

The Application of Various Fermented Malang Apple Water As A Source Of Natural Yeast for Sourdough Bread Processing

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ABSTRACT: The sourdough fermentation method is identified as a complex microbial ecosystem stimulant by the spontaneous natural water starters process. At the fermentation time, lactic acid bacteria and yeast confer the resulting characteristic bread features such as staling of bread, and palatability. With the scope of highlighting fermentation, the investigation of starter culture is relevant in order to determine starch fractions of flour. The present article employed water from apple local varieties of Malang as a mixture of natural yeast. The research was conducted in two stages. The first stage was to get the best apple variety to produce the optimum quality of sourdough starter. The second was applying the best sourdough starter at various concentrations in bread formulation. Parameters observed included yeast, and lactic acid bacteria counts, pH, Total Titratable Acidity (TTA), lactic acid content, hardness, loaf volume, specific volume, and sensory properties of sourdough bread. There was an influence on the treatment of making a sourdough starter with different types of apple fruit and showed the best treatment if sourdough starter of fermented *manalagi* apple water with a pH value of 4.5, TTA value of 2.71 mL, the number of lactic acid bacteria of 5.6×10^{10} CFU/mL, and the number of yeast of 2.62×10^{11} CFU/mL.

Keywords: fermentation, hardness, lactic acid bacteria, sensory properties, starter

INTRODUCTION

Nestled amidst the breathtaking landscapes of East Java, Indonesia, the city of Malang stands as a beacon of culture and natural abundance. Among its cherished treasures are the sprawling apple orchards that grace its surroundings. Malang's apples, celebrated for their exquisite flavor and versatility, have found their way into numerous culinary creations. In particular, an exciting avenue of exploration has emerged, one that harnesses the intrinsic potential of apples as a cornerstone ingredient in sourdough starter production. Beyond their gustatory delight, apples offer a treasure trove of compounds, making them an alluring choice for developing sourdough starters. Fresh apples, for instance, are renowned for their high pectin content, low-calorie profile, and a bounty of vitamins and antioxidants (Eberhardt, 2000).

Furthermore, when apple water undergoes the transformative process of fermentation, it metamorphoses into a reservoir of organic acids, notably lactic acid, accompanied by a pH level typically ranging from 3 to 4.5. This unique attribute endows it with a natural defense mechanism against the proliferation of harmful pathogenic bacteria (Cousin *et al.*, 2017). However, the

apple's role in baking extends far beyond its nutritional attributes. It harbors a culinary secret within its core—natural yeast, a microscopic organism that plays a pivotal role in the art of breadmaking (Graca *et al.*, 2015).

The generous sugar content within apples provides an ideal breeding ground for yeast, nurturing a symbiotic relationship between the fruit and the microorganism. Yeast, a fundamental component of bread fermentation, relies on carbohydrates, particularly sugars, for its growth and reproduction (Laaksonen *et al.*, 2017). While conventional sourdough production predominantly relies on fermented mixtures of flour and water to craft the starter, this study embarks on a novel journey by tapping into the potential of fermented apple water as an innovative foundation for the sourdough starter. The primary objective is to delve into the multifaceted enhancements that this unconventional approach can bring to the table to improve the microbiological, chemical, physical, and sensory properties of sourdough bread. The significance of this endeavor lies not only in its potential to elevate the sensory experience of bread but also in promoting sustainability and reduce reliance on commercial yeast while potentially offering health

Table 1. Formulation of sourdough sweet bread

Ingredients (%)	Percentage of Sourdough Starter				
	10%	15%	20%	25%	30%
Sourdough Starter	210	215	220	225	230
Bread flour	210	215	220	225	230
All Purpose flour	53	54	55	56	57
Sugar	66	67	68	69	70
Water	119	116	113	110	107
Whole milk	66	67	68	69	70
Salt	4	4	4	4	4
Butter	8	8	8	8	8

benefits through the inclusion of beneficial microorganisms.

MATERIALS AND METHODS

Materials

Whole-wheat flour, strong flour, all-purpose flour, organic cane sugar, salt, butter, and whole milk were obtained from a local supermarket. Four different types of apples (*manalagi*, *wang lin*, *anna*, and *rome beauty*) were purchased from a local farmer. Those apples had the same degree of ripeness (their harvest age are about 4-5 month). They were chosen because of their popularity and high demand in Malang. Materials for analysis included aquades, NaOH (Brataco Ltd., Indonesia), HClO₄ (Brataco Ltd.), PP indicators (Brataco Ltd.), sesame seeds (Karya Baru Indonesia Ltd.), PDA (Potato Dextrose Agar) and MRSA (deMann Rogosa Sharpe Agar) (Merck Chemicals and Life Sciences Ltd.).

Methods

Natural Fermentation of Apple Water

The apples were peeled and diced 2 × 2 cm in size, weighed 100 g, and then soaked in 250 mL of mineral water containing 10 g of dissolved organic cane sugar. The mixture was allowed to ferment naturally at room temperature for 5 days (Sangjin, 2012).

Sourdough Starter Production

Starter A made from a mixture of whole wheat flour and fermented apple-soaked water in a ratio of 1:1 (w/v). The mixture is then allowed to ferment naturally at room temperature for 24 hours or until doubled its size. Starter B is made from 100 g of Starter A, 100 mL of mineral water, 100 g of whole wheat flour, and 2 g of salt and then fermented at room temperature for 12 hours. Starter C is made from 100 g of Starter B, 100 mL of mineral water, 100 g of whole wheat flour, and 2 g of salt and then fermented at room temperature for 8 hours. Starter C was

the one used as a sourdough starter for making sourdough sweet bread (Sangjin, 2012).

Bread Making

The ingredients used per 250 g of total flour are shown in Table 1. All dry ingredients except salt were mixed with water and whole milk. The dough was rested for 3 hours to let the dough hydrate optimally. After 3 hours, sourdough starter, butter, and salt were added into the mixture and let it rest for 45 minutes. Dough stretching and folding were done 4 times every 45 minutes. Then, the mixture was moved to the bowl and allowed it to ferment for 3 hours at room temperature. After that, the dough undergone a proofing process in the refrigerator at 5 °C for 14 minutes. Then, the dough was gently moved into baking pans and baked at 200 °C for 15 minutes (Sangjin, 2012).

Microbiological Analysis of Sourdough Starter and Bread

Total lactic acid bacteria (LAB) and yeast count on the sourdough starter and bread were determined using standard microbiological dilution, plating, and enumeration techniques. Sample aliquot (1 g) was homogenized in 9 mL of sterile distilled water. From the appropriate nine-fold dilution, 1 mL of each dilution was plated out using pour plate method. Calculation of the number of lactic acid bacteria colonies was carried out on de Mann Rogosa Sharpe Medium (MRS) media. Potato Dextrose Agar was used as a media for isolation and enumeration of yeast (IAL, 2008).

Chemical Analysis of Sourdough Starter and Bread

The pH and Total Titrable Acidity (TTA) were determined on sourdough starter and bread by the method of Wu (2012). Briefly, sample aliquots (10 g) were homogenized with 90 ml of sterile distilled water. The pH value was recorded, and the acidity was titrated with 0.1

Table 2. Microbiological and chemical properties of sourdough starter

Samples	Yeast Population (CFU/ml)	LAB Population (CFU/ml)	pH	Titrable Acidity (TTA) (mL)
Manalagi	2.62×10^{11} b	5.6×10^{10} c	4.50 ^b	2.72 ^b
Wang Lin	2.49×10^{11} c	5.4×10^{10} bc	4.70 ^c	2.85 ^b
Anna	1.23×10^{11} a	5.9×10^{10} b	4.25 ^a	3.16 ^c
Rome Beauty	1.89×10^{11} c	7.7×10^{10} d	4.00 ^a	4.05 ^d

Means in each column with different superscripts represent a significant difference ($p < 0.05$) by Duncan Multiple Range Test (DMRT)

mol/L NaOH to a final pH of 8.6. The TTA was expressed in mL of 0.1 mol/L NaOH.

Lactic acid content on sourdough starter and bread was determined by the method of Alfonzo (2013). 10 g of samples were dissolved with 90 ml of sterile distilled water. 10 mL of sample solution were mixed with 5 mL of 0.1 mmol/L HClO₄. After centrifugation at 4000 rpm for 15 minutes at 15 °C, the mixture was added with distilled water until it reached a volume of 25 mL and filtered with 0.45 µm of cellulose membrane. Then, the lactic acid content of the mixture was read using HPLC.

Physical Analysis of Bread Samples

Bread hardness was measured by a texture analyzer (TZ-SX 500N). The bread samples were uniformly cut into cubes of 1.5 cm × 1.5 cm × 1.5 cm and pressed twice with a cylindrical probe, up to 40% of its initial height at a constant pressure speed of 1 mm/s. Hardness was defined as peak strength during the first compression cycle and expressed in units of kgf (kilogram-force).

Loaf volume was measured by the seeds displacement method. The seeds were poured into a container of known volume until the bottom was covered. The bread sample was then placed inside the container, followed by more seeds, which were leveled across the top with a spatula. The seeds inside the container were then transferred to a measuring cup to measure their volume. Loaf volume was calculated by subtracting the container volume from the volume of the seeds read in a measuring cup. The specific bread volume was calculated by dividing the loaf volume by the weight of the bread. (Hathorn, 2008)

The dough and the baked loaf were weighed and the percent weight loss was calculated as shown in Eq.

$$\% \text{ weight loss} = \frac{\text{Weight of dough} - \text{Weight of baked bread}}{\text{Weight of dough}} \times 100$$

Sensory Evaluation of Bread

Sensory evaluation of the bread was carried out within 24 hours of baking, using hedonic rating tests on 12 untrained panelists at the University of Muhammadiyah Malang. Evaluated attributes were appearance, taste, texture, aroma, crumb, and overall acceptability. Panelists scored for different properties with a maximum score of 9 for like immensely, 8-like very good, 7-like good, 6-like moderately, 5-neither like nor dislike, 4-dislike moderately, 3- dislike fairly, 2-dislike very much, and 1 for dislike extremely (Galla *et al.*, 2007).

Statistical Analysis

The statistical analyses were conducted using analysis of variance (ANOVA) procedures with a simple randomized design. Statistical differences in samples were tested for at $p \leq 0.05$. Duncan's multiple range test (DMRT) was used to differentiate between the mean values. All the analyses were done with SPSS software for Windows.

RESULT AND DISCUSSION

Microbiological and Chemical Properties of Starter

The microbiological and chemical properties of sourdough starter are presented in Table 2. The number of yeast and LAB in sourdough fermentation reached 10^{11} and 10^{10} CFU/mL, respectively, within 2 days in all dough types. The number of yeast is higher than that obtained by Ogunsakin *et al.* (2015) of spontaneous fermented sorghum meal (10^4 CFU/mL within 2 days) and by Komatsuzaki *et al.* (2019) of sourdough using wild yeast isolated from apple leaves (10^9 CFU/mL within 3 days). Starters made from various types of apples provide significantly different numbers of yeast, ranging from 1.23×10^{11} to 2.62×10^{11} CFU/mL. The Manalagi apple starter boasted the highest yeast count, followed by Wang Lin, Rome Beauty, and Anna, respectively. This variation correlated with the sugar content and sweetness level of the apples, as Manalagi, with the highest sugar content,

Table 3. Microbiological and chemical properties of sourdough bread

Samples	Yeast Population (CFU/ml)	LAB Population (CFU/ml)	pH	Lactic Acid Content (mg/kg)
0%*	0×10^{5a}	0×10^{5a}	5.89 ^a	0 ^a
10%	1.59×10^{7a}	2.5×10^{6a}	4.57 ^a	578.65 ^b
15%	1.76×10^{7b}	3.6×10^{6b}	4.67 ^b	956.91 ^c
20%	1.83×10^{7b}	4.5×10^{6bc}	4.69 ^b	1028.9 ^d
25%	1.74×10^{7b}	5.0×10^{6cd}	4.76 ^b	1082.10 ^d
30%	1.77×10^{7b}	5.8×10^{6d}	4.83 ^b	1274.95 ^d

Means in each column with different superscripts represent a significant difference ($p < 0.05$) by Duncan Multiple Range Test (DMRT)

*=Control, Commercial Instant Dry Yeast

harbored the most significant yeast population. Apples naturally host a multitude of yeasts on their peel, flesh, and seeds, which derive their sustenance through anaerobic fermentation, transforming various sugars like glucose and fructose into ethanol, methyl alcohols, methyl aldehydes, acetaldehyde, and other volatile compounds (McKay *et al.*, 2011). The dominant yeast species identified in fresh apples were *Candida sake* and *Pichia fermentans* (Graca, *et al.* 2015). Yeasts found in apples that are often used in industrial activities are *Saccharomyces cerevisiae*, *Hanseniaspora valbyensis*, *Brettanomyces / Dekkera* (Rodriguez, 2015).

The LAB populations in sourdough starter varied across apple varieties, ranging from 5.4×10^{10} to 7.7×10^{10} CFU/mL. These levels exceeded those reported by Komatsuzaki *et al.* (2019) for sourdough using LAB isolated from funa-sushi (10^8 CFU/mL within 2 days) and by Karrar *et al.* (2016) for *L. plantarum*-fermented sourdough (10^8 CFU/mL within 24 hours). Among the 4 types of fresh apples, Rome Beauty has a relatively high tartness level, leading to produce the highest number of LAB. The LAB found in the sourdough starter comes from apples and the fermented apple-soaked water used in sourdough preparation. Apples have approximately 100 million natural LAB on their skin and flesh (Wasserman, 2019). They are *Lactobacillus (para) collinoides*, *Lactobacillus diolivorans*, *Lactobacillus hilgardii*, *Lactobacillus casei*, *Lactobacillus brevis*, *Lactobacillus sicerae*, and *Lactobacillus suebicus* (Graca *et al.*, 2015; Puertas *et al.*, 2014).

Different types of apples significantly influenced the pH value of the sourdough starter, ranging from 4.0 to 4.7. This pH range aligns closely with values reported by Poutanen *et al.* (2009), which vary from 4.1 to 4.5. The

pH value is closely related to the organic acid content. A lower pH starter indicates that the organic acid content is also higher.

Apples with prominent tartness, like Rome Beauty and Anna, tend to have more organic acid content than the Wangling and Manalagi types. Harvest age and cultivation methods also determine the levels of organic acids in similar apples. Fresh ripe apples have varying TTA values ranging from 0.1 to 1.87% (Xu *et al.*, 2012; Nour *et al.*, 2015).

The TAT in sourdough starter with different apple varieties ranged from 2.72 to 4.05 mL. Apart from the organic acid content of the apples used, the TAT value is also contributed by the fermentation of yeast and lactic acid bacteria in the sourdough starter. In the current research, the TAT value is higher than that obtained (0.47 to 1.69 mL) by Ogunsakin *et al.* (2015) of spontaneous fermented sorghum meal but is lower than that obtained (10.60 to 20.60 mL) by Hanis-Syaswani *et al.*, (2018) of rice bran sourdoughs fermented with baker's yeast and/or lactic acid bacteria.

Spontaneous sourdough fermentation followed by further backslipping as in this study, contains mixtures of distinctive yeast and mesophilic LAB species and/or strains, thus representing a great diversity of natural starters for sourdough fermentation (De Vuyst *et al.*, 2014). The yeast species diversity in spontaneously fermented sourdoughs in a bakery environment is characterized by the presence of (in decreasing order of abundance) *S. cerevisiae*, *C. glabrata*, *P. kudriavzevii*, *Kazachstania unispora*, *W. anomalus*, and *C. humilis* (Harth *et al.*, 2016; Rizzelo *et al.*, 2015). While the LAB communities mostly consist of heterofermentative LAB

Table 4. Physical properties in sourdough bread

Samples	Hardness (N/mm ²)	Specific Volume (mL/g)	Weight loss (%)
0%*	1.78 ^b	1.96 ^d	37.19 ^d
10%	2.65 ^a	1.31 ^a	19.17 ^a
15%	4.30 ^a	1.42 ^b	20.83 ^{ab}
20%	3.95 ^a	1.95 ^d	28.33 ^c
25%	1.77 ^b	1.90 ^d	27.50 ^c
30%	1.84 ^b	1.65 ^c	22.92 ^b

Means in each column with different superscripts represent significant difference ($p < 0.05$) by Duncan Multiple Range Test (DMRT)

*=Control, Commercial Instant Dry Yeast

species, particularly of the genus *Lactobacillus* (Huys *et al.*, 2013; Gobbetti *et al.*, 2016)

Yeast and LAB have a synergistic relationship in food matrix fermentation. The ability of LAB and yeast to grow together is crucial in food fermentation (Wood, 2004). LAB provides an acidic environment for the growth of yeast, while yeast provides vitamins and other growth factor of LAB. LAB produces organic acids [in particular lactic acid (fresh acidity) and acetic acid (sharp acidity)] using soluble carbohydrates (maltose, sucrose, glucose, and fructose) in substrate or produced by yeast fermentation (Ganzle, 2014). In addition, yeasts prevent the accumulation of lactic acid from reaching a toxic concentration. The growth and metabolism of LAB usually inhibit the growth of normal spoilage flora of the matrix and of any bacterial pathogens that it may contain. (Mukisa, *et al.*, 2016).

Based on yeast and LAB counts, pH, and TTA values, the starter derived from Manalagi apples was identified as the most favorable among the tested varieties.

Microbiological and Chemical Properties of Sourdough Bread

The microbiological and chemical properties of sourdough bread are presented in Table 3. The difference in starter concentration significantly affected the amount of yeast on sourdough bread. The list of yeast species that are most commonly found in spontaneously developed sourdough bread, in decreasing order of abundance, i.e. *S. cerevisiae*, *C. humilis*, *Torulaspora delbrueckii*, *Wickerhamomyces anomalus*, *S. exiguus*, *Pichia kudriavzevii* and *Candida glabrata* (Legras *et al.*, 2007; Daniel *et al.*, 2011; Gullo *et al.*, 2003; Lhomme *et al.*, 2016).

The more starter added to the bread dough, the higher the number of yeast in it. However, the results showed a decrease in the number of yeast in bread with a starter concentration of 25% and 30%. This can occur because of the high concentration of lactic acid in bread. Stress response and the death of yeast cells can also be triggered by the presence of acetic acid (Sousa *et al.*, 2012).

The difference in starter concentration had a significant effect on the amount of LAB on sourdough bread. The more starter added to the bread dough, the higher the number of LAB in it, leading to an increase of lactic acid content in the bread. LAB in sourdough bread produces lactic acid through glycolysis. In addition, some LAB also produce acetic acid, CO₂, and ethanol. They play a very important role in natural preservation because they can inhibit the growth of other microbes by producing bacteriocin, which has anti-bacterial activity (Holtzel, 2000). Lactic acid is one of the organic acids, where the organic acids contained in sourdough bread can improve the taste of bread, increase gas retention, and help gluten swelling to provide a good texture (Paramithiotis *et al.*, 2005). The highest amount of lactic acid was obtained with the addition of 30% sourdough starter, while the lowest was shown with the addition of 10%

The pH value of bread decreased as the starter concentration increased due to lactic acid build-up. In addition to lactic acid, acetic acid, propionic acid, and amino acids are some acids found in sourdough that affect the pH value (Gullo, 2003). According to Vogelmann (2008), the pH of sourdough bread is strongly influenced by the duration of fermentation, but the pH of sourdough bread generally ranges from 3.5 to 5.

Table 5. Sensory properties of sourdough bread

Samples	Appearance	Taste	Aroma	Texture	Acceptability
0%*	7.17 ^c	7.42 ^d	7.50 ^c	7.00 ^c	7.42 ^c
10%	5.67 ^a	5.50 ^a	5.75 ^a	4.58 ^a	5.33 ^a
15%	6.50 ^b	6.25 ^b	6.67 ^b	6.25 ^b	6.33 ^b
20%	6.92 ^{bc}	6.75 ^{bc}	7.08 ^{bc}	6.83 ^{bc}	6.50 ^b
25%	7.08 ^{bc}	6.83 ^{bc}	7.25 ^{bc}	6.83 ^{bc}	6.67 ^b
30%	7.00 ^{bc}	6.92 ^{cd}	7.25 ^{bc}	7.08 ^c	6.58 ^b

Means in each column with different superscripts represent a significant difference ($p < 0.05$) by Duncan Multiple Range Test (DMRT)

*=Control, Commercial Instant Dry Yeast

Physical Properties of Sourdough Bread

The difference in starter concentration has a significant effect on the hardness value of sourdough bread (Table 4). Hardness analysis of bread was carried out on the same day after baking. The hardness value in sourdough bread is greatly influenced by the gluten because it has a very important role in trapping the CO₂ produced during the fermentation process.

Hardness values increase at a starter concentration of 10% to 15%. The highest hardness value was achieved at the starter concentration of 15%. Hardness values tend to decrease at concentrations of 20% to 30%. These results indicated that as the concentration of the starter increased, the CO₂ trapped in the gluten matrix of the dough was also increasing, triggering the addition of pores and producing a softer texture. Sourdough bread has a more elastic texture compared to ordinary white bread. The gluten content of the flour greatly influences the elastic texture of the sourdough bread. These breads use whole wheat flour, high protein wheat flour, and medium protein wheat flour. High-protein flour produces a more developed gluten network. (Arendt, 2007).

Sourdough bread with the addition of different natural yeast concentrations showed different weight loss and specific volume. A starter concentration of 20% produces the highest (28,33%) weight loss, while the lowest (19,17%) is given by a concentration of 10%. A previous study by Karrar *et al.* (2016) showed that the weight loss with a starter concentration of 10-20% was 13% to 18%. The highest weight loss produced by the starter cannot be higher than that produced by control bread using commercial yeast. Commercial yeast is well-known for its exceptional weight loss, so this yeast is often used in the bread baking in Indonesia.

The weight loss value reflects how much the dough expands by the CO₂ produced during fermentation. Sourdough bread with a starter concentration of 10% cannot expand due to the lack of gluten network and CO₂ produced. Control breads can expand the most because of the rapid activity of their commercial yeast in breaking down carbohydrates into simple sugars and producing CO₂.

The highest specific volume is also provided by control bread. Sourdough bread with a higher specific volume is strongly influenced by an increase in the CO₂ binding capacity of the dough, which is also closely related to the acidity of the sourdough bread itself. The acidity and starter concentration influenced the formation of the gluten network, which determined the structure of bread crumbs (Sanz, 2012). A previous study showed that the acidity of the dough had a negative effect on the volume and texture of sourdough bread (Gocmen, 2007). Therefore, the specific volume of bread with starter concentrations of 25% and 30% has decreased. Extreme acidity causes the yeast cells to die so that the bread is less able to expand.

Sensory properties of bread

The sensory evaluation based on appearance, taste, aroma, texture, and acceptability showed a significant difference among the sourdough bread samples (Table 5). The appearance of the sourdough sweet bread was tested based on its crumb and crust. All the sourdough bread samples showed similar crumb and brown crusts with the exception of control bread with commercial instant dry yeast. The brown crust in sourdough sweet bread is because of the Maillard reaction (Moroni *et al.*, 2009). Maillard reaction occurs between sugar and amino acids in the presence of heat due to the baking process resulting

in the browning of the crust of sourdough sweet bread. This process also resulted in the formation of aromas and flavors. (Brescia, *et al.*, 2007).

CONCLUSION

The study exploring the effects of different apple varieties and sourdough starter concentrations on the bread-making process revealed notable differences in microbiological, chemical, and sensory properties. The sourdough starter derived from fermented Manalagi apple-soaked water, boasting a pH level of 4.5, TTA of 2.72 mL of NaOH, a yeast population of 26.2×10^9 CFU/ml, and a population of lactic acid bacteria of 56×10^9 CFU/ml, was identified as the best among the tested varieties.

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