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IMPROVING GLUTEN-FREE BREAD PRODUCTION WITH TAPIOCA AND CORN FLOUR: PHYSICOCHEMICAL INSIGHTS

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Abstract: *Gluten-free bread production remains limited in the commercial market, mainly due to technical issues such as dough fragility and product weight loss during processing. This study aimed to evaluate the physicochemical properties of tapioca and corn flour as alternative raw materials for gluten-free bread formulation, and to assess their feasibility in large-scale production. The research utilized a comparative experimental design involving three flour samples: pure tapioca flour, pure corn flour, and a 1:1 blend of both. The physicochemical properties were analyzed through scanning electron microscopy (SEM) to observe starch and gluten granules, while protein content, water absorption capacity, and starch liquefaction numbers were quantified using standard AOAC methods. Results indicated that production without mechanical pulling or pressure yielded better texture and structure. A significant linear correlation ($p < 0.01$) was observed between gluten and protein content, as well as water absorption capacity. Additionally, starch content varied significantly across samples ($p < 0.05$), and strongly influenced liquefaction and hydration behavior ($p < 0.01$). These findings suggest that the appropriate physicochemical profiling of low-gluten flours supports the feasibility of consistent gluten-free bread production on a larger industrial scale.*

Key words: *starch, glutenin, glassy starch, rubbery starch, rubbery gluten.*

1. Introduction

The statement that gluten plays a significant role in determining the quality of bread has been an established concept to date. The viscoelastic matrix maintains the quality of the bread against excess stress and pulls which affect the selection of high-gluten ingredients. On the other hand, excessive gluten consumption has been linked to Celiac disease, Glutenataxia, Dermatitis herpetiformis [4].

The healthy living trend strongly influences the community's behavior towards the consumption of gluten-based bread. Currently, gluten-free bread has

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become an increasingly interesting product to explore further**.** However, despite the significant number of studies on gluten-free bread production, the large-scale production of such products remains limited**.** In this study, we mapped out 750 research papers from 2010-2024 regarding this matter as visualized in Figure 1. In 2010, there were many studies on the gluten-free diet that were published, which were then followed by a significant increase in the production of gluten-free bread from 2018 – 2024.

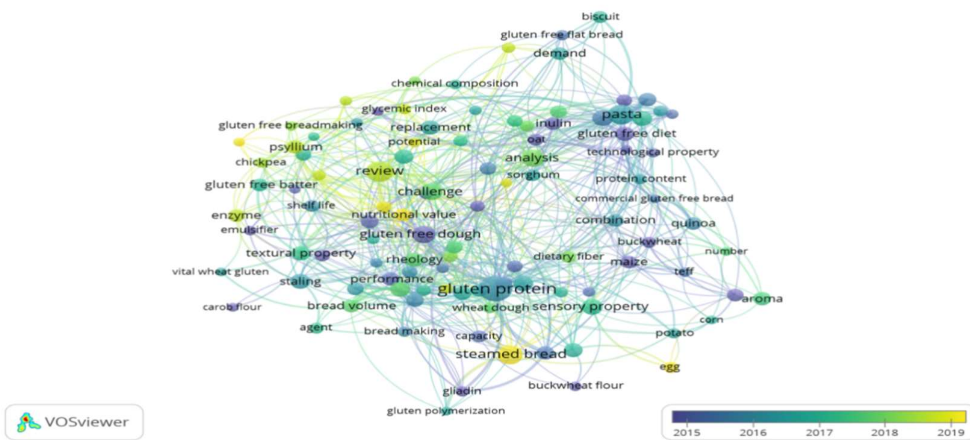


Fig. 1. *Map of research on the role of gluten on bread quality from 2010-2024. Data collection uses Harzing's Publish or Perish (Window GUI Edition) 7.31.3306.7768 and visualization uses VOSviewer version 1.6.16*

Figure 1 allows an easier classification of prior studies based on the method of gluten-free bread production by:

- replacing gluten with non-gluten component (*NGC*) ingredients, such as: rice [26, 32, 34], starch from seeds, fruit, plant extracts, seaweed [25], cornstarch [10], tapioca [7];
- modifying the gluten protein chemical reaction (*CMR*) using the phosphorylation method [29], glycosylation [3], deamidation of the amide groups from glutamine and asparagine to carboxylic, glutamate and aspartic acid groups [18]; acylation of amino groups is converted to amides [19, 20];
- use of Natural Hydrocolloids (*NH*), such as: xanthan gum [13, 14, 27], Guar gum [8, 23], Methylcellulose, Carboxy methyl cellulose gum [27], balagu seed gum, wild sage seed gum [30].

Despite the large body of references on the production of gluten-free bread, large-scale production remains constrained by various limitations**.** This study was performed to address this gap by conducting a survey with ten large companies to investigate their perceived constraints in large-scale gluten-free bread production. Two factors with the highest percentages have prevented the companies from scaling up gluten-free bread production: the brittleness of the dough due to the tensile process and excessive weight loss.

These two factors relate to the well-established concepts about the Physico-chemical properties of gluten and starch in flour [1, 2]. Gluten has high elastic properties at a moisture content of 6% if heated at 60-70°C or 15% without heating. These properties also apply to starch with a higher temperature threshold and moisture content. The addition of water causes elastic and springy properties and forms intermolecular bonds. When gluten is hydrated, irreversible cross-links are formed, rendering the starch in glass form unable to absorb water. When gluten is heated through the glass transition, it becomes rubbery and easily absorbs water.

In reference to Figure 2, gluten-free bread production mostly relies on starch elasticity and processing conditions in the rubbery starch (*RS*) area. Will high starch content provide adequate dough elasticity during the process? Can starch maintain a small mass loss? These characteristics were found in gluten [5]. To answer these questions, ingredients that are high in starch, i.e. tapioca and cornstarch, were compared to wheat flour. This test was carried out on machines that required pulling (flat wafer and wafer roll machines) and machines without the need for pulling (sponge and eclair making machines).

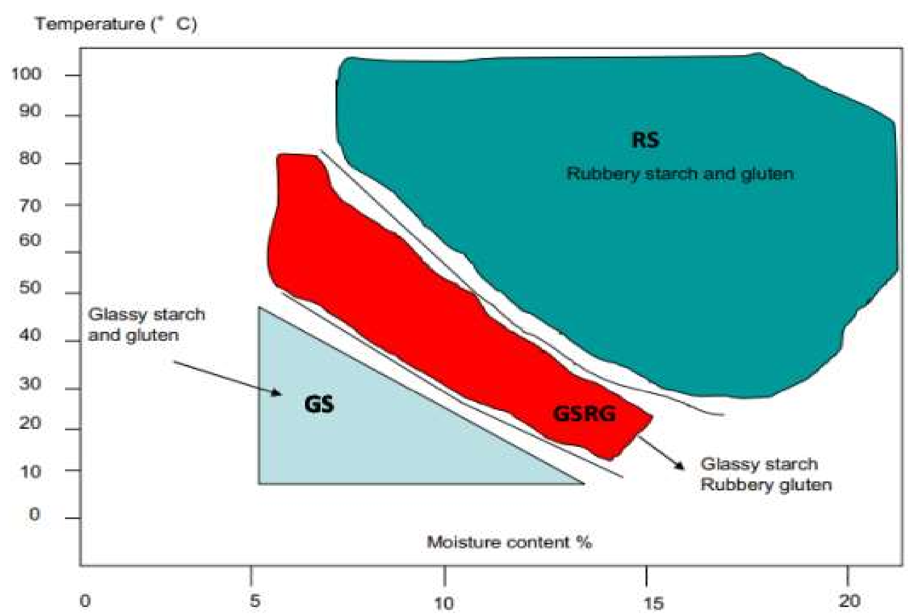


Fig. 2. *Physico-chemical behavior of starch and gluten during bread-making, illustrating transitions from glassy to rubbery phases that influence dough elasticity and water absorption [5, 31]*

This study compared the effectiveness of low-gluten flour (tapioca flour, cornstarch) to wheat flour in the manufacture of wafer flat products, wafer rolls, cakes, and eclairs. We examined the effectiveness of those ingredients based on the physical appearance of the product, weight loss, and the acceptability of the consumers.

2. Materials and Methods

2.1. Physicochemical Properties of Gluten-Free Dough

In this study, we investigated the physicochemical properties of gluten-free dough using several phases involving starch and gluten, namely Glassy Starch and Gluten (*GS*), Glassy Starch Rubbery Gluten (*GSRG*), and Rubbery Starch and Gluten (*RS*).

Glassy Starch and Gluten (*GS*): Refers to the combination of starch in a glassy form and gluten. In this phase, the starch remained rigid and unable to absorb water, while gluten provided some elasticity to the dough.

Glassy Starch Rubbery Gluten (*GSRG*): In this phase, the starch remained in its glassy form, but gluten had absorbed water and become more elastic. This allowed for the formation of a more flexible dough, though challenges in maintaining texture stability remained.

Rubbery Starch and Gluten (*RS*): In this phase, the starch was in a rubbery form, allowing the dough to absorb more water and resulting in a dough texture that was chewy and pliable, which is desirable in gluten-free bread products.

This explanation provides a clearer understanding of how these phases played a role in gluten-free bread production and were used in our analysis of gluten-free flour dough.

2.2. Sample Collection

Samples of wheat flour, corn flour, and tapioca flour were purchased from a local supermarket. These flours were selected based on their widespread commercial availability and frequent use in gluten-free food development. The analyses were conducted in two locations: physicochemical testing in Jakarta and machine-based baking trials in Bandung.

A. Dough Preparation

Three types of dough were prepared using the selected flours, with each formulation adjusted to contain 5, 10, and 15% water (w/w). These water levels were chosen to evaluate the impact of hydration on dough structure, as water content plays a critical role in starch gelatinization, gluten development, and dough machinability. A total of 9 dough samples (3 types × 3 water levels) were produced. The doughs were manually kneaded for consistency and thinly sliced to a thickness of approximately 2.5 mm. They were then grouped into three categories: *GS* (non-heated), *GSRG*, and *RS* (both heated at 60°C for 30 minutes). The heating temperature was selected to simulate low-heat drying and preserve the granule structure for microscopic observation.

B. Product Trials and Formulation Rationale

The wafer flat, wafer roll, sponge cake, and choux pastry formulations were selected as representative baked products with varying processing requirements and textures, to evaluate flour performance in real applications. These four products were manufactured using standard operating procedures from an industrial baking company. The recipes predominantly consisted of flour and water (comprising over 80% of the total formulation), with other ingredients (e.g., sugar, salt, eggs) excluded from this study due to their minimal proportion and limited impact on flour behavior.

2.3. Physical – Chemical Analysis

2.3.1. The Falling Numbers in the Flour

Determination of the drop rate correlated with α amylase in flour. The procedure was conducted based on the AACC 56-81.03 method [1] using the Perten Falling Numbers instrument expressed in seconds. The liquefaction numbers (*LN*) of the three flour samples are explained by the Equation (1).

$$LN = \frac{6000}{FN-50} \quad (1)$$

where:
LN is the liquefaction number;
FN – the falling number.

2.3.2. Gluten Content

The gluten analysis procedure was conducted according to AACC 38-12.02 [2], where 0.5 ml of NaCl (2%) was added to 10 g of dough samples to be mixed (1-3 min). The dough was set aside for 30 minutes before being washed with 2% NaCl, then filtered and dried. For obtaining the gluten content was applied Equation (2).

$$Gluten = \frac{weight\ gluten\ x^2}{weight\ sample} \cdot 100\ [\%] \quad (2)$$

2.3.3. Protein Content

The biuret method with was conducted at λ 595 nm UV-Vis on the spectrophotometer as proposed by Manzoor et al. [21].

2.3.4. Starch Content

The analysis of starch content following Mitchell [24], where carbohydrates are hydrolyzed into monosaccharides to reduce Cu^{2+} to Cu^{1+}. Meanwhile, the excess Cu^{2+} was titrated iodometrically.

2.3.5. Ash Content

The procedure of ash content measurement according to Marchall [22], where flour samples were roasted in an oven for 5-6 hours at 525°C. The ash content was then determined gravimetrically.

2.3.6. Water Absorption Capacity

To measure the water absorption capacity, two grams of sample were dissolved in 25 mL of distilled water in a centrifuge tube. The sample was set aside for 24 hours to absorb water before being centrifuged at 110 rpm for 30 minutes. The water phase above the sample was removed and the sample at the bottom was weighed. The difference in the weight of the sample residue to the initial sample weight showed the value of water absorption (g/g).

2.3.7. Scanning Electron Microscopy (SEM) Analysis

The SEM procedure used in this study was adapted from Koga et al. [15]. The structure of starch and gluten granules was analyzed using SEM (ZEISS, type EVOMA10).

2.4. Statistical Analysis

The ANOVA test was carried out on the IBM SPSS Statistic 26 software to measure the average value, range of variation, standard deviation. Furthermore, the post-hoc Tukey HSD test was also done to examine the differences in psychochemical test results. The psychological correlation of the samples was measured using the Pearson Correlation (2-tailed).

2.5. Sensory Evaluation

The four types of baked products were evaluated by 100 randomly selected respondents. Sensory attributes (taste, shape, texture, and color) were rated using a five-point Likert scale: 1 = dislike very much, 2 = dislike, 3 = neutral, 4 = like, 5 = like very much.

3. Results

The physico-chemical changes of starch and gluten in the bread-making process in the three samples are presented in Figure 3. The addition of 5% water without any heating treatment (*GS*) did not bring any changes in the gluten and starch of the three samples. The starch remained hard and glassy. The tapioca flour and cornstarch dough did not stick together evenly and there were cracks on several sides. Wheat flour dough looked quite sticky and sticks to each other on all sides of the dough. The SEM observation was conducted at 100 µm zone.

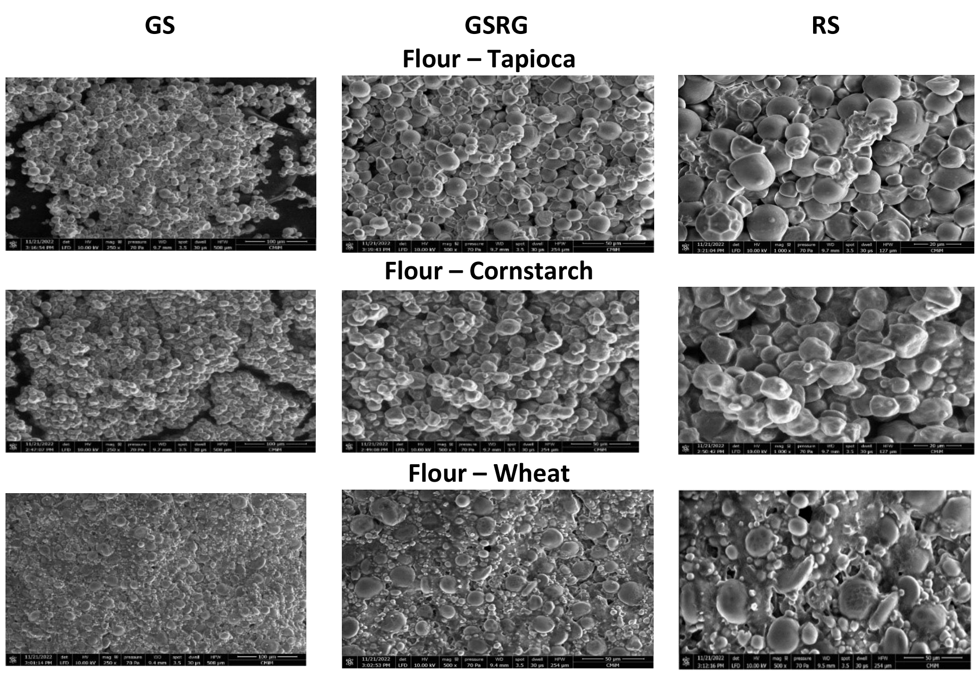


Fig. 3. *Physical changes in flour due to moisture content and heating. At 5% moisture content without heating will cause glassy starch (GS), heating at 60°C for 30 minutes will produce glassy starch and rubbery gluten (GSRG), if the moisture content is 10% and rubbery starch (RS) will occur at 15% moisture*

The addition of 10% water with heating at 60°C for 30 minutes was conducted, focusing on the glassy starch and rubbery gluten condition. In this phase, gluten usually undergoes physical changes, from hard to flexible or a specific sign of gluten melting that renders it sticky. Such condition is only seen in wheat flour, tapioca flour and cornstarch, while no change was found in other ingredients. Although gluten was also found in tapioca flour and cornstarch, the content was small and it did not significantly determine the stickiness of the dough as shown by the cracks on several sides for tapioca flour and cornstarch.

In the rubbery starch (*RS*) phase, with a water content of 15% and heating at 60°C for 30 minutes, the starch went through a transition period in its glass form, melted on each side, and formed more sticky dough. This condition was obvious in wheat flour, where the melting of starch was clearly visible, added with the residual melting of gluten after passing through the GSRG phase. The melting of starch in tapioca flour and cornstarch was not obvious, but its elastic properties were visible from the stickiness of the dough when touched.

The differences of the three samples in the psychochemical analysis are presented in Table 1. The starch content for the three samples was significantly different ($p < 0.05$). On the other side, no significant difference was found for the ash content ($p > 0.05$). The protein content, falling number (*FN*), liquefaction number (*LN*), wet gluten, and water absorption capacity were significantly different for wheat flour ($p < 0.05$), while no significant differences were found between tapioca flour and cornstarch.

Physico-chemical tests of tapioca flour, cornstarch and wheat flour Table 1

Analysis	Tapioca	Cornstarch	Wheat
Protein [%]	1.40 ± 0.52^{a}	1.66 ± 0.57^{a}	10.66 ± 057^{b}
Starch content [%]	78.66 ± 3.21^{b}	85.33 ± 1.52^{a}	68.33 ± 1.52^{c}
Ash content [%]	0.20 ± 0.10^{a}	0.13 ± 0.05^{a}	0.116 ± 0.02^{a}
Falling number at 14% moisture content [Sec.]	223.33 ± 5.77^{a}	220.00 ± 26.45^{a}	315 ± 5.00^{b}
Liquefaction number	34.33 ± 1.15^{b}	35.83 ± 5.20^{b}	22.33 ± 1.57^{a}
Wet gluten [%]	0.27 ± 0.15^{a}	0.23 ± 0.05^{a}	23 ± 1.00^{b}

Note: ANOVA test using Tukey HSD Post-Hoc, results are displayed in Mean ± SD. Significant level at $p < 0.05$, the same superscript indicates no significant.

The correlation tests done in this study showed that each indicator influenced one another at a significant level of 0.01 ($p < 0.01$), except for the ash content. Gluten has a linear effect on water absorption capacity, falling number (*FN*), and protein content. Starch content has a linear effect on liquefaction number (*LN*) and water absorption capacity as seen in Table 2.

Test the influence between variables using the Pearson Correlation method Table 2

Correlation	Protein	Starch	Ash	*FN*	*LN*	Wet gluten	Water absorption capacity
Protein	1	-.879**	-.369	.960**	-.918**	.995**	-.987**
Starch	-.879**	1	.288	-.854**	.837**	-.895**	.888**
Ash	-.369	.288	1	-.409	.388	-.363	.480
Falling number (*FN*)	.960**	-.854**	-.409	1	-.991**	.959**	-.955**
Liquefaction number (*LN*)	-.918**	.837**	.388	-.991**	1	-.918**	.917**
Wet gluten	.995**	-.895**	-.363	.959**	-.918**	1	.985**
Water absorption capacity	-.987**	.888**	.480	-.955**	.917**	.985**	1

Before conducting a trial of flour dough in a bread making machine, we also conducted an organoleptic test (shape, color, texture) on thin dough (±1 mm) which was heated at 120-130°C for 30 minutes, whose results are shown in Figure 4.

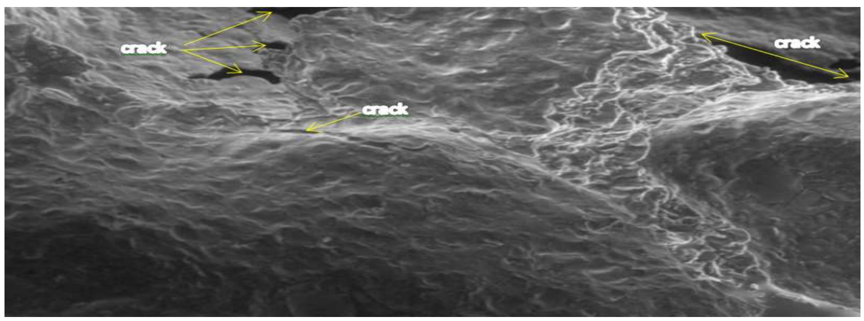
The tapioca dough layer cracked and broken on several sides. It has a textually hard property but it is brittle. This also happens with the cornstarch mixture but the tears in the cornstarch are larger at one point. The layer of wheat flour did not have any cracks and damages. The texture of the flour coating was considered elastic in the range of 2-3 minutes, but cornstarch and tapioca showed elasticity of only < 1 min.

The results of the dough trial on flat wafer, wafer roll, sponge cake, and choux production machines can be seen in Figure 5 and Table 3. Wafer flat products basically require an elastic dough character when compression is applied. In tapioca flour, there is still a perforated surface layer on each side irregularly. A similar condition also occurred in the cornstarch dough, but the cornstarch was more dominated by an uneven (thin) surface that extended following the wafer mold. In wheat flour, the surface layer was even with no holes and a thin elongated surface. No significant difference was identified in weight loss in the case of tapioca and cornstarch ($p > 0.05$), but significant difference was found ($p < 0.05$) in wheat flour.

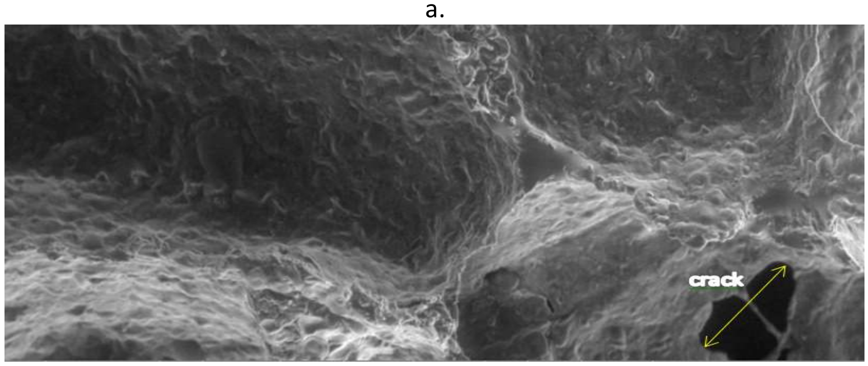
Wafer roll is a product with a pull and roll system, requiring ductile and elastic layers. Pores were found in the tapioca dough layer with a varying diameter (0.3 - 2 mm). During the rolling process, the dough often broke and the layers failed to stick. Pores were also found in the cornstarch layer, but large and elongated tears during the pulling and rolling process occurred frequently. Whereas, the texture of this dough wafer skin was very hard and brittle. Wheat flour wafer roll skin layers looked tight and pores were rarely found. The pulling and rolling of the skin layers did not break the layers which remained firmly attached. Weight loss in tapioca flour and cornstarch was not significantly different (p

> 0.05), when compared with tapioca flour where a significant difference ($p < 0.05$) was found.

Sponge cake in the making does not require pulling and pressure as changes in shape occur during the baking process. Pores of diameters between 2 - 5 mm were found in the sponge cake made from tapioca and cornstarch, while small and flat pores are displayed by sponge cakes made from wheat flour. Despite their physically different appearance, the weight loss of the three samples was not significantly different ($p > 0.05$).



a.



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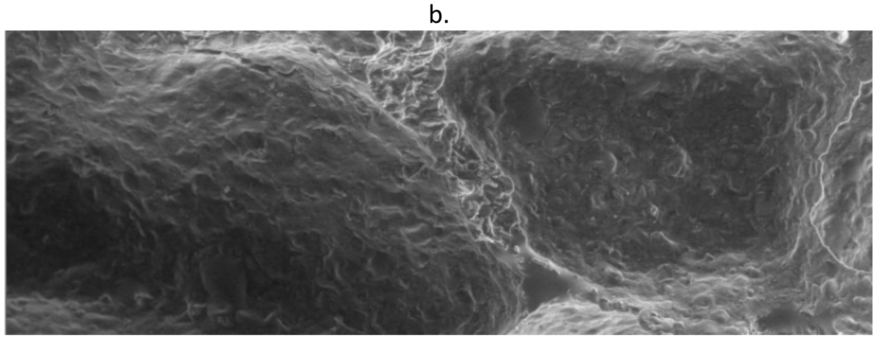

c.

Fig. 4. *Organoleptic evaluation of thin-layer dough after high-temperature heating Visual comparison of tapioca flour (a.), corn flour (b.), and wheat flour (c.) to assess texture and surface characteristics relevant to gluten-free baking applications*

Fig. 5. *Comparison of physical and functional characteristics of different flour samples used in various gluten-free product types to evaluate their performance in dough elasticity and baking outcomes*

For the choux pastry products, the three flours had almost similar physical appearance. There is a big hole in the middle of the cake that hallmarks this cake. Hence, choux pastry is often filled with paste. There was no significant difference ($p > 0.05$) found in the weight loss in the three flours.

The product preference test that involved 100 randomly selected respondents showed the following results. Wheat flour-based ingredients (W) for all product variants showed an average preference score ranging between 4 - 5 which dominates in point of shape, color, textures, and taste (> 85%).

Tapioca flour (*T*), with regard to taste in all product variants dominated on a scale of 4, meaning that the taste of the cake made from tapioca was still acceptable. For the texture of the wafer roll and wafer flat products, the scores ranged between 2 and 3, indicating that the texture of the products was not very much liked by the respondents. On the other hand, sponge cake and choux products showed a high preference score of 4. In general, tapioca flour can be used to make sponge cake and choux products, but when it is used for wafer flat and wafer roll products, its brittle texture should be considered (Figure 6).

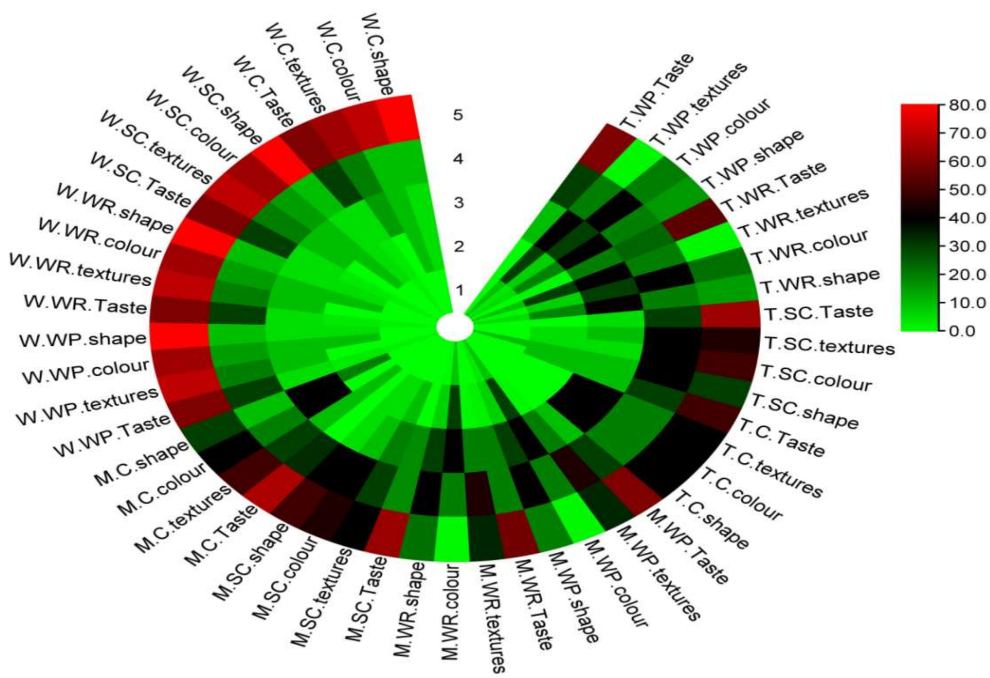

Fig. 6. *Respondents' level of liking for the organoleptic of the product. Using a Likert scale (1= dislike very much; 2= dislike; 3= neutral; 4= like; 5= like very much) for wafer flat (WP), wafer roll (WR), sponge cake (SC) and choux (C) products made from wheat (W), tapioca (T) and cornstarch (M)*

The cornstarch-based ingredient (*M*) showed similar outcome as tapioca flour. For wafer flat and wafer roll products, many respondents did not like the texture and shape, but the taste was preferred (scoring 4). Cornstarch as the ingredient of sponge cake and choux was easily accepted by respondents, on account in characteristics which almost resemble the wheat flour-based products.

4. Discussion

The dough formation is characterized by the formation of a network of gluten and starch, where flour that contains protein produces protein fibrils to form a cohesive dough [11]. The order of the molecular level is not yet a common agreement, but almost all researchers agree to the function of the dough. The addition of water makes the dough unite and become homogeneous. When absorption of water occurs, the hydration process will produce a new surface (powder particles) to the water. In wet dough, the protein network will wrap the starch granules to produce minimal cavities and cracks [33]. This condition only occurred in wheat flour, where cracks appeared for ingredients that are low in gluten (tapioca and cornstarch) in the *GS*, *GSRG*, and *RS* phases. These findings are relevant for guiding industrial applications, particularly in optimizing formulations for gluten-free dough systems where structural stability is a challenge.

Starch is a biopolymer of amylose and amylopectin. Amylose is a hard and strong gel, while amylopectin is easily dispersed into water with soft and elastic texture. There is a strong correlation between the amylose content in starch and its hardness [17]. The greater the amylose content, the more fine the fibers wrapping the granules (Figure 3) as found in cornstarch. The tough and inflexible properties were visible in the layers of tapioca flour and cornstarch dough for wafer roll and wafer flat products. Without the pulling process, the thin layers of the mixture of the two samples indicated the presence of large pores (Figures 2a and 2b). This suggests limitations in using certain gluten-free flours for products requiring flexibility or structural strength during mechanical processing.

The protein groups that affect the bread making are glutenin and gliadin [16]. The polypeptide bonds consisting of glutenin, gliadin and water are the remaining elastic mass in the glassy starch phase and loss of starch weight [9]. The making of dough usually relies on the addition of water to obtain changes in starch and gluten. The dough must be elastic because there will be a thinning process that will reduce the amount of O_2 trapped in the dough. For low-gluten ingredients, such a condition is not easily formed even though reaching the *RS* phase requires heating the dough up or adding water. These treatments affected the viscosity of the bread dough. High water content will cause brittleness and mass loss in the oven. This study shows that tapioca flour and cornstarch form large and brittle pores when the layer is made thin. Understanding these physicochemical transitions is important for scaling up production processes that aim to retain desired textural qualities in gluten-free bread products.

The gluten content correlates with viscosity and elasticity [12]. This study also found that the gluten content has a strong influence ($p < 0.01$) on the water absorption capacity. Viscosity and elasticity are high due to high water absorption. Falling number (*FN*) is a predictor of flour quality, where low FN indicates high α amylase. *FN* and *α* amylase activities were not found to be linearly correlated according to Perten [28]. Therefore, Perten [28] replaced *FN* with *LN* to make it linear and to make the detection of flour damage due to α amylase activity easier. Furthermore, this study also found that the LN value had a positive correlation ($p < 0.01$) on the water absorption capacity in terms of dilution (flour elasticity). These insights offer practical implications for flour selection and formulation adjustments in gluten-free baking systems.

The results and explanation of this study trigger the understanding that low-gluten flour can be used optimally on a large scale for products that do not require pulling, rolling and compression processes in the making [6]. The quality of wheat flour bread is superior to low gluten flour. The concept of psycho-chemistry of starch and gluten in flour [5] only applies when the gluten content is high. The glassy starch look was not clearly seen at the *GS* and GSRB stages, as well as rubbery starch which only occurred in the RS stage for low gluten flour. Therefore, when selecting gluten-free flours one must consider their structural behaviour during specific processing stages, especially in large-scale production setups.

5. Conclusions

Gluten significantly affects the quality of dough and bread products. The results of the study indicate that wheat flour (high gluten) produces a cohesive and elastic dough structure, with the highest water absorption value of 4.1 ± 1.0% and a wet gluten content of 23 ± 1.0%. In contrast, tapioca flour and cornstarch, which have low gluten content (< 0.27%), produce dough with a brittle and cracked texture, especially in products that require stretching and pressing, such as wafer and wafer roll.

The statistical analysis shows that the gluten content has a significant positive linear correlation with protein content ($r = 0.995$, $p < 0.01$), Falling Number ($r = 0.960$, $p < 0.01$), and water absorption capacity ($r = 0.985$, $p < 0.01$). Meanwhile, starch content significantly affects the Liquefaction Number ($r = -0.854$, $p < 0.01$) and water absorption capacity ($r = 0.888$, $p < 0.01$). The amylose content in starch is correlated with increased dough hardness and the formation of fine starch granules (diameter ± 8-10 µm), as observed through SEM testing.

Consumer preference tests ($n = 100$) show that wheat-based bread receives the highest scores for shape, colour, texture, and taste (> 85% of respondents rated 4-5). Tapioca flour and cornstarch are still suitable for products such as sponge cake and choux, with an average preference score of 4. However, for wafer and wafer roll products, both ingredients showed weaknesses in texture and elasticity, with preference scores of 2-3.

Overall, low-gluten flours like tapioca and cornstarch can be optimally utilized for products that do not require stretching and pressing during production, such as sponge cake and choux, while wheat flour remains the preferred choice for bread products that require a more cohesive and elastic structure.

Acknowledgements

This research is dedicated to the memory of Ayu Suryani, who passed away during the course of this study. Your spirit and friendship will always be remembered. May you rest peacefully.

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