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The Effect of *Saccharomyces Cerevisiae* Concentration and Fermentation Time on Chemical and Functional Properties of Duck Egg Flour

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***Abstract*— Duck egg flour is a form of processed product from whole eggs through a drying process. The production process influences egg flour's quality. Fermentation of egg flour before drying is one approach to preserve its quality. Fermenting eggs with yeast prevents the Maillard reaction and improve duck egg flour quality. This study aimed to examine the effect of *Saccharomyces cerevisiae* concentration and fermentation time on duck egg flour's chemical and functional properties. This research used a nested design in a completely random design with fermentation times of 12 and 24 hours and concentrations of 0.2%, 0.4%, and 0.6%. The results indicated that fermented duck egg flour with a 0.2% concentration of *Saccharomyces cerevisiae* for 24 hours generated the optimum chemical and functional properties (4.43% ash content, 13.74% protein content, 134.9% carbohydrate content, 6.45% water content, 169.33% foaming power, and 44.68% foaming stability).**

***Keywords*—Duck egg; fermentation; *S. cerevisiae*; chemical properties; functional properties.**

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I. INTRODUCTION

In complement to chicken eggs, there is a growing need for duck eggs since they include key nutrients for human needs. Per 100 g, eggs have nutritional advantages over other poultry eggs, including 162 kcal, 12.1 g of protein, 12.1 g of protein fat, 0.68 g of carbohydrates, 56 mg of calcium, 2.1 mg of iron, 0.3 mg of vitamin B6, 1.86 mg of pantothenic acid, 0.16 mg of thiamine, 674 IU of vitamin A, 1.3–4 mg of vitamin E, and 0.2 mg of vitamin B12 [1].

The egg's essential components are the egg white and the yolk. Whites and yolks of eggs are used in the food processing industry. The white and yolk component has practical capabilities in the food processing sector and bakery because it has properties to expand dough, impart color and flavor, and function as an emulsifier. Egg yolk is an effective emulsifier because it contains surface-active substances, such as *lecithin*, *cholesterol*, and *lecito-protein*. Cholesterol forms a water-in-oil (w/o) emulsion, whereas lecithin forms an oil-in-water (o/w) emulsion [2]. Egg white contains *ovomucin, globulin*, *ovomucoid*, and *ovalbumin*, which increase foaming power. The greater the amount of trapped air, the more rigid and less flowable the foam will become. The protein ovomucin determines the stability of foam [3]. Ovomucin has the ability as a foaming agent. Ovomucin will trap air and then stabilize the foam. Thus, in the foaming process, the ovomucin structure opens, traps air, and closes again.[4]

Large-scale use of egg whites and yolks results in volume requirements, maximum handling, and a reduction in the functional qualities of egg whites [5]. Producing egg flour-based products is one possible treatment. Changing the product's liquid to flour can extend its shelf life without compromising quality and make it more convenient to store.

Yeast *Saccharomyces cerevisiae* is the most significant source of hydrolase and invertase enzyme capable of converting sugar into ethanol. The hydrolase enzyme catalyzes the hydrolysis of sucrose (disaccharide) into glucose (monosaccharide), whereas the invertase enzyme converts glucose into ethanol. The form of *Saccharomyces cerevisiae* cells is oval or circular. The consistent shape of the cell facilitates its identification. With a cell diameter of around 0.0005 cm, facultative microorganisms have a large cell diameter [6]. This study examines the influence of *Saccharomyces cerevisiae* concentration and fermentation time on duck egg flour's chemical and functional qualities.

II. Materials and Method

A. Materials

The main ingredients used in this study were 1500 mL egg yolk and white from Mojosari duck eggs in Majalaya, *Saccharomyces cerevisiae*, Malt Extract Broth (MEB), Malt Extract Agar (MEA), 5% citric acid, distilled water, Bovine Serum Albumin (BSA), lowry A, lowry B, acetic acid buffer pH 5, Anthrone reagent, lead acetate ($Pb(CH_3COO)_2$), 0.1 N NaOH, sodium oxalate ($Na_2C_2)_4$), glucose standard, $CaCO_3$, Whatman paper No. 2, and 70% and 80% alcohol.

B. Methods

1) To produce the liquid starter, we followed the protocols by Nasir et al. [7]:

- A total of 2–3 oses of pure *Saccharomyces cerevisiae* yeast isolate were inoculated on 5 ml MEB media, then incubated at 27°C for 24 hours (culture 1).
- A total of 0.1 ml (0.1%) of culture 1 was taken, then inoculated on 100 ml MEB, then incubated at 27°C for 48 hours; and
- Yeast *Saccharomyces cerevisiae* liquid starter is ready to use.

2) To make duck egg flour, we follow the procedures by Pérez-Reyes et al. [8]:

- Egg whites and yolks are mixed by shaking until they are homogenous.
- The pH is then adjusted. If the pH of the liquid egg is above 5, % citric acid is added to the egg until it reaches pH 5 for the process of making duck egg flour.
- The whole egg liquid is pasteurized in a water bath at 60°C.
- The fermentation time has used in an incubator for 12 hours and 24 hours at a temperature of 27°C at different levels of *Saccharomyces cerevisiae* concentration (0.2%, 0.4%, and 0.6%).
- Drying was carried out using the pan-drying method at 45°C for 48 hours in an electric oven.
- For the milling, the dried samples were then ground using a blender, and the duck egg flour was filtered with an 80-mesh sieve so that the texture of the duck egg flour became smoother.

3) Ash Content: For the ash content, we follow the methods by Sun et al. [9]. Approximately 5 g of egg white flour sample was placed in an ashing furnace and burned until grey ash or constant weight was achieved. The ashing was performed in two stages, the first at temperatures of about 400°C and the second at 500°C for two hours. Ashes were cooled in a desiccator before weighing using the following formula:

$$\%\ Ash\ content = \frac{Ash\ weight\ (g)}{Sample\ weight\ (g)} \text{x } 100\%$$

4) Protein content: We followed Betti et al. [10] procedures for protein content determination. Protein content was measured by using the Lowry method. Five grams of sample were added with 40 ml of distilled water, and the resulting extract was filtered. After 10 minutes of centrifugation at 11.000 rpm, the precipitated protein was separated from the supernatant. The protein precipitate is then redissolved with an acetic acid pH 5.0 buffer to a volume of 10 ml. Then, 4 ml of protein sample was collected, and the method was carried out in the same way as in the standard curve preparation treatment, beginning with the addition of Lowry B reagent, etc. Using the above standard curve, the protein content of the absorbance obtained from the sample solution was then measured. The sample dilution was also taken into account. The following formula was used to compute the protein's weight and percent protein content:

$$Protein\ weight\ (g) = Volume\ of\ sample \times Protein\ cons.of\ the\ sample$$

$$\%\ Protein\ content = \frac{Protein\ weight\ (g)}{Sample\ weight} \text{x } 100\%$$

5) Carbohydrate content: Carbohydrate content was determined using the Anthrone method [9]. The sample was mashed using 250 ml of distilled water. The dilution was performed until the 8th dilution. Samples were pipetted into a test tube for blanks and 0 samples, i.e., 0.2, 0.4, 0.6, 0.8, and 1.0 ml of standard sugar solution. The distilled water was added until the total volume is 1.0 ml each. Five ml of anthrone reagent was added to each tube. The sample was boiled for 12 minutes and cooled down. The samples were tested using a Spectrophotometer (CARY 50 UV) with a wavelength of 630 nm.

6) Water content: Water content determination followed the method by Sun et al. [9]. 2 grams of white egg flour are placed in an aluminum cup. The cup is placed in an air blast oven (YENACO YNC 30L) at a temperature of 105-110°C for 3 hours. After 3 hours, the cup is removed, cooled in a desiccator for 15 minutes, and then weighed. The procedures are repeated until a constant weight is obtained. The following equation is used to calculate the water content:

$$\%\ Water\ content = \frac{Initial\ weight - Final\ weight\ (g)}{Initial\ weight} \text{x } 100\%$$

7) Foaming power and stability: We adhere to Ho et al. [11] methods for determining the foaming power and stability. The rehydrated product was shaken at speeds of three (680–700 rpm) for five minutes using a Philips mixer. The foam was flattened with a spatula, and its volume was determined. After allowing the foam to rest for one hour, the produced drain volume was measured. The following equation calculates the formula for foaming power and foaming stability:

$$Foaming\ power = \frac{Foaming\ volume}{Egg\ white\ volume} \text{x } 100\%$$

$$Foaming\ stability = 100\% - \left(\frac{Seepage}{Foaming\ volume} x\ 100\%\right)$$

III. Results and Discussions

A. Effects on Chemical Properties of Duck Egg Flour

Different Saccharomyces cerevisiae concentrations in the fermentation process showed different results on duck egg flour's chemical properties (ash content, moisture content, and protein content). This difference can be seen in figure 1.

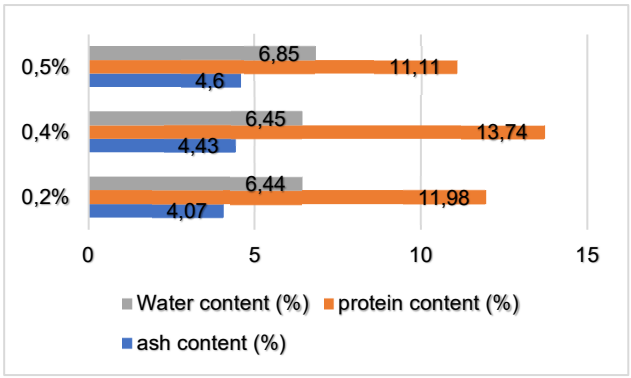

Fig. 1 Chemical properties of different concentrations *Saccharomyces cerevisiae*

1) Ash content. Based on the obtained average ash concentration, the egg flour meets the quality standards for ash content, which, according to the 1996 SNI for egg flour, the maximum ash level in egg flour is 5 %. According to Abreha et al. [12], the results indicate the same results as macro and micro-mineral, calcium, magnesium, potassium, sodium, phosphorus, iron, zinc, copper, and manganese.

The addition of *Saccharomyces cerevisiae* to the produced duck egg flour had no discernible effect, likely due to the high mineral content of duck egg flour. Minerals influence fermentation, causing the substance to undergo physical and chemical changes [13]. Macroelements in raw vegetables were higher compared to fermented vegetables.

The proportion of ash produced tends to rise with the duration of fermentation and the amount of yeast administered. Yeast can produce phytase enzymes that degrade phytic acid (which binds numerous minerals) into phosphorus and inositol [14]. By breaking down phytic acid, certain minerals (magnesium, iron, calcium, and zinc) become available. Therefore, the ash content tends to increase as yeast concentration increases.

2) Protein content. Fig. 1 compares the fermentation treatment with a concentration of 0.2% *Saccharomyces cerevisiae* that produced the same result. These results demonstrate that adding *Saccharomyces cerevisiae* yeast concentration to duck egg flour had no discernible effect. This occurs due to the yeast's digestion of the egg's protein into amino acids. The fermentation process reduces large compounds (proteins) to little compounds (amino acids), making them easier to digest. Except for protein, amino acids cannot be quantified in the Lowry method for analyzing protein levels.

According to Santos et al [15], an increase in pH above the optimal pH for the growth of *Saccharomyces cerevisiae* provided insufficient carbon sources in the medium, which led to the breakdown of protein in the medium required for its metabolic activity. The metabolic process of protein will produce urea and ammonium ions, which can raise the pH.

The protein content of duck egg flour differed between 12 and 24 hours of *Saccharomyces cerevisiae* fermentation, as shown in Fig. 2. The protein content decreases as fermentation duration increases. It is hypothesized that an increase in pH impedes yeast's ability to rebuild glucose properly, causing the protein to bind to glucose and raise egg sugar levels. Tape yeast may ferment carbohydrates or sugar in egg flour into carbon dioxide and water, and if both are present in the same solution, it will dissolve and create compounds quickly [16]. The decomposition of H+ ions by carbonic acid causes the egg's pH to rise.

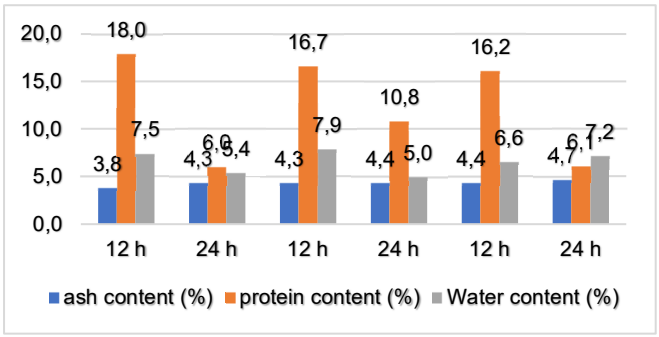

Fig. 2 Chemical properties on different fermentation time

3) Carbohydrate content. As the concentration of *Saccharomyces cerevisiae* increased, the data showed a carbohydrate rise. The rise is believed to be due to a suboptimal effect of yeast growth during fermentation, which prevents adequate glucose reduction in eggs. Essentially, fermentation minimizes the glucose in eggs so that the Maillard reaction can be slowed down. Studies by [17] showed that *Saccharomyces cerevisiae* could reduce egg glucose levels by releasing hydrogen ions from egg glucose with the aid of the zymase to produce alcohol and carbon dioxide in aerobic conditions by oxidizing glucose and binding its carbon atoms to oxygen.

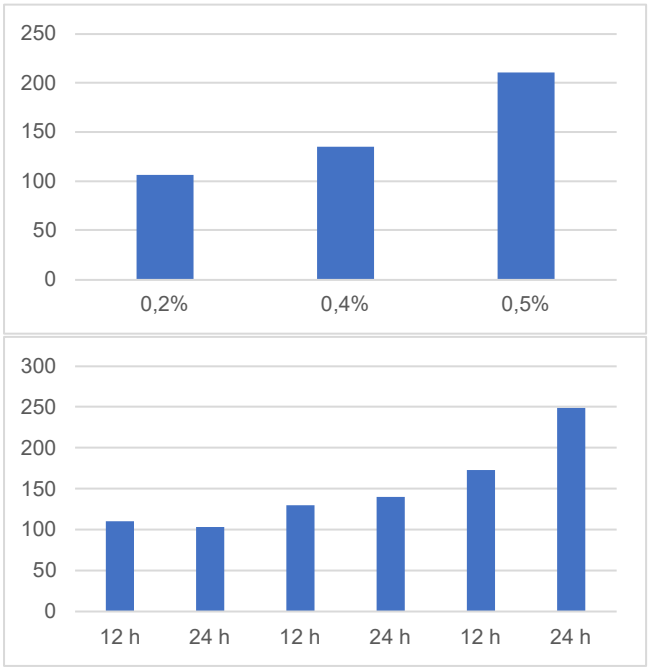

Fig. 3 Chemical properties of Carbohydrate content on different concentrations and fermentation time

The carbohydrate content of duck egg flour varied between 12 and 24 hours of *Saccharomyces cerevisiae* fermentation of duck eggs (Fig. 3). At a concentration of 0.2%, yeast can efficiently reconstruct glucose, as evidenced by the drop in carbohydrate levels after 12 hours of fermentation (103.04%) and 24 hours of fermentation (110.09 %). The administration of *K. lactis* during the production of white egg flour can suppress the Maillard reaction caused by heating during the drying process by decreasing glucose levels [18].

Concentrations of 0.4% and 0.6% at 24 hours of fermentation increased the carbohydrate content of duck egg flour. This shows that *Saccharomyces cerevisiae* was unable to decrease glucose during fermentation. Due to the high carbohydrate content in eggs, the likelihood of Maillard reaction during heating is increased. According to Pujimulyati, Andiwarsana, and Suprapti [19], the maximum amount of reduced sugar in duck egg flour is 0.5%, but the total amount of reduced sugar is greater than 0.5%, as determined by this study. Overall carbohydrate content increased as fermentation time increased.

4) Water content. The water content of duck egg flour produced by *Saccharomyces cerevisiae* at varied concentrations and fermentation periods ranged from 5.00 to 7.47 % (Fig. 1). There was an increase in water content as the concentration of *Saccharomyces cerevisiae*. This may have been caused by the fermentation process that turns glucose into water. During fermentation, the tertiary structure of ovomucin molecules was transformed into secondary and primary structures. During the fermentation process, structural changes led to the formation of more active groups in the free position, resulting in a greater capacity to bind water. According to Chen et al. [20], the egg white separates into two layers throughout the fermentation process. The thin layer underneath the thick layer is a precipitate, whereas the thick layer is rich in glycoproteins and ovomucin. This gelatinous layer will become watery if fermented for an extended period, impacting the drying process. However, increasing the concentration to 0.4% did not make a difference. This may be due to the optimal fermentation process by *Saccharomyces cerevisiae* in each treatment, so the glycolysis process produces volatile water similarly. Syainah [21] research indicates that *Saccharomyces cerevisiae* is capable of aerobic respiration, turning glucose into carbon dioxide and water. Fermentation will enhance the separation of water that evaporates during heating from other components.

The longer fermentation would last, the less water content (Fig. 3). Following prior research by Nusa, Suarti, and Marbun [22] the longer the fermentation period, the lower the water content. This is due to the decreasing activity of *Saccharomyces cerevisiae,* causing the produced water content to be reduced. During the fermentation process by *Saccharomyces cerevisiae*, glucose is converted into carbon dioxide (CO_2) and water (H_2O), decreasing the dry matter's water content.

The treatment with a concentration of 0.4 % that was fermented for twenty-four hours produced the lowest water content at 5.00 %. This water content is to the Food and Drug Administration's recommendation that the maximum water content of whole egg flour (combined egg white and egg yolk) be 5%. Following prior research by Nusa, Suarti, and Marbun [22], the treatment with a concentration of 0.4% produced the highest water content in quail egg albumin flour at 6.75%, and a fermentation time of 24 hours had the highest water content at 6.02%.

B. Effects on Functional Properties of Duck Egg Flour

Different of Saccharomyces cerevisiae concentrations in the fermentation process showed different results on the functional properties (foaming stability and foaming power) of duck egg flour. This difference can be seen in figure 1.

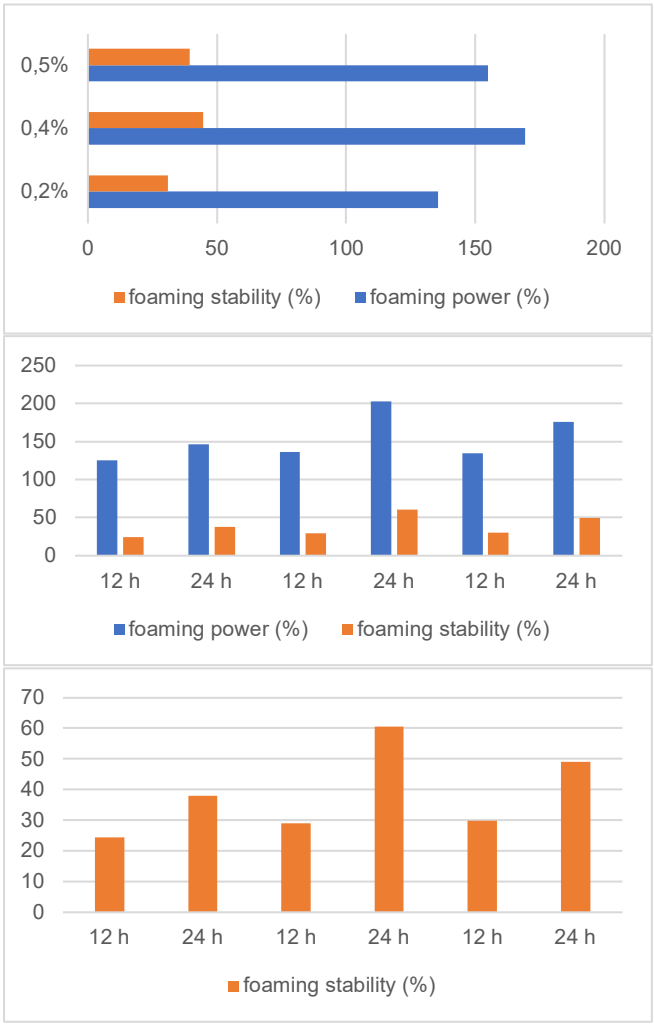

Fig. 4 Functional properties of different concentrations and fermentation time

1) Foaming power. Globulin is one of the egg white protein fractions that can stimulate foam development, whereas ovomucin-lysozyme complex, ovalbumin, and conalbumin can stabilize foam when heated [23]. There was an increase in foaming power at 0.4% treatment versus 0.2% treatment. There is a tendency for the foaming power to grow as the concentration of *Saccharomyces cerevisiae* rises [24], [25]. As a result of the entrapped air in the protein molecules caused by the shaking process, the foam will form. Shaking will cause the bonds in the egg protein molecule to loosen, resulting in a longer protein chain that can bind air [26].

However, there was a decrease at the 0.6% treatment compared to the 0.4% treatment; this may be owing to the fermentation process by yeast activity at higher concentrations causing an excessive breakdown of glucose into carbon dioxide and water. The more water produced, the more difficult it will be for the foam to form, and the egg flour's glucose content might increase the foaming strength created [8], [27]. The addition of 0.4% concentration produced the greatest amount of foam, 163.33%. Following previous research by Nusa, Suarti, and Marbun [22], adding 0.4% *tempe* yeast provided the best foaming power of 356.740% in quail egg albumin flour.

The foaming power of duck egg flour differed between 12 and 24 hours of *Saccharomyces cerevisiae* fermentation (Fig. 4). The longer the fermentation period, the greater the foaming power. Fermentation for 24 hours can increase quail egg albumin flour because microorganisms can break down glucose into large amounts of CO_2, and the substrate used by microorganisms is still readily available, expanding the foam produced [22].

Fermentation for 24 hours on duck egg albumin flour produced greater foaming power than fermentation for 12 hours [19]. Longer fermentation will trigger the lysozyme complex compound with ovomucin to increase the pH close to the isoelectric point of lysozyme so that it can increase duck egg flour's foaming power. The rise in foaming power was caused by transforming the ovomucin compound's structure from tertiary to secondary and even primary. The tertiary structure of a protein is a form of protein that occurs due to the folding of secondary proteins, resulting in globules. The treatment with a concentration of 0.4% that was fermented for 24 hours produced the highest foaming power (202.67%). Fermentation for 24 hours had the highest quail foaming power of 345.70% in egg albumin flour [22].

2) Foaming stability. Egg whites easily create foam because the links between protein molecules are already broken, making it simple to bind air during shaking [28], [29]. Yeast's fermentation mechanism degrades protein macromolecules into smaller molecules [30]. A lesser number of molecules will increase surface area, resulting in stronger and more stable interactions between molecules. During the foam generation process, protein is a surface-active substance capable of stabilizing the foam. The stability of duck egg flour foam differed between 12 and 24 hours of *Saccharomyces cerevisiae* fermentation (Fig. 4). The longer the fermentation period, the more stable the foam will be. Due to the decrease in water content during fermentation, foam formation is enhanced. Due to the transformation of glucose into carbon dioxide and water, the water content of dry matter decreases. The longer the fermentation, up to 24 hours, the smaller the foam generated, resulting in a high stability result. Prolonged heating alters the viscosity of foam-forming proteins, particularly ovomucin protein, which contributes to the stability of foam in egg flour [31]. 0.4% solution fermented for 24 hours produced the highest foam stability (60.66%).

IV. Conclusion

Based on the results of the research on the effect of *Saccharomyces cerevisiae* concentration and fermentation time on the chemical and functional properties of duck egg flour, it can be concluded that the treatment of 0.2%- and 24-hours fermentation time of *Saccharomyces cerevisiae* resulted in the best chemical and functional properties of duck egg flour.

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