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The effect combination concentration and fermentation time of *Lactococcus lactis* with *Saccharomyces cerevisiae* on the functional properties of Duck egg flour

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Abstract
Duck egg flour is one of the final products obtained from the processing of eggs through drying. The fermentation process can applied to eggs to extend their shelf life, maintain the quality of duck egg flour, and prevent Maillard reactions during the drying process. This study aimed to investigate the effect concentration and fermentation time of *Lactococcus lactis* and *Saccharomyces cerevisiae* on the functional properties (foaming capacity, foam stability, and emulsification capacity) of ducks egg flour. The research conducted experimentally using a Nested Complete Randomized Design with fermentation times of 12 and 24 hours nested within concentrations of 0.4% *Saccharomyces cerevisiae* and 4%, 6%, and 8% *Lactococcus lactis*. The results indicate that adding 6% *Lactococcus lactis* and 0.4% *Saccharomyces cerevisiae* to duck egg flour with 24-hour fermentation showed the best foaming capacity, foam stability, and emulsification capacity (40.45%, 21.91%, and 0.995%, respectively).

Keyword: *Lactococcus lactis*, duck egg flour, fermentation

Introduction

Duck eggs contain a high level of animal protein that is easily digestible and are often use as an alternative source of protein besides meat. Duck eggs from farms generally come in various weights, ranging from 64 grams as the lowest to 88 grams as the highest, with the ideal weight around 70 – 79 grams (Sumaryani & Permatasari, 2020) [15]. Per 100 grams of duck egg weight, contains 185 kcal of energy, 12.81 g of protein, 13.77 g of total fat, 1.45 g of carbohydrates, 64 mg of calcium, 3.85 mg of iron, 0.25 mg of vitamin B6, 1.86 mg of pantothenic acid, 0.16 mg of thiamine, 674 IU of vitamin A, 1.34 mg of vitamin E, 0.2 mg of niacin, and 5.4 mg of vitamin B12 (Huang & Lin, 2011) [5].

The processing of duck eggs into flour is one way to extend their shelf life without compromising their nutritional value. Egg flour, specifically duck egg flour, can prolong its storage life, optimize storage space, prevent damage due to impact, and make rehydration and usage more convenient. Duck egg flour can be produced through drying processes, more precisely, by the pan-drying method (thin-layer drying), which employs an oven for drying. One drawback of the pan-drying method is the potential occurrence of Maillard reactions during the drying process, which can affect the odor, taste, and color of duck egg flour. These reactions are caused by glucosamine reactions (the bonding of protein and carbohydrate), and can be prevented through fermentation before the drying process. Fermentation can reduce glucose content in the eggs and minimize the likelihood of browning.

The fermentation of whole eggs can be influenced by several factors, including fermentation time and the concentration of the starter used (Pratama, Wulandari, & Balia, 2023) [9]. The use of *Saccharomyces cerevisiae* can transform glucose in the eggs, preventing it from binding with carbohydrates and thus reducing the occurrence of Maillard reactions. Maillard reactions are browning reactions caused by the interaction of aldehyde groups from carbohydrates with amino groups from the egg's protein constituents (Cui *et al*., 2012) [3].

Saccharomyces cerevisiae has a high conversion rate of sugar to ethanol and produces hydrolyze and invertase enzymes. Hydrolyase enzymes break down sucrose into glucose, and invertase enzymes convert glucose into ethanol (Pratama *et al*., 2019) [8]. The growth of *Saccharomyces cerevisiae* is influenced by factors such as pH, with a growth range between 2.0 – 8.6 and an optimal pH range between 4.5 – 5.0 (Wiratmaja, Bagus, Kusuma, & Winaya, 2011) [19]. Therefore, the egg's pH is adjusted to create optimal conditions for yeast growth and fermentation.

Lactococcus lactis is a probiotic bacterium that produces lactic acid from various sugars, including galactose, glucose, fructose, lactose, maltose, mannose, N-acetylglucosamine, ribose, and trehalose (Teuber, 1995) [16]. *Lactococcus lactis* is used in this study because it is a type of lactic acid bacterium capable of rapidly producing lactic acid and has a higher proteolytic activity compared to other lactic acid bacteria, providing a more optimal effect (Piraino, Zotta, Ricciardi, McSweeney, & Parente, 2008) [7] (Garabal, Rodríguez-Alonso, & Centeno, 2008) [4].

Saccharomyces cerevisiae and *Lactococcus lactis* exhibit different growth curve patterns. *Saccharomyces cerevisiae* experiences a lag phase from hours 1 to 2, followed by a log or exponential phase from hours 3 to 6, a stationary phase from hours 7 to 14, and a death phase from hours 15 to 24 (Salari & Salari, 2017) [12]. On the other hand, *Lactococcus lactis* undergoes a lag phase at hour 1, followed by a log or exponential phase from hours 2 to 5, a stationary phase starting at hours 5 to 6, and a death phase from hours 7 to 11 (Chen, Shen, Ingvar Hellgren, Jensen, & Solem, 2015) [2]. Both growth curve patterns indicate that *Lactococcus lactis* transitions more rapidly through phases from lag to death comparing to *Saccharomyces cerevisiae.*

The use of *Saccharomyces cerevisiae* in combination with *Lactococcus lactis* and varying fermentation times is believed to enhance the functional properties (foaming capacity, foam stability, and emulsification capacity) of duck egg flour. However, research on the combined use of *Saccharomyces cerevisiae* and *Lactococcus lactis* and the fermentation time's impact on the functional properties of duck egg flour is still limited, necessitating further research on this subject.

Materials and Methods

The study utilized duck eggs aged less than five days, totaling 95 eggs, as its primary ingredient. Saccharomyces cerevisiae, isolated from Fermipan, and Lactococcus lactis, in isolated agar stab form, were essential microbial components. Various agar and broth media, including Yeast Malt Agar (YMA), Yeast Malt Broth (YMB), de Man Rogosa and Sharpe Agar (MRSA), and de Man Rogosa and Sharpe Broth (MRSB), were used to cultivate and isolate the microorganisms. Additionally, citric acid, palm oil, lemon juice, salt, sugar, plastic wrap, plastic clips, aluminum foil, and deionized water (Aquades) were utilized in the experimental procedures to achieve the research objectives effectively.

Research Method

1. Preparation of Liquid Starter for *Saccharomyces cerevisiae* (Modified from (Wahono, Damayanti, Rosyida, & Sadyastuti, 2011) [18]

A pure culture of *Saccharomyces cerevisiae* yeast isolates (2-3 osse) is inoculated into 5 mL of Yeast Malt Broth (YMB) and then incubated aerobically at 27℃ for 24 hours (culture 1). A 0.1 mL (0.1%) portion of culture 1 is taken and inoculated into 100 mL of YMB, followed by another aerobic incubation at 27℃ for 48 hours. The liquid yeast starter of *Saccharomyces cerevisiae* is now ready for use.

2. Procedure for Bacterial Culture Propagation of *Lactococcus lactis* (Sulastri & Manguntungi, 2020) [14]

A volume of 5 µl of *Lactococcus lactis* bacterial stock culture is inoculated into 5 mL of de Man Rogosa and Sharpe Broth (MRSB), then incubated aerobically at 37℃ for 24 hours.

3. Procedure for Making Duck Egg Flour (Whole Egg) (Pratama *et al*., 2019) [8]

Egg Selection, The first step in making duck egg flour involves the careful selection of eggs based on criteria such as weight, age, shape, shell cleanliness, and shell integrity. The chosen eggs typically weigh between 50 to 60 grams. Egg Preparation, Selected duck eggs with 1 to 4 days of storage age are thoroughly cleaned with flowing water, ensuring that the shells are free from contaminants. They are then dried with a clean cloth to prevent any bacterial contamination of the egg content. Egg Separation, The eggs are gently cracked, and the eggshells are separated from the egg contents. Mixing, The egg yolks and egg whites are mixed thoroughly using a hand mixer until a homogenous mixture is achieved. pH Adjustment, If the whole egg liquid has a pH above 6.5, a 5% citric acid solution is added to lower the pH to approximately 5.0. Pasteurization, The whole egg liquid is pasteurized by immersing it in a water bath at 60°C for 3 minutes. Fermentation, The egg mixture undergoes fermentation for either 12 or 24 hours at 30°C, using an incubator. This fermentation process involves the addition of liquid *Saccharomyces cerevisiae* culture at a concentration of 0.4% and three different concentrations of *Lactococcus lactis*, which are 4%, 6%, and 8%. Drying, The mixture is then dried using the pan-drying method in an electric oven at 45°C for 48 hours, with a thickness of approximately 5 mm. The expected yield ranges between 20% to 30%. Milling, After drying, the mixture is removed and milled using a blender, followed by grinding with a pestle and mortar to achieve a finer texture. Packaging, The duck egg flour is carefully packaged using plastic clips to preserve its quality and protect it from damage. Observation, Functional tests are conducted to evaluate the flour's performance, including its foaming capacity, foam stability, and emulsification properties. This comprehensive procedure ensures the production of high-quality duck egg flour while extending its shelf life and enhancing its functional characteristics.

Analysis Procedure

1. Foaming Capacity and Foam Stability of Duck Egg Flour (Pratama *et al*., 2019) [8] with the following steps:

a. Dehydration Product: Take a 20-gram sample for rehydration using a hand mixer at a speed of 680 - 700 rpm for 5 minutes. The sample-to-water ratio depends on the obtained yield.
b. Foam Formation: The foam formed is leveled with a spatula, and its volume is measured.
c. Foam Stability: Allow the foam to stand for one hour, then measure the volume of the resulting residue.
d. Foaming Capacity and Foam Stability Calculation: The foaming capacity and foam stability are calculated based on the formula presented by Stadelman and Cotterill (1995).

$$\text{Foaming Capacity} = \frac{\text{final volume} - \text{initial volume}}{\text{initial volume}} \times 100\%$$

$$\text{Foam Stability} = 100\% - \frac{\text{drainage}}{\text{foam volume}} \times 100\%$$

2. Emulsifying power of duck egg flour through the mayonnaise-making process (Usman, Wulandari, & Suradi, 2016) [17] with the following steps::

a. Compose the ingredients, which include 78% (117 g) of palm oil, 9% (13.5 g) of duck egg flour (whole egg), 7% (10.5 g) of lemon juice, 1% (1.5 g) of salt, and 5% (7.5 g) of sugar, for a total of 150 g, and mix them using a mixer.
b. Combine the palm oil with the other ingredients, excluding the lemon juice. Add 1/5 of the palm oil at the beginning and gradually add the rest.
c. Add the lemon juice to the mixture after the oil and other ingredients have been thoroughly mixed. Mix for 4 minutes.
d. Place the mixture in a container of known volume and heat it in an oven at 45℃ for 1 hour.
e. Let the mixture cool in a refrigerator at 5℃ for 1 hour.
f. Calculate the emulsifying power of duck egg flour based on the presence or absence of separation in the emulsion, using the formula employed by Suciati *et al*. (2022).

$$\%\text{Emulsifying power} = 100 - \frac{b}{a}$$

Note:
a = total volume of the emulsion ingredients
b = volume of the separated phase

Result and Dicussion

Table 1: Combinations of *Lactococcus lactis* concentration with *Saccharomyces cerevisae* and fermentation time on the functional properties of duck egg flour

Observed Variables	Level of Concentration		
	$P_{1...}$	$P_{2...}$	$P_{3...}$
Foaming Capacity (%)	24,11[a]	30,15[ab]	19,95[a]
Foam Stability (%)	58,72[a]	21,91[a]	17,40[a]
Emulsifying power (%)	0,993[a]	0,994[ab]	0,995[b]

Observed Variables	Level of Time					
	$P_{1.1}$	$P_{1.2}$	$P_{2.1}$	$P_{2.2}$	$P_{3.1}$	$P_{3.2}$
Foaming Capacity (%)	25,82[a]	22,39[a]	19,80[a]	40,45[b]	12,40[a]	27,49[a]
Foam Stability (%)	72,19[b]	45,25[b]	21,53[a]	22,28[a]	22,41[a]	12,38[a]
Emulsifying power (%)	0,993[a]	0,993[a]	0,992[a]	0,995[b]	0,995[b]	0,994[ab]

The Influence of Treatments on the Foaming Capacity of Duck Egg Flour

Functional properties of eggs, essential ingredients in food products, depend on various characteristics, with foaming capacity being particularly important. Foaming capacity measures the volume of foam generated when egg flour is hydrated and whipped for a certain period. The data presented in Table 1 indicate that the average foaming capacity of duck egg flour, resulting from different combinations of *Lactococcus lactis* concentrations and fermentation durations, ranges from 12.40% to 40.45%. It is noteworthy that the concentration of *Lactococcus lactis* has a consistent impact on the foaming capacity of duck egg flour. However, the nested factor of fermentation duration within each concentration level has a significant influence on the foaming capacity of duck egg flour.

Foam formation in egg flour occurs due to the presence of proteins, where the molecular protein bonds open, causing protein chains to elongate. Air enters between these protein chains and becomes trapped within them, increasing in volume. The proteins considered instrumental in egg foam formation are globulin and ovalbumin. Globulin helps reduce surface tension, thus aiding in foam formation, while ovalbumin assists globulin in creating a strong foam structure. Ovalbumin is most effective at forming foam at a pH of 3.7-4.0, while other proteins work best at pH levels between 6.5 and 9.5 (Quan & Benjakul, 2019) [10].

The treatment involving *Lactococcus lactis* resulted in a decrease in comparison to egg flour without *Lactococcus lactis*. This effect can be attributed to the high activity of yeast and bacteria during fermentation. Yeast continuously produces invertase enzymes that hydrolyze sucrose into glucose and fructose throughout the fermentation process. These two sugar metabolites are an energy source needed by *Lactococcus lactis* to produce lactic acid during bacterial growth. The high production of lactic acid leads to a decrease in pH, causing protein denaturation and a reduction in the total protein content (Yusmarini, Indrati, Utami, & Marsono, 2010) [20].

Table 1. also presents data on the influence of the fermentation time of duck egg flour combined with *Lactococcus lactis* for 12 and 24 hours. The data indicate differences in the foaming ability of duck egg flour. The longer the fermentation process, the higher the foam volume generated during the rehydration of duck egg flour. A 24-hour fermentation of duck egg flour results in better foaming ability compared to a 12-hour fermentation. The longer fermentation time can trigger changes in the structure of ovomucin compounds from tertiary to secondary or primary structures, enhancing the foaming ability of duck egg flour.

The highest foaming ability of duck egg flour, reaching 40.45%, was obtained from the treatment with 6% *Lactococcus lactis* concentration, fermented for 24 hours. The addition of 0.4% *Saccharomyces cerevisiae* and 6% *Lactococcus lactis* is the most suitable option to achieve the optimal foaming ability of duck egg flour, consistent with the results of a study conducted by (Ndife, Ejikeme, & Amaechi, 2010) [6] on the foaming ability of standard chicken egg flour without treatment, which also reached 40%. On the other hand, the lower foaming ability in the results of other treatments may be due to the relatively lower concentration of yeast and bacteria, suboptimal yeast and bacterial activity, or yeast and bacteria entering the death phase.

The Effect of Treatments on the Foam Stability of Duck Egg Powder

Functional properties of eggs required for their use as a food ingredient include not only foaming ability but also foam stability. Foam stability is the measurement of the ability of the foam structure to remain intact after being left undisturbed for 60 minutes. The average foam stability of duck egg powder from three concentration treatments and two nested time treatments is presented in Table 1.

The data in Table 1. shows that the average foam stability of duck egg powder resulting from various concentration and time treatments using *Lactococcus lactis* ranged from 12.38% to 72.19%. Ovomusin, a protein found in eggs, is considered the foam stabilizer. Ovomusin can form an insoluble layer in water, thus stabilizing the foam (Quan & Benjakul, 2019) [10]. Treatment with 6% and 8% *Lactococcus lactis* concentrations resulted in a decrease compared to the 4% concentration treatment. This may be due to protein denaturation caused by the high production of lactic acid and the egg drying process using heat for 48 hours. Protein denaturation leads to a reduction in the ovomusin protein content in the egg powder, making it weaker and no longer capable of binding water to create a stable foam structure.

Protein denaturation is caused by the proteolytic activity of lactic acid bacteria, where lactic acid bacteria produce protease and peptidase enzymes capable of breaking down protein chains into peptides and other free amino acids in duck egg powder, which can be used for the bacteria's survival (Yusmarini *et al*., 2010) [20]. Some lactic acid bacteria strains, including *Lactococcus lactis*, are known to have a proteolytic system that allows them to grow on protein-rich substrates like duck egg powder (Savijoki, Ingmer, & Varmanen, 2006) [13].

Foam stability is also influenced by various factors such as egg age, temperature and duration of whipping, pH, and the presence of added ingredients (Quan & Benjakul, 2019) [10]. The older the egg, the disassociation between ovomusin and

lysozyme occurs, leading to the breaking of disulfide bonds and making the egg white become thinner.

The longer the fermentation process, the lower the stability of the foam structure formed by whipping rehydrated duck egg powder. Fermentation for 24 hours with duck egg powder results in foam stability that is not better than fermentation for 12 hours because the longer fermentation time can trigger increased production of lactic acid bacteria, raising the pH and causing protein denaturation. The highest foam stability of duck egg powder, reaching 72.19%, was achieved with the treatment of 4% *Lactococcus lactis* concentration fermented for 12 hours.

The Influence of Treatment on the Emulsifying Capacity of Duck Egg Powder

Functional properties of eggs, such as their emulsifying capacity, are crucial in various food applications. Emulsifying capacity represents the ability of egg proteins to create and stabilize emulsions, which are mixtures of two immiscible liquids like oil and water. The data in Table 1 demonstrates the average emulsifying capacity of duck egg powder resulting from different concentration treatments and fermentation times by *Lactococcus lactis*. The emulsifying capacity values range from 0.992% to 0.995%.

Eggs contain lecithin (phospholipid), lipoprotein, and protein. The role of lecithin in eggs is as a strong emulsifier that can bind water and oil (Quan & Benjakul, 2019) [10]. Lecithin is a phospholipid that is an essential component of cell membranes found in almost all varieties of living organisms (Bot, Cossuta, & O'Mahony, 2021) [1]. The role of lipoprotein in eggs is as an emulsion stabilizer because lipoprotein can interact on the surface of fat globules and form a protective layer (Quan & Benjakul, 2019) [10]. Lipoprotein is an emulsifier with two groups, namely the hydrophilic polar group and the lipophilic group that is non-polar.

Fermentation for 24 hours on duck egg powder results in emulsifying power not significantly different from fermentation for 12 hours because the proteolytic activity of *Lactococcus lactis* continues to increase in tandem with the increase in biomass and yeast cell activity. This leads to an increase in the level of incomplete protein, so the emulsifying power generated by duck egg powder in this study is not like the emulsifying power of fresh eggs or eggs without the addition of *Lactococcus lactis*

In essence, the addition of *Saccharomyces cerevisiae* can enhance the protein substrate because yeast biomass and yeast cells act as a single-cell protein (SCP) agent, increasing during the fermentation period (Raita, Kusnere, Spalvins, & Blumberga, 2022) [11]. The chemical composition of *Saccharomyces cerevisiae* consists of approximately 50-52% crude protein, 30-37% carbohydrates, 4-5% fat, and 8-17% minerals. The high protein content in *Saccharomyces cerevisiae* leads to an increase in the protein content of duck egg powder. However, the addition of *Lactococcus lactis* results in lactic acid bacteria with the ability to perform proteolytic activities that can reduce the pH value and suppress the protein content of duck egg powder, contradicting the ability possessed by *Saccharomyces cerevisiae* to increase the protein content of duck egg powder.

In addition to the fermentation time, emulsifying power and emulsion stability in egg powder, as well as fresh eggs, are also influenced by the percentage of lecithin present in egg yolk. Lecithin is one of the protein contents in eggs that undergo denaturation as a result of proteolytic activity carried out by protease enzymes from *Lactococcus lactis*, a lactic acid bacteria. This proteolysis reduces the percentage of lecithin in egg powder, so the smaller the percentage of lecithin in egg powder, the lower the emulsifying power and emulsion stability.

Conclusion

1. The concentration of a combination of Lactococcus lactis and Saccharomyces cerevisiae affects foam power, foam stability, and provides the same result in the emulsifying power of duck egg powder.
2. The use of a concentration of 6% *Lactococcus lactis* and 0.4% *Saccharomyces cerevisiae*, along with a 24-hour fermentation period, results in a foam power of 40.45%, foam stability of 21.91%, and emulsifying power of 0.995.

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