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Crude gelatin from jellyfish for storage stability of frozen maize tortilla bread

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Abstract

To support food security, a frozen tortilla bread was prepared from local Madura corn with crude jellyfish gelatin as cryoprotectant. The purpose of this study was to determine the effect of jellyfish gelatin concentration on the quality of Madura frozen tortilla bread during storage at low temperatures. This research was conducted using a completely randomized factorial design with two factors: jellyfish gelatin concentration (0, 5 and 10%) and storage time (0, 7, 14 and 21 days). The research parameters analyzed were colour, hardness, elongation, tensile strength, staling rate, water content, water activity and hedonic sensory characteristics. Results showed that there was no change in elongation, tensile strength, water activity, and moisture content at the beginning and end of storage. Gelatin concentration increased hardness, preference towards taste and mouthfeel, and overall preference; while reduced L value, elongation, tensile strength, water activity, and preference towards colour. Meanwhile, storage time increased the L and a values, hardness, and preference towards taste and decreased the value of b, preference toward colour, texture and overall preference. Interaction of two factors did not affect staling rate, a value, b value, and preference toward aroma, but showed a significant effect on hardness, elongation, and tensile strength. In conclusion, crude jellyfish gelatin can be used to maintain the physical, chemical, and sensory qualities of frozen tortilla bread due to low-temperature storage.

1. Introduction

Frozen healthy bread products including frozen corn tortilla bread, showed a good future despite the pandemic. The pandemic has changed people's lifestyles in regard to cooking their own food, and frozen bread is a new choice (globalnewswire.com, 2021). Therefore, after the lockdown was lifted, the demand for frozen healthy bread increased (globalnewswire.com). Market research reported that demand for bake-off technology (BOT) is rising and projected to grow at a CAGR of 4.3% during 2020-2025 (Researchandmarkets, 2020), with European countries' market being well-established, and America is the next market. In the form of unfermented frozen dough (UFD), partially baked frozen bread (PBF), and partially baked unfrozen bread (PBUF), it offers convenience and less labour to in-store bakeries, restaurants, cafés, and other food services. Frozen bread supports baking on sale which provides a fresher product, and more desirable textural dan organoleptic properties. In the case of corn tortilla bread, its consumption in the US significantly increased during the pandemic, and the demand was hard to fulfil (aldianews.com, 2017). It is the second most wanted bread in the US, partly due to its low-fat content (aldianews.com, 2017).

Although corn tortilla bread is popular, it lacks lysine and tryptophan (Huang *et al*., 2006), and shows lower protein, thiamin, riboflavin, and niacin, when compared to enriched wheat bread (Saldana and Brown, 2006). Corn-based diet was reported to lead to a low concentration of glutamic acid, lysine, tyrosine, and histidine in the cerebellum, but did not affect the physiological state in rats (Velázquez *et al*., 1993). The corn tortilla was rich in resistant starch, and its content increased by nixtamalization (Rojas-Molina *et al*., 2020). However, starch retrogradation takes place during frozen storage, increased friability (Rojas-Molina *et al*., 2020) and firmness due to stronger hydrogen bonds in the starch-lipid complex (Rojas-Molina *et al*., 2020). The formation of ice crystals and its subsequent re-crystallization (Bueso *et al.,* 2006) further affected tortilla bread friability. To slow down and prevent staling

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of tortillas, the addition of an anti-freezing agent is needed.

The anti-freezing agent prevents ice crystal growth and minimizes damage and aggregation of protein myofibril during freezing and thawing (Ustun and Turhan, 2015). One of the potential cryoprotectants is gelatin. Pig skin gelatin increases the specific volume of frozen dough, decreases the hardness of the crumb, and maintains fermentation stability (Yu *et al.,* 2019). However, pig gelatin does not suit some groups of religious groups. Jellyfish gelatin is an alternative to pork skin gelatin as a cryoprotectant. It is extracted from collagen (Hsieh *et al*., 2001) which comprises about 35% of jellyfish protein (Nining, 2020). As jellyfish is an underutilised resource in some countries including Indonesia, the purpose of this study was to the utilization of its crude gelatin as a cryoprotectant for frozen tortilla bread.

2. Materials and methods

2.1 Crude jellyfish gelatin extraction

Fresh jellyfish were collected from a fisherman in Bangkalan Regency, Indonesia. Gelatin extraction was carried out following a method previously used by Ayudiarti *et al*. (2019). The species was unknown. It was then washed with running water and then cut into small pieces of 2×2 cm. Jellyfish were ground with 1:2 of deionized water and heated at a temperature of 60ºC for 5 hrs using a hot plate. The mixture of ingredients was filtered using a filter cloth to separate jellyfish flesh from the washing liquid. The second filtration was carried out using a vacuum pump. The filtrate was reheated using a hot plate at 60ºC for 3 hrs. The concentrated filtrate was then dried in a drying oven at 50ºC for 24 hrs. The dried extract was ground and sifted with a 60 mesh sieve and then kept in an airtight container until use.

2.2 Preparation of corn flour

A local variety of Madura corn called Talango variety was purchased as dried corn on the cob, from a farmer in Talango island. Kernels were then removed from cobs. Dried corn kernels were first sorted, washed, and nixtamalised in limewater (5% of corn) using low heat for 45 mins. Nixtamalization was carried out by first putting cleaned and washed corn kernels in a large pan about 5 times the volume of corn. Tap water was added about three times the volume of corn. Lime, as much as 5% (w/w) of corn weight was then added to the mixture. The mixture was then cooked at low heat for 45 mins until the outer skin of the kernel was easily removed by gentle rubbing of fingers. The mixture was then let to stand for 24 hrs, after which it was washed with running water to completely remove lime, and then drained. Nixtamalised corn was ground in an electric grinder, then dried in a cabinet dryer at 60ºC for 24 hrs, and sifted using a 60 mesh sieve. Corn flour was kept in an airtight container until use.

2.3 Tortilla bread making

Tortilla bread was made by mixing nixtamalised corn flour, crude jellyfish gelatin (0, 5, 10%), garlic powder (5%) and water (90% of flour). All raw material was mixed using an electric mixer at low speed until it formed an even and smooth dough. It was then shaped into a ball that weighed around 20 g. The dough balls were flattened to form a round shape of about 1 mm thick. The round shape dough was then baked in a pan over low heat. The baking process was carried out until a cavity was formed in the centre of the dough. The tortilla was cooled at room temperature, then packed in an oriented polypropylene (OPP) plastic bag (40 cm x 60 cm; thickness 30 microns), sealed and stored in a freezer. Samples were withdrawn every seven days for three weeks period.

2.4 Colour analysis

The colour of tortilla bread was measured using a colour reader (CR-10, Konica Minolta, Japan). Data were taken from the centre of each piece of tortilla bread. Results were expressed as L (brightness), a (red-greenness) and b (yellow-blueness).

2.5 Hardness, elongation, tensile strength analysis

Prior to textural analysis, tortilla bread was cut into 35×70 mm strips. The hardness of tortilla bread was measured using the Texture Profile Analysis method in a texture analyzer (TAXT-Plus, Stable Micro System, Surrey, UK) using a cylinder probe P/35 at the following settings: pre-test speed 5 mm/s, test Speed 2 mm/s, post test speed 10 mm/s, distance 30 mm, time 5 s, trigger force 5 g. Elongation and tensile strength were measured using Tensile Grips A/TG using the following settings: pre-test speed 1 mm/sec, test speed 1 mm/s, post test speed 5 mm/s, distance 30 mm, time 5 s, trigger force 5 g. Measurement was repeated fifteen times for each sample.

2.6 Staling rate analysis

Staling rate was calculated as the average increase in hardness within 21 days of storage.

2.7 Moisture content and water activity analysis

The moisture content of frozen tortilla bread was determined by the oven method (105°C, weighed every 3 hrs until constant weight). The water activity of frozen tortilla bread was measured using a water activity meter (Hygropalm HP23 AW A Set 40, Rotronic, Singapore).

2.8 Sensory analysis

A hedonic sensory analysis was carried out using 15 semi-trained panellists to give their preference towards aroma, colour, taste, mouth feel, and overall preference, on a hedonic scale of 1 to 5. Panellists were students of the University Trunojoyo of Madura, who had completed the lesson on the principle of the sensory test and were familiar with tortilla bread. Panelists were asked to indicate their preference on a 1-5 scale, where 1 for 'do not like very much', 2 for 'do not like', 4 for 'like', and 5 for 'like very much'. Panellists were also asked to describe the sensory characteristics of tortilla bread where needed. Preference sensory tests could be carried out using semi-trained panellists instead of untrained panellists, especially when descriptive terms would be needed from them (Ruiz-Capillas *et al*., 2021). The number of panellists depended on dissimilarities among samples, and the objective of the studies (Ruiz-Capillas *et al*., 2021).

2.9 Data analysis

Data was processed using a statistical package SPSS for Windows, Version 16.0. Released 2007 (Chicago, SPSS Inc.). An analysis of variance was performed, and a mean comparison was conducted using Duncan's multiple range test ($\alpha \leq 0.05$). Non-parametric Mann-Whitney test was employed to identify statistical differences ($p \leq 0.05$) among preferences for sensory parameters.

3. Results and discussion

3.1 Colour analysis

The value of L and a of tortilla bread increased with prolonged storage (Table 1). Value b, on the contrary, decreased as storage progressed, although, at the end of the experiment, there was no significant difference in the b value from that at the beginning of the experiment. The colour of maize reflected the presence of carotenoids (Lozano-Alejo *et al*., 2007), with zeaxanthin and lutein being major carotenoids in maize (Kuhnen *et al*., 2011). Although storage time is one of the factors to degrade carotenoids, it did not seem to occur in our work, since there was relatively no loss of colour, which seemed due to the freezing temperature of storage. As there was no loss of colour, the oxidation process of carotenoids (Rodriguez-Amaya, 1999; Lozano-Alejo *et al*., 2007; Calvo-Brenes and O'Hare, 2020; Urbina *et al*., 2021) did not seem to occur in the sample. Freezing (Dias *et al*., 2014) or room temperature (Lin and Chen, 2005) storage did not alter carotenoids. An increased L and a value were shown previously as the effect of gelatin (Urbina *et al*., 2021). Furthermore, the food matrix could protect carotenoids from destruction (Dias *et al*., 2014).

3.2 Hardness, elongation, tensile strength analysis

The incorporation of jellyfish gelatin resulted in harder tortilla bread (3887, 4971, and 5187 g for tortilla bread with 0, 5, and 10% gelatin, respectively) (Table 3). Storage time increased hardness (3031.7 g at the beginning of storage, and 5837.3 g at the end of storage), as expected. An increase in hardness during storage was due to retrogradation and water-binding led to an increase in density (Yu *et al*., 2019). More gelatin also increases water absorption and density of dough, thus increasing hardness (Ayudiarti *et al*., 2017; Yu *et al*., 2019). The incorporation of hydrocolloids gave two opposite effects (Guarda *et al*., 2004): an increase in stiffness due to reduced swelling of starch and a weakening of starch network. The effect of the interaction between starch and hydrocolloids on the hardness of the blend was specific and depended on the types and concentration of both materials (Subaric *et al*., 2011; Acosta *et al*., 2015; Rodrigues-Castellanos *et al*., 2015; Yu *et al*., 2019). Hardness was related to crosslinking between the starch and hydrocolloids, and the extent of retrogradation (Muhrbeck and Eliasson, 1991).

The three-dimensional structure between starch and hydrocolloid during prolonged frozen storage changed over time, with thinner and shorter strands in the network (Muadiad and Sirivongpaisal, 2021). Hardness seems to correlate to the water-binding properties of the system, that corn starch stored at freezing temperature needed incorporation of material with water-binding ability, to maintain hardness (Woo *et al*., 2021). Types of hydrocolloids added to the starch system showed a different effect on the textural properties of the network (BeMiller, 2008; Rodriguez-Castellanos *et al*., 2015).

Table 1. Physical and chemical characteristics of frozen maize tortilla bread as the effect of crude jellyfish gelatin concentration.

Gelatin Concentration (%)	L	a	b	Hardness (g)	Elongation (%)	Tensile Strength (N)	Water Activity
0	$+25.93^{b}$	8.79	36.52	3887^{a}	95.82^{c}	14.10^{c}	0.94^{c}
5	$+24.71^{a}$	9.01	36.71	4971^{b}	94.16^{b}	11.68^{b}	0.88^{b}
10	$+25.42^{ab}$	9.26	37.16	5187^{b}	92.02^{a}	8.68^{a}	0.81^{a}

Values with different superscripts within the same column are statistically significantly different at 95% confidence level.

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The addition of several hydrocolloids could improve the textural characteristics of the starch system (BeMiller, 2008; Rodrigues-Castellanos *et al*., 2015).

Gelatin addition reduced the elongation of tortilla bread (95.82, 94.16, and 92.02% for tortilla bread containing 0, 5, and 10% gelatin, respectively) (Table 1). However, although elongation changed during storage, there was no difference between elongation at the start (94.65) and the end of storage (95.03) (Table 2). Elongation in tortilla bread seemed to be easily altered by changes in matrix composition. This was not as previously reported, where elongation seemed not as sensitive as tensile strength or other textural parameters in responding to changes in protein concentration in the starch-protein system (Al-Hassan and Norziah, 2012). In their report, while tensile strength was lowered by an increase in protein concentration, elongation was not changed (Al-Hassan and Norziah, 2012). The difference between our result in theirs may be caused by a different type of starch and protein used in our works and in their experiment.

Gelatin concentration, as expected, reduced tensile strength (14.10, 11.68, and 8.68 for tortilla bread with 0, 5, and 10% gelatin, respectively) (Table 1). The phenomenon was due to the binding of free water by gelatin in the tortilla, to reduce starch crystallization, rendering a more flexible network (Muadiadi and Sirivongpisal, 2021). Protein in the starch-protein system improved resistance to break and extensibility (Acosta *et al*., 2015). At the end of three weeks of storage, the tensile strength of the tortilla did not differ from that at the beginning of storage (Table 2). This, again, showed the effect of gelatin in maintaining the flexibility of the tortilla at freezing temperatures. As a hydrocolloid, gelatin has a high capacity for binding water, and the bound water leads to better flexibility in the starch system (Woo *et al.*, 2021). The gelatin in the corn tortilla matrix was very likely to determine tensile strength and overall flexibility (Rodrigues-Castellanos *et al*., 2015). There was a significant effect of interaction between gelatin concentration and storage time on hardness, elongation, and tensile strength (Table 3).

3.3 Staling rate analysis

The staling rate of frozen tortilla bread was not affected by gelatin concentration, storage time, and interaction between the two factors. The reason was not clear. The staling process was considered a complicated mechanism (Gray and BeMiller, 2003), including crosslinking of starch and protein and lipid, water migration microscopically and macroscopically, and crystallization of starch (Gray and BeMiller, 2003). Gray and BeMiller (2003) also summarised that above 0°C, higher temperature retarded staling, the same was true for freezing temperature. In our work, either gelatin or

Table 2. Physical and chemical characteristics of frozen maize tortilla bread as the effect of storage time.

Storage Time (day)	L	a	b	Hardness (g)	Elongation (%)	Tensile Strength (N)	Water Activity	Moisture Content (%)
0	$+24.26^{b}$	$+7.07^{a}$	$+38.13^{bc}$	3031.7^{a}	94.65^{bc}	9.77^{a}	0.87^{ab}	22.74^{ab}
7	$+20.99^{a}$	$+8.51^{b}$	$+33.39^{a}$	4957.5^{b}	94.42^{c}	10.88^{a}	0.89^{ab}	24.01^{b}
14	$+26.91^{c}$	$+10.01^{c}$	$+39.15^{c}$	4911.9^{a}	91.92^{a}	14.68^{b}	0.85^{a}	19.15^{a}
21	$+29.25^{d}$	$+10.50^{c}$	$+36.52^{b}$	5837.3^{c}	95.03^{c}	10.62^{a}	0.90^{b}	25.44^{b}

Values with different superscripts within the same column are statistically significantly different at 95% confidence level.

Table 3. Physical and chemical characteristics of frozen maize tortilla bread as the effect of interaction between storage time and gelatin concentration.

Storage Time (day)	Gelatin Concentration (%)	Hardness (g)	Elongation (%)	Tensile Strength (N)
0	0	2477^{a}	95.32^{efg}	12.31^{c}
	5	3437^{a}	94.41^{de}	8.97^{ab}
	10	3181^{a}	94.21^{d}	8.04^{a}
7	0	4126^{bc}	96.69^{h}	12.34^{c}
	5	4808^{c}	95.64^{fg}	11.29^{bc}
	10	5909^{d}	90.92^{b}	9.01^{ab}
14	0	3010^{a}	95.35^{efg}	19.03^{d}
	5	5920^{d}	91.95^{c}	13.65^{c}
	10	5806^{d}	88.45^{a}	11.36^{bc}
21	0	5937^{d}	95.92^{gh}	12.73^{c}
	5	5720^{d}	94.64^{def}	12.81^{c}
	10	5855^{d}	94.52^{de}	6.32^{a}

Values with different superscripts within the same column are statistically significantly different at 95% confidence level.

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freezing temperature, or both, possibly hindered staling. Interaction among food components in the matrix influenced staling (Weber, 2000), and this was not easy to comprehend using the method employed in our work.

3.4 Moisture content and water activity analysis

Moisture content in frozen tortilla bread was not significantly changed during storage (Table 2). Gelatin concentration did not have any significant effect on moisture content. Low pressure in the freezer caused low humidity and consequently enhanced moisture loss from the food matrix. In our work, water vaporization from the tortilla seemed to be minimized, likely due to the strong water binding of gelatin in the tortilla.

Tortilla bread with higher gelatin concentration showed lower water activity (Table 1). Tortilla bread with no gelatin indicated water activity of 0.96, while those with 5 and 10% gelatin were 0.88 and 0.81, respectively. Gelatin is a hydrocolloid capable of binding to water and makes it unavailable for microbial growth. A similar result was reported previously, where frozen wheat bread also showed a decrease in water activity as the effect of hydrocolloid concentration added into the dough (Bárcenas *et al*., 2004). In a food matrix where protein is present such as in maize, water activity was further reduced due to water binding competition between protein and starch (Schiraldi *et al*., 1996; Bárcenas *et al*., 2004). Frozen tortilla bread maintained water activity during storage (Table 2). Low humidity in the freezer, and water binding by gelatin, might play a role in keeping low water activity of frozen tortilla bread.

3.5 Sensory analysis

There were differences among samples in the preference toward all sensory parameters (colour, taste, texture/mouthfeel, and overall preference), except aroma (Table 4). The highest score (3.87) on preference towards colour was shown by most samples containing no gelatin. Gelatin tended to reduce preference toward colour (Table 4), since pigment was not removed completely in jellyfish gelatin (Ayudiarti *et al.*, 2017). The highest scores for preference towards taste (4.0) and overall preference (3.95) were shown by samples containing 5% gelatin at day 14 of storage (Table 4). The highest score for preference towards texture/mouthfeel was shown by samples containing 10% gelatin on day 14, as well as samples with no gelatin on day 21 (Table 4). Therefore, gelatin seemed to increase preference for taste, texture, and overall characteristics (Table 4). Panellists thought that frozen Madura corn tortilla bread without the addition of jellyfish gelatin tasted bland, while that with the addition of 10% gelatin tasted very salty. The salty taste of jellyfish gelatin was caused by sodium chloride (Bonaccorsi *et al.*, 2020).

Results of the hedonic sensory analysis showed that aroma was not different among samples (Table 4). Strong maize aroma was predominant in frozen tortilla bread and subsequently did not lead to any difference in the aroma of all samples. Tortillas made from a traditional process gave a stronger maize aroma which was preferred by consumers (Iuga *et al*., 2019). Consequently, maize smell was an important factor in determining consumers' choice of tortilla bread (Iuga *et al*., 2019). Iuga *et al*. (2019) indicated that consummers' choice of tortilla bread is mainly determined by textural properties (tensile strength, breaking distance, elasticity), tooth packing, masticability; and sweet or salty taste. Meanwhile, colour was the least considered when choosing a tortilla for purchase (Iuga *et al*., 2019).

4. Conclusion

The incorporation of crude jellyfish gelatin into frozen tortilla bread maintained its moisture content, water activity, tensile strength, and elongation, and prevented staling, while increasing brightness and redness, during three weeks of storage. Crude gelatin in

Table 4. Sensory preference score of frozen maize tortilla bread,

Storage Time (day)	Gelatin Concentration (%)	Aroma	Color	Taste	Texture	Overall
0	0	3.47	3.87^{c}	2.67^{a}	2.73^{abc}	3.13^{abc}
	5	3.67	3.67^{c}	3.13^{abc}	3.00^{bc}	3.47^{abc}
	10	3.6	3.87^{c}	3.13^{abc}	3.40^{c}	3.87^{c}
7	0	3.67	3.8^{bc}	2.27^{a}	2.07^{a}	2.93^{ab}
	5	2.93	3.67^{bc}	3.73^{bc}	3.13^{bc}	3.87^{c}
	10	2.8	3.60^{bc}	3.53^{bc}	3.60^{c}	3.73^{bc}
14	0	3.8	3.87^{bc}	3.00^{ab}	2.93^{abc}	3.47^{abc}
	5	3.67	3.40^{bc}	4.00^{c}	3.53^{c}	3.93^{c}
	10	3.4	3.20^{abc}	3.60^{bc}	3.13^{bc}	3.47^{abc}
21	0	3.27	3.47^{bc}	3.73^{bc}	3.60^{c}	3.60^{bc}
	5	3.6	3.00^{ab}	2.40^{a}	2.20^{ab}	2.67^{a}
	10	3.4	2.60^{a}	3.13^{abc}	3.20^{c}	3.00^{ab}

Values with different superscripts within the same column are statistically significantly different at 95% confidence level.

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frozen tortilla bread improved preference towards taste, texture, and overall likeness, but reduced preference towards colour.

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