisture content of pork loin and belly rib. However, several researchers also found that freezing
130 significantly ($P < 0.05$) influenced the moisture content of the fried potato (Adedeji and Ngadi, 2017), lamb
131 (Coombs *et al.*, 2017), and pork ham (Medic *et al.*, 2018). According to Fennema (1985), water in food
132 converts to ice crystals during freezing at -18°C and easily removed from food when the food is dried or
133 fried; so that the moisture content decreases.

134 Oil absorption analysis was conducted to measure the ability of cassava sticks to absorb oil during
135 frying by using a modifying thermogravimetric method (Mohamed *et al.*, 1988). Table 1 showed that
136 frozen storage duration significantly increased ($P < 0.05$) the oil absorption of cassava sticks. Cassava stick

137 stored frozen for 2 months has the highest oil absorption (58.45%) but has no significant difference with
138 cassava stick stored frozen for 3 months (57.84%). In Adedeji's and Ngadi's (2017) study, oil absorption
139 also showed a significant increase in fried potato that was stored frozen. However, they also found that
140 there was no interaction between storage duration and freezing method, which contradicts this study.

141 According to Adedeji *et al.* (2009), moisture loss during frying affects the amount of oil absorbed in
142 fried food. This statement is in accordance with Fellows (1990) statement, where there is an oil transfer
143 into the product to replace evaporated water during frying. In addition, moisture evaporation from fried
144 food during frying damages the cellular structure of plant tissues, resulting in an increase in porosity which
145 contributes to oil absorption (Mellema, 2003; Rimac-Brncic *et al.*, 2004; Dana and Saguy, 2006). On the
146 other hand, during frozen storage, all water in food converts to ice crystal and causes volume expansion
147 which is characterized by the damage to cell membranes during ice crystal formation (Fennema, 1985;
148 Celli *et al.*, 2016). This volume expansion causes the water in cassava sticks to be easier to evaporate
149 during frying and increases oil absorption.

150 3.2. Hardness

151 In this study, hardness was chosen as the analyzed texture attribute to evaluate the impact of frozen
152 storage duration on the texture of cassava sticks. According to Szczesniak (2002), hardness is the force
153 required to change the shape of material due to its resistance to resist deformation. The effect of frozen
154 storage duration on the hardness of cassava sticks is presented in Figure 1. As shown in that figure, frozen
155 storage duration significantly affected ($P < 0.05$) the hardness of the cassava sticks. Cassava stick that was
156 stored frozen for 3 months has the highest force (2.54) among other samples (0.63 – 1.41). This results
157 were in accordance with the results of Adedeji and Ngadi (2017). In that study, they found a marginal
158 increase in the hardness of fried potatoes as the frozen storage duration increased. Therefore, the
159 increase of fried cassava sticks' hardness might be due to the retrogradation of cassava starch during
160 frozen storage. This statement was supported by a similar study by Yu *et al.* (2010), where the hardness

161 of cooked rice increased continually with the amylopectin retrogradation during frozen storage. Based on
162 those findings, amylopectin retrogradation contributed to the hardness increase during storage on the
163 high starch food products.

164 3.3. Free fatty acid and peroxide value

165 This study assessed the percentage of free fatty acid and peroxide values to monitor the fat
166 oxidation in cassava sticks during frozen storage. The increase of free fatty acid and peroxide value
167 indicates the rancidity of food products that produce off-flavors (Maity *et al.*, 2012) and might reduce
168 consumers' preferences. The free fatty acid of cassava stick was expressed as the percentage of palmitic
169 acid since the cooking oil used to fry was palm oil. Meanwhile, the peroxide value as the primary oxidation
170 product of fat oxidation was expressed as meq peroxide/kg fat. The percentage of free fatty acid and
171 peroxide value of fried cassava sticks is shown in Figure 2. A significant increased ($P<0.05$) of free fatty
172 acid was recorded during 2 months of frozen storage (0.13 %) and had no significant difference ($P>0.05$)
173 with cassava sticks stored frozen for 3 months (0.14%). On the other hand, the peroxide value of cassava
174 stick was also significantly increased ($P<0.05$) as the frozen storage duration increased (0 – 34.53 meq
175 peroxide/ kg fat). Similar results were also reported by some studies where frozen storage duration
176 increased the free fatty acid and peroxide value of potato strips (Kizito *et al.*, 2017), pork meat (Medic *et*
177 *al.*, 2018), and ready-to-fry vegetable snacks (Maity *et al.*, 2012).

178 According to Crosa *et al.* (2014), free fatty acids are formed through the nucleophilic attack at
179 triacylglycerol's ester bond and have been associated with the undesirable odors and taste of food
180 products. Furthermore, the formation of hydroperoxides during storage generally occurred during the
181 early stage of oxidation (Fennema, 1985) and later decomposed to other secondary oxidation products
182 such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (Kizito *et al.*, 2017; Medic *et al.*, 2018).
183 Another factor that might increase the formation of hydroperoxides was the contact of cassava stick with

184 oxygen before and after storage; since oxygen is one of the reactants that caused fat oxidation (Giri and
185 Mangaraj, 2012).

186 3.4. Colour properties

187 Colour is generally considered an important attribute that significantly affects the physical
188 properties of food, the perception of consumers, and determines the nutritional quality of food products
189 (Hutchings, 2002; Patras *et al.*, 2011). In this study, the colour of cassava sticks is shown in Table 2 and
190 was measured using Hunter's L*, a*, and b* colour attributes. The L* value expressed the degree of
191 lightness, where the highest value was measured in the cassava stick stored frozen for 3 months (75.20).
192 However, the results were not significantly different ($P>0.05$) with cassava stick that was not stored frozen
193 (71.55) and significantly different ($P<0.05$) with other samples (66.52 – 67.98). The different results were
194 shown on the a* value, which expressed the degree of redness (+a*) and greenness (-a*) of cassava sticks.
195 All samples had no significant difference ($P>0.05$) on the degree of redness (2.94 – 4.62). Meanwhile, the
196 b* value expressed the degree of yellowness (+b*) and blueness (-b*). The results show that the b* values
197 of cassava stick (25.77) that stored frozen for 2 months was significantly lower ($P<0.05$) than other
198 samples (30.79 – 32.08).

199 Research by Oner and Wall (2012) also found that frozen storage significantly influenced
200 ($P<0.05$) the colour of French fries. The colour change of fried cassava sticks during frozen storage duration
201 might be due to the surface moisture desiccation (Maity *et al.*, 2012). The amount of moisture loss during
202 the frying process also influence the colour of fried cassava stick since moisture loss is associated with
203 crust formation and accelerates the Maillard browning (Sahin, 2000). The Maillard browning occurs due
204 to the reaction between amino acid and reducing sugar during frying and as the result, increases the
205 coloration of yellow, red, and brown colour in fried food (Pedreschi *et al.*, 2005). Another factor that might
206 influence the crust colour of cassava sticks was the heat transfer rate in the cassava sticks during frying
207 (Maity *et al.*, 2012).

208 3.5. Sensory evaluation

209 The sensory evaluation results of cassava sticks stored frozen in different storage durations are
210 shown as a spider plot in Figure 3. According to the results, the highest score for the colour attribute was
211 the 0-month storage cassava sticks (5.60) and significantly difference ($P < 0.05$) with other samples (3.20 –
212 4.96). The decrease in panelists' colour preference was due to the colour changes in cassava sticks that
213 tend to be darker and brownish than the regular cassava sticks. Meanwhile, on the hardness attribute,
214 the panelists' preference significantly decreased ($P < 0.05$) as the frozen storage duration increased (5.30
215 – 4.20). This result was in accordance with the objective analysis on hardness, where frozen storage
216 duration significantly increased the hardness of cassava sticks due to the starch retrogradation. The next
217 sensory attribute tested was the crispiness and the results were quite varied. Cassava sticks stored frozen
218 for 0 months (4.88) had no significant difference ($P > 0.05$) with 2 months stored cassava stick (4.66) but
219 showed significant difference ($P < 0.05$) with cassava sticks stored frozen for 1 month (3.86) and 3 months
220 (3.68). This result indicated that panelists perceived that frozen storage duration influenced the crispiness
221 of fried cassava sticks. Furthermore, the frozen storage duration significantly influenced ($P < 0.05$) the
222 panelists' preferences on the aroma of cassava sticks. The aroma of cassava sticks tended to be different
223 for each treatment and might be influenced by the formation of hydroperoxides and free fatty acids. The
224 last sensory attribute evaluated was the taste of cassava sticks. According to the result, the frozen storage
225 duration had no significant difference ($P > 0.05$) on the panelists' perception of the taste of cassava sticks
226 (4.16 – 4.88). Overall, the average score of panelists' preference showed that frozen storage duration did
227 not significantly affect ($P > 0.05$) panelists' acceptance of the cassava stick.

228 4. Conclusion

229 Frozen storage can be used to extend the shelf life of food products. However, the use of frozen
230 storage on cassava sticks affects its physical and chemical properties. Different frozen storage duration
231 influenced the oil absorption of cassava sticks, though the moisture content showed no significant change.

232 The effect of frozen storage duration was also significant on the texture of cassava sticks, especially on
233 hardness. Another studied quality characteristic, such as cassava stick surface colour, was substantially
234 declined during the frozen storage. The free fatty acid and peroxide value of cassava sticks was gradually
235 increased with elongation of frozen storage duration. According to the sensory evaluation results, frozen
236 storage duration influenced all of the sensory attributes besides the taste of cassava sticks. Cassava sticks
237 stored frozen for 3 months were still acceptable by the panelists and the acceptance was defined as
238 neither like nor dislike.

239 **Conflict of interest**

240 The authors declare no conflict of interest.

241 **Acknowledgments**

242 This study was financially supported by the Lembaga Penelitian dan Pengabdian Masyarakat (LPPM)
243 Widya Mandala Surabaya Catholic University through PPPG Research Grants 2015/2016.

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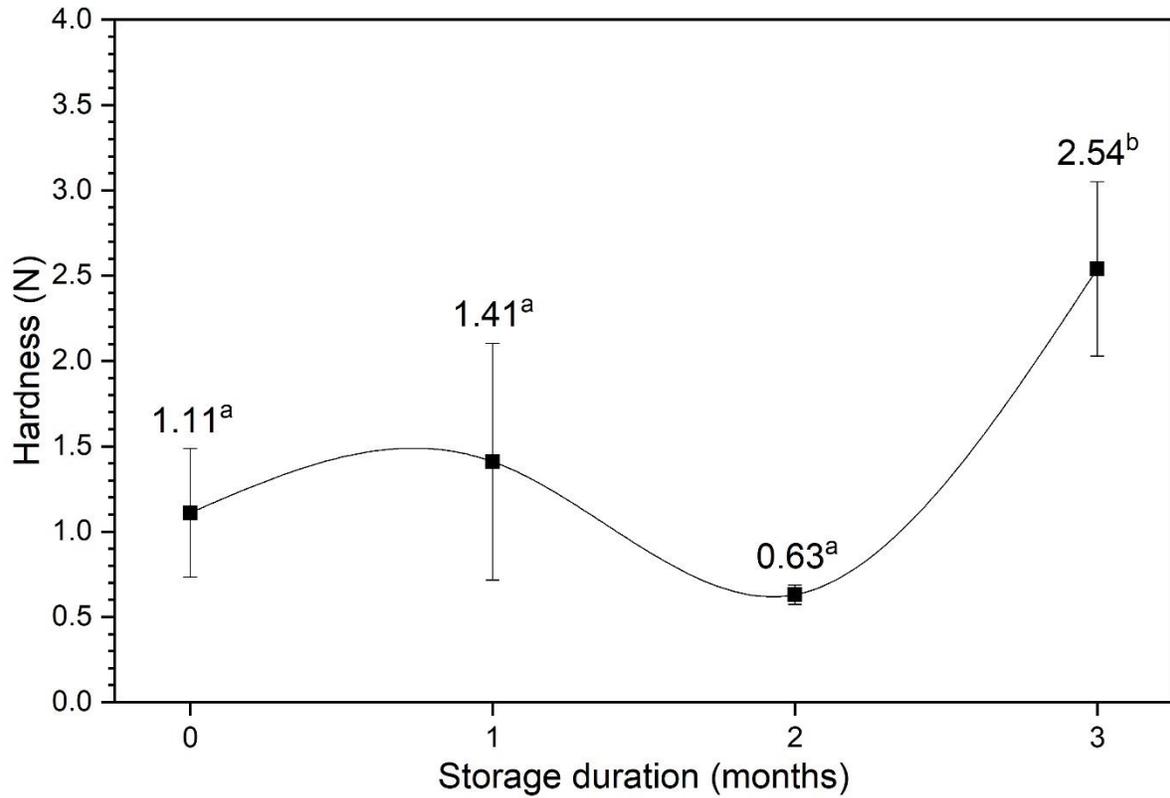
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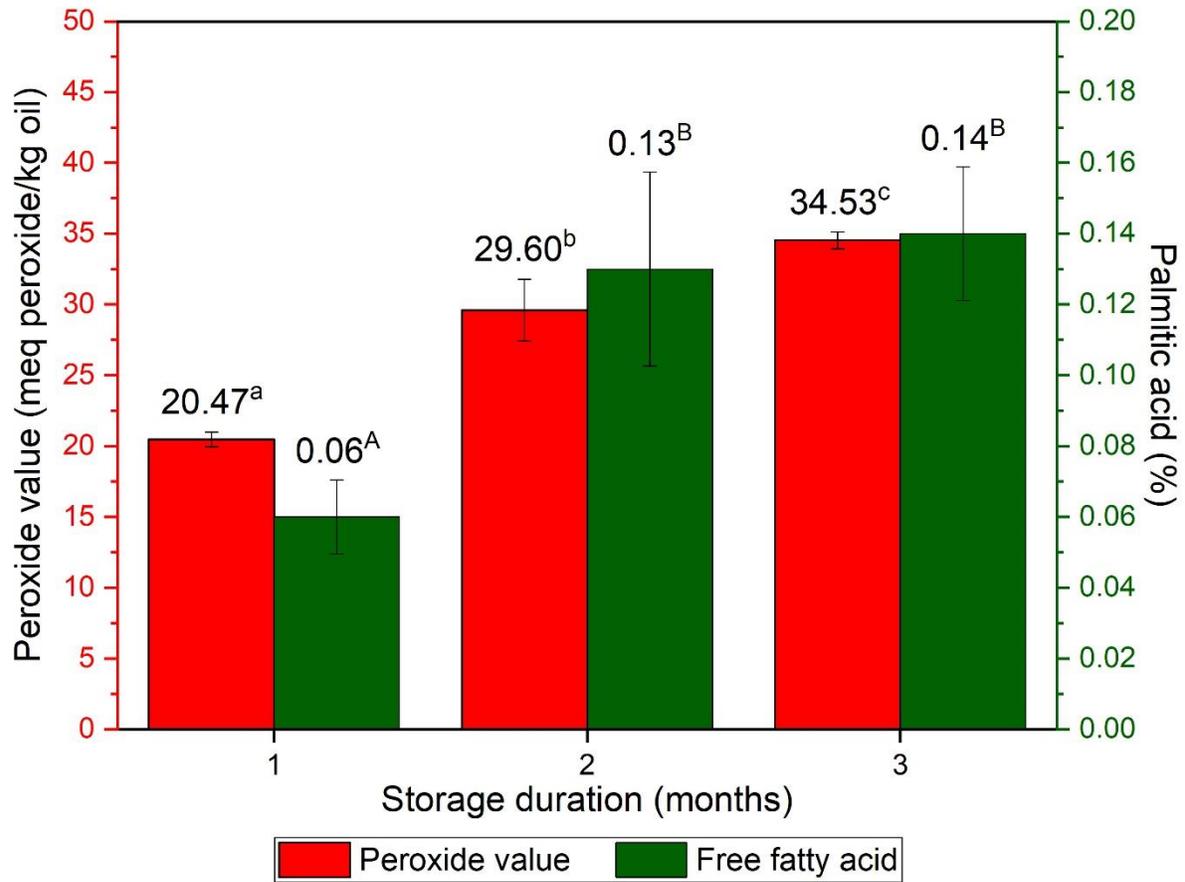
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318 Figure 1. Hardness of cassava stick stored frozen in different storage duration. Means \pm standard
319 deviation (n = 3 for each group) in the same column followed by the different letter are significantly
320 different (P<0.05). The statistical significance was evaluated by one-way ANOVA followed by DMRT test

321 ($\alpha = 0.05$).

322

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324

325 Figure 2. Free fatty acid and peroxide value of cassava stick stored frozen in different storage duration.

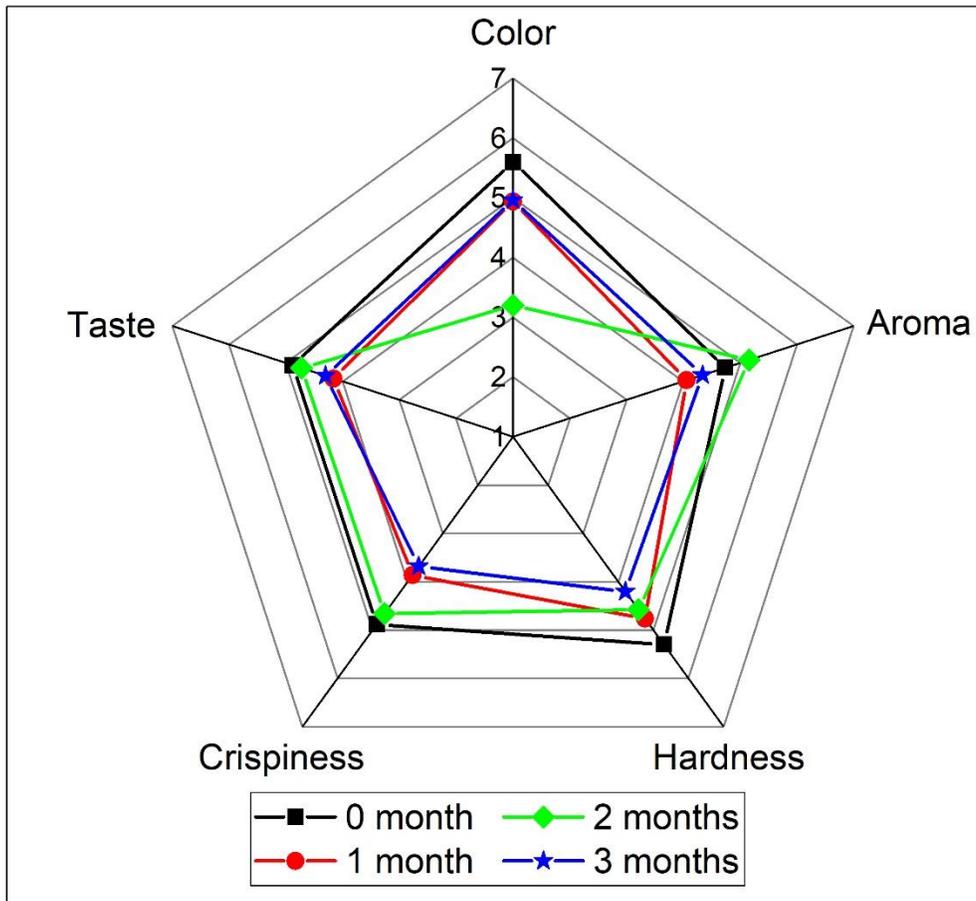
326 Means \pm standard deviation (n = 3 for each group) in the same column followed by the different letter

327 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA

328

followed by DMRT test ($\alpha = 0.05$).

329



330

331 Figure 3. Sensory evaluation of cassava stick stored frozen in different storage duration. Means \pm
 332 standard deviation (n = 100 for each group) in the same column followed by the different letter are
 333 significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA followed by
 334 DMRT test ($\alpha = 0.05$).

335

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338

339 **List of Tables**

340 Table 1. Moisture content and oil absorption of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Moisture content (%)	Oil absorption (%)
0	45.13 ± 7.46 ^a	20.87 ± 15.91 ^a
1	48.83 ± 3.27 ^a	25.66 ± 9.51 ^a
2	28.68 ± 7.95 ^a	58.45 ± 2.47 ^b
3	27.75 ± 10.13 ^a	57.94 ± 24.71 ^b

341 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter
 342 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA
 343 followed by DMRT test (α = 0.05).

344 Table 2. Colour of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Colour		
	L*	a*	b*
0	71.55 ± 4.08 ^{ab}	2.95 ± 0.78 ^a	32.08 ± 3.02 ^b
1	67.98 ± 2.78 ^a	4.62 ± 1.93 ^a	30.79 ± 1.87 ^b
2	66.52 ± 1.15 ^a	3.21 ± 0.37 ^a	25.77 ± 0.98 ^a
3	75.20 ± 1.80 ^b	4.01 ± 0.30 ^a	31.40 ± 1.66 ^b

345 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter
 346 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA
 347 followed by DMRT test (α = 0.05).

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8.

Bukti konfirmasi revisi artikel yang harus dilakukan

(26 Maret 2022)

Manuscript ID: FR-IFC-005 - chat x +

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F Food Research <foodresearch.my@outlook.com> to me Mar 26, 2022, 6:31 PM

Dear Dr. Chatarina Yayuk Trisnawati,

Please revise Figure 3 spelling of the word 'color' to 'colour'.

Best regards,
Son Radu, PhD
Chief Editor

From: Chatarina Yayuk Trisnawati, STP., MP. <chatarina@ukwms.ac.id>
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22 oxidation also occurred during storage, marked by a significant increase ($P < 0.05$) of free fatty acid (0.06
23 to 0.14%) and peroxide value (0 to 34.53 mg peroxide/kg lipid) on three months of frozen storage. Thus,
24 this study concludes that frozen storage duration affected the physical and chemical properties of cassava
25 sticks. Moreover, cassava sticks stored frozen for three months were acceptable for panelists with neither
26 like nor dislike (4.30) average acceptance, and had no significant difference ($P > 0.05$) with other samples.

27 **Keywords:** Cassava, Cassava stick, Frozen storage duration

28 **1. Introduction**

29 Cassava (*Manihot esculenta* Crantz) is one of the most consumed crops in the world, especially in
30 Sub-Saharan Africa (SSA) and in developing countries of Asia such as Cambodia, Vietnam, and Laos (Benesi
31 *et al.*, 2004; International Atomic Energy Agency, 2018). Cassava is considered as the substitute for rice as
32 the primary carbohydrate source and can be consumed in various ways (fried, boiled, or steamed). Besides
33 carbohydrates, cassava is also rich in calcium (50 mg/100 g), phosphorus (40 mg/100 g), vitamin C (25
34 mg/100g) and contains a significant amount of thiamine, riboflavin, and nicotinic acid (International
35 Atomic Energy Agency, 2018). In Indonesia, cassava is generally processed into traditional food such as
36 *tiwul*, *gatot*, *gaplek*, *gethuk*, *tapai*, and cassava chips. Furthermore, it can also be processed into a modern
37 food such as cassava stick.

38 Cassava stick has similar characteristics to French fries due to its shoestring-like shape, golden
39 brown colour, crunchy exterior, and fluffy interior. Those characteristics are obtained through several
40 processes such as blanching, drying, frying, and each step affects the quality of the final product (Lamberg
41 *et al.*, 1990). Other processes are also required to extend shelf life since cassava sticks are classified as
42 perishable food. Among other processes that might be employed for long-term preservation (high
43 pressure, infrared irradiation, pulsed electric field, and ultrasound), freezing or frozen storage is still one

44 of the most used methods (Rahman and Velez-Ruiz, 2007). Generally, frozen storage temperature is 0°F
45 (-18°C) and even colder depending on the type of the food (WFLO Commodity Storage Manual, 2008).

46 Several studies showed that storing food (fruit, meat, and French fries) under freezing temperatures
47 affects the physical and chemical properties (Sattar *et al.*, 2015; Celli *et al.*, 2016; Medic *et al.*, 2018).
48 However, based on authors' knowledge, there is no study reported about the effect of frozen storage on
49 the cassava sticks properties. Therefore, this study aimed to investigate the physical and chemical changes
50 on cassava sticks during frozen storage. In addition, the sensory evaluation of cassava sticks during frozen
51 storage was also studied.

52 **2. Materials and methods**

53 *2.1. Materials*

54 Cassava tubers (*Manihot esculenta* Crantz) used in this study were yellow cassava (Malang 2),
55 purchased from a local market in Malang, Indonesia. The cooking oil was purchased from a market in
56 Surabaya, Indonesia. All chemicals and other reagents used in this study were analytical grades.

57 *2.2. Preparation of cassava stick*

58 Cassava tubers were washed two times using tap water to remove physical contaminations. The
59 cleaned cassava tubers were then peeled, cut into strips of 4 x 1 x 1 cm, and soaked in 0.1% (w/v) CaCl₂
60 for 15 min to increase the crispiness of the cassava stick. Next, the soaked cassava sticks were steamed in
61 a steamer (98°C, 15 min) and cooled to room temperature before being pre-fried (170°C, 30 s) in cassava
62 sticks : cooking oil ratio of 1:12 (w/v) using a deep fryer (Fritel Professional 35 SICO, Belgium). Later, the
63 pre-fried cassava sticks were vacuum packed in a polypropylene plastic bag using a vacuum sealer
64 (Maksindo DZ300, Indonesia) and frozen at -20°C for different storage duration (0, 1, 2, 3 months) in a
65 chest freezer (Modena MD 45, Italy). The frozen cassava sticks were then thawed in a water bath (15 min)
66 and fried for 2 min at the same condition as the pre-frying process. At the end of frying, the fryer basket

67 was immediately shaken for 10 seconds and sticks were cooled to room temperature before analyzing the
68 physicochemical properties.

69 2.3. *Moisture content*

70 All fried cassava stick samples were minced before immediately analyzing the moisture content.
71 The moisture content was determined using a drying oven (Binder ED 53, Germany) with the
72 thermogravimetric method (AOAC, 2006) for 3-5 hours at 105 ± 2 °C.

73 2.4. *Oil absorption analysis*

74 The oil absorption analysis was determined according to the method by Mohammed *et al.* (1988).
75 Approximately 5 g of minced pre-fried and fried cassava sticks were oven-dried at 105°C for 3-5 hours and
76 weighed until a stable weight of the sample was obtained. The oil absorption was calculated using the
77 following equation:

$$78 \text{ Oil absorption (\%)} = \frac{W_2 - W_1}{W_1} \times 100\%$$

79 where W_1 is the weight of pre-fried cassava stick (g) and W_2 is the weight of fried cassava stick (g).

80 2.5. *Hardness*

81 Hardness as the selected texture characteristics were determined using a TA-XT plus (Stable Micro
82 System, UK) equipped with a three-point bend rig using compression test mode with the following
83 conditions: pre-test speed= 2 mm/s, test speed= 0.5 mm/s, post-test speed= 10 mm/s, and distance= 10
84 mm. The hardness of fried cassava stick was expressed as Newton and determined as the maximum force
85 required to compress the sample.

86 2.6. *Free fatty acid analysis*

87 The free fatty acid of cassava sticks was analyzed using a method by Sudarmadji *et al.* (1984).
88 Approximately 28.2 g of fried cassava sticks were minced before adding 50 mL of neutralized alcohol and
89 2 mL phenolphthalein. The mixture was then titrated with 0.1 N NaOH until a stable pink colour occurred

90 for 30 s. The percentage of free fatty acid was expressed as the percentage of palmitic acid and calculated
91 using the following equation:

$$92 \quad \text{Palmitic acid (\%)} = \frac{V_{\text{NaOH}} \times N_{\text{NaOH}} \times \text{Mr}}{W \times 1000} \times 100\%$$

93 where V_{NaOH} is the titre of NaOH (mL), N_{NaOH} is the concentration of NaOH (N), Mr is the molecular
94 weight of palmitic acid (256.42 g/mol), and W is the sample weight (g).

95 2.7. Peroxide value

96 An iodometric titration method was used to analyze the peroxide value as the primary oxidation
97 product (Sudarmadji *et al.*, 1984). Briefly, 5 g of fried cassava sticks were mixed with 30 mL acetic acid –
98 chloroform (3:2) and 0.5 mL saturated aqueous potassium iodide solution was added. Later, the mixture
99 was shaken vigorously for 1 minute before 30 mL distilled water was added. The mixture was titrated with
100 0.1 M $\text{Na}_2\text{S}_2\text{O}_3$ until the yellow colour had almost disappeared. Next, 0.5 mL of 1% (w/v) starch indicator
101 solution was added and the titration continued until the blue colour disappeared. The peroxide value was
102 calculated using the following formula:

$$103 \quad \text{Peroxide value (meq peroxide/kg oil)} = \frac{V_{\text{thio}} \times N_{\text{thio}} \times 1000}{W}$$

104 where V_{thio} is the titre of $\text{Na}_2\text{S}_2\text{O}_3$ (mL), N_{thio} is the concentration of $\text{Na}_2\text{S}_2\text{O}_3$, and W is the sample
105 weight (g).

106 2.8. Colour

107 The colour of fried cassava stick was identified using a colour reader (Colour Reader Minolta, CR-
108 10). The L^* value (0 = blackness, 100 = whiteness), a^* value (+ = redness, - = greenness), and b^* value (+ =
109 yellowness, - = blueness) of each sample were observed.

110 2.9. Sensory evaluation

111 In this study, the Hedonic Scale Scoring (preferred test) was used to measure the level of preference
112 and acceptance of the product by consumers (Kusuma *et al.*, 2017). The sensory evaluation was evaluated

113 by 100 untrained panelists. The panelists were students of the Faculty of Agricultural Technology, Widya
114 Mandala Catholic University Surabaya. The sensory attributes tested were colour, hardness, crispiness,
115 aroma, and taste. A scoring system of 1-7 points was used to represent the sensory characteristics of each
116 sample. On this scale, 1 was defined as dislike extremely, 2 was defined as dislike moderately, 3 was
117 defined as dislike slightly, 4 was defined as neither like nor dislike, 5 was defined as like slightly, 6 was
118 defined as like moderately, and 7 was defined as like extremely.

119 2.10. Statistical analysis

120 All experiments were analyzed at least three times in triplicate and represented as mean values
121 \pm SD. Statistical analyses were performed using SPSS for Windows (version 19.0, SPSS Inc., USA) and
122 compared with Duncan Multiple Range Test (DMRT) at $P < 0.05$.

123 3. Results and discussion

124 3.1. Moisture content and oil absorption

125 The moisture content of fried cassava sticks is shown in Table 1. Based on the result, different frozen
126 storage durations have no significant difference ($P > 0.05$) on the moisture content of cassava sticks, even
127 though there was a downward trend in the moisture content of frozen cassava sticks for 2 and 3 months.
128 Similar results were reported by Medic *et al.* (2018), where frozen storage duration had no significant on
129 the moisture content of pork loin and belly rib. However, several researchers also found that freezing
130 significantly ($P < 0.05$) influenced the moisture content of the fried potato (Adedeji and Ngadi, 2017), lamb
131 (Coombs *et al.*, 2017), and pork ham (Medic *et al.*, 2018). According to Fennema (1985), water in food
132 converts to ice crystals during freezing at -18°C and easily removed from food when the food is dried or
133 fried; so that the moisture content decreases.

134 Oil absorption analysis was conducted to measure the ability of cassava sticks to absorb oil during
135 frying by using a modifying thermogravimetric method (Mohamed *et al.*, 1988). Table 1 showed that
136 frozen storage duration significantly increased ($P < 0.05$) the oil absorption of cassava sticks. Cassava stick

137 stored frozen for 2 months has the highest oil absorption (58.45%) but has no significant difference with
138 cassava stick stored frozen for 3 months (57.84%). In Adedeji's and Ngadi's (2017) study, oil absorption
139 also showed a significant increase in fried potato that was stored frozen. However, they also found that
140 there was no interaction between storage duration and freezing method, which contradicts this study.

141 According to Adedeji *et al.* (2009), moisture loss during frying affects the amount of oil absorbed in
142 fried food. This statement is in accordance with Fellows (1990) statement, where there is an oil transfer
143 into the product to replace evaporated water during frying. In addition, moisture evaporation from fried
144 food during frying damages the cellular structure of plant tissues, resulting in an increase in porosity which
145 contributes to oil absorption (Mellema, 2003; Rimac-Brncic *et al.*, 2004; Dana and Saguy, 2006). On the
146 other hand, during frozen storage, all water in food converts to ice crystal and causes volume expansion
147 which is characterized by the damage to cell membranes during ice crystal formation (Fennema, 1985;
148 Celli *et al.*, 2016). This volume expansion causes the water in cassava sticks to be easier to evaporate
149 during frying and increases oil absorption.

150 3.2. Hardness

151 In this study, hardness was chosen as the analyzed texture attribute to evaluate the impact of frozen
152 storage duration on the texture of cassava sticks. According to Szczesniak (2002), hardness is the force
153 required to change the shape of material due to its resistance to resist deformation. The effect of frozen
154 storage duration on the hardness of cassava sticks is presented in Figure 1. As shown in that figure, frozen
155 storage duration significantly affected ($P < 0.05$) the hardness of the cassava sticks. Cassava stick that was
156 stored frozen for 3 months has the highest force (2.54) among other samples (0.63 – 1.41). This results
157 were in accordance with the results of Adedeji and Ngadi (2017). In that study, they found a marginal
158 increase in the hardness of fried potatoes as the frozen storage duration increased. Therefore, the
159 increase of fried cassava sticks' hardness might be due to the retrogradation of cassava starch during
160 frozen storage. This statement was supported by a similar study by Yu *et al.* (2010), where the hardness

161 of cooked rice increased continually with the amylopectin retrogradation during frozen storage. Based on
162 those findings, amylopectin retrogradation contributed to the hardness increase during storage on the
163 high starch food products.

164 3.3. Free fatty acid and peroxide value

165 This study assessed the percentage of free fatty acid and peroxide values to monitor the fat
166 oxidation in cassava sticks during frozen storage. The increase of free fatty acid and peroxide value
167 indicates the rancidity of food products that produce off-flavors (Maity *et al.*, 2012) and might reduce
168 consumers' preferences. The free fatty acid of cassava stick was expressed as the percentage of palmitic
169 acid since the cooking oil used to fry was palm oil. Meanwhile, the peroxide value as the primary oxidation
170 product of fat oxidation was expressed as meq peroxide/kg fat. The percentage of free fatty acid and
171 peroxide value of fried cassava sticks is shown in Figure 2. A significant increased ($P<0.05$) of free fatty
172 acid was recorded during 2 months of frozen storage (0.13 %) and had no significant difference ($P>0.05$)
173 with cassava sticks stored frozen for 3 months (0.14%). On the other hand, the peroxide value of cassava
174 stick was also significantly increased ($P<0.05$) as the frozen storage duration increased (0 – 34.53 meq
175 peroxide/ kg fat). Similar results were also reported by some studies where frozen storage duration
176 increased the free fatty acid and peroxide value of potato strips (Kizito *et al.*, 2017), pork meat (Medic *et*
177 *al.*, 2018), and ready-to-fry vegetable snacks (Maity *et al.*, 2012).

178 According to Crosa *et al.* (2014), free fatty acids are formed through the nucleophilic attack at
179 triacylglycerol's ester bond and have been associated with the undesirable odors and taste of food
180 products. Furthermore, the formation of hydroperoxides during storage generally occurred during the
181 early stage of oxidation (Fennema, 1985) and later decomposed to other secondary oxidation products
182 such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (Kizito *et al.*, 2017; Medic *et al.*, 2018).
183 Another factor that might increase the formation of hydroperoxides was the contact of cassava stick with

184 oxygen before and after storage; since oxygen is one of the reactants that caused fat oxidation (Giri and
185 Mangaraj, 2012).

186 3.4. Colour properties

187 Colour is generally considered an important attribute that significantly affects the physical
188 properties of food, the perception of consumers, and determines the nutritional quality of food products
189 (Hutchings, 2002; Patras *et al.*, 2011). In this study, the colour of cassava sticks is shown in Table 2 and
190 was measured using Hunter's L*, a*, and b* colour attributes. The L* value expressed the degree of
191 lightness, where the highest value was measured in the cassava stick stored frozen for 3 months (75.20).
192 However, the results were not significantly different ($P>0.05$) with cassava stick that was not stored frozen
193 (71.55) and significantly different ($P<0.05$) with other samples (66.52 – 67.98). The different results were
194 shown on the a* value, which expressed the degree of redness (+a*) and greenness (-a*) of cassava sticks.
195 All samples had no significant difference ($P>0.05$) on the degree of redness (2.94 – 4.62). Meanwhile, the
196 b* value expressed the degree of yellowness (+b*) and blueness (-b*). The results show that the b* values
197 of cassava stick (25.77) that stored frozen for 2 months was significantly lower ($P<0.05$) than other
198 samples (30.79 – 32.08).

199 Research by Oner and Wall (2012) also found that frozen storage significantly influenced
200 ($P<0.05$) the colour of French fries. The colour change of fried cassava sticks during frozen storage duration
201 might be due to the surface moisture desiccation (Maity *et al.*, 2012). The amount of moisture loss during
202 the frying process also influence the colour of fried cassava stick since moisture loss is associated with
203 crust formation and accelerates the Maillard browning (Sahin, 2000). The Maillard browning occurs due
204 to the reaction between amino acid and reducing sugar during frying and as the result, increases the
205 coloration of yellow, red, and brown colour in fried food (Pedreschi *et al.*, 2005). Another factor that might
206 influence the crust colour of cassava sticks was the heat transfer rate in the cassava sticks during frying
207 (Maity *et al.*, 2012).

208 3.5. *Sensory evaluation*

209 The sensory evaluation results of cassava sticks stored frozen in different storage durations are
210 shown as a spider plot in Figure 3. According to the results, the highest score for the colour attribute was
211 the 0-month storage cassava sticks (5.60) and significantly difference ($P < 0.05$) with other samples (3.20 –
212 4.96). The decrease in panelists' colour preference was due to the colour changes in cassava sticks that
213 tend to be darker and brownish than the regular cassava sticks. Meanwhile, on the hardness attribute,
214 the panelists' preference significantly decreased ($P < 0.05$) as the frozen storage duration increased (5.30
215 – 4.20). This result was in accordance with the objective analysis on hardness, where frozen storage
216 duration significantly increased the hardness of cassava sticks due to the starch retrogradation. The next
217 sensory attribute tested was the crispiness and the results were quite varied. Cassava sticks stored frozen
218 for 0 months (4.88) had no significant difference ($P > 0.05$) with 2 months stored cassava stick (4.66) but
219 showed significant difference ($P < 0.05$) with cassava sticks stored frozen for 1 month (3.86) and 3 months
220 (3.68). This result indicated that panelists perceived that frozen storage duration influenced the crispiness
221 of fried cassava sticks. Furthermore, the frozen storage duration significantly influenced ($P < 0.05$) the
222 panelists' preferences on the aroma of cassava sticks. The aroma of cassava sticks tended to be different
223 for each treatment and might be influenced by the formation of hydroperoxides and free fatty acids. The
224 last sensory attribute evaluated was the taste of cassava sticks. According to the result, the frozen storage
225 duration had no significant difference ($P > 0.05$) on the panelists' perception of the taste of cassava sticks
226 (4.16 – 4.88). Overall, the average score of panelists' preference showed that frozen storage duration did
227 not significantly affect ($P > 0.05$) panelists' acceptance of the cassava stick.

228 **4. Conclusion**

229 Frozen storage can be used to extend the shelf life of food products. However, the use of frozen
230 storage on cassava sticks affects its physical and chemical properties. Different frozen storage duration
231 influenced the oil absorption of cassava sticks, though the moisture content showed no significant change.

232 The effect of frozen storage duration was also significant on the texture of cassava sticks, especially on
233 hardness. Another studied quality characteristic, such as cassava stick surface colour, was substantially
234 declined during the frozen storage. The free fatty acid and peroxide value of cassava sticks was gradually
235 increased with elongation of frozen storage duration. According to the sensory evaluation results, frozen
236 storage duration influenced all of the sensory attributes besides the taste of cassava sticks. Cassava sticks
237 stored frozen for 3 months were still acceptable by the panelists and the acceptance was defined as
238 neither like nor dislike.

239 **Conflict of interest**

240 The authors declare no conflict of interest.

241 **Acknowledgments**

242 This study was financially supported by the Lembaga Penelitian dan Pengabdian Masyarakat (LPPM)
243 Widya Mandala Surabaya Catholic University through PPPG Research Grants 2015/2016.

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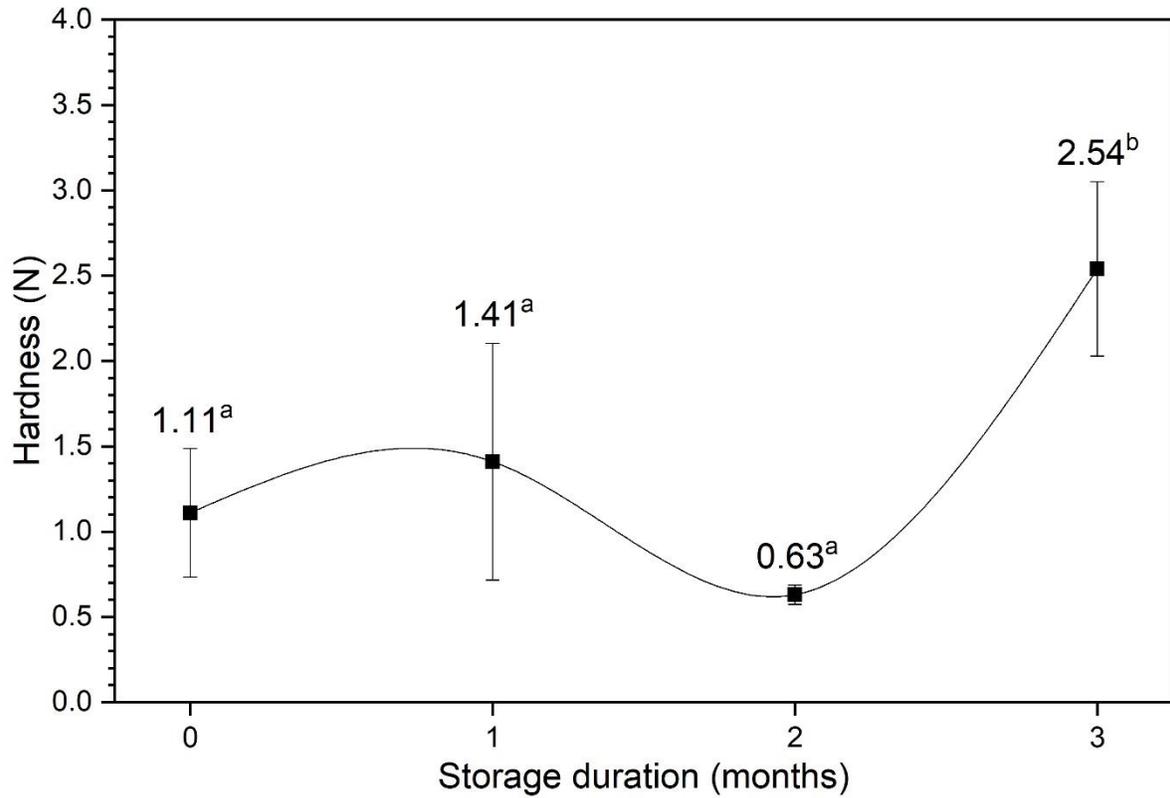
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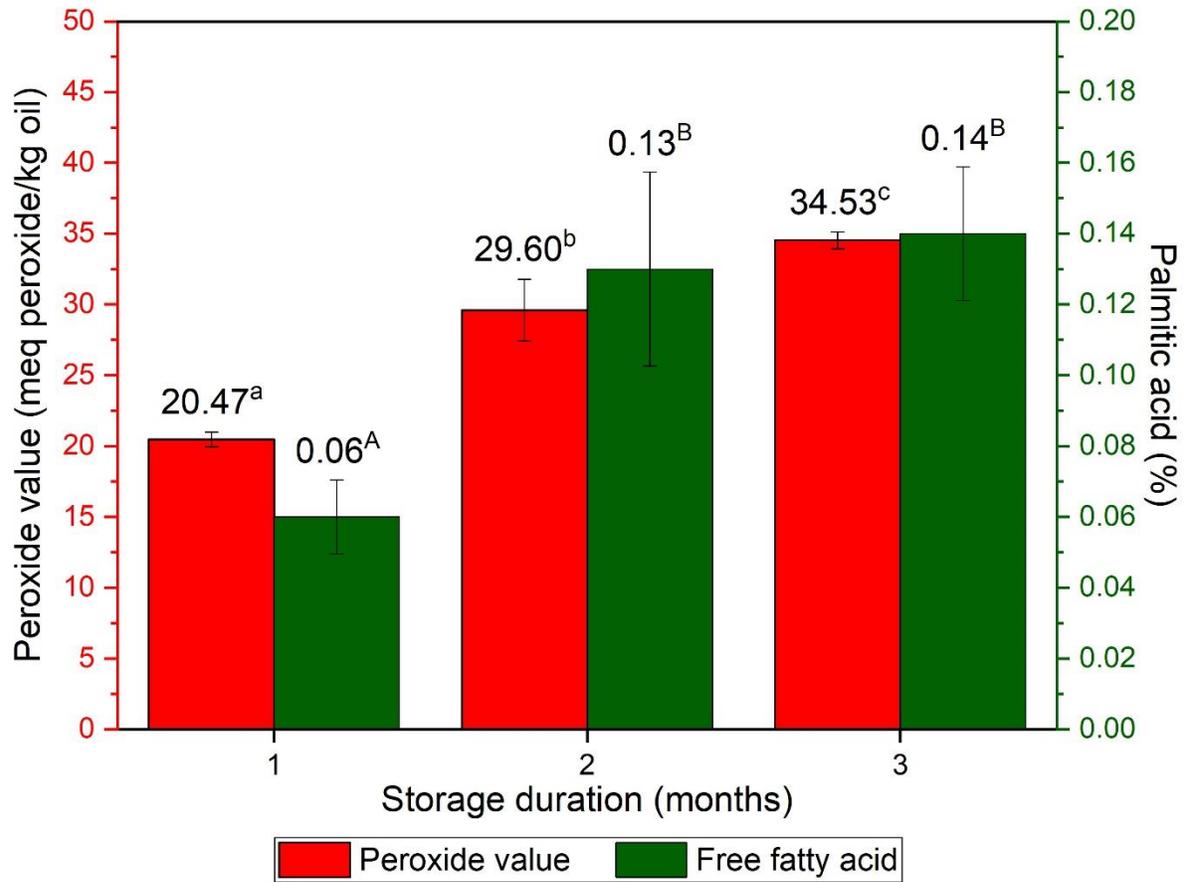
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318 Figure 1. Hardness of cassava stick stored frozen in different storage duration. Means \pm standard
319 deviation (n = 3 for each group) in the same column followed by the different letter are significantly
320 different (P<0.05). The statistical significance was evaluated by one-way ANOVA followed by DMRT test

321 ($\alpha = 0.05$).

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325 Figure 2. Free fatty acid and peroxide value of cassava stick stored frozen in different storage duration.

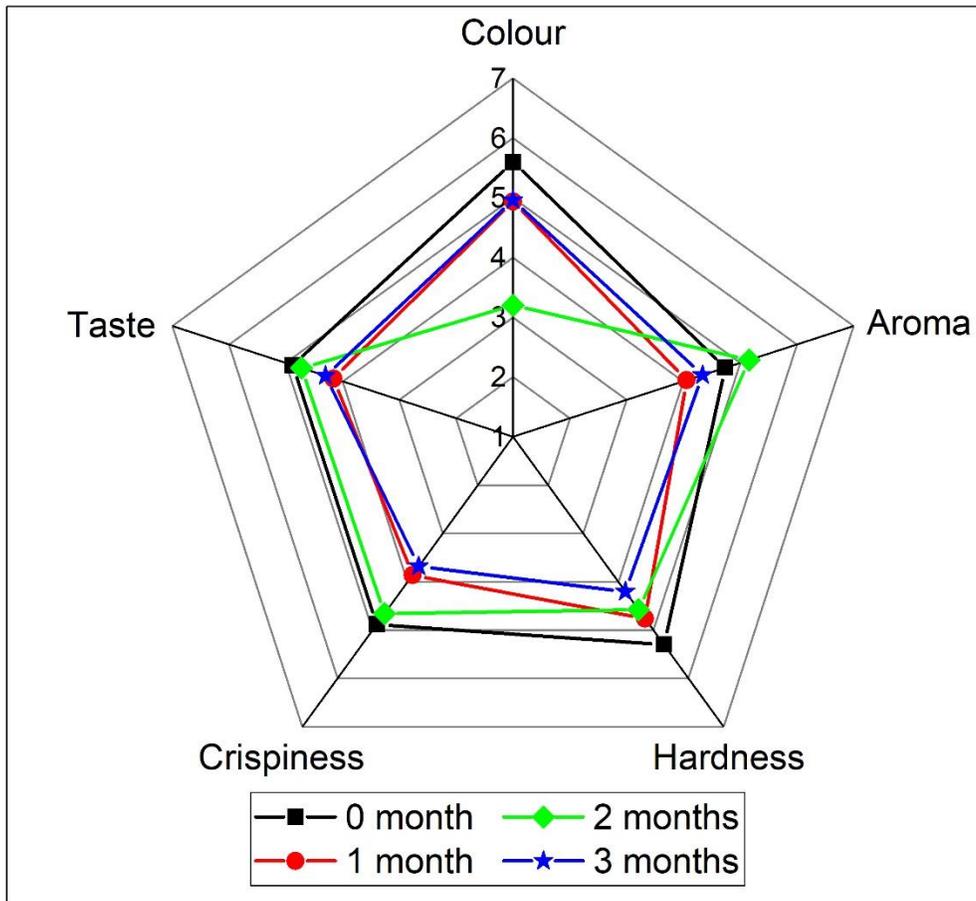
326 Means \pm standard deviation (n = 3 for each group) in the same column followed by the different letter

327 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA

328

followed by DMRT test ($\alpha = 0.05$).

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331 Figure 3. Sensory evaluation of cassava stick stored frozen in different storage duration. Means \pm
 332 standard deviation (n = 100 for each group) in the same column followed by the different letter are
 333 significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA followed by
 334 DMRT test ($\alpha = 0.05$).

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339 **List of Tables**

340 Table 1. Moisture content and oil absorption of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Moisture content (%)	Oil absorption (%)
0	45.13 ± 7.46 ^a	20.87 ± 15.91 ^a
1	48.83 ± 3.27 ^a	25.66 ± 9.51 ^a
2	28.68 ± 7.95 ^a	58.45 ± 2.47 ^b
3	27.75 ± 10.13 ^a	57.94 ± 24.71 ^b

341 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter
 342 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA
 343 followed by DMRT test (α = 0.05).

344 **Table 2.** Colour of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Colour		
	L*	a*	b*
0	71.55 ± 4.08 ^{ab}	2.95 ± 0.78 ^a	32.08 ± 3.02 ^b
1	67.98 ± 2.78 ^a	4.62 ± 1.93 ^a	30.79 ± 1.87 ^b
2	66.52 ± 1.15 ^a	3.21 ± 0.37 ^a	25.77 ± 0.98 ^a
3	75.20 ± 1.80 ^b	4.01 ± 0.30 ^a	31.40 ± 1.66 ^b

345 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter
 346 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA
 347 followed by DMRT test (α = 0.05).

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Bukti konfirmasi submit revisi artikel keempat dan balasannya

(28 Maret 2022)

22 oxidation also occurred during storage, marked by a significant increase ($P < 0.05$) of free fatty acid (0.06
23 to 0.14%) and peroxide value (0 to 34.53 mg peroxide/kg lipid) on three months of frozen storage. Thus,
24 this study concludes that frozen storage duration affected the physical and chemical properties of cassava
25 sticks. Moreover, cassava sticks stored frozen for three months were acceptable for panelists with neither
26 like nor dislike (4.30) average acceptance, and had no significant difference ($P > 0.05$) with other samples.

27 **Keywords:** Cassava, Cassava stick, Frozen storage duration

28 **1. Introduction**

29 Cassava (*Manihot esculenta* Crantz) is one of the most consumed crops in the world, especially in
30 Sub-Saharan Africa (SSA) and in developing countries of Asia such as Cambodia, Vietnam, and Laos (Benesi
31 *et al.*, 2004; International Atomic Energy Agency, 2018). Cassava is considered as the substitute for rice as
32 the primary carbohydrate source and can be consumed in various ways (fried, boiled, or steamed). Besides
33 carbohydrates, cassava is also rich in calcium (50 mg/100 g), phosphorus (40 mg/100 g), vitamin C (25
34 mg/100g) and contains a significant amount of thiamine, riboflavin, and nicotinic acid (International
35 Atomic Energy Agency, 2018). In Indonesia, cassava is generally processed into traditional food such as
36 *tiwul*, *gatot*, *gaplek*, *gethuk*, *tapai*, and cassava chips. Furthermore, it can also be processed into a modern
37 food such as cassava stick.

38 Cassava stick has similar characteristics to French fries due to its shoestring-like shape, golden
39 brown colour, crunchy exterior, and fluffy interior. Those characteristics are obtained through several
40 processes such as blanching, drying, frying, and each step affects the quality of the final product (Lamberg
41 *et al.*, 1990). Other processes are also required to extend shelf life since cassava sticks are classified as
42 perishable food. Among other processes that might be employed for long-term preservation (high
43 pressure, infrared irradiation, pulsed electric field, and ultrasound), freezing or frozen storage is still one

44 of the most used methods (Rahman and Velez-Ruiz, 2007). Generally, frozen storage temperature is 0°F
45 (-18°C) and even colder depending on the type of the food (WFLO Commodity Storage Manual, 2008).

46 Several studies showed that storing food (fruit, meat, and French fries) under freezing temperatures
47 affects the physical and chemical properties (Sattar *et al.*, 2015; Celli *et al.*, 2016; Medic *et al.*, 2018).
48 However, based on authors' knowledge, there is no study reported about the effect of frozen storage on
49 the cassava sticks properties. Therefore, this study aimed to investigate the physical and chemical changes
50 on cassava sticks during frozen storage. In addition, the sensory evaluation of cassava sticks during frozen
51 storage was also studied.

52 **2. Materials and methods**

53 *2.1. Materials*

54 Cassava tubers (*Manihot esculenta* Crantz) used in this study were yellow cassava (Malang 2),
55 purchased from a local market in Malang, Indonesia. The cooking oil was purchased from a market in
56 Surabaya, Indonesia. All chemicals and other reagents used in this study were analytical grades.

57 *2.2. Preparation of cassava stick*

58 Cassava tubers were washed two times using tap water to remove physical contaminations. The
59 cleaned cassava tubers were then peeled, cut into strips of 4 x 1 x 1 cm, and soaked in 0.1% (w/v) CaCl₂
60 for 15 min to increase the crispiness of the cassava stick. Next, the soaked cassava sticks were steamed in
61 a steamer (98°C, 15 min) and cooled to room temperature before being pre-fried (170°C, 30 s) in cassava
62 sticks : cooking oil ratio of 1:12 (w/v) using a deep fryer (Fritel Professional 35 SICO, Belgium). Later, the
63 pre-fried cassava sticks were vacuum packed in a polypropylene plastic bag using a vacuum sealer
64 (Maksindo DZ300, Indonesia) and frozen at -20°C for different storage duration (0, 1, 2, 3 months) in a
65 chest freezer (Modena MD 45, Italy). The frozen cassava sticks were then thawed in a water bath (15 min)
66 and fried for 2 min at the same condition as the pre-frying process. At the end of frying, the fryer basket

67 was immediately shaken for 10 seconds and sticks were cooled to room temperature before analyzing the
68 physicochemical properties.

69 2.3. *Moisture content*

70 All fried cassava stick samples were minced before immediately analyzing the moisture content.
71 The moisture content was determined using a drying oven (Binder ED 53, Germany) with the
72 thermogravimetric method (AOAC, 2006) for 3-5 hours at 105 ± 2 °C.

73 2.4. *Oil absorption analysis*

74 The oil absorption analysis was determined according to the method by Mohammed *et al.* (1988).
75 Approximately 5 g of minced pre-fried and fried cassava sticks were oven-dried at 105°C for 3-5 hours and
76 weighed until a stable weight of the sample was obtained. The oil absorption was calculated using the
77 following equation:

$$78 \text{ Oil absorption (\%)} = \frac{W_2 - W_1}{W_1} \times 100\%$$

79 where W_1 is the weight of pre-fried cassava stick (g) and W_2 is the weight of fried cassava stick (g).

80 2.5. *Hardness*

81 Hardness as the selected texture characteristics were determined using a TA-XT plus (Stable Micro
82 System, UK) equipped with a three-point bend rig using compression test mode with the following
83 conditions: pre-test speed= 2 mm/s, test speed= 0.5 mm/s, post-test speed= 10 mm/s, and distance= 10
84 mm. The hardness of fried cassava stick was expressed as Newton and determined as the maximum force
85 required to compress the sample.

86 2.6. *Free fatty acid analysis*

87 The free fatty acid of cassava sticks was analyzed using a method by Sudarmadji *et al.* (1984).
88 Approximately 28.2 g of fried cassava sticks were minced before adding 50 mL of neutralized alcohol and
89 2 mL phenolphthalein. The mixture was then titrated with 0.1 N NaOH until a stable pink colour occurred

90 for 30 s. The percentage of free fatty acid was expressed as the percentage of palmitic acid and calculated
91 using the following equation:

$$92 \quad \text{Palmitic acid (\%)} = \frac{V_{\text{NaOH}} \times N_{\text{NaOH}} \times \text{Mr}}{W \times 1000} \times 100\%$$

93 where V_{NaOH} is the titre of NaOH (mL), N_{NaOH} is the concentration of NaOH (N), Mr is the molecular
94 weight of palmitic acid (256.42 g/mol), and W is the sample weight (g).

95 2.7. Peroxide value

96 An iodometric titration method was used to analyze the peroxide value as the primary oxidation
97 product (Sudarmadji *et al.*, 1984). Briefly, 5 g of fried cassava sticks were mixed with 30 mL acetic acid –
98 chloroform (3:2) and 0.5 mL saturated aqueous potassium iodide solution was added. Later, the mixture
99 was shaken vigorously for 1 minute before 30 mL distilled water was added. The mixture was titrated with
100 0.1 M $\text{Na}_2\text{S}_2\text{O}_3$ until the yellow colour had almost disappeared. Next, 0.5 mL of 1% (w/v) starch indicator
101 solution was added and the titration continued until the blue colour disappeared. The peroxide value was
102 calculated using the following formula:

$$103 \quad \text{Peroxide value (meq peroxide/kg oil)} = \frac{V_{\text{thio}} \times N_{\text{thio}} \times 1000}{W}$$

104 where V_{thio} is the titre of $\text{Na}_2\text{S}_2\text{O}_3$ (mL), N_{thio} is the concentration of $\text{Na}_2\text{S}_2\text{O}_3$, and W is the sample
105 weight (g).

106 2.8. Colour

107 The colour of fried cassava stick was identified using a colour reader (Colour Reader Minolta, CR-
108 10). The L^* value (0 = blackness, 100 = whiteness), a^* value (+ = redness, - = greenness), and b^* value (+ =
109 yellowness, - = blueness) of each sample were observed.

110 2.9. Sensory evaluation

111 In this study, the Hedonic Scale Scoring (preferred test) was used to measure the level of preference
112 and acceptance of the product by consumers (Kusuma *et al.*, 2017). The sensory evaluation was evaluated

113 by 100 untrained panelists. The panelists were students of the Faculty of Agricultural Technology, Widya
114 Mandala Catholic University Surabaya. The sensory attributes tested were colour, hardness, crispiness,
115 aroma, and taste. A scoring system of 1-7 points was used to represent the sensory characteristics of each
116 sample. On this scale, 1 was defined as dislike extremely, 2 was defined as dislike moderately, 3 was
117 defined as dislike slightly, 4 was defined as neither like nor dislike, 5 was defined as like slightly, 6 was
118 defined as like moderately, and 7 was defined as like extremely.

119 2.10. Statistical analysis

120 All experiments were analyzed at least three times in triplicate and represented as mean values
121 \pm SD. Statistical analyses were performed using SPSS for Windows (version 19.0, SPSS Inc., USA) and
122 compared with Duncan Multiple Range Test (DMRT) at $P < 0.05$.

123 3. Results and discussion

124 3.1. Moisture content and oil absorption

125 The moisture content of fried cassava sticks is shown in Table 1. Based on the result, different frozen
126 storage durations have no significant difference ($P > 0.05$) on the moisture content of cassava sticks, even
127 though there was a downward trend in the moisture content of frozen cassava sticks for 2 and 3 months.
128 Similar results were reported by Medic *et al.* (2018), where frozen storage duration had no significant on
129 the moisture content of pork loin and belly rib. However, several researchers also found that freezing
130 significantly ($P < 0.05$) influenced the moisture content of the fried potato (Adedeji and Ngadi, 2017), lamb
131 (Coombs *et al.*, 2017), and pork ham (Medic *et al.*, 2018). According to Fennema (1985), water in food
132 converts to ice crystals during freezing at -18°C and easily removed from food when the food is dried or
133 fried; so that the moisture content decreases.

134 Oil absorption analysis was conducted to measure the ability of cassava sticks to absorb oil during
135 frying by using a modifying thermogravimetric method (Mohamed *et al.*, 1988). Table 1 showed that
136 frozen storage duration significantly increased ($P < 0.05$) the oil absorption of cassava sticks. Cassava stick

137 stored frozen for 2 months has the highest oil absorption (58.45%) but has no significant difference with
138 cassava stick stored frozen for 3 months (57.84%). In Adedeji's and Ngadi's (2017) study, oil absorption
139 also showed a significant increase in fried potato that was stored frozen. However, they also found that
140 there was no interaction between storage duration and freezing method, which contradicts this study.

141 According to Adedeji *et al.* (2009), moisture loss during frying affects the amount of oil absorbed in
142 fried food. This statement is in accordance with Fellows (1990) statement, where there is an oil transfer
143 into the product to replace evaporated water during frying. In addition, moisture evaporation from fried
144 food during frying damages the cellular structure of plant tissues, resulting in an increase in porosity which
145 contributes to oil absorption (Mellema, 2003; Rimac-Brncic *et al.*, 2004; Dana and Saguy, 2006). On the
146 other hand, during frozen storage, all water in food converts to ice crystal and causes volume expansion
147 which is characterized by the damage to cell membranes during ice crystal formation (Fennema, 1985;
148 Celli *et al.*, 2016). This volume expansion causes the water in cassava sticks to be easier to evaporate
149 during frying and increases oil absorption.

150 3.2. Hardness

151 In this study, hardness was chosen as the analyzed texture attribute to evaluate the impact of frozen
152 storage duration on the texture of cassava sticks. According to Szczesniak (2002), hardness is the force
153 required to change the shape of material due to its resistance to resist deformation. The effect of frozen
154 storage duration on the hardness of cassava sticks is presented in Figure 1. As shown in that figure, frozen
155 storage duration significantly affected ($P < 0.05$) the hardness of the cassava sticks. Cassava stick that was
156 stored frozen for 3 months has the highest force (2.54) among other samples (0.63 – 1.41). This results
157 were in accordance with the results of Adedeji and Ngadi (2017). In that study, they found a marginal
158 increase in the hardness of fried potatoes as the frozen storage duration increased. Therefore, the
159 increase of fried cassava sticks' hardness might be due to the retrogradation of cassava starch during
160 frozen storage. This statement was supported by a similar study by Yu *et al.* (2010), where the hardness

161 of cooked rice increased continually with the amylopectin retrogradation during frozen storage. Based on
162 those findings, amylopectin retrogradation contributed to the hardness increase during storage on the
163 high starch food products.

164 3.3. Free fatty acid and peroxide value

165 This study assessed the percentage of free fatty acid and peroxide values to monitor the fat
166 oxidation in cassava sticks during frozen storage. The increase of free fatty acid and peroxide value
167 indicates the rancidity of food products that produce off-flavors (Maity *et al.*, 2012) and might reduce
168 consumers' preferences. The free fatty acid of cassava stick was expressed as the percentage of palmitic
169 acid since the cooking oil used to fry was palm oil. Meanwhile, the peroxide value as the primary oxidation
170 product of fat oxidation was expressed as meq peroxide/kg fat. The percentage of free fatty acid and
171 peroxide value of fried cassava sticks is shown in Figure 2. A significant increased ($P<0.05$) of free fatty
172 acid was recorded during 2 months of frozen storage (0.13 %) and had no significant difference ($P>0.05$)
173 with cassava sticks stored frozen for 3 months (0.14%). On the other hand, the peroxide value of cassava
174 stick was also significantly increased ($P<0.05$) as the frozen storage duration increased (0 – 34.53 meq
175 peroxide/ kg fat). Similar results were also reported by some studies where frozen storage duration
176 increased the free fatty acid and peroxide value of potato strips (Kizito *et al.*, 2017), pork meat (Medic *et*
177 *al.*, 2018), and ready-to-fry vegetable snacks (Maity *et al.*, 2012).

178 According to Crosa *et al.* (2014), free fatty acids are formed through the nucleophilic attack at
179 triacylglycerol's ester bond and have been associated with the undesirable odors and taste of food
180 products. Furthermore, the formation of hydroperoxides during storage generally occurred during the
181 early stage of oxidation (Fennema, 1985) and later decomposed to other secondary oxidation products
182 such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (Kizito *et al.*, 2017; Medic *et al.*, 2018).
183 Another factor that might increase the formation of hydroperoxides was the contact of cassava stick with

184 oxygen before and after storage; since oxygen is one of the reactants that caused fat oxidation (Giri and
185 Mangaraj, 2012).

186 3.4. Colour properties

187 Colour is generally considered an important attribute that significantly affects the physical
188 properties of food, the perception of consumers, and determines the nutritional quality of food products
189 (Hutchings, 2002; Patras *et al.*, 2011). In this study, the colour of cassava sticks is shown in Table 2 and
190 was measured using Hunter's L*, a*, and b* colour attributes. The L* value expressed the degree of
191 lightness, where the highest value was measured in the cassava stick stored frozen for 3 months (75.20).
192 However, the results were not significantly different ($P>0.05$) with cassava stick that was not stored frozen
193 (71.55) and significantly different ($P<0.05$) with other samples (66.52 – 67.98). The different results were
194 shown on the a* value, which expressed the degree of redness (+a*) and greenness (-a*) of cassava sticks.
195 All samples had no significant difference ($P>0.05$) on the degree of redness (2.94 – 4.62). Meanwhile, the
196 b* value expressed the degree of yellowness (+b*) and blueness (-b*). The results show that the b* values
197 of cassava stick (25.77) that stored frozen for 2 months was significantly lower ($P<0.05$) than other
198 samples (30.79 – 32.08).

199 Research by Oner and Wall (2012) also found that frozen storage significantly influenced
200 ($P<0.05$) the colour of French fries. The colour change of fried cassava sticks during frozen storage duration
201 might be due to the surface moisture desiccation (Maity *et al.*, 2012). The amount of moisture loss during
202 the frying process also influence the colour of fried cassava stick since moisture loss is associated with
203 crust formation and accelerates the Maillard browning (Sahin, 2000). The Maillard browning occurs due
204 to the reaction between amino acid and reducing sugar during frying and as the result, increases the
205 coloration of yellow, red, and brown colour in fried food (Pedreschi *et al.*, 2005). Another factor that might
206 influence the crust colour of cassava sticks was the heat transfer rate in the cassava sticks during frying
207 (Maity *et al.*, 2012).

208 3.5. *Sensory evaluation*

209 The sensory evaluation results of cassava sticks stored frozen in different storage durations are
210 shown as a spider plot in Figure 3. According to the results, the highest score for the colour attribute was
211 the 0-month storage cassava sticks (5.60) and significantly difference ($P < 0.05$) with other samples (3.20 –
212 4.96). The decrease in panelists' colour preference was due to the colour changes in cassava sticks that
213 tend to be darker and brownish than the regular cassava sticks. Meanwhile, on the hardness attribute,
214 the panelists' preference significantly decreased ($P < 0.05$) as the frozen storage duration increased (5.30
215 – 4.20). This result was in accordance with the objective analysis on hardness, where frozen storage
216 duration significantly increased the hardness of cassava sticks due to the starch retrogradation. The next
217 sensory attribute tested was the crispiness and the results were quite varied. Cassava sticks stored frozen
218 for 0 months (4.88) had no significant difference ($P > 0.05$) with 2 months stored cassava stick (4.66) but
219 showed significant difference ($P < 0.05$) with cassava sticks stored frozen for 1 month (3.86) and 3 months
220 (3.68). This result indicated that panelists perceived that frozen storage duration influenced the crispiness
221 of fried cassava sticks. Furthermore, the frozen storage duration significantly influenced ($P < 0.05$) the
222 panelists' preferences on the aroma of cassava sticks. The aroma of cassava sticks tended to be different
223 for each treatment and might be influenced by the formation of hydroperoxides and free fatty acids. The
224 last sensory attribute evaluated was the taste of cassava sticks. According to the result, the frozen storage
225 duration had no significant difference ($P > 0.05$) on the panelists' perception of the taste of cassava sticks
226 (4.16 – 4.88). Overall, the average score of panelists' preference showed that frozen storage duration did
227 not significantly affect ($P > 0.05$) panelists' acceptance of the cassava stick.

228 **4. Conclusion**

229 Frozen storage can be used to extend the shelf life of food products. However, the use of frozen
230 storage on cassava sticks affects its physical and chemical properties. Different frozen storage duration
231 influenced the oil absorption of cassava sticks, though the moisture content showed no significant change.

232 The effect of frozen storage duration was also significant on the texture of cassava sticks, especially on
233 hardness. Another studied quality characteristic, such as cassava stick surface colour, was substantially
234 declined during the frozen storage. The free fatty acid and peroxide value of cassava sticks was gradually
235 increased with elongation of frozen storage duration. According to the sensory evaluation results, frozen
236 storage duration influenced all of the sensory attributes besides the taste of cassava sticks. Cassava sticks
237 stored frozen for 3 months were still acceptable by the panelists and the acceptance was defined as
238 neither like nor dislike.

239 **Conflict of interest**

240 The authors declare no conflict of interest.

241 **Acknowledgments**

242 This study was financially supported by the Lembaga Penelitian dan Pengabdian Masyarakat (LPPM)
243 Widya Mandala Surabaya Catholic University through PPPG Research Grants 2015/2016.

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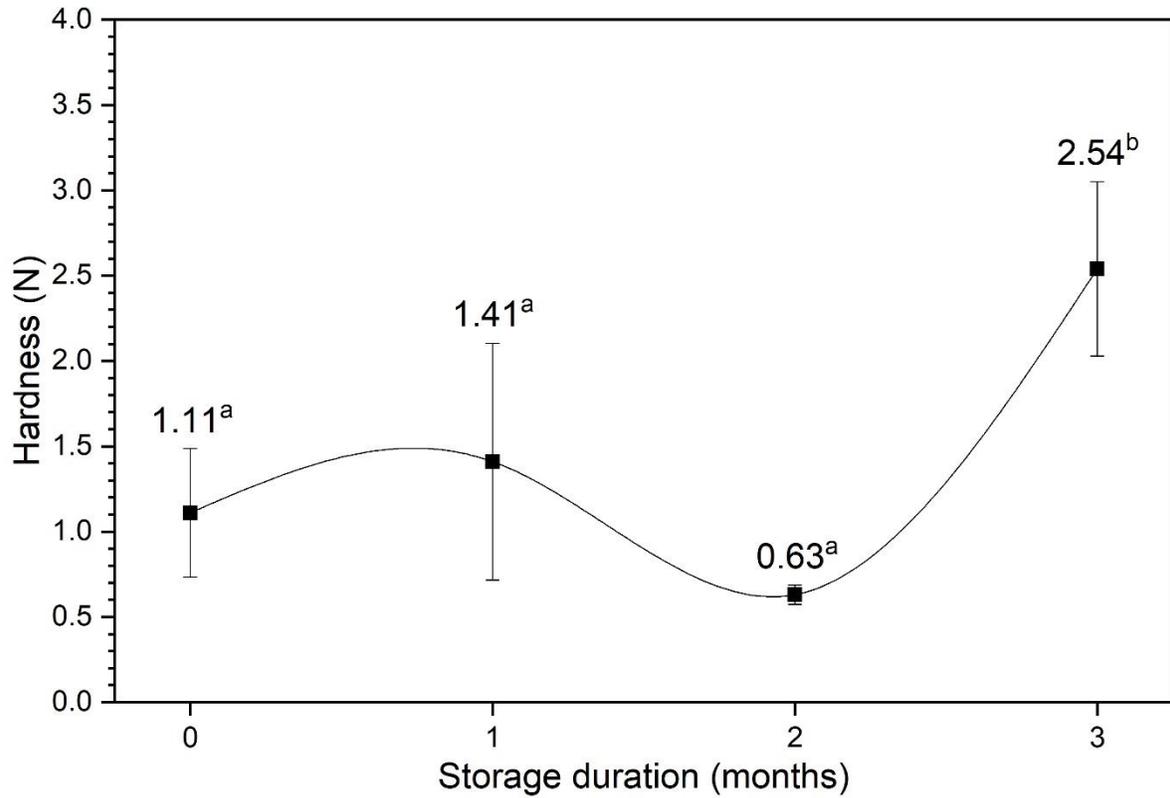
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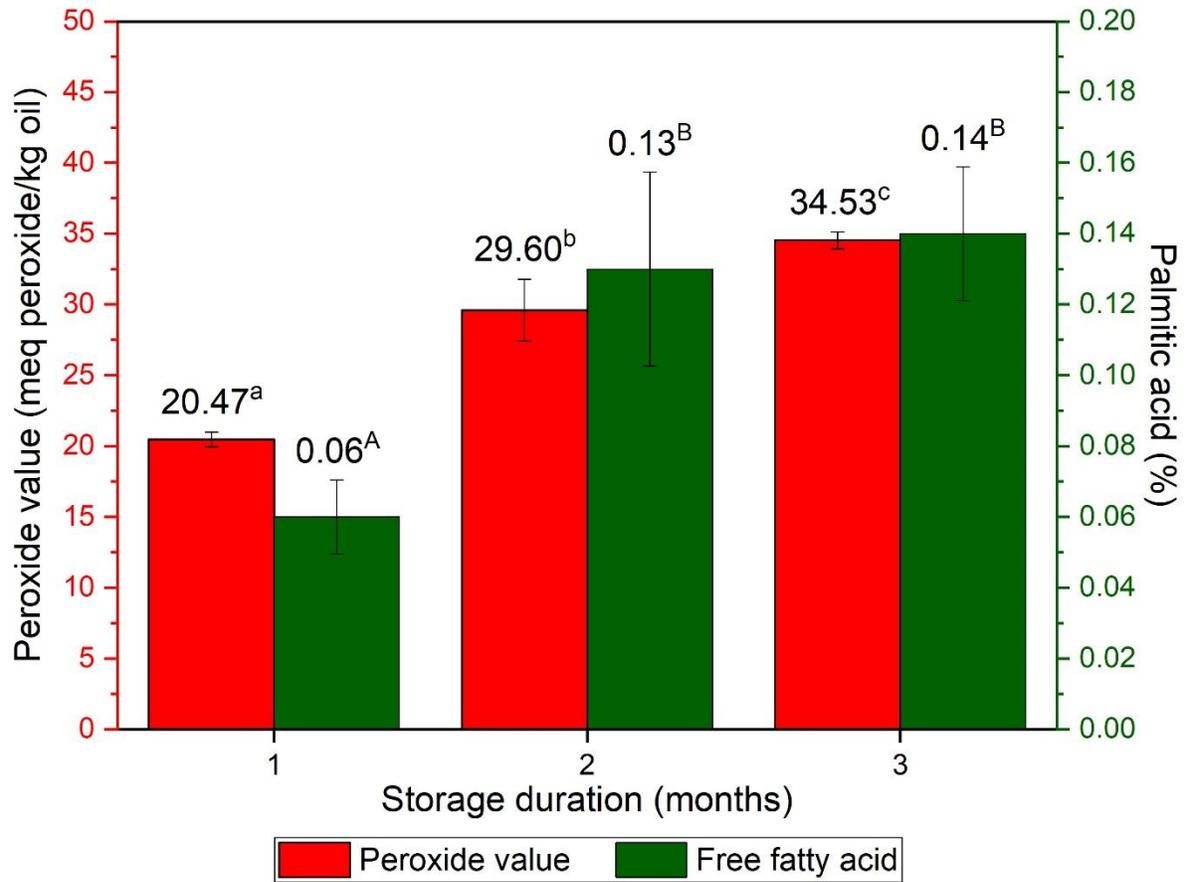
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318 Figure 1. Hardness of cassava stick stored frozen in different storage duration. Means \pm standard
319 deviation (n = 3 for each group) in the same column followed by the different letter are significantly
320 different (P<0.05). The statistical significance was evaluated by one-way ANOVA followed by DMRT test

321 ($\alpha = 0.05$).

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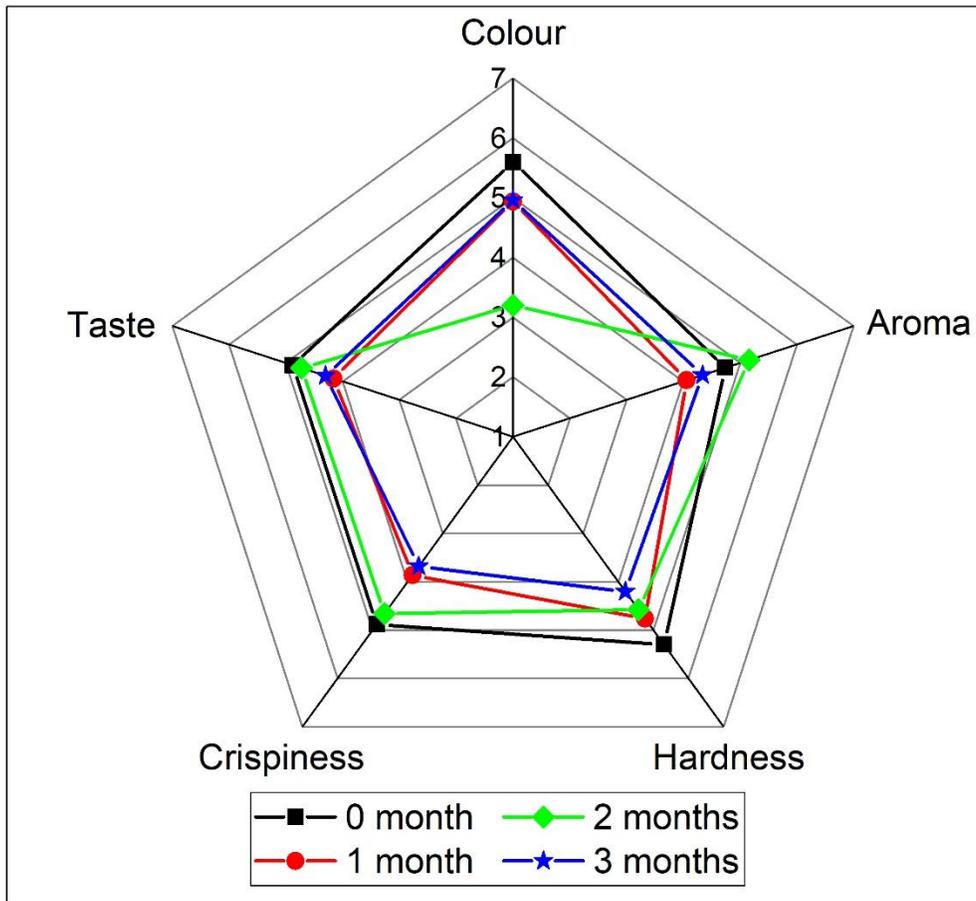
325 Figure 2. Free fatty acid and peroxide value of cassava stick stored frozen in different storage duration.

326 Means \pm standard deviation (n = 3 for each group) in the same column followed by the different letter

327 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA

328 followed by DMRT test ($\alpha = 0.05$).

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331 Figure 3. Sensory evaluation of cassava stick stored frozen in different storage duration. Means \pm
 332 standard deviation (n = 100 for each group) in the same column followed by the different letter are
 333 significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA followed by
 334 DMRT test ($\alpha = 0.05$).

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338

339 **List of Tables**

340 Table 1. Moisture content and oil absorption of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Moisture content (%)	Oil absorption (%)
0	45.13 ± 7.46 ^a	20.87 ± 15.91 ^a
1	48.83 ± 3.27 ^a	25.66 ± 9.51 ^a
2	28.68 ± 7.95 ^a	58.45 ± 2.47 ^b
3	27.75 ± 10.13 ^a	57.94 ± 24.71 ^b

341 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter
 342 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA
 343 followed by DMRT test (α = 0.05).

344 **Table 2.** Colour of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Colour		
	L*	a*	b*
0	71.55 ± 4.08 ^{ab}	2.95 ± 0.78 ^a	32.08 ± 3.02 ^b
1	67.98 ± 2.78 ^a	4.62 ± 1.93 ^a	30.79 ± 1.87 ^b
2	66.52 ± 1.15 ^a	3.21 ± 0.37 ^a	25.77 ± 0.98 ^a
3	75.20 ± 1.80 ^b	4.01 ± 0.30 ^a	31.40 ± 1.66 ^b

345 Means ± standard deviation (n = 3 for each group) in the same column followed by the different letter
 346 are significantly different (P<0.05). The statistical significance was evaluated by one-way ANOVA
 347 followed by DMRT test (α = 0.05).

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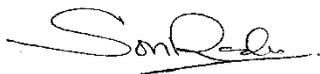
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Effects of frozen storage duration on the physicochemical and sensory properties of cassava sticks

*Trisnawati, C.Y., Sutedja, A.M. and Kaharso, V.C.

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Article history:

Received: 5 February 2022

Received in revised form: 28

March 2022

Accepted: 31 March 2022

Available Online:

Keywords:

Cassava,

Cassava stick,

Frozen storage duration

DOI:

Abstract

Cassava sticks is a processed food made from cassava that has similar characteristics to French fries. However, cassava sticks are also classified as perishable food, so it is required to employ other processes that might increase its preservation, such as frozen storage. In this study, the cassava stick was stored at -20°C for three months to investigate its effects on the physicochemical and sensory properties of cassava sticks. The effects of frozen storage duration were monitored every month carried out in three replications. A randomized block design with a single factor was used as the experimental design. According to the results, storing cassava sticks under frozen conditions significantly increased ($P<0.05$) the oil absorption and had no effect ($P>0.05$) on the moisture content. A significant alteration ($P<0.05$) in texture was observed through the increase of cassava stick hardness from 1.11 to 2.54 N. Frozen storage duration also influenced ($P<0.05$) the lightness and yellowness, but not the redness ($P>0.05$) of cassava sticks. Fat oxidation also occurred during storage, marked by a significant increase ($P<0.05$) of free fatty acid (0.06 to 0.14%) and peroxide value (0 to 34.53 mg peroxide/kg lipid) on three months of frozen storage. Thus, this study concludes that frozen storage duration affected the physical and chemical properties of cassava sticks. Moreover, cassava sticks stored frozen for three months were acceptable for panelists with neither like nor dislike (4.30) average acceptance, and had no significant difference ($P>0.05$) with other samples.

1. Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most consumed crops in the world, especially in Sub-Saharan Africa (SSA) and in developing countries of Asia such as Cambodia, Vietnam, and Laos (Benesi *et al.*, 2004; International Atomic Energy Agency, 2018). Cassava is considered a substitute for rice as the primary carbohydrate source and can be consumed in various ways (fried, boiled, or steamed). Besides carbohydrates, cassava is also rich in calcium (50 mg/100 g), phosphorus (40 mg/100 g), vitamin C (25 mg/100g) and contains a significant amount of thiamine, riboflavin, and nicotinic acid (International Atomic Energy Agency, 2018). In Indonesia, cassava is generally processed into traditional food such as *tiwul*, *gatot*, *gapek*, *gethuk*, *tapai*, and cassava chips. Furthermore, it can also be processed into a modern food such as cassava stick.

Cassava stick has similar characteristics to French fries due to its shoestring-like shape, golden brown colour, crunchy exterior, and fluffy interior. Those characteristics are obtained through several processes

such as blanching, drying, frying, and each step affects the quality of the final product (Lamberg *et al.*, 1990). Other processes are also required to extend shelf life since cassava sticks are classified as perishable food. Among other processes that might be employed for long-term preservation (high pressure, infrared irradiation, pulsed electric field, and ultrasound), freezing or frozen storage is still one of the most used methods (Rahman and Velez-Ruiz, 2007). Generally, frozen storage temperature is 0°F (-18°C) and even colder depending on the type of food (WFLO Commodity Storage Manual, 2008).

Several studies showed that storing food (fruit, meat, and French fries) under freezing temperatures affects the physical and chemical properties (Sattar *et al.*, 2015; Celli *et al.*, 2016; Medic *et al.*, 2018). However, based on authors' knowledge, there is no study reported about the effect of frozen storage on the cassava sticks properties. Therefore, this study aimed to investigate the physical and chemical changes on cassava sticks during frozen storage. In addition, the sensory evaluation of

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cassava sticks during frozen storage was also studied.

2. Materials and methods

2.1 Materials

Cassava tubers (*Manihot esculenta* Crantz) used in this study were yellow cassava (Malang 2), purchased from a local market in Malang, Indonesia. The cooking oil was purchased from a market in Surabaya, Indonesia. All chemicals and other reagents used in this study were analytical grades.

2.2 Preparation of cassava stick

Cassava tubers were washed two times using tap water to remove physical contaminations. The cleaned cassava tubers were then peeled, cut into strips of 4×1×1 cm, and soaked in 0.1% (w/v) CaCl₂ for 15 mins to increase the crispiness of the cassava stick. Next, the soaked cassava sticks were steamed in a steamer (98°C, 15 mins) and cooled to room temperature before being pre-fried (170°C, 30 s) in cassava sticks : cooking oil ratio of 1:12 (w/v) using a deep fryer (Fritel Professional 35 SICO, Belgium). Later, the pre-fried cassava sticks were vacuum packed in a polypropylene plastic bag using a vacuum sealer (Maksindo DZ300, Indonesia) and frozen at -20°C for different storage duration (0, 1, 2, 3 months) in a chest freezer (Modena MD 45, Italy). The frozen cassava sticks were then thawed in a water bath (15 mins) and fried for 2 mins at the same condition as the pre-frying process. At the end of frying, the fryer basket was immediately shaken for 10 s and sticks were cooled to room temperature before analyzing the physicochemical properties.

2.3 Moisture content

All fried cassava stick samples were minced before immediately analyzing the moisture content. The moisture content was determined using a drying oven (Binder ED 53, Germany) with the thermogravimetric method (AOAC, 2006) for 3-5 hrs at 105±2°C.

2.4 Oil absorption analysis

The oil absorption analysis was determined according to the method by Mohamed *et al.* (1988). Approximately 5 g of minced pre-fried and fried cassava sticks were oven-dried at 105°C for 3-5 hrs and weighed until a stable weight of the sample was obtained. The oil absorption was calculated using the following equation:

$$\text{Oil absorption (\%)} = \frac{W_2 - W_1}{W_1} \times 100\%$$

where W₁ is the weight of pre-fried cassava stick (g) and W₂ is the weight of fried cassava stick (g).

2.5 Hardness

Hardness as the selected texture characteristics were determined using a TA-XT plus (Stable Micro System, UK) equipped with a three-point bend rig using compression test mode with the following conditions: pre-test speed= 2 mm/s, test speed= 0.5 mm/s, post-test speed= 10 mm/s, and distance= 10 mm. The hardness of fried cassava stick was expressed as Newton and determined as the maximum force required to compress the sample.

2.6 Free fatty acid analysis

The free fatty acid of cassava sticks was analyzed using a method by Sudarmadji *et al.* (1984). Approximately 28.2 g of fried cassava sticks were minced before adding 50 mL of neutralized alcohol and 2 mL phenolphthalein. The mixture was then titrated with 0.1 N NaOH until a stable pink colour occurred for 30 s. The percentage of free fatty acid was expressed as the percentage of palmitic acid and calculated using the following equation:

$$\text{Palmitic acid (\%)} = \frac{V_{\text{NaOH}} \times N_{\text{NaOH}} \times Mr}{W \times 1000} \times 100\%$$

where V_{NaOH} is the titre of NaOH (mL), N_{NaOH} is the concentration of NaOH (N), Mr is the molecular weight of palmitic acid (256.42 g/mol), and W is the sample weight (g).

2.7 Peroxide value

An iodometric titration method was used to analyze the peroxide value as the primary oxidation product (Sudarmadji *et al.*, 1984). Briefly, 5 g of fried cassava sticks were mixed with 30 mL acetic acid – chloroform (3:2) and 0.5 mL saturated aqueous potassium iodide solution was added. Later, the mixture was shaken vigorously for 1 min before 30 mL of distilled water was added. The mixture was titrated with 0.1 M Na₂S₂O₃ until the yellow colour had almost disappeared. Next, 0.5 mL of 1% (w/v) starch indicator solution was added and the titration continued until the blue colour disappeared. The peroxide value was calculated using the following formula:

$$\text{Peroxide value (meq peroxide/kg oil)} = \frac{V_{\text{thio}} \times N_{\text{thio}} \times 1000}{W}$$

where V_{thio} is the titre of Na₂S₂O₃ (mL), N_{thio} is the concentration of Na₂S₂O₃, and W is the sample weight (g).

2.8 Colour

The colour of fried cassava stick was identified using a colour reader (Colour Reader Minolta, CR-10). The L* value (0 = blackness, 100 = whiteness), a* value (+ = redness, - = greenness), and b* value (+ = yellowness, -

= blueness) of each sample were observed.

2.9 Sensory evaluation

In this study, the Hedonic Scale Scoring (preferred test) was used to measure the level of preference and acceptance of the product by consumers (Kusuma *et al.*, 2017). The sensory evaluation was evaluated by 100 untrained panelists. The panelists were students of the Faculty of Agricultural Technology, Widya Mandala Catholic University Surabaya. The sensory attributes tested were colour, hardness, crispiness, aroma, and taste. A scoring system of 1-7 points was used to represent the sensory characteristics of each sample. On this scale, 1 was defined as dislike extremely, 2 was defined as dislike moderately, 3 was defined as dislike slightly, 4 was defined as neither like nor dislike, 5 was defined as like slightly, 6 was defined as like moderately, and 7 was defined as like extremely.

2.10 Statistical analysis

All experiments were analyzed at least three times in triplicate and represented as mean values \pm SD. Statistical analyses were performed using SPSS for Windows (version 19.0, SPSS Inc., USA) and compared with Duncan Multiple Range Test (DMRT) at $P < 0.05$.

3. Results and discussion

3.1 Moisture content and oil absorption

The moisture content of fried cassava sticks is shown in Table 1. Based on the result, different frozen storage durations have no significant difference ($P > 0.05$) on the moisture content of cassava sticks, even though there was a downward trend in the moisture content of frozen cassava sticks for 2 and 3 months. Similar results were reported by Medic *et al.* (2018), where frozen storage duration had no significant effect on the moisture content of pork loin and belly rib. However, several researchers also found that freezing significantly ($P < 0.05$) influenced the moisture content of the fried potato (Adedeji and Ngadi, 2017), lamb (Coombs *et al.*, 2017), and pork ham (Medic *et al.*, 2018). According to Fennema (1985), water in food converts to ice crystals during freezing at -18°C and is easily removed from food when the food is dried or fried, causing the moisture content to decrease.

Oil absorption analysis was conducted to measure the ability of cassava sticks to absorb oil during frying by using a modified thermogravimetric method (Mohamed *et al.*, 1988). Table 1 shows that the storage duration significantly increased ($P < 0.05$) the oil absorption of cassava sticks. Cassava stick stored frozen for 2 months has the highest oil absorption (58.45%) but has no significant difference with cassava stick stored frozen for

3 months (57.84%). In a study conducted by Adedeji and Ngadi (2017), oil absorption also showed a significant increase in fried potato that was stored frozen. However, they also found that there was no interaction between storage duration and freezing method, which contradicts this study.

Table 1. Moisture content and oil absorption of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Moisture content (%)	Oil absorption (%)
0	45.13 \pm 7.46 ^a	20.87 \pm 15.91 ^a
1	48.83 \pm 3.27 ^a	25.66 \pm 9.51 ^a
2	28.68 \pm 7.95 ^a	58.45 \pm 2.47 ^b
3	27.75 \pm 10.13 ^a	57.94 \pm 24.71 ^b

Values are presented as mean \pm SD (n = 3 for each group). Values with different superscript are significantly different ($P < 0.05$) by one-way ANOVA followed by DMRT test ($\alpha = 0.05$).

According to Adedeji *et al.* (2009), moisture loss during frying affects the amount of oil absorbed in fried food. This statement is in accordance with Fellows (1990), where oil transfers into the product to replace evaporated water during frying. In addition, moisture evaporation from fried food during frying damages the cellular structure of plant tissues, resulting in an increase in porosity which contributes to oil absorption (Mellema, 2003; Rimac-Brncic *et al.*, 2004; Dana and Saguy, 2006). On the other hand, during frozen storage, all water in food converts to ice crystal and causes volume expansion which is characterized by the damage to cell membranes during ice crystal formation (Fennema, 1985; Celli *et al.*, 2016). This volume expansion causes the water in cassava sticks to be easier to evaporate during frying and increases oil absorption.

3.2 Hardness

In this study, hardness was chosen as the analyzed texture attribute to evaluate the impact of frozen storage duration on the texture of cassava sticks. According to Szczesniak (2002), hardness is the force required to change the shape of material due to its resistance to resist deformation. The effect of frozen storage duration on the hardness of cassava sticks is presented in Figure 1. As shown in that figure, frozen storage duration significantly affected ($P < 0.05$) the hardness of the cassava sticks. Cassava stick that was stored frozen for 3 months has the highest force (2.54) among other samples (0.63 – 1.41). These results were in accordance to Adedeji and Ngadi (2017). They found a marginal increase in the hardness of fried potatoes as the frozen storage duration increased. Therefore, the increase in fried cassava stick hardness might be due to the retrogradation of cassava starch during frozen storage. This statement was supported by a similar study by Yu *et*

al. (2010), where the hardness of cooked rice increased continually with the amylopectin retrogradation during frozen storage. Based on those findings, amylopectin retrogradation contributed to the hardness increase during storage on the high starch food products.

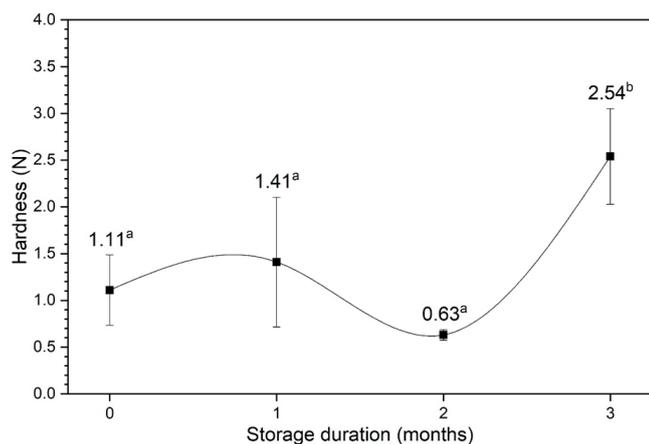


Figure 1. Hardness of cassava stick stored frozen in different storage duration. Values are presented as mean±SD (n = 3 for each group). Values with different superscript are significantly different (P<0.05) by one-way ANOVA followed by DMRT test ($\alpha = 0.05$).

3.3 Free fatty acid and peroxide value

This study assessed the percentage of free fatty acid and peroxide values to monitor the fat oxidation in cassava sticks during frozen storage. The increase of free fatty acid and peroxide value indicates the rancidity of food products that produce off-flavors (Maity *et al.*, 2012) and might reduce consumers' preferences. The free fatty acid of cassava stick was expressed as the percentage of palmitic acid since the cooking oil used to fry was palm oil. Meanwhile, the peroxide value as the primary oxidation product of fat oxidation was expressed as meq peroxide/kg fat. The percentage of free fatty acid and peroxide value of fried cassava sticks is shown in Figure 2. A significant increased (P<0.05) of free fatty acid was recorded during 2 months of frozen storage (0.13 %) and had no significant difference (P>0.05) with cassava sticks stored frozen for 3 months (0.14%). On the other hand, the peroxide value of cassava stick was also significantly increased (P<0.05) as the frozen storage duration increased (0 – 34.53 meq peroxide/ kg fat). Similar results were also reported by some studies where frozen storage duration increased the free fatty acid and peroxide value of potato strips (Kizito *et al.*, 2017), pork meat (Medic *et al.*, 2018), and ready-to-fry vegetable snacks (Maity *et al.*, 2012).

According to Crosa *et al.* (2014), free fatty acids are formed through the nucleophilic attack at triacylglycerol's ester bond and have been associated with the undesirable odors and taste of food products. Furthermore, the formation of hydroperoxides during storage generally occurred during the early stage of

oxidation (Fennema, 1985) and later decomposed to other secondary oxidation products such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (Kizito *et al.*, 2017; Medic *et al.*, 2018). Another factor that might increase the formation of hydroperoxides was the contact of cassava stick with oxygen before and after storage; since oxygen is one of the reactants that caused fat oxidation (Giri and Mangaraj, 2012).

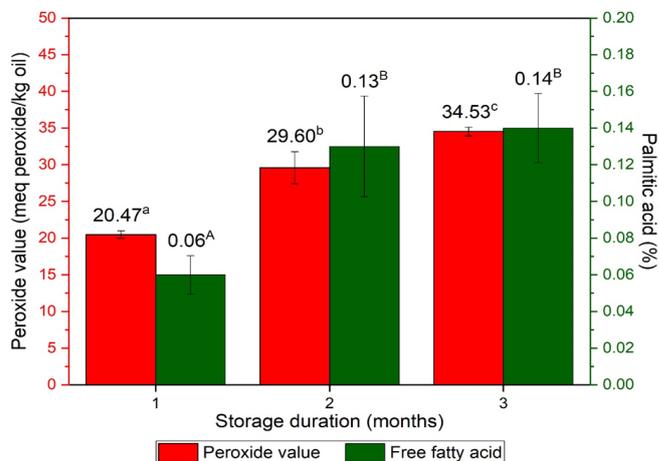


Figure 2. Free fatty acid and peroxide value of cassava stick stored frozen in different storage duration. Values are presented as mean±SD (n = 3 for each group). Values with different superscript are significantly different (P<0.05) by one-way ANOVA followed by DMRT test ($\alpha = 0.05$).

3.4 Colour properties

Colour is generally considered an important attribute that significantly affects the physical properties of food, the perception of consumers, and determines the nutritional quality of food products (Hutchings, 2002; Patras *et al.*, 2011). In this study, the colour of cassava sticks is shown in Table 2 and was measured using Hunter's L*, a*, and b* colour attributes. The L* value expressed the degree of lightness, where the highest value was measured in the cassava stick stored frozen for 3 months (75.20). However, the results were not significantly different (P>0.05) with cassava stick that was not stored frozen (71.55) and significantly different (P<0.05) with other samples (66.52 – 67.98). The different results were shown on the a* value, which expressed the degree of redness (+a*) and greenness (-a*) of cassava sticks. All samples had no significant difference (P>0.05) on the degree of redness (2.94 – 4.62). Meanwhile, the b* value expressed the degree of yellowness (+b*) and blueness (-b*). The results show that the b* values of cassava stick (25.77) that stored frozen for 2 months was significantly lower (P<0.05) than other samples (30.79 – 32.08).

Research by Oner and Wall (2012) also found that frozen storage significantly influenced (P<0.05) the colour of French fries. The colour change of fried cassava sticks during frozen storage duration might be

due to the surface moisture desiccation (Maity *et al.*, 2012). The amount of moisture loss during the frying process also influence the colour of fried cassava stick since moisture loss is associated with crust formation and accelerates the Maillard browning (Sahin, 2000). The Maillard browning occurs due to the reaction between amino acid and reducing sugar during frying and results in the increase in coloration of yellow, red, and brown in fried food (Pedreschi *et al.*, 2005). Another factor that might influence the crust colour of cassava sticks was the heat transfer rate in the cassava sticks during frying (Maity *et al.*, 2012).

Table 2. Colour of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Colour		
	L*	a*	b*
0	71.55±4.08 ^{ab}	2.95±0.78 ^a	32.08±3.02 ^b
1	67.98±2.78 ^a	4.62±1.93 ^a	30.79±1.87 ^b
2	66.52±1.15 ^a	3.21±0.37 ^a	25.77±0.98 ^a
3	75.20±1.80 ^b	4.01±0.30 ^a	31.40±1.66 ^b

Values are presented as mean±SD (n = 3 for each group). Values with different superscript are significantly different (P<0.05) by one-way ANOVA followed by DMRT test ($\alpha = 0.05$).

3.5 Sensory evaluation

The sensory evaluation results of cassava sticks stored frozen in different storage durations are shown as a spider plot in Figure 3. According to the results, the highest score for the colour attribute was the 0-month storage cassava sticks (5.60) and significantly different (P<0.05) with other samples (3.20 – 4.96). The decrease in panelists' colour preference was due to the colour changes in cassava sticks that tend to be darker and brownish than the regular cassava sticks. Meanwhile, on the hardness attribute, the panelists' preference significantly decreased (P<0.05) as the frozen storage duration increased (5.30 – 4.20). This result was in accordance with the objective analysis on hardness, where frozen storage duration significantly increased the hardness of cassava sticks due to the starch retrogradation. The next sensory attribute tested was the crispiness and the results were quite varied. Cassava sticks stored frozen for 0 months (4.88) had no significant difference (P>0.05) compared to 2 months stored cassava stick (4.66) but showed a significant difference (P<0.05) with cassava sticks stored frozen for 1 month (3.86) and 3 months (3.68). This result indicated that panelists perceived that frozen storage duration influenced the crispiness of fried cassava sticks. Furthermore, the frozen storage duration significantly influenced (P<0.05) the panelists' preferences on the aroma of cassava sticks. The aroma of cassava sticks

tended to be different for each treatment and might be influenced by the formation of hydroperoxides and free fatty acids. The last sensory attribute evaluated was the taste of cassava sticks. According to the result, the frozen storage duration had no significant difference (P>0.05) on the panelists' perception of the taste of cassava sticks (4.16 – 4.88). Overall, the average score of panelists' preference showed that frozen storage duration did not significantly affect (P>0.05) panelists' acceptance of the cassava stick.

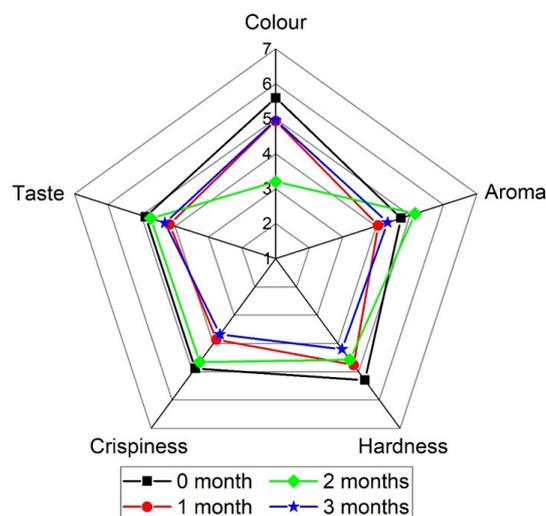


Figure 3. Sensory evaluation of cassava stick stored frozen in different storage duration. Values are presented as mean±SD (n = 3 for each group). Values with different superscript are significantly different (P<0.05) by one-way ANOVA followed by DMRT test ($\alpha = 0.05$).

4. Conclusion

Frozen storage can be used to extend the shelf life of food products. However, the use of frozen storage on cassava sticks affects its physical and chemical properties. Different frozen storage duration influenced the oil absorption of cassava sticks, though the moisture content showed no significant change. The effect of frozen storage duration was also significant on the texture of cassava sticks, especially on hardness. Another studied quality characteristic, such as cassava stick surface colour, substantially declined during the frozen storage. The free fatty acid and peroxide value of cassava sticks was gradually increased with the elongation of frozen storage duration. According to the sensory evaluation results, frozen storage duration influenced all of the sensory attributes besides the taste of cassava sticks. Cassava sticks stored frozen for 3 months were still acceptable by the panelists and the acceptance was defined as neither like nor dislike.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This study was financially supported by the Lembaga Penelitian dan Pengabdian Masyarakat (LPPM) Widya Mandala Surabaya Catholic University through PPPG Research Grants 2015/2016.

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Effects of frozen storage duration on the physicochemical and sensory properties of cassava sticks

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Article history:

Received: 5 February 2022

Received in revised form: 28

March 2022

Accepted: 31 March 2022

Available Online:

Keywords:

Cassava,

Cassava stick,

Frozen storage duration

DOI:

Abstract

Cassava sticks is a processed food made from cassava that has similar characteristics to French fries. However, cassava sticks are also classified as perishable food, so it is required to employ other processes that might increase its preservation, such as frozen storage. In this study, the cassava stick was stored at -20°C for three months to investigate its effects on the physicochemical and sensory properties of cassava sticks. The effects of frozen storage duration were monitored every month carried out in three replications. A randomized block design with a single factor was used as the experimental design. According to the results, storing cassava sticks under frozen conditions significantly increased ($P<0.05$) the oil absorption and had no effect ($P>0.05$) on the moisture content. A significant alteration ($P<0.05$) in texture was observed through the increase of cassava stick hardness from 1.11 to 2.54 N. Frozen storage duration also influenced ($P<0.05$) the lightness and yellowness, but not the redness ($P>0.05$) of cassava sticks. Fat oxidation also occurred during storage, marked by a significant increase ($P<0.05$) of free fatty acid (0.06 to 0.14%) and peroxide value (0 to 34.53 mg peroxide/kg lipid) on three months of frozen storage. Thus, this study concludes that frozen storage duration affected the physical and chemical properties of cassava sticks. Moreover, cassava sticks stored frozen for three months were acceptable for panelists with neither like nor dislike (4.30) average acceptance, and had no significant difference ($P>0.05$) with other samples.

1. Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most consumed crops in the world, especially in Sub-Saharan Africa (SSA) and in developing countries of Asia such as Cambodia, Vietnam, and Laos (Benesi *et al.*, 2004; International Atomic Energy Agency, 2018). Cassava is considered a substitute for rice as the primary carbohydrate source and can be consumed in various ways (fried, boiled, or steamed). Besides carbohydrates, cassava is also rich in calcium (50 mg/100 g), phosphorus (40 mg/100 g), vitamin C (25 mg/100g) and contains a significant amount of thiamine, riboflavin, and nicotinic acid (International Atomic Energy Agency, 2018). In Indonesia, cassava is generally processed into traditional food such as *tiwul*, *gatot*, *gapek*, *gethuk*, *tapai*, and cassava chips. Furthermore, it can also be processed into a modern food such as cassava stick.

Cassava stick has similar characteristics to French fries due to its shoestring-like shape, golden brown colour, crunchy exterior, and fluffy interior. Those characteristics are obtained through several processes

such as blanching, drying, frying, and each step affects the quality of the final product (Lamberg *et al.*, 1990). Other processes are also required to extend shelf life since cassava sticks are classified as perishable food. Among other processes that might be employed for long-term preservation (high pressure, infrared irradiation, pulsed electric field, and ultrasound), freezing or frozen storage is still one of the most used methods (Rahman and Velez-Ruiz, 2007). Generally, frozen storage temperature is 0°F (-18°C) and even colder depending on the type of food (WFLO Commodity Storage Manual, 2008).

Several studies showed that storing food (fruit, meat, and French fries) under freezing temperatures affects the physical and chemical properties (Sattar *et al.*, 2015; Celli *et al.*, 2016; Medic *et al.*, 2018). However, based on authors' knowledge, there is no study reported about the effect of frozen storage on the cassava sticks properties. Therefore, this study aimed to investigate the physical and chemical changes on cassava sticks during frozen storage. In addition, the sensory evaluation of

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cassava sticks during frozen storage was also studied.

2. Materials and methods

2.1 Materials

Cassava tubers (*Manihot esculenta* Crantz) used in this study were yellow cassava (Malang 2), purchased from a local market in Malang, Indonesia. The cooking oil was purchased from a market in Surabaya, Indonesia. All chemicals and other reagents used in this study were analytical grades.

2.2 Preparation of cassava stick

Cassava tubers were washed two times using tap water to remove physical contaminations. The cleaned cassava tubers were then peeled, cut into strips of 4×1×1 cm, and soaked in 0.1% (w/v) CaCl₂ for 15 mins to increase the crispiness of the cassava stick. Next, the soaked cassava sticks were steamed in a steamer (98°C, 15 mins) and cooled to room temperature before being pre-fried (170°C, 30 s) in cassava sticks : cooking oil ratio of 1:12 (w/v) using a deep fryer (Fritel Professional 35 SICO, Belgium). Later, the pre-fried cassava sticks were vacuum packed in a polypropylene plastic bag using a vacuum sealer (Maksindo DZ300, Indonesia) and frozen at -20°C for different storage duration (0, 1, 2, 3 months) in a chest freezer (Modena MD 45, Italy). The frozen cassava sticks were then thawed in a water bath (15 mins) and fried for 2 mins at the same condition as the pre-frying process. At the end of frying, the fryer basket was immediately shaken for 10 s and sticks were cooled to room temperature before analyzing the physicochemical properties.

2.3 Moisture content

All fried cassava stick samples were minced before immediately analyzing the moisture content. The moisture content was determined using a drying oven (Binder ED 53, Germany) with the thermogravimetric method (AOAC, 2006) for 3-5 hrs at 105±2°C.

2.4 Oil absorption analysis

The oil absorption analysis was determined according to the method by Mohamed *et al.* (1988). Approximately 5 g of minced pre-fried and fried cassava sticks were oven-dried at 105°C for 3-5 hrs and weighed until a stable weight of the sample was obtained. The oil absorption was calculated using the following equation:

$$\text{Oil absorption (\%)} = \frac{W_2 - W_1}{W_1} \times 100\%$$

where W₁ is the weight of pre-fried cassava stick (g) and W₂ is the weight of fried cassava stick (g).

2.5 Hardness

Hardness as the selected texture characteristics were determined using a TA-XT plus (Stable Micro System, UK) equipped with a three-point bend rig using compression test mode with the following conditions: pre-test speed= 2 mm/s, test speed= 0.5 mm/s, post-test speed= 10 mm/s, and distance= 10 mm. The hardness of fried cassava stick was expressed as Newton and determined as the maximum force required to compress the sample.

2.6 Free fatty acid analysis

The free fatty acid of cassava sticks was analyzed using a method by Sudarmadji *et al.* (1984). Approximately 28.2 g of fried cassava sticks were minced before adding 50 mL of neutralized alcohol and 2 mL phenolphthalein. The mixture was then titrated with 0.1 N NaOH until a stable pink colour occurred for 30 s. The percentage of free fatty acid was expressed as the percentage of palmitic acid and calculated using the following equation:

$$\text{Palmitic acid (\%)} = \frac{V_{\text{NaOH}} \times N_{\text{NaOH}} \times Mr}{W \times 1000} \times 100\%$$

where V_{NaOH} is the titre of NaOH (mL), N_{NaOH} is the concentration of NaOH (N), Mr is the molecular weight of palmitic acid (256.42 g/mol), and W is the sample weight (g).

2.7 Peroxide value

An iodometric titration method was used to analyze the peroxide value as the primary oxidation product (Sudarmadji *et al.*, 1984). Briefly, 5 g of fried cassava sticks were mixed with 30 mL acetic acid – chloroform (3:2) and 0.5 mL saturated aqueous potassium iodide solution was added. Later, the mixture was shaken vigorously for 1 min before 30 mL of distilled water was added. The mixture was titrated with 0.1 M Na₂S₂O₃ until the yellow colour had almost disappeared. Next, 0.5 mL of 1% (w/v) starch indicator solution was added and the titration continued until the blue colour disappeared. The peroxide value was calculated using the following formula:

$$\text{Peroxide value (meq peroxide/kg oil)} = \frac{V_{\text{thio}} \times N_{\text{thio}} \times 1000}{W}$$

where V_{thio} is the titre of Na₂S₂O₃ (mL), N_{thio} is the concentration of Na₂S₂O₃, and W is the sample weight (g).

2.8 Colour

The colour of fried cassava stick was identified using a colour reader (Colour Reader Minolta, CR-10). The L* value (0 = blackness, 100 = whiteness), a* value (+ = redness, - = greenness), and b* value (+ = yellowness, -

= blueness) of each sample were observed.

2.9 Sensory evaluation

In this study, the Hedonic Scale Scoring (preferred test) was used to measure the level of preference and acceptance of the product by consumers (Kusuma *et al.*, 2017). The sensory evaluation was evaluated by 100 untrained panelists. The panelists were students of the Faculty of Agricultural Technology, Widya Mandala Catholic University Surabaya. The sensory attributes tested were colour, hardness, crispiness, aroma, and taste. A scoring system of 1-7 points was used to represent the sensory characteristics of each sample. On this scale, 1 was defined as dislike extremely, 2 was defined as dislike moderately, 3 was defined as dislike slightly, 4 was defined as neither like nor dislike, 5 was defined as like slightly, 6 was defined as like moderately, and 7 was defined as like extremely.

2.10 Statistical analysis

All experiments were analyzed at least three times in triplicate and represented as mean values \pm SD. Statistical analyses were performed using SPSS for Windows (version 19.0, SPSS Inc., USA) and compared with Duncan Multiple Range Test (DMRT) at $P < 0.05$.

3. Results and discussion

3.1 Moisture content and oil absorption

The moisture content of fried cassava sticks is shown in Table 1. Based on the result, different frozen storage durations have no significant difference ($P > 0.05$) on the moisture content of cassava sticks, even though there was a downward trend in the moisture content of frozen cassava sticks for 2 and 3 months. Similar results were reported by Medic *et al.* (2018), where frozen storage duration had no significant effect on the moisture content of pork loin and belly rib. However, several researchers also found that freezing significantly ($P < 0.05$) influenced the moisture content of the fried potato (Adedeji and Ngadi, 2017), lamb (Coombs *et al.*, 2017), and pork ham (Medic *et al.*, 2018). According to Fennema (1985), water in food converts to ice crystals during freezing at -18°C and is easily removed from food when the food is dried or fried, causing the moisture content to decrease.

Oil absorption analysis was conducted to measure the ability of cassava sticks to absorb oil during frying by using a modified thermogravimetric method (Mohamed *et al.*, 1988). Table 1 shows that the storage duration significantly increased ($P < 0.05$) the oil absorption of cassava sticks. Cassava stick stored frozen for 2 months has the highest oil absorption (58.45%) but has no significant difference with cassava stick stored frozen for

3 months (57.84%). In a study conducted by Adedeji and Ngadi (2017), oil absorption also showed a significant increase in fried potato that was stored frozen. However, they also found that there was no interaction between storage duration and freezing method, which contradicts this study.

Table 1. Moisture content and oil absorption of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Moisture content (%)	Oil absorption (%)
0	45.13 \pm 7.46 ^a	20.87 \pm 15.91 ^a
1	48.83 \pm 3.27 ^a	25.66 \pm 9.51 ^a
2	28.68 \pm 7.95 ^a	58.45 \pm 2.47 ^b
3	27.75 \pm 10.13 ^a	57.94 \pm 24.71 ^b

Values are presented as mean \pm SD (n = 3 for each group). Values with different superscript are significantly different ($P < 0.05$) by one-way ANOVA followed by DMRT test ($\alpha = 0.05$).

According to Adedeji *et al.* (2009), moisture loss during frying affects the amount of oil absorbed in fried food. This statement is in accordance with Fellows (1990), where oil transfers into the product to replace evaporated water during frying. In addition, moisture evaporation from fried food during frying damages the cellular structure of plant tissues, resulting in an increase in porosity which contributes to oil absorption (Mellema, 2003; Rimac-Brncic *et al.*, 2004; Dana and Saguy, 2006). On the other hand, during frozen storage, all water in food converts to ice crystal and causes volume expansion which is characterized by the damage to cell membranes during ice crystal formation (Fennema, 1985; Celli *et al.*, 2016). This volume expansion causes the water in cassava sticks to be easier to evaporate during frying and increases oil absorption.

3.2 Hardness

In this study, hardness was chosen as the analyzed texture attribute to evaluate the impact of frozen storage duration on the texture of cassava sticks. According to Szczesniak (2002), hardness is the force required to change the shape of material due to its resistance to resist deformation. The effect of frozen storage duration on the hardness of cassava sticks is presented in Figure 1. As shown in that figure, frozen storage duration significantly affected ($P < 0.05$) the hardness of the cassava sticks. Cassava stick that stored frozen for 3 months has the highest force (2.54) among other samples (0.63 – 1.41). These results were in accordance to Adedeji and Ngadi (2017). They found a marginal increase in the hardness of fried potatoes as the frozen storage duration increased. Therefore, the increase in fried cassava stick hardness might be due to the retrogradation of cassava starch during frozen storage. This statement was supported by a similar study by Yu *et*

al. (2010), where the hardness of cooked rice increased continually with the amylopectin retrogradation during frozen storage. Based on those findings, amylopectin retrogradation contributed to the hardness increase during storage on the high starch food products.

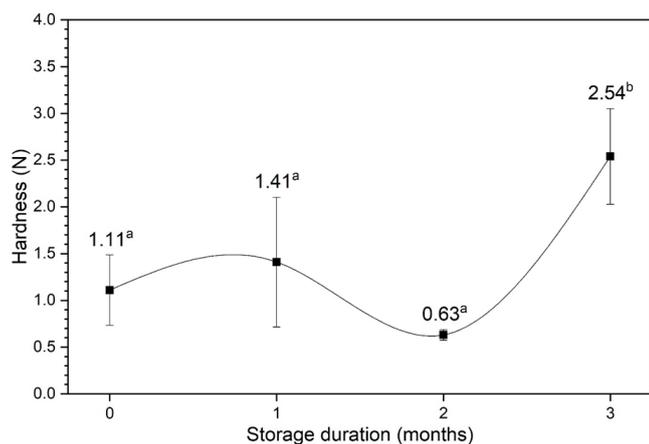


Figure 1. Hardness of cassava stick stored frozen in different storage duration. Values are presented as mean±SD (n = 3 for each group). Values with different superscript are significantly different (P<0.05) by one-way ANOVA followed by DMRT test ($\alpha = 0.05$).

3.3 Free fatty acid and peroxide value

This study assessed the percentage of free fatty acid and peroxide values to monitor the fat oxidation in cassava sticks during frozen storage. The increase of free fatty acid and peroxide value indicates the rancidity of food products that produce off-flavors (Maity *et al.*, 2012) and might reduce consumers' preferences. The free fatty acid of cassava stick was expressed as the percentage of palmitic acid since the cooking oil used to fry was palm oil. Meanwhile, the peroxide value as the primary oxidation product of fat oxidation was expressed as meq peroxide/kg fat. The percentage of free fatty acid and peroxide value of fried cassava sticks is shown in Figure 2. A significant increased (P<0.05) of free fatty acid was recorded during 2 months of frozen storage (0.13 %) and had no significant difference (P>0.05) with cassava sticks stored frozen for 3 months (0.14%). On the other hand, the peroxide value of cassava stick was also significantly increased (P<0.05) as the frozen storage duration increased (0 – 34.53 meq peroxide/ kg fat). Similar results were also reported by some studies where frozen storage duration increased the free fatty acid and peroxide value of potato strips (Kizito *et al.*, 2017), pork meat (Medic *et al.*, 2018), and ready-to-fry vegetable snacks (Maity *et al.*, 2012).

According to Crosa *et al.* (2014), free fatty acids are formed through the nucleophilic attack at triacylglycerol's ester bond and have been associated with the undesirable odors and taste of food products. Furthermore, the formation of hydroperoxides during storage generally occurred during the early stage of

oxidation (Fennema, 1985) and later decomposed to other secondary oxidation products such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (Kizito *et al.*, 2017; Medic *et al.*, 2018). Another factor that might increase the formation of hydroperoxides was the contact of cassava stick with oxygen before and after storage; since oxygen is one of the reactants that caused fat oxidation (Giri and Mangaraj, 2012).

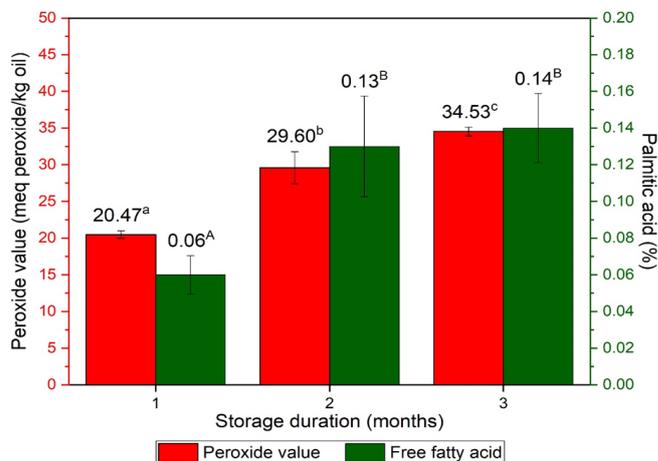


Figure 2. Free fatty acid and peroxide value of cassava stick stored frozen in different storage duration. Values are presented as mean±SD (n = 3 for each group). Values with different superscript are significantly different (P<0.05) by one-way ANOVA followed by DMRT test ($\alpha = 0.05$).

3.4 Colour properties

Colour is generally considered an important attribute that significantly affects the physical properties of food, the perception of consumers, and determines the nutritional quality of food products (Hutchings, 2002; Patras *et al.*, 2011). In this study, the colour of cassava sticks is shown in Table 2 and was measured using Hunter's L*, a*, and b* colour attributes. The L* value expressed the degree of lightness, where the highest value was measured in the cassava stick stored frozen for 3 months (75.20). However, the results were not significantly different (P>0.05) with cassava stick that was not stored frozen (71.55) and significantly different (P<0.05) with other samples (66.52 – 67.98). The different results were shown on the a* value, which expressed the degree of redness (+a*) and greenness (-a*) of cassava sticks. All samples had no significant difference (P>0.05) on the degree of redness (2.94 – 4.62). Meanwhile, the b* value expressed the degree of yellowness (+b*) and blueness (-b*). The results show that the b* values of cassava stick (25.77) that stored frozen for 2 months was significantly lower (P<0.05) than other samples (30.79 – 32.08).

Research by Oner and Wall (2012) also found that frozen storage significantly influenced (P<0.05) the colour of French fries. The colour change of fried cassava sticks during frozen storage duration might be

due to the surface moisture desiccation (Maity *et al.*, 2012). The amount of moisture loss during the frying process also influence the colour of fried cassava stick since moisture loss is associated with crust formation and accelerates the Maillard browning (Sahin, 2000). The Maillard browning occurs due to the reaction between amino acid and reducing sugar during frying and results in the increase in coloration of yellow, red, and brown in fried food (Pedreschi *et al.*, 2005). Another factor that might influence the crust colour of cassava sticks was the heat transfer rate in the cassava sticks during frying (Maity *et al.*, 2012).

Table 2. Colour of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Colour		
	L*	a*	b*
0	71.55±4.08 ^{ab}	2.95±0.78 ^a	32.08±3.02 ^b
1	67.98±2.78 ^a	4.62±1.93 ^a	30.79±1.87 ^b
2	66.52±1.15 ^a	3.21±0.37 ^a	25.77±0.98 ^a
3	75.20±1.80 ^b	4.01±0.30 ^a	31.40±1.66 ^b

Values are presented as mean±SD (n = 3 for each group). Values with different superscript are significantly different (P<0.05) by one-way ANOVA followed by DMRT test ($\alpha = 0.05$).

3.5 Sensory evaluation

The sensory evaluation results of cassava sticks stored frozen in different storage durations are shown as a spider plot in Figure 3. According to the results, the highest score for the colour attribute was the 0-month storage cassava sticks (5.60) and significantly different (P<0.05) with other samples (3.20 – 4.96). The decrease in panelists' colour preference was due to the colour changes in cassava sticks that tend to be darker and brownish than the regular cassava sticks. Meanwhile, on the hardness attribute, the panelists' preference significantly decreased (P<0.05) as the frozen storage duration increased (5.30 – 4.20). This result was in accordance with the objective analysis on hardness, where frozen storage duration significantly increased the hardness of cassava sticks due to the starch retrogradation. The next sensory attribute tested was the crispiness and the results were quite varied. Cassava sticks stored frozen for 0 months (4.88) had no significant difference (P>0.05) compared to 2 months stored cassava stick (4.66) but showed a significant difference (P<0.05) with cassava sticks stored frozen for 1 month (3.86) and 3 months (3.68). This result indicated that panelists perceived that frozen storage duration influenced the crispiness of fried cassava sticks. Furthermore, the frozen storage duration significantly influenced (P<0.05) the panelists' preferences on the aroma of cassava sticks. The aroma of cassava sticks

tended to be different for each treatment and might be influenced by the formation of hydroperoxides and free fatty acids. The last sensory attribute evaluated was the taste of cassava sticks. According to the result, the frozen storage duration had no significant difference (P>0.05) on the panelists' perception of the taste of cassava sticks (4.16 – 4.88). Overall, the average score of panelists' preference showed that frozen storage duration did not significantly affect (P>0.05) panelists' acceptance of the cassava stick.

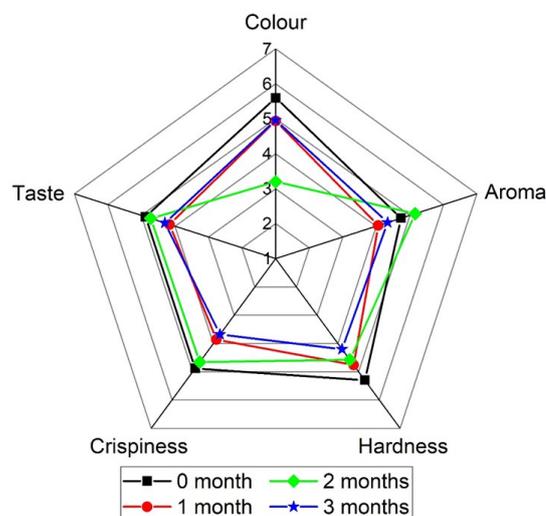


Figure 3. Sensory evaluation of cassava stick stored frozen in different storage duration. Values are presented as mean±SD (n = 3 for each group). Values with different superscript are significantly different (P<0.05) by one-way ANOVA followed by DMRT test ($\alpha = 0.05$).

4. Conclusion

Frozen storage can be used to extend the shelf life of food products. However, the use of frozen storage on cassava sticks affects its physical and chemical properties. Different frozen storage duration influenced the oil absorption of cassava sticks, though the moisture content showed no significant change. The effect of frozen storage duration was also significant on the texture of cassava sticks, especially on hardness. Another studied quality characteristic, such as cassava stick surface colour, substantially declined during the frozen storage. The free fatty acid and peroxide value of cassava sticks was gradually increased with the elongation of frozen storage duration. According to the sensory evaluation results, frozen storage duration influenced all of the sensory attributes besides the taste of cassava sticks. Cassava sticks stored frozen for 3 months were still acceptable by the panelists and the acceptance was defined as neither like nor dislike.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This study was financially supported by the Lembaga Penelitian dan Pengabdian Masyarakat (LPPM) Widya Mandala Surabaya Catholic University through PPPG Research Grants 2015/2016.

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Effects of frozen storage duration on the physicochemical and sensory properties of cassava sticks

*Trisnawati, C.Y., Sutedja, A.M. and Kaharso, V.C.

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Article history:

Received: 5 February 2022

Received in revised form: 28

March 2022

Accepted: 31 March 2022

Available Online:

Keywords:

Cassava,

Cassava stick,

Frozen storage duration

DOI:

Abstract

Cassava sticks is a processed food made from cassava that has similar characteristics to French fries. However, cassava sticks are also classified as perishable food, so it is required to employ other processes that might increase its preservation, such as frozen storage. In this study, the cassava stick was stored at -20°C for three months to investigate its effects on the physicochemical and sensory properties of cassava sticks. The effects of frozen storage duration were monitored every month carried out in three replications. A randomized block design with a single factor was used as the experimental design. According to the results, storing cassava sticks under frozen conditions significantly increased ($P<0.05$) the oil absorption and had no effect ($P>0.05$) on the moisture content. A significant alteration ($P<0.05$) in texture was observed through the increase of cassava stick hardness from 1.11 to 2.54 N. Frozen storage duration also influenced ($P<0.05$) the lightness and yellowness, but not the redness ($P>0.05$) of cassava sticks. Fat oxidation also occurred during storage, marked by a significant increase ($P<0.05$) of free fatty acid (0.06 to 0.14%) and peroxide value (0 to 34.53 mg peroxide/kg lipid) on three months of frozen storage. Thus, this study concludes that frozen storage duration affected the physical and chemical properties of cassava sticks. Moreover, cassava sticks stored frozen for three months were acceptable for panelists with neither like nor dislike (4.30) average acceptance, and had no significant difference ($P>0.05$) with other samples.

1. Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most consumed crops in the world, especially in Sub-Saharan Africa (SSA) and in developing countries of Asia such as Cambodia, Vietnam, and Laos (Benesi *et al.*, 2004; International Atomic Energy Agency, 2018). Cassava is considered a substitute for rice as the primary carbohydrate source and can be consumed in various ways (fried, boiled, or steamed). Besides carbohydrates, cassava is also rich in calcium (50 mg/100 g), phosphorus (40 mg/100 g), vitamin C (25 mg/100g) and contains a significant amount of thiamine, riboflavin, and nicotinic acid (International Atomic Energy Agency, 2018). In Indonesia, cassava is generally processed into traditional food such as *tiwul*, *gatot*, *gapek*, *gethuk*, *tapai*, and cassava chips. Furthermore, it can also be processed into a modern food such as cassava stick.

Cassava stick has similar characteristics to French fries due to its shoestring-like shape, golden brown colour, crunchy exterior, and fluffy interior. Those characteristics are obtained through several processes

such as blanching, drying, frying, and each step affects the quality of the final product (Lamberg *et al.*, 1990). Other processes are also required to extend shelf life since cassava sticks are classified as perishable food. Among other processes that might be employed for long-term preservation (high pressure, infrared irradiation, pulsed electric field, and ultrasound), freezing or frozen storage is still one of the most used methods (Rahman and Velez-Ruiz, 2007). Generally, frozen storage temperature is 0°F (-18°C) and even colder depending on the type of food (WFLO Commodity Storage Manual, 2008).

Several studies showed that storing food (fruit, meat, and French fries) under freezing temperatures affects the physical and chemical properties (Sattar *et al.*, 2015; Celli *et al.*, 2016; Medic *et al.*, 2018). However, based on authors' knowledge, there is no study reported about the effect of frozen storage on the cassava sticks properties. Therefore, this study aimed to investigate the physical and chemical changes on cassava sticks during frozen storage. In addition, the sensory evaluation of

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cassava sticks during frozen storage was also studied.

2. Materials and methods

2.1 Materials

Cassava tubers (*Manihot esculenta* Crantz) used in this study were yellow cassava (Malang 2), purchased from a local market in Malang, Indonesia. The cooking oil was purchased from a market in Surabaya, Indonesia. All chemicals and other reagents used in this study were analytical grades.

2.2 Preparation of cassava stick

Cassava tubers were washed two times using tap water to remove physical contaminations. The cleaned cassava tubers were then peeled, cut into strips of 4×1×1 cm, and soaked in 0.1% (w/v) CaCl₂ for 15 mins to increase the crispiness of the cassava stick. Next, the soaked cassava sticks were steamed in a steamer (98°C, 15 mins) and cooled to room temperature before being pre-fried (170°C, 30 s) in cassava sticks : cooking oil ratio of 1:12 (w/v) using a deep fryer (Fritel Professional 35 SICO, Belgium). Later, the pre-fried cassava sticks were vacuum packed in a polypropylene plastic bag using a vacuum sealer (Maksindo DZ300, Indonesia) and frozen at -20°C for different storage duration (0, 1, 2, 3 months) in a chest freezer (Modena MD 45, Italy). The frozen cassava sticks were then thawed in a water bath (15 mins) and fried for 2 mins at the same condition as the pre-frying process. At the end of frying, the fryer basket was immediately shaken for 10 s and sticks were cooled to room temperature before analyzing the physicochemical properties.

2.3 Moisture content

All fried cassava stick samples were minced before immediately analyzing the moisture content. The moisture content was determined using a drying oven (Binder ED 53, Germany) with the thermogravimetric method (AOAC, 2006) for 3-5 hrs at 105±2°C.

2.4 Oil absorption analysis

The oil absorption analysis was determined according to the method by Mohamed *et al.* (1988). Approximately 5 g of minced pre-fried and fried cassava sticks were oven-dried at 105°C for 3-5 hrs and weighed until a stable weight of the sample was obtained. The oil absorption was calculated using the following equation:

$$\text{Oil absorption (\%)} = \frac{W_2 - W_1}{W_1} \times 100\%$$

where W₁ is the weight of pre-fried cassava stick (g) and W₂ is the weight of fried cassava stick (g).

2.5 Hardness

Hardness as the selected texture characteristics were determined using a TA-XT plus (Stable Micro System, UK) equipped with a three-point bend rig using compression test mode with the following conditions: pre-test speed= 2 mm/s, test speed= 0.5 mm/s, post-test speed= 10 mm/s, and distance= 10 mm. The hardness of fried cassava stick was expressed as Newton and determined as the maximum force required to compress the sample.

2.6 Free fatty acid analysis

The free fatty acid of cassava sticks was analyzed using a method by Sudarmadji *et al.* (1984). Approximately 28.2 g of fried cassava sticks were minced before adding 50 mL of neutralized alcohol and 2 mL phenolphthalein. The mixture was then titrated with 0.1 N NaOH until a stable pink colour occurred for 30 s. The percentage of free fatty acid was expressed as the percentage of palmitic acid and calculated using the following equation:

$$\text{Palmitic acid (\%)} = \frac{V_{\text{NaOH}} \times N_{\text{NaOH}} \times Mr}{W \times 1000} \times 100\%$$

where V_{NaOH} is the titre of NaOH (mL), N_{NaOH} is the concentration of NaOH (N), Mr is the molecular weight of palmitic acid (256.42 g/mol), and W is the sample weight (g).

2.7 Peroxide value

An iodometric titration method was used to analyze the peroxide value as the primary oxidation product (Sudarmadji *et al.*, 1984). Briefly, 5 g of fried cassava sticks were mixed with 30 mL acetic acid – chloroform (3:2) and 0.5 mL saturated aqueous potassium iodide solution was added. Later, the mixture was shaken vigorously for 1 min before 30 mL of distilled water was added. The mixture was titrated with 0.1 M Na₂S₂O₃ until the yellow colour had almost disappeared. Next, 0.5 mL of 1% (w/v) starch indicator solution was added and the titration continued until the blue colour disappeared. The peroxide value was calculated using the following formula:

$$\text{Peroxide value (meq peroxide/kg oil)} = \frac{V_{\text{thio}} \times N_{\text{thio}} \times 1000}{W}$$

where V_{thio} is the titre of Na₂S₂O₃ (mL), N_{thio} is the concentration of Na₂S₂O₃, and W is the sample weight (g).

2.8 Colour

The colour of fried cassava stick was identified using a colour reader (Colour Reader Minolta, CR-10). The L* value (0 = blackness, 100 = whiteness), a* value (+ = redness, - = greenness), and b* value (+ = yellowness, -

= blueness) of each sample were observed.

2.9 Sensory evaluation

In this study, the Hedonic Scale Scoring (preferred test) was used to measure the level of preference and acceptance of the product by consumers (Kusuma *et al.*, 2017). The sensory evaluation was evaluated by 100 untrained panelists. The panelists were students of the Faculty of Agricultural Technology, Widya Mandala Catholic University Surabaya. The sensory attributes tested were colour, hardness, crispiness, aroma, and taste. A scoring system of 1-7 points was used to represent the sensory characteristics of each sample. On this scale, 1 was defined as dislike extremely, 2 was defined as dislike moderately, 3 was defined as dislike slightly, 4 was defined as neither like nor dislike, 5 was defined as like slightly, 6 was defined as like moderately, and 7 was defined as like extremely.

2.10 Statistical analysis

All experiments were analyzed at least three times in triplicate and represented as mean values \pm SD. Statistical analyses were performed using SPSS for Windows (version 19.0, SPSS Inc., USA) and compared with Duncan Multiple Range Test (DMRT) at $P < 0.05$.

3. Results and discussion

3.1 Moisture content and oil absorption

The moisture content of fried cassava sticks is shown in Table 1. Based on the result, different frozen storage durations have no significant difference ($P > 0.05$) on the moisture content of cassava sticks, even though there was a downward trend in the moisture content of frozen cassava sticks for 2 and 3 months. Similar results were reported by Medic *et al.* (2018), where frozen storage duration had no significant effect on the moisture content of pork loin and belly rib. However, several researchers also found that freezing significantly ($P < 0.05$) influenced the moisture content of the fried potato (Adedeji and Ngadi, 2017), lamb (Coombs *et al.*, 2017), and pork ham (Medic *et al.*, 2018). According to Fennema (1985), water in food converts to ice crystals during freezing at -18°C and is easily removed from food when the food is dried or fried, causing the moisture content to decrease.

Oil absorption analysis was conducted to measure the ability of cassava sticks to absorb oil during frying by using a modified thermogravimetric method (Mohamed *et al.*, 1988). Table 1 shows that the storage duration significantly increased ($P < 0.05$) the oil absorption of cassava sticks. Cassava stick stored frozen for 2 months has the highest oil absorption (58.45%) but has no significant difference with cassava stick stored frozen for

3 months (57.84%). In a study conducted by Adedeji and Ngadi (2017), oil absorption also showed a significant increase in fried potato that was stored frozen. However, they also found that there was no interaction between storage duration and freezing method, which contradicts this study.

Table 1. Moisture content and oil absorption of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Moisture content (%)	Oil absorption (%)
0	45.13 \pm 7.46 ^a	20.87 \pm 15.91 ^a
1	48.83 \pm 3.27 ^a	25.66 \pm 9.51 ^a
2	28.68 \pm 7.95 ^a	58.45 \pm 2.47 ^b
3	27.75 \pm 10.13 ^a	57.94 \pm 24.71 ^b

Values are presented as mean \pm SD (n = 3 for each group). Values with different superscript are significantly different ($P < 0.05$) by one-way ANOVA followed by DMRT test ($\alpha = 0.05$).

According to Adedeji *et al.* (2009), moisture loss during frying affects the amount of oil absorbed in fried food. This statement is in accordance with Fellows (1990), where oil transfers into the product to replace evaporated water during frying. In addition, moisture evaporation from fried food during frying damages the cellular structure of plant tissues, resulting in an increase in porosity which contributes to oil absorption (Mellema, 2003; Rimac-Brncic *et al.*, 2004; Dana and Saguy, 2006). On the other hand, during frozen storage, all water in food converts to ice crystal and causes volume expansion which is characterized by the damage to cell membranes during ice crystal formation (Fennema, 1985; Celli *et al.*, 2016). This volume expansion causes the water in cassava sticks to be easier to evaporate during frying and increases oil absorption.

3.2 Hardness

In this study, hardness was chosen as the analyzed texture attribute to evaluate the impact of frozen storage duration on the texture of cassava sticks. According to Szczesniak (2002), hardness is the force required to change the shape of material due to its resistance to resist deformation. The effect of frozen storage duration on the hardness of cassava sticks is presented in Figure 1. As shown in that figure, frozen storage duration significantly affected ($P < 0.05$) the hardness of the cassava sticks. Cassava stick that was stored frozen for 3 months has the highest force (2.54 N) among other samples (0.63 – 1.41 N). These results were in accordance to Adedeji and Ngadi (2017). They found a marginal increase in the hardness of fried potatoes as the frozen storage duration increased. Therefore, the increase in fried cassava stick hardness might be due to the retrogradation of cassava starch during frozen storage. This statement was supported by a similar study by Yu *et*

al. (2010), where the hardness of cooked rice increased continually with the amylopectin retrogradation during frozen storage. Based on those findings, amylopectin retrogradation contributed to the hardness increase during storage on the high starch food products.

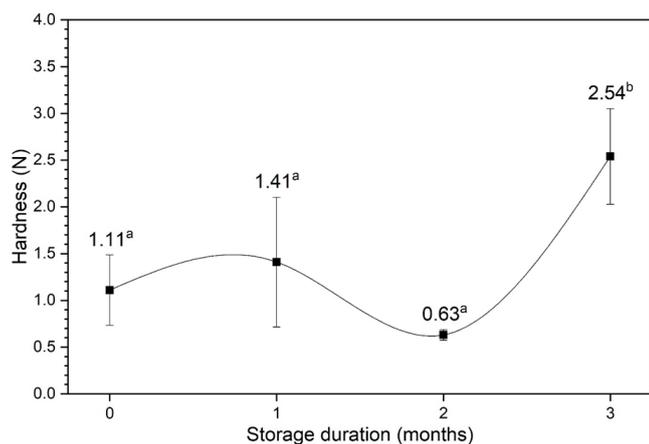


Figure 1. Hardness of cassava stick stored frozen in different storage duration. Values are presented as mean±SD (n = 3 for each group). Values with different superscript are significantly different (P<0.05) by one-way ANOVA followed by DMRT test ($\alpha = 0.05$).

3.3 Free fatty acid and peroxide value

This study assessed the percentage of free fatty acid and peroxide values to monitor the fat oxidation in cassava sticks during frozen storage. The increase of free fatty acid and peroxide value indicates the rancidity of food products that produce off-flavors (Maity *et al.*, 2012) and might reduce consumers' preferences. The free fatty acid of cassava stick was expressed as the percentage of palmitic acid since the cooking oil used to fry was palm oil. Meanwhile, the peroxide value as the primary oxidation product of fat oxidation was expressed as meq peroxide/kg fat. The percentage of free fatty acid and peroxide value of fried cassava sticks is shown in Figure 2. A significant increased (P<0.05) of free fatty acid was recorded during 2 months of frozen storage (0.13 %) and had no significant difference (P>0.05) with cassava sticks stored frozen for 3 months (0.14%). On the other hand, the peroxide value of cassava stick was also significantly increased (P<0.05) as the frozen storage duration increased (0 – 34.53 meq peroxide/ kg fat). Similar results were also reported by some studies where frozen storage duration increased the free fatty acid and peroxide value of potato strips (Kizito *et al.*, 2017), pork meat (Medic *et al.*, 2018), and ready-to-fry vegetable snacks (Maity *et al.*, 2012).

According to Crosa *et al.* (2014), free fatty acids are formed through the nucleophilic attack at triacylglycerol's ester bond and have been associated with the undesirable odors and taste of food products. Furthermore, the formation of hydroperoxides during storage generally occurred during the early stage of

oxidation (Fennema, 1985) and later decomposed to other secondary oxidation products such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (Kizito *et al.*, 2017; Medic *et al.*, 2018). Another factor that might increase the formation of hydroperoxides was the contact of cassava stick with oxygen before and after storage; since oxygen is one of the reactants that caused fat oxidation (Giri and Mangaraj, 2012).

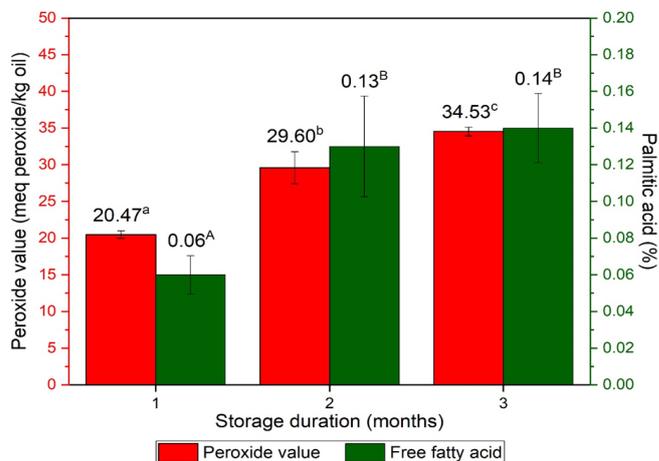


Figure 2. Free fatty acid and peroxide value of cassava stick stored frozen in different storage duration. Values are presented as mean±SD (n = 3 for each group). Values with different superscript are significantly different (P<0.05) by one-way ANOVA followed by DMRT test ($\alpha = 0.05$).

3.4 Colour properties

Colour is generally considered an important attribute that significantly affects the physical properties of food, the perception of consumers, and determines the nutritional quality of food products (Hutchings, 2002; Patras *et al.*, 2011). In this study, the colour of cassava sticks is shown in Table 2 and was measured using Hunter's L*, a*, and b* colour attributes. The L* value expressed the degree of lightness, where the highest value was measured in the cassava stick stored frozen for 3 months (75.20). However, the results were not significantly different (P>0.05) with cassava stick that was not stored frozen (71.55) and significantly different (P<0.05) with other samples (66.52 – 67.98). The different results were shown on the a* value, which expressed the degree of redness (+a*) and greenness (-a*) of cassava sticks. All samples had no significant difference (P>0.05) on the degree of redness (2.94 – 4.62). Meanwhile, the b* value expressed the degree of yellowness (+b*) and blueness (-b*). The results show that the b* values of cassava stick (25.77) that stored frozen for 2 months was significantly lower (P<0.05) than other samples (30.79 – 32.08).

Research by Oner and Wall (2012) also found that frozen storage significantly influenced (P<0.05) the colour of French fries. The colour change of fried cassava sticks during frozen storage duration might be

due to the surface moisture desiccation (Maity *et al.*, 2012). The amount of moisture loss during the frying process also influence the colour of fried cassava stick since moisture loss is associated with crust formation and accelerates the Maillard browning (Sahin, 2000). The Maillard browning occurs due to the reaction between amino acid and reducing sugar during frying and results in the increase in coloration of yellow, red, and brown in fried food (Pedreschi *et al.*, 2005). Another factor that might influence the crust colour of cassava sticks was the heat transfer rate in the cassava sticks during frying (Maity *et al.*, 2012).

Table 2. Colour of cassava stick stored frozen in different storage duration

Frozen storage duration (months)	Colour		
	L*	a*	b*
0	71.55±4.08 ^{ab}	2.95±0.78 ^a	32.08±3.02 ^b
1	67.98±2.78 ^a	4.62±1.93 ^a	30.79±1.87 ^b
2	66.52±1.15 ^a	3.21±0.37 ^a	25.77±0.98 ^a
3	75.20±1.80 ^b	4.01±0.30 ^a	31.40±1.66 ^b

Values are presented as mean±SD (n = 3 for each group). Values with different superscript are significantly different (P<0.05) by one-way ANOVA followed by DMRT test ($\alpha = 0.05$).

3.5 Sensory evaluation

The sensory evaluation results of cassava sticks stored frozen in different storage durations are shown as a spider plot in Figure 3. According to the results, the highest score for the colour attribute was the 0-month storage cassava sticks (5.60) and significantly different (P<0.05) with other samples (3.20 – 4.96). The decrease in panelists' colour preference was due to the colour changes in cassava sticks that tend to be darker and brownish than the regular cassava sticks. Meanwhile, on the hardness attribute, the panelists' preference significantly decreased (P<0.05) as the frozen storage duration increased (5.30 – 4.20). This result was in accordance with the objective analysis on hardness, where frozen storage duration significantly increased the hardness of cassava sticks due to the starch retrogradation. The next sensory attribute tested was the crispiness and the results were quite varied. Cassava sticks stored frozen for 0 months (4.88) had no significant difference (P>0.05) compared to 2 months stored cassava stick (4.66) but showed a significant difference (P<0.05) with cassava sticks stored frozen for 1 month (3.86) and 3 months (3.68). This result indicated that panelists perceived that frozen storage duration influenced the crispiness of fried cassava sticks. Furthermore, the frozen storage duration significantly influenced (P<0.05) the panelists' preferences on the aroma of cassava sticks. The aroma of cassava sticks

tended to be different for each treatment and might be influenced by the formation of hydroperoxides and free fatty acids. The last sensory attribute evaluated was the taste of cassava sticks. According to the result, the frozen storage duration had no significant difference (P>0.05) on the panelists' perception of the taste of cassava sticks (4.16 – 4.88). Overall, the average score of panelists' preference showed that frozen storage duration did not significantly affect (P>0.05) panelists' acceptance of the cassava stick.

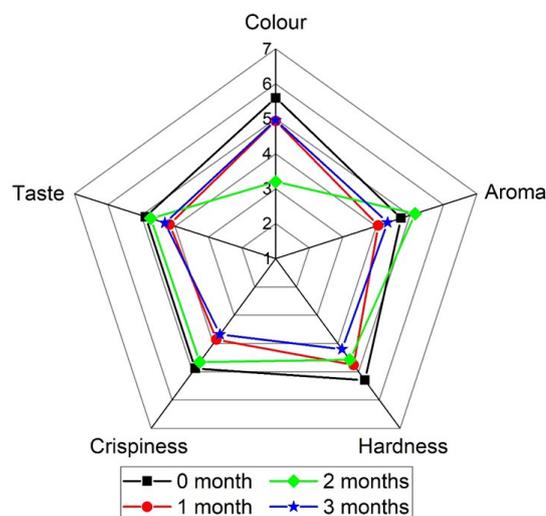


Figure 3. Sensory evaluation of cassava stick stored frozen in different storage duration. Values are presented as mean±SD (n = 3 for each group). Values with different superscript are significantly different (P<0.05) by one-way ANOVA followed by DMRT test ($\alpha = 0.05$).

4. Conclusion

Frozen storage can be used to extend the shelf life of food products. However, the use of frozen storage on cassava sticks affects its physical and chemical properties. Different frozen storage duration influenced the oil absorption of cassava sticks, though the moisture content showed no significant change. The effect of frozen storage duration was also significant on the texture of cassava sticks, especially on hardness. Another studied quality characteristic, such as cassava stick surface colour, substantially declined during the frozen storage. The free fatty acid and peroxide value of cassava sticks was gradually increased with the elongation of frozen storage duration. According to the sensory evaluation results, frozen storage duration influenced all of the sensory attributes besides the taste of cassava sticks. Cassava sticks stored frozen for 3 months were still acceptable by the panelists and the acceptance was defined as neither like nor dislike.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This study was financially supported by the Lembaga Penelitian dan Pengabdian Masyarakat (LPPM) Widya Mandala Surabaya Catholic University through PPPG Research Grants 2015/2016.

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