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# Effect of *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* Fermentation on the Pasting Properties of Sweet Potato (*Ipomoea batatas*) Flour

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**The modification effect of fermentation on the pasting properties of flours enhances their potential as functional ingredients in product development. As such, the fermentation of sweet potato (SP) may alter its pasting properties and thus enhance its application for new food product development. The aim of this study was to determine the effect of starter culture and fermentation time variations on the pasting profile and amylose content of SP flour. The starters used were *Leuconostoc mesenteroides*, *Saccharomyces cerevisiae*, and a paired culture of *Leuconostoc mesenteroides and Saccharomyces cerevisiae* with a fermentation time of 24, 48, 72, and 96 h. Results obtained showed that fermentation starter variation had a significant effect ($p < 0.01$) on some pasting properties and amylose content of the SP. The highest peak viscosity of 1204 Brabender units (BU) was obtained from samples fermented with the paired culture of *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae*. Based on these results, fermented SP flour possesses the potential to be applied to products that require a thickening property.**

Keywords: fermented sweet potato flour, *Leuconostoc meseneroides*, pasting properties, *Saccharomyces cerevisiae*

## INTRODUCTION

Processing sweet potatoes (SP) into flour provides an advantage as an alternate source of industrial raw materials and a flour substitute. Utilization of SP flour as a composite with wheat flour in the preparation of bread and biscuits (Mais 2008; Etudaiye *et al*. 2015; Yuliana *et al*. 2018a; Ayo-Omogie 2021), pasta (Saleh *et al*. 2017), and noodles (Ginting and Yulifanti 2015) has been reported. Although SP flour can be used as a substitute for wheat flour for raw materials of several food products, the use of this flour still presents some limitations. SP starch does not possess desirably high viscosity values on pasting and gelatinization (Garcia 1993). To improve its properties and suitability, SP flour needs modification to enhance its industrial application. Starch and flour are usually modified to effect changes in cooking characteristics, increase swelling power, gelatinization temperature, viscosity, process stability, decrease retrogradation, and improve solubility properties (Kaur *et al*. 2016; Onyango 2016; Manuhara *et al*. 2017; Ma *et al*. 2022).

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One of the methods for SP flour modification is fermentation. Several studies have reported that lactic acid fermentation showed beneficial effects on the physicochemical properties of SP flour such as the altered expansion ability of SP starch and flour during baking (Yuliana *et al*. 2018a), the decrease in the broken rate of noodles (Yuliana *et al*. 2018b), and a significant increase in the hardness and extension of noodles (Liao and Wu 2017). Also, some functional groups such as hydroxy, aldehydes, alcohol, and carboxy detected in the fermented samples of SP flour can serve as antioxidants, inhibit spoilage organisms, and increase the shelf-life of SP products (Ajayi *et al*. 2019).

Alteration of starch during fermentation with significant development of physicochemical characteristics and properties of flour has been documented (Ajayi *et al*. 2016; Liao and Wu 2017; Yuliana *et al*. 2017; Velly *et al*. 2022). Improving SP flour's functional characteristics as an effect of fermentation allows the tailoring of specific attributes of fermented SP flour, including pasting behavior. The pasting property is an essential indicator of the quality of starch or flour and is very important for its processing and utilization (BeMiller 2011; Liao and Wu 2017); hence, understanding the pasting properties of SP flour is necessary to better predict the functional properties of processed foods. Although fermentation has been demonstrated to enhance the functional properties of SP flour, the information on how the SP pasting properties change during fermentation has not been extensively reported. Adequate characterization of SP flour in terms of pasting properties will be beneficial to develop more applications. Considering the importance of the pasting properties, it is necessary to determine the relationship between the effect of starter type and fermentation time on the fermented SP flour paste properties. In this study, the modification was carried out by using a starter of *Leuconostoc mesenteroides* and a paired culture of *Leuconostoc mesenteroides–Saccharomyces cerevisiae* to determine the changes in the pasting profile of SP flour and lay the basis for formulating future applications of these flours.

## MATERIALS AND METHODS

### Sources of Materials

The local SP Ciceh white variety, harvested 100 d after planting, was purchased from a farm at Metro, Lampung. *Leuconostoc mesenteroides* FNCC 0023 were obtained from Pusat Studi Pangan dan Gizi, University of Gadjah Mada. A yeast starter (*Saccharomyces cerevisiae*) was prepared from commercial "Ragi roti" powder. Chemicals were obtained from Sigma and Merck.

### Sample Preparation

The lactic starter culture was cultivated by inoculating 1 mL of pure culture into 9 mL of MRS broth and incubated at a temperature of 37 °C for 48 h. A total of 10 mL of the suspension was then put into 90 mL of sterile MRS Broth and incubated for 24 h at 37 ºC. The number of cells in this preparation was approximately $10^6$ colony-forming units (CFU) per mL. Plate counts were performed to determine the CFU. A yeast starter culture (*Saccharomyces cerevisiae*) was prepared by watering 1 g of commercial "Ragi roti" powder in sterilized bottles containing 100 mL of sterile distilled water.

### Preparation of Fermented SP Flour

The fermentation method was referred to by Yuliana *et al.* (2017). The SP were divided into five representative lots, with each lot weighing about 1.5 kg. The SPes were peeled, washed thoroughly in clean tap water, sliced using a Hobart slicer into 1-mm thickness, and packed into a clean 5-L plastic container with a lid. A 4-L sterile saline solution composed of 3% sodium chloride and 1% sucrose was then added to the container. Three lots were each inoculated with the respective starters [including *Leuconostoc mesenteroides* (Lc), *Saccharomyces cerevisiae* (Y), and paired culture of *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* (LcY)] at 5% cell suspension containing $10^6$ cells/mL of the fermenting medium. The fermentation treatment with the mixed starter was carried out by adding 2.5% of *Leuconostoc mesenteroides* and 2.5% of *Saccharomyces cerevisiae* to the fermentation volume.

The 4$^{th}$ lot was allowed to ferment spontaneously (Sp) without starter culture inoculation, whereas the 5$^{th}$ lot that served as the control was not fermented. The fermentation process was held at 30 ± 2 °C for 0, 24, 48, 72, and 96 h under anaerobic conditions. After the fermentation process was completed, the SP slices were washed by passing the slice under running tap water to reduce the level of acidity, drained, and dried in an oven (Jouan, German) at a temperature of 60 °C for 10–12 h until the moisture content reached 6–10%. The dried SP chips were then powdered into flour using a Hammer Mill (Retsch GmbH model 5667 HAAN type SK1 Nr 71266 West Germany) and sieved using an 80-mesh screen (Retsch). All fermented SP flours were packed in sealed polyethylene bags for further analysis.

### Analysis

The pasting properties of the flours were evaluated using a Micro Visco-Amylo-Graph (Brabender OHG, Duisburg, Germany) according to a previous report (Yuliana *et al.* 2018a). Flour suspension (10%) was put into an amylograph bowl, then rotated at 75 revolutions per min while increasing the temperature from 30 to 95 ºC at a rate of 1.5 ºC/min. The temperature was then maintained at 95 ºC for 20 min, then lowered to 50 ºC at a rate of 1.5 ºC/min. The recorded parameters were pasting temperature (PT), peak viscosity (PV), minimum viscosity (MV) or trough viscosity, final viscosity (FV), and peak time (P Time). Breakdown viscosity (BV) was calculated as the difference between PV minus MV, whereas total setback viscosity (TSV) was determined as the FV minus MV. All determinations were performed in duplicate. Amylose content was determined using the amylose-iodine method described by Yuan *et al*. (2007) Sampling amylose standards are read at 620 nm with a spectrophotometer UV-Vis 1800 (Shimadzu, Japan). Plot the absorbance of the sample against the pure potato amylose standard curve was used for calculations. The value of pH was done using a pH meter (Lovibond, German).

### Statistical Analysis

The experiments were ordered in a randomized block design. Data were analyzed using the two-way analysis of variance, and the differences between means were determined using the orthogonal comparison and polynomial test.

## RESULTS AND DISCUSSION

### Degree of Acidity (pH)

The starter and duration of fermentation significantly decreased the pH (Figure 1). In this study, inoculation of the starter significantly decreased the pH of the SP flour from 5.5 to 3.4–4.12. Among starters, fermentation using *Leuconostoc mesenteroides* (LcY) had a relatively lower pH decrease that was not significant ($p > 0.05$) than that of using *Saccharomyces cerevisiae* (Y) alone (Table 1). It was probably because there was a competition between *Leuconostoc mesenteroides* (Lc) and *Saccharomyces* (Y) that influenced the growth of that LAB and, thus, may decrease lactic acid production. This may be corroborated by the findings of Ajayi *et al*. (2016), who reported lower pH values in SP fermented without *Saccharomyces cerevisiae*. According to Gobbetti *et al*. (1994), the lactic acid bacteria-yeast co-cultures may alter bacterial cell output and lactic and acetic acid production *via* carbohydrate metabolism.

### Amylose Content

The effect of starter culture and fermentation time variations on the amylose content of SP is presented in Figure 2. The results showed that the starter treatment

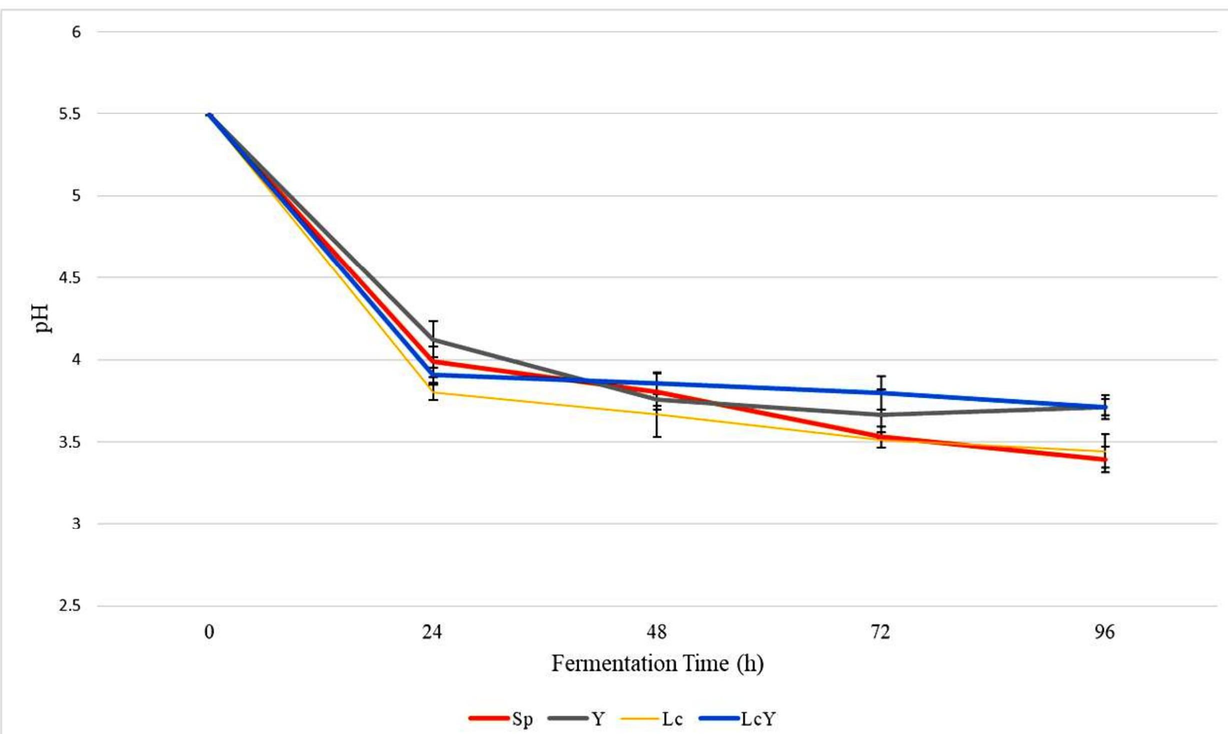


**Figure 1.** pH changes in fermented SP as affected by starter and fermentation time. [*Sp*] spontaneously fermented SP flour; [*Lc*] SP fermented with single culture of *Leuconostoc mesenteroides*; [*Y*] SP fermented with single culture of *Saccharomyces cerevisiae*; [*LcY*] SP fermented with mixed culture of *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae*. pH of control = 5.5.

**Table 1.** Orthogonal comparison and polynomial significance on pH among starters and fermentation time.

| Starter | Significance |
|---|---|
| [C1] Control *vs.* Sp, Y, Lc, LcY | ** |
| [C2] Sp *vs.* Y | ** |
| [C3] Sp *vs.* Lc | * |
| [C4] Y *vs.* LcY | ns |
| [C5] Lc *vs.* LcY | ** |
| Fermentation time | |
| [C6] Linear | ns |
| [C7] Quadratic | ** |

[*] Significant ($p < 0.05$); [**] significant ($p < 0.01$); [ns] not significant
$Y_{Sp} = 0.1888x^2 – 1.5985x + 6.7607$ ($R^2 = 0.9367$)
$Y_{Lc} = 0.2307x^2 – 1.8233x + 6.9147$ ($R^2 = 0.9122$)
$Y_{Y} = 0.2217x^2 – 1.7323x + 6.9087$ ($R^2 = 0.9663$)
$Y_{LcY} = 0.2136x^2 – 1.6491x + 6.7507$ ($R^2 = 0.8775$)

**Table 2.** Orthogonal comparison and polynomial significance on amylose content among starters and fermentation time.

| Starter | Significance |
|---|---|
| [C1] Control *vs.* Sp, Y, Lc, LcY | ** |
| [C2] Sp *vs.* Y | ** |
| [C3] Sp *vs.* Lc | ** |
| [C4] Y *vs.* LcY | ** |
| [C5] Lc *vs.* LcY | ** |
| Fermentation time | |
| [C6] Linear | ns |
| [C7] Quadratic | ** |

[**] Significant ($p < 0.01$); [ns] not significant
$Y_{Sp} = –1.0857x^2 + 8.1423x + 26.44$ ($R^2 = 0.8479$)
$Y_{Lc} = –1.2586x^2 + 9.4294x + 25.369$ ($R^2 = 0.8549$)
$Y_{Y} = y = –1.126x^2 + 8.814x + 25.715$ ($R^2 = 0.8926$)
$Y_{LcY} = –1.3174x^2 + 10.216x + 24.742$ ($R^2 = 0.8748$)

and fermentation time had significantly increased the amylose content (40.05–43.94%) of fermented white SP flour. There were significant differences in amylose content among the type starters treatment (Table 2). Among starters, fermentation with mixed *Leuconostoc mesenteroides–Saccharomyces cerevisiae* (LcY) culture resulted in the highest percent amylose content. The amylose content of white SP increased quadratically with the longer fermentation time.

Lactic acid bacteria such as *Leuconostoc mesenteroides* and yeast such as *Saccharomyces cerevisiae* have amylolytic enzymes such as amylase and pullulanase (glucoamylase) (Setiarto *et al*. 2015; Pretorius *et al*. 1991; Latorre-García *et al*. 2005; Petkova *et al*. 2020). Pullulanase activity causes the branched α-1,6 glycosidic bond in the amylopectin chain to break, resulting in oligosaccharides with shorter degrees of polymerization (Moradi *et al*. 2014; Rahma *et al*. 2017). The continuous depolymerization of amylopectin by organic acid increases amylose concentrations (Kasemsuwan *et al*. 1995; Bian *et al*. 2022). As a result, the amount of amylose increases with fermentation time. Zhou *et al*. (2015) reported that higher amylose content of SP starch led to the rise of setback value, and the reduction of breakdown value led to high shear resistance. These properties could be suitable for various food and non-food applications such as biodegradable packaging materials, as well as resistant starch-rich food that functions as health-promoting food (Zhong *et al*. 2022).

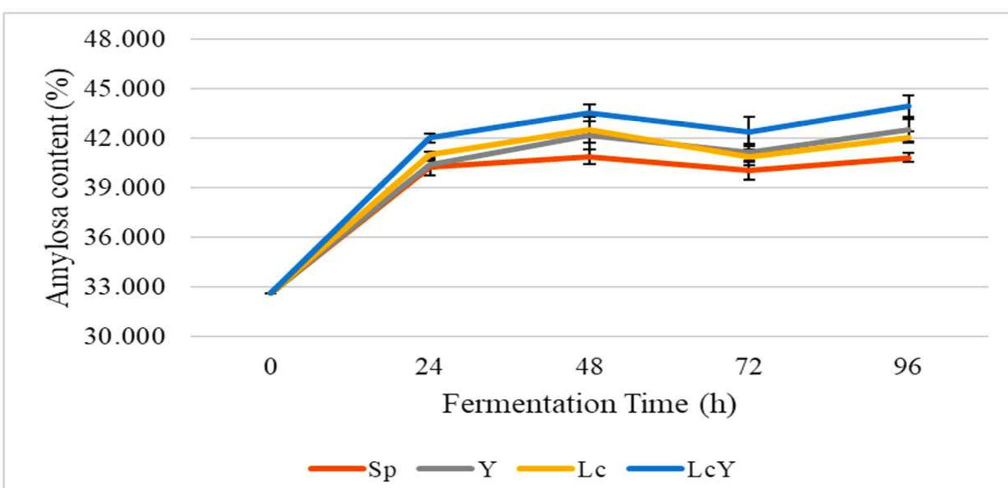


**Figure 2.** Amylose content (%) of fermented SP as affected by starters and fermentation time. [*Sp*] spontaneously fermented SP flour; [*Lc*] SP fermented with single culture of *Leuconostoc mesenteroides*; [*Y*] SP fermented with single culture of *Saccharomyces cerevisiae*; [*LcY*] SP fermented with mixed culture of *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae*. Amylose content of control = 32.59%.

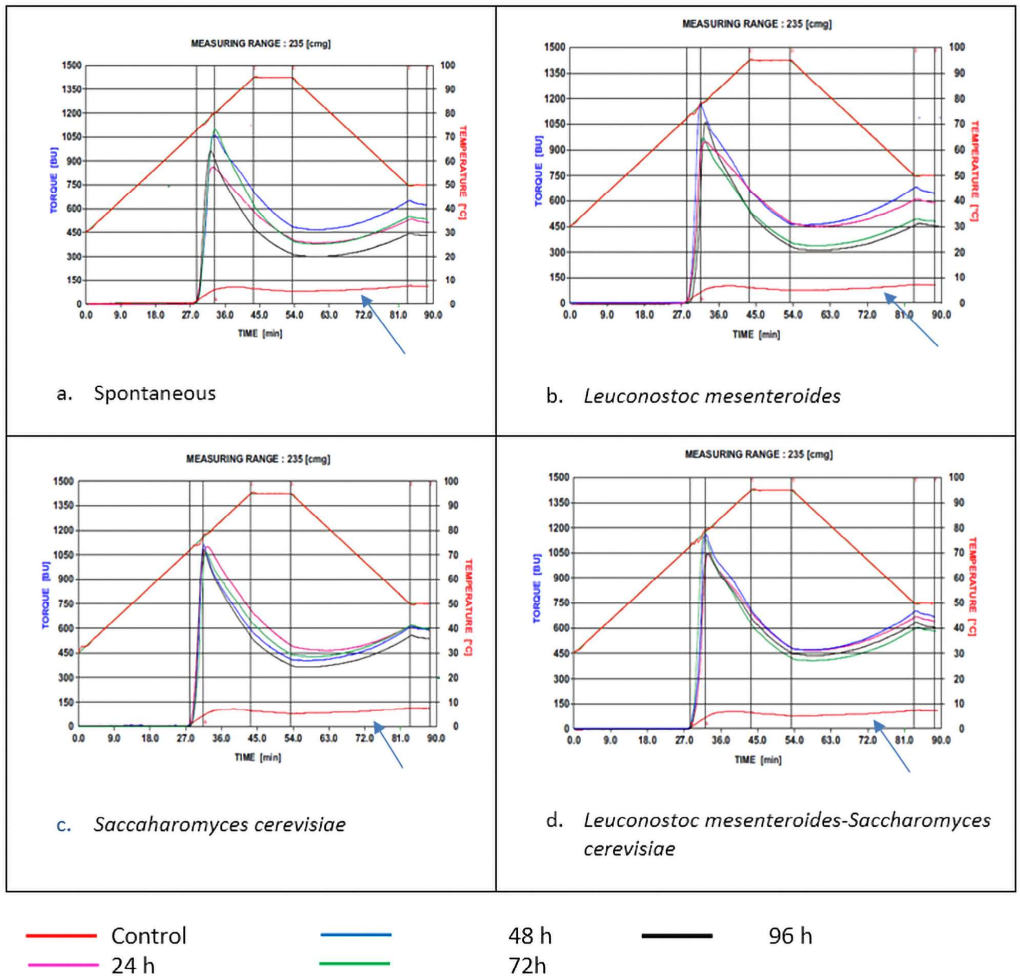


**Figure 3.** Viscoamylogram of fermented SP flours using different starters.

**Pasting Profile**

The pasting profiles of SP flour fermented with different starters of *Leuconostoc mesenteroides* (Lc), *Saccharomyces cerevisiae* (Y), paired culture of *Leuconostoc mesenteroides*, and *Saccharomyces cerevisiae* (LcY)] and control (arrows) are shown in Figures 3a–d. Each pasting parameter is summarized in Figures 4–7. The pasting profile of SP flour was significantly ($p \leq 0.05$) affected by the fermentation process. Figures 1a–d illustrate a sudden increase in viscosity (900–1200 BU) in the fermented flour with different starters as compared to a sloping graph (150 BU) in the control sample. The pasting profiles of the fermented flour had similar trends, where there was a sharp PV despite the different values. The magnitudes of increase in the PV compared to unfermented SP are 8.5–10. Meanwhile, lactic acid fermentation of SP resulted in a slight increase of PV (Figure 1b) compared to spontaneous fermentation, with a new maximum PV ~ 1150 Brabender units (BU) reached after samples were treated for 48 h. Interestingly, beyond 48 h fermentation, a more drastic decrease in FV was seen. Fermentation with *Saccharomyces cerevisiae* (Y) (Figure 3c) resulted in a uniform PV regardless of fermentation time. A similar Type A pasting profile of spontaneously fermented SP for 48–72 h (Figure 3a) was obtained with higher FV than SP fermented with *Leuconostoc mesenteroides* (Lc) (Figure 3b). Again, fermentation for 96 h resulted in the lowest value of FV. Combined fermentation with LcY (Figure 3d) resulted in the highest PV for samples after 48–72 h treatment. The high FV values of the fermented samples are approximate of several unmodified SP starches (Chen *et al*. 2003).

The pasting profiles of fermented SP are comparable to those of native starch. SP starch is commonly characterized as having a Type A pasting profile with a sharp PV, followed by shear thinning and viscosity breakdown and ultimately low cold paste viscosity (Collado *et al*. 1999). Meanwhile, the effect of fermentation time on the profile of flour pasta also varies depending on the pasta parameters, which are further described as follows.

**Initial Temperature of Gelatinization**

Results of initial gelatinization temperature as presented in Figure 4 show that the type of starter culture had no significant effect ($p > 0.05$), whereas fermentation time had a significant effect ($p \leq 0.05$) on the initial gelatinization temperature (°C) of fermented SP flour, and there was no interaction between the two factors. Further tests of orthogonal polynomials showed that the initial gelatinization temperature (°C) of fermented white SP flour slightly increased linearly with the duration of fermentation. Meanwhile, the orthogonal comparison showed that there was no significant initial gelatinization between the control and within starters (Table 3).

During the fermentation process, there were significant ($p \leq 0.01$) changes in the initial gelatinization temperature between 0–96 h, but there was no significant difference ($p > 0.01$) among the starters (Table 3). The slight linear increase in initial gelatinization may be due to acid produced by the lactic acid bacteria during the fermentation of the substrate. A significant ($p \leq 0.05$) increase in acidity was indicated by a decrease in the pH from 5.48 (initial fermentation) to 3.39 (96 h fermentation) (Figure 1). The actual change caused by acid production in the starch granules would also have changed the gelatinization temperature. In our previous study, spontaneous fermentation significantly increased ($p \leq 0.05$) initial gelatinization temperature compared to unfermented SP (Yuliana *et al*. 2014). However, the results of the present study provide new insights into the effect of fermentation time variation on the gelatinization temperature of SP. Data suggest that fermentation after 48 h results in flour with increased initial gelatinization temperature. Our findings agree with those of a study on maize starches (Knutson 1990) and rice flour (Saif *et al*. 2003), which discovered that gelatinization temperatures increased as amylose levels increased. During fermentation time, the amylose content increased linearly (Figure 2).

**Table 3.** Orthogonal comparison and polynomial significance on initial gelatinization among starters and fermentation time.

| Starter | Significance |
|---|---|
| [C1] Control *vs*. Sp, Y, Lc, LcY | ns |
| [C2] Sp *vs*. Y | ns |
| [C3] Sp *vs*. Lc | ns |
| [C4] Y *vs*. LcY | ns |
| [C5] Lc *vs*. LcY | ns |
| Fermentation time | |
| [C6] Linear | ** |
| [C7] Quadratic | ns |

[**] Significant ($\alpha = 0.01$); [ns] not significant
$Y_{Sp} = 0.25x + 71.63$ ($R^2 = 0.9356$)
$Y_{Lc} = 0.171x + 71593$ ($R^2 = 0.64$)
$Y_{Y} = 0.0652x^2 + 0.519x + 71.41$ ($R^2 = 0.87$)
$Y_{LcY} = 0.189x + 71.699$ ($R^2 = 0.73$)

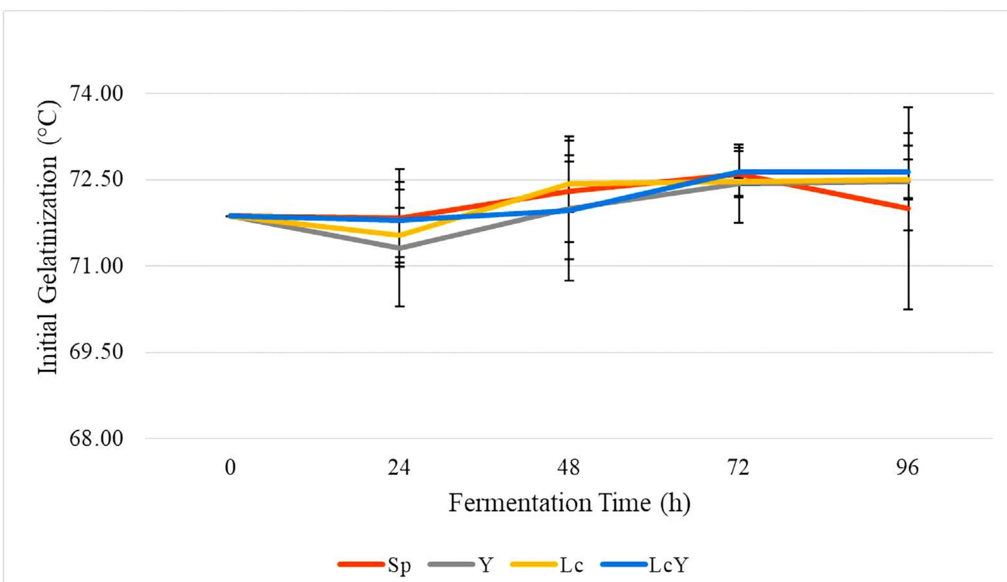


**Figure 4.** Initial gelatinization temperature of fermented SP flour as affected by starter and fermentation time. [Sp] spontaneously fermented SP flour; [Lc] SP fermented with single culture of *Leuconostoc mesenteroides*; [Y] SP fermented with single culture of *Saccharomyces cerevisiae*; [LcY] SP fermented with mixed culture of *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae*. Initial gelatinization temperature of control = 71.87 ± 1.09 ºC.

The SP flour with a high initial gelatinization temperature has an impact on the longer cooking time compared to the SP flour, which has a low initial gelatinization temperature. The gelatinization temperatures of all fermentation starter treatments (71.63–72.80 ºC) are lower than the PT of SP fermented flour reported by Ayo-Omogie (2021) within the value of 98.5 ºC.

**Peak Viscosity (PV)**

The results presented in Figure 5 showed that the starters and duration of fermentation treatment had a significant effect ($p \leq 0.05$) on the PV value of fermented white SP flour with no interaction between the two factors. The PV of fermented SP flour slightly increased in a quadratic trend with the duration of fermentation. Further results showed that the PV levels of SP flour were significantly different $p \leq 0.05$ between starters and control. Fermentation using single culture of *Saccharomyces cerevisiae* (Y) caused significantly higher ($p \leq 0.05$) PV of SP flour as compared to the spontaneously fermented sample, as shown in Table 4. Likewise, the lactic acid bacteria treatment was significantly different ($p \leq 0.05$) compared to the paired starter of bacterium and yeast. There was no significant difference between spontaneous and *Leuconostoc mesenteroides*, and between *Saccaharomyces cerevisiae* and mixed starter of *Leuconostoc mesenteroides* and yeast.

Results obtained (Figure 5) show that although lower PV values were observed in the fermented SP reported in the present study as compared to native SP starch, the trends were similar (a Type A pasting profile). Chen *et al*. (2003) reported that the PV of a 4% (w/v) starch suspension of three SP varieties averaged 500 BU and increased to 1500–2100 BU for a 6% (w/v) suspension. In this study, the recorded PV of unfermented SP (control) is < 150 BU.

**Table 4.** Orthogonal comparison and polynomial significance on peak viscosity among starters and fermentation time.

| Starter | Significance |
|---|---|
| [C1] Control *vs.* Sp, Y, Lc, LcY | ** |
| [C2] Sp *vs.* Y | ** |
| [C3] Sp *vs.* Lc | ns |
| [C4] Y *vs.* LcY | ns |
| [C5] Lc *vs.* LcY | ** |
| Fermentation time | |
| [C6] Linear | ns |
| [C7] Quadratic | ** |

[**] Significant ($p < 0.01$); [ns] not significant
$Y_{Sp} = –138.93x^2 + 1009.3x – 698.6$ ($R^2 = 0.97$)
$Y_{Lc} = –137.64x^2 + 1004.4x – 639.5$ ($R^2 = 0.87$)
$Y_{Y} = –139.14x^2 + 1039.9x – 663.2$ ($R^2 = 0.87$)
$Y_{LcY} = –158.14x^2 + 1145.5x – 760.4$ ($R^2 = 0.89$)

Fermentation significantly increased the PV from 130 BU to 1000–1200 BU.

Fermentation of SP flour with the mixed starter of *Leuconostoc mesenteroides* and yeast (LcY) resulted in the highest increase in PV compared to others, with a new maximum PV ~ 1150 BU reached for samples treated for 48 h. Meanwhile, spontaneous fermentation had the same PV as the sample fermented with *Leuconostoc mesenteroides* (Lc). Fermentation using yeast (Y and LcY) possessed significantly higher ($p \leq 0.05$) PV as compared to the control, spontaneous, or bacterially (Lc) fermented samples. The presence of yeast in the inoculum probably exerted synergistic effects on lactic acid (LcY) to promote significant modifications in SP starch. The association of lactic acid bacteria and yeast through synergism, either neutralized or assimilated lactic acid, was reported in

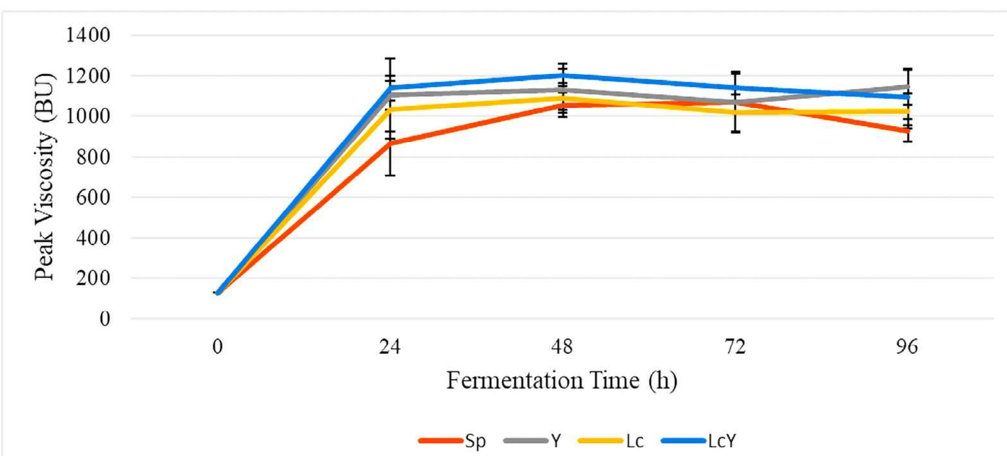


**Figure 5.** Peak viscosity of fermented SP flour as affected by starter and fermentation time. [Sp] spontaneously fermented SP flour; [Lc] SP fermented with single culture of *Leuconostoc mesenteroides*; [Y] SP fermented with single culture of *Saccharomyces cerevisiae*; [LcY] SP fermented with mixed culture of *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae*. Peak viscosity of control = 130 BU.

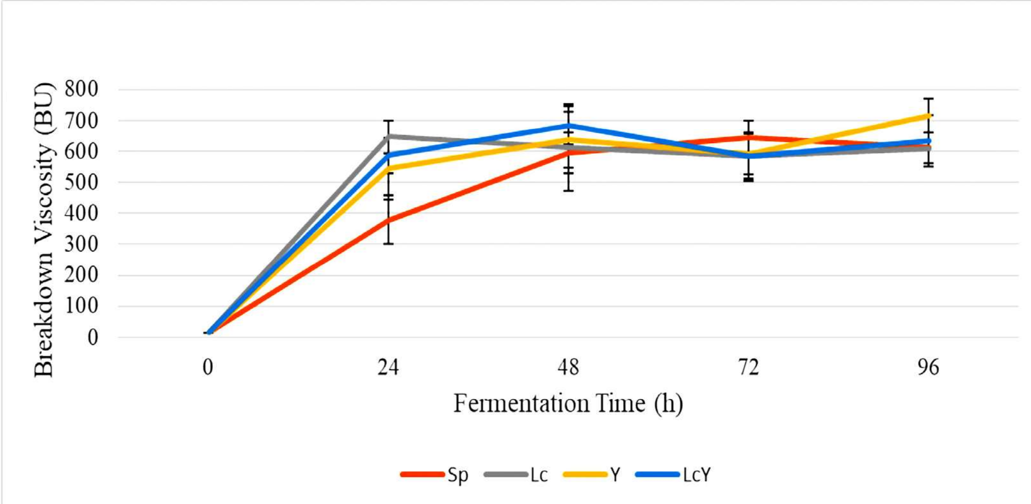


**Figure 6.** Breakdown viscosity of fermented SP flour as affected by starter and time. [*Sp*] spontaneously fermented SP flour; [*Lc*] SP fermented with single culture of *Leuconostoc mesenteroides*; [*Y*] SP fermented with single culture of *Saccharomyces cerevisiae*; [*LcY*] SP fermented with mixed culture of *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae*. Breakdown viscosity of control = 14 BU.

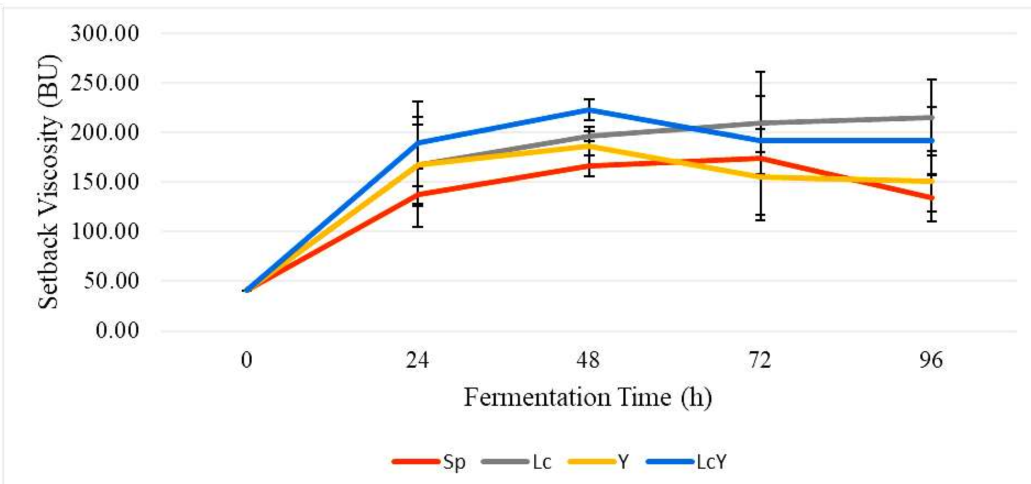


**Figure 7.** Total setback viscosity of fermented SP flour as affected by starter and fermentation time. [*Sp*] spontaneously fermented SP flour; [*Lc*] SP fermented with single culture of *Leuconostoc mesenteroides*, [*Y*] SP fermented with single culture of *Saccharomyces cerevisiae*, [*LcY*] SP fermented with mixed culture of *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae*. Setback viscosity of control = 40.67 BU.

some studies (Istiqomah *et al.* 2019; Adesulu-Dahunsi *et al*. 2020; Hu e*t al*. 2022). Ye *et al*. (2019) observed that fermentation affects the physicochemical properties of SP starch by modifying the structure of starch molecules. An increase in PV of flour as affected by fermentation has been severally reported (Yuliana *et al.* 2014; Oloyede *et al*. 2016; Ye *et al*. 2019; Silva *et al*. 2021). The quadratic trend observed for PV indicates limits on the PV for fermented SP flour; subsequent treatments may be needed to reach PV > 1500 BU reported for some SP cultivars (Chen *et al*. 2003). PV may be correlated with product quality with high PV values necessary to develop paste with desirable texture gel strength (Alamu *et al*. 2017). The relatively high PV of fermented SP flour in this study is indicative that the flour may be suitable for products requiring comparable gel strength and elasticity such as gluten-free baked products.

**Breakdown Viscosity (BV)**

The results showed that the starter and duration of fermentation significantly affected the BV of fermented SP flour. The BV of the samples increased quadratically as the fermentation took place. Fermentation by inoculation with

**Table 5.** Orthogonal comparison and polynomial significance on breakdown viscosity among starters and fermentation time.

| Starter | Significance |
|---|---|
| [C1] Control *vs.* Sp, Y, Lc, LcY | ** |
| [C2] Sp *vs.* Y | ns |
| [C3] Sp *vs.* Lc | * |
| [C4] Y *vs.* LcY | ns |
| [C5] Lc *vs.* LcY | ns |
| Fermentation time | |
| [C6] Linear | ns |
| [C7] Quadratic | ** |

[*] Significant ($p < 0.05$); [**] significant ($p < 0.01$); [ns] not significant
$Y_{Sp} = -72.071x^2 + 573.73x - 470.4$ ($R^2 = 0.99$)
$Y_{Lc} = -73.929x^2 + 580.07x - 435.4$ ($R^2 = 0.89$)
$Y_Y = -84.71x^2 + 629.29x - 461.8$ ($R^2 = 0.86$)
$Y_{LcY} = 158.14x^2 + 1145.5x - 760.4$ ($R^2 = 0.89$)

**Table 6.** Orthogonal comparison and polynomial significance on setback viscosity among starters and fermentation time.

| Starter | Significance |
|---|---|
| [C1] Control *vs.* Sp, Y, Lc, LcY | ** |
| [C2] Sp *vs.* Y | ns |
| [C3] Sp *vs.* Lc | * |
| [C4] Y *vs.* LcY | ns |
| [C5] Lc *vs.* LcY | ns |
| Fermentation time | |
| [C6] Linear | ns |
| [C7] Quadratic | ** |

[*] Significant ($p < 0.05$); [**] significant ($p < 0.01$); [ns] not significant
$Y_{Sp} = -20.929x^2 + 148.07x - 83.2$ ($R^2 = 0.99$)
$Y_{Lc} = -22.143x^2 + 153.66x - 77.4$ ($R^2 = 0.86$)
$Y_Y = -15.5x^2 + 127.7x - 55$ ($R^2 = 0.83$)
$Y_{LcY} = -26.286x^2 + 187.51x - 104.6$ ($R^2 = 0.88$)

starters resulted in a higher BV than that without the starter (spontaneous) except for Y, whose BV is statistically equivalent to that of Sp. Treatment with the addition of LcY resulted in a similar BV value to Y or Lc alone.

Fermentation resulted in greater magnitudes of BV in the SP flours than the unfermented flour (control). In comparison to the spontaneously fermented SP, sample Lc had higher BV, especially after 24 h of fermentation. BV is related to how well starch granules withstand heating. In other words, high breakdown starch indicates poorer resistance to heat, and BV represents the resistance of the starch paste to heat and shear (Guo *et al*. 2018; Bento *et al*. 2020). The ability to withstand this heating and shear stress is crucial for many procedures (Alamu *et al*. 2017). Starches with high breakdown are likely to produce unstable pastes (Singh *et al*. 2006). Thus, the lowest BV must be observed to optimize the starter used and fermentation time to produce fermented SP flour.

**Total Setback Viscosity (TSV)**

Similar to BV, the results showed that the starter treatment has a significant effect on the TSV value of fermented white SP flour. However, there are no significant differences in TSV among starters except between Sp and Lc. TSV of white SP fermented flour increased in a quadratic trend as the fermentation time increased.

TSV is related to amylose content and reflects the retrogradation of starch (Oloyede *et al*. 2016). The higher the setback value, the lower the retrogradation during the cooling of the product made from flour (James and Nwabueze 2014). Supporting data (Figure 2; Table 2) showed that amylose content in fermented SP is higher compared to the control, and among the starters, LcY had the highest amylose content at 48 h. This may positively impact the TSV. Apparently, the findings showed that at 48 h fermentation, SP flour fermented with a paired culture of LcY had a higher TSV than the control, Sp, and Y-fermented samples. Flour with a high TSV will possess less tendency to retrogradation and syneresis and may find use in wheat-supplemented composite flours for the production of noodles, bread, and vermicelli (Marston *et al*. 2016).

## CONCLUSION

The type of starter treatment significantly affected the pasting profile (except the initial gelatinization temperature), amylose content, and the pH value of the fermenting liquid. Fermentation using a mixed culture of *Leuconostoc mesenteroides-Saccharomyces cerevisiae* produced higher PV, BV, setback viscosity, and amylose content than the control. Fermentation time caused significant ($p \leq 0.05$) variation in the pasting profile (except breakdown and peak viscosities), pH, and amylose content of fermented SP flour. Based on its highest PV of 1204 BU, SP flour fermented with a mixed culture of *Leuconostoc mesenteroides–Saccharomyces cerevisiae* may be suitable for application in products that require high viscosity. Lactic acid fermentation appears to affect pasting properties after heating (BV and TSV), and more studies can be conducted to determine structural changes in starch and other proximates.

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