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Research article

CHARACTERISTICS OF AMYLUM, AMYLOGRAPH, AND SWELLING POWER OF YELLOW PUMPKIN FLOUR WITH DIFFERENT FERMENTATION TIMES

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Abstract

Yellow pumpkin flour has disadvantages, including reduced fluffiness and a strong water-binding capacity. This study aimed to analyze the effects of fermentation duration on the amylum characteristics, amylograph properties, and swelling power of yellow pumpkin flour to determine the optimal fermentation time. A randomized experimental design with one factor—fermentation duration—was used, consisting of six treatments (0 to 5 days of fermentation), with each treatment repeated four times. The parameters measured included amylum, amylose, amylopectin, amylograph, swelling power, pH, solubility, and moisture content. The results indicated that fermentation time significantly affected most parameters except gelatinization time. Extended fermentation improved amylograph properties by enhancing gelatinization and retrogradation, although paste stability during heating decreased. However, prolonged fermentation did not enhance the flour's ability to expand, but it did increase water-binding capacity. The study concluded that one-day fermentation was optimal, as it improved gelatinization, retrogradation, and solubility while maintaining a high swelling power in yellow pumpkin flour. This fermentation period produced flour with improved functional properties, making it more suitable for various food applications. Future research should explore further modifications to enhance expansion properties while maintaining the benefits of fermentation on yellow pumpkin flour's quality.

1. Introduction

Yellow pumpkin flour has advantages including its long shelf life, ease of use in food formulations (e.g., bread products, cakes, instant noodles, flakes, rice, pasta, and composite flour), and its role as a natural coloring agent (Budianto et al., 2019; Husna et al., 2020; Pramono et al., 2021; Zhavira & Rizqiati, 2020). Additionally, yellow pumpkin flour is a convenient source of β-carotene supplementation in food products. It enhances color, taste, and moisture of bread product (Pereira et al., 2020). However, it also has some less desirable characteristics, such as being prone to clumping, limited ability to expand, and a slight tendency to bind water (Yanuwardana et al., 2013). Efforts to improve the properties of yellow pumpkin flour have focused on modifying its functional properties to achieve better characteristics (Triyani et al., 2013). Methods of flour modification include the use of acids and enzymes, such as those employed in the production of porous starch (Purwitasari et al., 2023; Witasari et al., 2024). While enzyme-based modification is relatively safer, it is often costly (Budiarti et al., 2020; Rasbawati et al., 2014; Villa et al., 2013). On the other hand, acid-based modification has drawbacks, including being less environmentally friendly and potentially toxic when accumulated in the body (Sulastri et al., 2016; Rasbawati et al., 2014).

Fermentation is an alternative method for modifying starch, as it can alter macromolecular structures and improve the functional properties of pumpkin flour (Kumari et al., 2021). It enhances the surface of starch granules and disrupts their ordered structures, leading to better functionality (Yuliana et al., 2023). Fermentation-induced changes in flour characteristics include increased viscosity, improved gelatinization ability, higher rehydration and solubility, and enhanced resistance to damage (Putri, 2019; Siletty et al., 2022). Lactic acid bacteria and yeast are commonly used as fermentation starters. Lactic acid fermentation has been shown to improve the physical properties and texture of corn flour noodles by altering the amorphous regions of starch granules and the chemical components of starch (Yuan et al., 2008). In yellow pumpkin fermentation, the starch content is reduced due to the amylolytic activity of microbes like *Lactobacillus plantarum* (Tedom et al., 2019).

In this study, *Saccharomyces cerevisiae* was used as the fermentation starter for producing fermented pumpkin flour. Previous studies demonstrated that *S. cerevisiae* exhibited higher amylum degradation ability than *Lactobacillus* sp., with fermented flour containing 48.83% amylum content compared to 49.11% in flour fermented with *Lactobacillu*s sp. (Nainggolan et al., 2019). The duration of the fermentation process is a critical factor affecting the quality of fermented flour. Longer fermentation time increases microbial activity, resulting in greater starch degradation, viscosity, and solubility (Anggraeni & Sudarminto Setyo, 2014). Research by (Hidayat et al., 2018) showed that extended fermentation liberated starch granules from the fiber matrix, leading to more intense granule breakdown and an increase in the water solubility index in *S. cerevisiae* fermented rice flour. To date, there is limited research on the effect of fermentation time using *S. cerevisiae* on the characteristics of yellow pumpkin flour. Therefore, this study was conducted to investigate the impact of *S. cerevisiae* fermentation time on the characteristics of amylum, amylograph, and swelling power of yellow pumpkin flour and to determine the optimal fermentation curation for its production.

2. Materials and methods

2.1. Materials

2.1.1. Materials

The materials used in the study included yellow pumpkin varieties of Bokor obtained from Pati, Central Java, instant dry yeast *S. cerevisiae* (Fermipan), aquades, aluminum foil, HCL, NaOH, anthrone reagents, glucose, amylose, ethanol, acetic acid, and I-KI solution.

2.1.2. Tools

The tools used in the study included biosafety cabinet, beaker glasses, glass jars, Erlenmeyer flasks, pots, measuring flasks, measuring pipettes, test tubes, petri dishes, cooking utensils, incubators, slicers, autoclaves, counting chambers, cabinet dryers, choppers, 60-mesh sieves, cuvettes, ovens, desiccators, analytical scales, vortex mixers, water baths, digital thermometers, centrifuge tubes, micropipettes, centrifuges, UV-Vis spectrophotometers, digital pH meters, and rapid visco analyzers (RVA).

2.2. Research Design

The study used a randomized design with one factor, namely the length of fermentation time. There were six treatments, and each treatment was repeated 4 times. The treatments chosen were as follows: P1 (without fermentation as a control), P2 (1-day fermentation), P3 (2-day fermentation), P4 (3-day fermentation), P5 (4-day fermentation), P6 (5-day fermentation).

2.3. Methods

2.3.1. Starter Preparation

The starter of *S. cerevisiae* was prepared using the counting chamber method. A total of 5 g of instant dry yeast was mixed with 1,000 g of yellow pumpkin that had been pasteurized at 75°C for 3 minutes in a beaker glass. Aquades (in a 3:2 ratio to the yellow pumpkin) was added, stirred until evenly distributed, and covered with aluminum foil (Yani & Akbar, 2018). The mixture was then incubated at 30°C for 12 hours until a microorganism cell density of 106 CFU/ml was obtained (Tandrianto et al., 2014).

2.3.2. Yellow Pumpkin Fermentation

The yellow pumpkin was fermented using the method of (Sari et al., 2024) with modification. The pasteurized yellow pumpkin was placed in a sterilized glass jar. Fermentation was carried out by inoculating 3% of the starter by the weight of the yellow pumpkin, followed by the addition of aquades in a 3:2 ratio to the weight of the pumpkin. The mixture was stirred until evenly mixed. The fermentation process was conducted in an incubator at 30°C for 1, 2, 3, 4, and 5 days.

2.3.3. Fermented Yellow Pumpkin Flour Preparation

The fermentation results were filtered to separate the yellow pumpkin from the water. The yellow pumpkin was dried using a food dehydrator at 60°C for 24 hours (Herlina et al., 2021). After drying, the pumpkin was mashed using a chopper and sieved through a 60-mesh sieve (Triyani et al., 2013).

2.3.4. Analysis of Fermented Yellow Pumpkin Flour

The parameters tested in this study included the following: water content measured using thermogravimetry and amylum content determined following established methods (Joy et al., 2021); amylose levels and amylograph properties measured based on (Pasca et al., 2022); amylopectin levels were calculated as the difference between amylum and amylose levels; swelling power were determined using the Leach method as described by (Budiarti et al., 2020); pH measurement conducted using a pH meter (Putri, 2019); and solubility measured using the method described by (Winarti et al., 2022).

2.4. Data analysis

The software used for data analysis was SPSS. The data obtained were analyzed to determine whether the treatments had a significant effect using one-way ANOVA with a significance level of 5%. If a significant effect was found, the analysis was followed by the Duncan Multiple Range Test (DMRT) test at a significance level of 5%.

3. Results and discussions

The results of this study are presented in Figures 1, Table 1, and Table 2.

3.1. Composition Characteristics of Starch

The characteristics of amylum, amylose, and amylopectin in fermented yellow pumpkin flour are summarized in Figure 1. The duration of fermentation had a significant effect ($P<0.05$) on the amylum, amylose, and amylopectin levels of yellow pumpkin flour. The Duncan test results indicated that fermentation treatments were significantly different from the control (unfermented flour), and the length of fermentation also resulted in significant differences. Fermentation decreased the levels of amylum and amylopectin but increased the amylose content in yellow pumpkin flour.

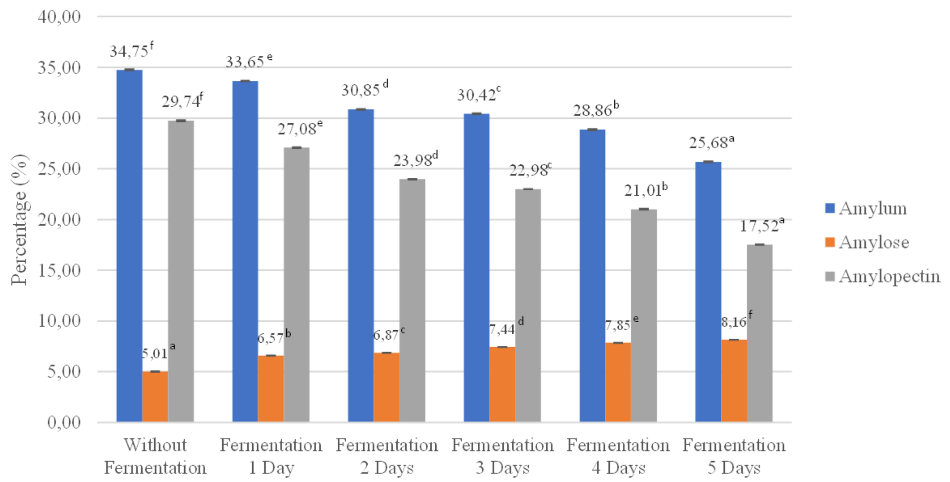

Figure 1. The characteristics of amylum, amylose, and amylopectin of fermented yellow pumpkin flour

The decrease in amylum levels was attributed to the increased production of amylase and diastase enzymes by *S. cerevisiae* during prolonged fermentation. These enzymes hydrolyzed maltose into glucose, reducing the amylum content in the flour (Yani & Akbar, 2018; Wibawanti et al., 2021). The amylum content of fermented yellow pumpkin flour (*S. cerevisiae*, 33.65%) was lower than the findings of (Fiqtinovri, 2020) for fermented elephant cassava mocaf flour (*Saccharomyces* sp., 84.44%) but higher than (Hervelly et al., 2019) for fermented sweet potato flour (*Bacillus subtilis*, 26.79%).

The increase in amylose levels was caused by the breakdown of amylum during fermentation due to amylase and pullulanase activity. These enzymes hydrolyzed specific bonds in starch converting amylopectin into short-chain amylose (Pasca et al., 2022). The breakdown of α-1,6-glycosidic bonds in amylopectin's branch chains resulted in a straight chain amylose, thereby increasing its levels (Winarti et al., 2022). Pullulanase also hydrolyzed branching α-1,6 glycosidic bonds, which contributed to the decrease in amylopectin levels (Setiarto & Widhyastuti, 2016). In this study, prolonged fermentation increased the amylose content. According to (Sumardiono et al., 2019), higher amylose content reduces the swelling capacity of starch. This finding aligns with the results shown in Table 1.

3.2. Pasting and Gelatinization Properties

The starch paste viscosity in flour served as a predictive indicator for determining the quality of the flour. According to (Setiarto & Widhyastuti, 2016), good amylograph characteristics of flour include stability, heat resistance, and strong setback viscosity, which are essential for forming the gel structure of gelatinized starch paste. The amylograph characteristics of fermented yellow pumpkin flour are presented in Table 1. The fermentation time significantly affected ($P<0.05$) the amylograph parameters, including gelatinization temperature, peak viscosity, trough viscosity, breakdown viscosity, setback viscosity, and final viscosity of yellow pumpkin flour. However, it did not significantly influence the gelatinization time ($P>0.05$).

Duncan test at a 5% significance level As the fermentation time increased, the peak, trough, setback, and final viscosity values increased, while the breakdown viscosity and gelatinization temperature decreased. According to (Wahjuningsih et al., 2024), breakdown viscosity is defined as the stability of starch under thermal conditions, where lower values indicate greater stability. Viscosity, on the other hand, refers to a liquid's resistance to flow, which is measured by the rate at which the liquid travels through a glass tube (Wijayanti et al., 2024). These findings demonstrated improvements in the amylograph properties of fermented yellow pumpkin flour.

The modification in the microstructure of starch granules by Saccharomyces cerevisiae contributed to these changes. Structural alternations in the granules, caused by acid and enzymatic actions during the fermentation, resulted in enhanced granule erosion and chain length shortening of starch molecules (Yuliana et al., 2023).

Unlike the gelatinization temperature of breadfruit, which remained unchanged after fermentation with *L. plantarum* (Wayantika et al., 2019), the reduction in gelatinization temperature in fermented yellow pumpkin flour was likely caused by the weakening of starch granule structures during the soaking phase of fermentation. This aligns with (Aini et al., 2010), who noted that gelatinization begins with the breakdown of the amorphous region of starch granules as hydrogen bonds are disrupted, resulting in leaching and lower gelatinization temperatures.

Table 1. Pasting and Gelatinization Properties

Sample	Gelatinization Temperature	Gelatinization Time (Minutes)	Peak Viscosity (BU/cP)	Trought Viscosity (BU/cP)	Breakdown viscosity *(BU/Cp)*	Setback Viscosity (BU/cP)	Final Viscosity (BU/cP)
Control	87.55±0.632[c]	13,00±1,414[a]	1207,00±8,641[a]	1205,00±4,203[a]	2.00±0.136[a]	843,00±8,524[a]	2048,00±6,683[a]
1 Day	74.60±0.441[a]	13,00±2,160[a]	4410.00±8,602[b]	4426,00±5,100[b]	-15,00±3,742[b]	2303,00±2,450[b]	6729,00±7,483[b]
2 Days	75.40±0.078[b]	13,00±1,633[a]	4598,00±14,306[c]	5220,00±7,483[d]	-13,00±2,160[bc]	3563,00±3,560[c]	8783,00±4,082[d]
3 Days	74.60±0.136[a]	13.00±0.817[a]	5207,00±2,944[d]	5392,00±5,100[f]	-10.00±0.817[c]	3839,00±11,576[d]	8447,00±4,967[c]
4 Days	75,70±0,220[b]	13,00±1,633[a]	5377,00±8,165[f]	5319,00±5,888[e]	-15,00±1,633[b]	4007,00±4,320[e]	9399,00±8,602[e]
5 Days	75.40±0.078[b]	13,00±2,828[a]	5296,00±6,481[e]	4608,00±5,100[c]	-23,00±3,560[a]	4265,00±7,483[f]	9584,00±5,010[f]

Data were expressed as means ± SD, in which different superscripts in the same line significantly differ ($p<0.05$) among the various proportions.

The ease of gelatinization in fermented flour was supported by (Fiqtinovri, 2020), who explained that a lower gelatinization temperature corresponds to more efficient water absorption and starch gelatinization at lower temperatures. Additionally, the decrease in gelatinization temperature was supported by changes in amylose and amylopectin levels (Table 1), as the fermentation process increased their concentration. Increased amylose levels and altered amylopectin content contributed to lowering the gelatinization temperature (Rahman et al., 2018).

The increase in peak viscosity observed in fermented yellow pumpkin flour may have resulted from the reduction in particle size due to starch degradation by *S. cerevisiae*. Smaller particles with larger surface areas absorbed water more effectively, leading to higher peak viscosity. (Anggraeni & Sudarminto Setyo, 2014) stated that the viscosity of fermented sweet potato flour increased with fermentation duration. During gelatinization, the amylose structure of starch diffused as hydrogen bonds between amylose and amylopectin broke, resulting in a rise in viscosity. This increase continued until the peak viscosity was reached, after which the starch granule structure broke down, forming a starch paste when heated to 93°C (Setiarto & Widhyastuti, 2016) However, after fermentation periods exceeding four days, a decrease in the peak viscosity of yellow pumpkin flour was observed. This decrease was suspected to be due to increased amylose levels in the flour, which inhibited viscosity. During gelatinization, amylose molecules exited the starch granules and formed amylose-fat inclusion complexes. The formation of these complexes reduced the amylose's ability to bond, form gels, and retrograde, thereby inhibiting the rate of viscosity increase during heating. Additionally, the reduction in the peak viscosity was attributed to the lower pH of the flour during extended fermentation, as supported by the pH data in Table 3, which indicated a consistent decline in pH with increasing fermentation time (Nadhira & Cahyana, 2023).

Fermentation also reduced the breakdown viscosity of yellow pumpkin flour. Lower breakdown viscosity indicated greater stability of the starch under hot conditions. However, extremely low breakdown viscosity suggested a high level of starch granules destruction, which reduced the paste's stability during heating and cooking (Agustin, 2011; Aini et al., 2016). As shown in Table 2., the breakdown viscosity of fermented yellow pumpkin flour was very low, implying significant destruction of amylum granules. This result aligned with (Setiarto & Widhyastuti, 2016), who reported that fermentation decreased the heat resistance properties of gadung starch.

Setback viscosity increased with longer fermentation times, indicating an enhanced retrogradation in yellow pumpkin flour. The fermented yellow pumpkin flour exhibited a higher tendency to retrograde as fermentation duration increased. This was likely due to the higher amylose content in the flour, as noted in the study findings. Amylose was more prone to retrogradation because it recrystallizes easily when exposed to water. Increased amylose levels were one of the primary factors contributing to the high setback viscosity observed in the study. Lastly, fermentation increased the final viscosity of yellow pumpkin flour. Starch with higher final viscosity demonstrated a better ability to form a gel resistant to shear forces, making it more stable during the stirring and cooking processes (Pasca et al., 2022).

Table 2. Swelling Power, pH, Solubility & Moisture Content Characteristics

Treatment	Swelling Power (%)	pH	Solubility (%)	Moisture Content (%)
Control	24,20±0,058^{f}	5.13±0.118^{d}	3.81±0.048^{a}	14.01±2.413^{c}
Fermentation 1 Day	18,13±0,100^{e}	4.07±0.029^{c}	5.35±0.092^{b}	9.06±0.499^{b}
Fermentation 2 Days	16.42±0.113^{d}	4.08±0.013^{c}	5.45±0.096^{b}	8.44±0.846^{ab}
Fermentation 3 Days	15.20±0.113^{c}	3.98±0.005^{b}	6.50±0.116^{c}	8.43±0.453^{ab}
Fermentation 4 Days	14.93±0.083^{b}	3.86±0.019^{a}	6.79±0.068^{d}	7.83±1,264^{ab}
Fermentation 5 Days	12.47±0.061^{a}	3.83±0.005^{a}	7.32±0.051^{e}	6.61±0.554^{a}

Data were expressed as means ± SD, in which different superscripts in the same line significantly differ ($p<0.05$) among the various proportions.

3.3. Physicochemical characteristics

Swelling power was an important characteristic affecting flour quality, as it reflected the ability of starch to expand and indicated the strength of the association force within starch granules, correlating with the composition of amylum. Table 2 showed that the swelling power of yellow pumpkin flour ranged from 12.47 to 24.20%. The results indicated that the fermentation time had a significant effect ($P<0.05$) on the swelling power of yellow pumpkin flour. Duncan's further test at a 5% significance level revealed that fermented samples differed significantly from unfermented ones, and the length of fermentation time also caused significant differences in swelling. Longer fermentation times resulted in a decrease in the swelling power of yellow pumpkin flour. Kumari et al. (2021) explained that the reduction in starch content, particularly amylopectin, played a key role in lowering swelling power. Factors such as amylose to amylopectin ratio, chain length, and molecular weight distribution also influenced swelling power. Higher heating temperature increased crystal formation, enhancing granule stability and reducing swelling capacity (Sarifah et al., 2021). This could also be attributed to amylose forming complexes with lipids and the linear chains of amylopectin, which limits amylose release and decreases starch granule swelling (Kumoro et al., 2019).

Fermentation's ability to reduce swelling power in yellow pumpkin flour was found to be less pronounced than the effect of chemical modification using acetic acid. The addition of acetic acid was reported to increase swelling power by weakening hydrogen bonds in starch, allowing water to penetrate the starch granules more easily, leading to greater expansion (Triyani et al., 2013). Conversely, fermentation with lactic acid soaking demonstrated a decrease in swelling power as fermentation duration increased. The lower pH caused by lactic acid was associated with the formation of carbonyl and carboxyl groups, which accelerated amylose degradation and reduced swelling power (Yanuwardana et al., 2013; Villa et al., 2013). Table 3 supported this, showing that longer fermentation times corresponded to lower pH levels in yellow pumpkin flour. Moreover, prolonged fermentation decreases nutritional content and reduces bacterial activity in breaking down starch into amylose, which significantly affects the swelling power (Rahma et al., 2017).

Solubility referred to the ability of solids to dissolve in water and was influenced by the sugar content of the flour. Table 1 indicated that the solubility of yellow pumpkin flour ranged from 3.81 to 7.32%. The results showed that fermentation time significantly affected ($P<0.05$) the solubility of yellow pumpkin flour. Duncan's test at a 5% significance level demonstrated significant differences between fermented and unfermented samples, as well as between different fermentation durations. Longer fermentation times increased the solubility of yellow pumpkin flour. This increase was attributed to the release of simple sugars (Al-Baarri et al., 2018) from the hydrolysis of amylum by microorganisms. Higher sugar content in the flour enhanced water binding, thereby increasing solubility (Fauzi et al., 2023). Additionally, higher temperatures caused increased solubility by damaging starch granules, transforming crystalline regions into amorphous ones and allowing more amylose to leach out (Sarifah et al., 2021). Higher solubility was considered beneficial as it indicated better water-binding capacity (Purwanto et al., 2013). Furthermore, higher solubility is associated with improved digestibility (Retnowati et al., 2018).

The decrease in the pH of fermented yellow pumpkin flour with longer fermentation time was attributed to increased total acidity. This aligns with the findings of (Al-Baarri et al., 2024), who explained that microbial activity levels and the number of microbes increase with fermentation time, leading to the production of acid metabolites as a result of microbial metabolism. Longer fermentation durations enhanced yeast activity, leading to the breakdown of glucose into alcohol and organic acids, which reduced pH (Yani & Akbar, 2018). Hydrolysis of starch granules by *S. cerevisiae* also produced organic acids, particularly lactic acid, which further decreased pH levels (Anggraeni & Sudarminto Setyo, 2014). The fermentation process in flour typically leads to a decrease in pH, making the flour more acidic. For instance, during the fermentation process in making MOCAF, the acidity is primarily due to the production of lactic acid by lactic acid bacteria (LAB), which is a byproduct of starch hydrolysis (Endrina et al., 2018; Swastawati et al., 2019).

The moisture content of unfermented yellow pumpkin flour (Table 1) was 14%, whereas fermented samples had lower moisture content, ranging from 9.06% to 6.61%. These values were within acceptable limits compared to SNI 3751-2018 standard, which set maximum moisture content for wheat flour at 14.5%, cassava flour at 12%, rice flour at 13%, and corn flour at 10% (Purwanto et al., 2013). Low moisture content also indicates a high bulk density (Rachma et al., 2018). Moisture content plays a crucial role in starch quality. When the moisture content exceeds 13%, it becomes undesirable, as it increases the risk of microbial spoilage, which can negatively impact storage safety and stability (Nuswantari, 2022). The results showed that fermentation time had a significant effect ($P<0.05$) on the moisture content of yellow pumpkin flour. Duncan's further test indicated that fermentation treatments were significantly different from unfermented samples, and fermentation duration also caused significant difference in moisture content. Longer fermentation times resulted on lower moistire content. This reduction was attributed to the degradation of starch by microorganisms, which decreased the material's water retention capacity. The moisture content of fermented yellow pumpkin flour is relatively lower compared to gadung flour fermented with *M. racemosus* and *L. plantarum*, which has a moisture content of approximately 12% (Kumoro et al., 2020). Prolonged enzymatic activity during fermentation released more bound water, softening and increasing porosity in the material. These structural changes facilitated water evaporation during drying, further reducing moisture content (Anggraeni & Sudarminto Setyo, 2014).

4. Conclusions

This study concluded that longer fermentation times decreased the levels of amylum and amylopectin while increasing the amylose content in yellow pumpkin flour. The amylograph properties of yellow pumpkin flour improved with longer fermentation, as evidenced by increases in peak, trough, breakdown, setback, and final viscosity, while the gelatinization temperature decreased. Prolonged fermentation also resulted in reductions in swelling power, pH, and moisture content, whereas solubility increased. One-day fermentation was chosen because it improved gelatinization, retrogradation, and solubility, while maintaining a high swelling power in yellow pumpkin flour.

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