International Journal of Multidisciplinary Education and Research
www.multidisciplinaryjournals.in
ISSN: 2455-4588
Received: 21-11-2023, Accepted: 06-12-2023, Published: 22-12-2023
Volume 8, Issue 4, 2023, Page No. 127-130

The effect of combination of *Saccharomyces cerevisiae* and *Lactococcus lactis* concentration on length of fermentation time on water content, protein content and color of duck egg flour

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Abstract
Egg flour is an alternative method to preserving duck eggs. Drying can extend the shelf life of an egg without reducing nutritional quality and save storage space. This study aims to determine the effect of a combination of *Saccharomyces cerevisiae* and *Lactococcus lactis* concentration and the length of fermentation time on the chemical and organoleptic properties of duck egg flour. This study used an experimental method with a Completely Randomized Design of Nested Patterns with 4%, 6%, and 8% concentration of *Lactococcus lactis* nested on 12 and 24 hours of fermentation time. The results showed that the length of fermentation time had no significant effect ($P>0.05$) on the moisture content, protein content, redness value, and yellowness value. However, a significant effect ($P<0.05$) due to the length of fermentation was found on the lightness value. The concentration of *Lactococcus lactis* nested in the length of fermentation time did not significantly affect the moisture content, protein content, and color value. The results showed that the length of fermentation time of 12 hours with a concentration of *Lactococcus lactis* 6% produced egg flour with a moisture content of 8.25%, protein content of 18.16%, lightness value of 20.84, redness value of 33.15 and yellowness value of 13.28, while the length of fermentation time of 24 hours with a concentration of *Lactococcus lactis* 8% produced egg flour with a moisture content of 6.37%, protein content of 23.67%, lightness value of 22.73, redness value of 31.60, yellowness value of 11.84

Keywords: Duck egg flour, water content, protein content, color, fermentation, *Lactococcus lactis, Saccharomyces cerevisiae*

Introduction

Eggs are one of the high-nutrition food ingredients produced by poultry, rich in protein, fats, vitamins, and minerals. The protein content in eggs is predominantly found in the egg yolk (ovavitelin) and egg white (ovalbumin). The protein content in 100 grams of duck eggs is approximately 13.1 grams. In Indonesia, the production of duck eggs in 2020 reached 332,907 tons [1]. Indonesian people commonly process duck eggs into salted eggs. However, an alternative method for processing duck eggs is turning them into duck egg powder.

The production of egg powder can extend the shelf life of the powder without compromising its nutritional quality and functional properties, while also saving storage space. In the process of making egg powder, there is a drying stage for the eggs, which can lead to the Maillard reaction. The Maillard reaction is a process that changes the color of food materials to dark brown due to the reaction between the aldehyde groups in reducing sugars and the amino groups in proteins. This reaction can affect the color, aroma, taste, and lower the pH of the food material. The Maillard reaction can be minimized by fermenting the eggs before drying.

Fermentation on flour can be carried out by adding *Saccharomyces cerevisiae* yeast and lactic acid bacteria *Lactococcus lactis*. The fermentation process can transform glucose in the eggs, producing carbon dioxide and water, thus preventing the Maillard reaction. *Lactococcus lactis* can increase the protein content in the flour because it possesses proteolytic activity. Additionally, *Lactococcus lactis* can also alter the macro and microstructure of the eggs, reducing the moisture content in the flour. The duration of the fermentation process can also affect the physicochemical properties of the flour. The combination of *Saccharomyces cerevisiae* and *Lactococcus lactis* can expedite the fermentation process because lactic acid bacteria can grow faster than yeast.

The production process of duck egg powder with the addition of a combination of different concentrations of *Saccharomyces cerevisiae* and *Lactococcus lactis* during various fermentation times has not been extensively studied. Therefore, this research is aimed at determining the influence of the combination of *Saccharomyces cerevisiae* and *Lactococcus lactis* concentrations at different fermentation times on the moisture content, protein content, and color of duck egg powder.

Material and Methods

Materials

The materials used include fresh duck eggs aged 1 - 3 days, the *Lactococcus lactis* FNCC 0120 lactic acid bacteria culture, *Saccharomyces cerevisiae*, 5% citric acid, Yeast Malt Broth (YMB), Yeast Malt agar (YMA), Man Rogosa Sharp Broth (MRSB), Man Rogosa Sharp Agar (MRSA), and distilled water (aquades). The materials used in the analysis include Bovine Serum Albumin (BSA), Lowry reagent A, Lowry reagent B, and acetic acid buffer at pH 5.

Methods

The preparation of Duck Egg Powder [2] begins with the selection of eggs based on their integrity and cleanliness, with eggs being no older than 1 - 3 days. The eggs are then cracked and the eggshells are separated from the egg contents. Subsequently, the egg yolks and egg whites are mixed and stirred until homogenous. After homogenization, the liquid eggs are tested for their pH value, and if the pH is above 5, 5% citric acid is added. The liquid eggs are then pasteurized for 2.5 - 3 minutes at 60°C using a water bath. After pasteurization, 0.4% *Saccharomyces cerevisiae* and

Lactococcus lactis with various concentrations of 4%, 6%, and 8% are added to the liquid eggs, followed by fermentation at 30°C for 12 and 24 hours. Once fermentation is complete, the liquid eggs are dried in an oven using the pan drying method for 48 hours at 45°C. The dried eggs are then ground using a blender and filtered. Finally, they are packed using plastic clips before conducting tests for moisture content, protein content, and color in the duck egg powder.

Water content

The moisture content of duck egg powder is tested using the gravimetric method according to [3]. A 2-gram sample of duck egg powder is weighed in a porcelain dish. Subsequently, the sample is dried in an electric oven at 105°C for 3 hours. After drying, the sample is cooled in a desiccator for 15 minutes and then weighed. The drying process is repeated, and the sample is cooled in a desiccator every half hour, and weighed until a constant weight is obtained. The data obtained is then calculated using the following formula:

$$water\ content\ (\%) = \frac{wet\ weight - weight\ after\ drying}{wet\ weight} x100\%$$

Protein content

The protein content of duck egg powder is determined using the Lowry method according to [4]. A 5-gram sample is weighed and then added to distilled water (aquadest) to create an extract, which is then filtered. Next, the precipitated protein is centrifuged at 11,000 rpm for 10 minutes. The protein residue is dissolved in acetic acid buffer at pH 5.0 to reach a volume of 10 ml. Then, 4 ml of the protein sample is taken and reacted with Lowry reagents A and B. Subsequently, the protein content is determined from the absorbance obtained from the sample solution using a standard curve. The data obtained is then calculated using the following formula:

Protein weight = Vsample x cons. Protein sample

$$protein\ content\ (\%) = \frac{protein\ weight}{sampel\ weight} x100\%$$

Color

The duck egg powder's color is tested using a Portable Colorimeter CS10 according to [5]. The testing is performed by setting the colorimeter to the measurement mode and placing it perpendicular to the surface of the sample. Then, the test button is pressed until the machine produces a sound. The test results are displayed on the device's screen in the format of L* (lightness), a* (redness), and b* (yellowness). The testing is repeated three times, and the average values are recorded.

Result and Discussion

Water content

The treatment various concentrations of *Lactococcus lactis* and different fermentation durations resulted in average moisture content levels ranging from 4.63% to 8.25%. Based on Table 1, the moisture content of duck egg powder obtained falls within the quality requirements for moisture content according to the Indonesian National Standard [6], which has a maximum moisture content of 8%, as well as the United Nations Economic Commission for Europe [7], which sets the maximum moisture content for egg powder at 5%.

The reduction in moisture content in duck egg powder occurs because during the fermentation process, *Lactococcus lactis* can create hydrogen bonds, which reduces the water molecule bonds and decreases the interaction between the molecular groups and water molecules [8]. Additionally, adding lactic acid bacteria can lower the pH value of the material, leading to a decrease in moisture content, as supported by [9], who stated that lower pH values result in reduced moisture content. Fermentation by lactic acid bacteria, combined with the addition of *Saccharomyces cerevisiae*, can break down organic compounds, producing alcohol, CO2, H2O, and energy in the form of heat. The heat generated raises the material's temperature, causing water to evaporate.

During the fermentation process, the enzymatic activity of *Lactococcus lactis* leads to an increase in the amount of bound water, resulting in the material becoming porous. This porous state can enhance the evaporation of water during the drying process [10]. The duration of the fermentation process also provides an opportunity for *Lactococcus lactis* to grow and develop by utilizing the free water in the material, consequently reducing the moisture content [11].

Table 1: The effect of concentration of *Lactococcus lactis* nested in fermentation times of 12 and 24 hours on water content, protein content and color

Variable	P1		P2		P3	
	12 hour	24 hour	12 hour	24 hour	12 hour	24 hour
Water content (%)	5,50^a	7,13^a	8,25^a	5,13^a	4,63^a	6,38^a
Protein content (%)	21,10^a	17,10^a	18,17^a	16,17^a	19,26^a	23,67^a
Lightness	19,37^a	21,51^a	20,85^a	22,22^a	18,75^a	22,73^b
Redness	31,98^a	31,03^a	33,16^a	32,01^a	31,49^a	31,61^a
Yellowness	12,66^a	11,30^a	13,29^a	12,63^a	12,49^a	11,84^a

Protein content

The average protein content of duck egg powder obtained from the addition of various concentrations of *Lactococcus lactis* and different fermentation durations ranges from 16.17% to 23.67%. Based on the data in Table 1, the protein content obtained is not significantly different. This occurs because during the fermentation, both microbes utilize the same energy source. According to [12], during fermentation, *Lactococcus lactis* utilizes glucose in the egg to grow and produce lactic acid through the Embden-Meyerhof Pathway (EMP). The addition of *Saccharomyces cerevisiae* during fermentation also utilizes glucose to produce energy, alcohol, and CO_2, which can limit the optimal performance of *Lactococcus lactis* in increasing protein [13, 14].

The addition of *Lactococcus lactis* during egg fermentation can increase the protein content. Lactic acid bacteria produce peptidoglycan through the cell wall during the fermentation process. Peptidoglycan consists of glycoproteins, which are proteins bound to oligosaccharides, and lipoproteins, which are proteins bound to lipids. *Lactococcus lactis* also has good proteolytic abilities to break down egg proteins into peptides and amino acids [14]. Saccharomyces cerevisiae also plays a role in increasing the protein content in the material because yeast itself is a single-cell protein containing a significant amount of crude protein, around 50-52% [15].

The duration of fermentation provides an opportunity for *Lactococcus lactis* to utilize carbohydrates to produce energy and CO_2, thereby increasing the proportion of protein in the total mass and causing nitrogen to become concentrated [16, 17]. According to [18], *Lactococcus lactis* also produces protease enzymes and lactate dehydrogenase (LDH) enzymes, both of which are proteins.

Color

Based on the data in Table 1, the lightness values of the duck egg range from 18.75 to 22.73. The brightness of duck egg powder is influenced by the Maillard reaction that occurs during drying, which causes the material's color to become brown or dark due to the formation of melanoidin pigments [19]. The addition of *Lactococcus lactis* during egg fermentation can break down glucose in the egg, preventing reactions between carbonyl compounds, especially reducing sugars, and compounds containing free amino acids.

A longer fermentation duration results in higher lightness values. This is because a longer duration allows lactic acid bacteria to work optimally in breaking down glucose in the egg. This breakdown of glucose can prevent the Maillard reaction, thus increasing the brightness of duck egg powder.

The average values of redness and yellowness in Table 1 range from 31.03 to 33.16 and 11.30 to 13.29, respectively. The reddish-yellow color in duck egg powder can be influenced by naturally occurring pigments in eggs, namely carotenoid pigments that provide yellow, orange, and red colors [20]. The redness value may be due to the presence of cantaxanthin in fresh eggs, which imparts a red-orange color. According to [21], cantaxanthin pigments can be influenced by duck feed such as shrimp or shellfish.

The yellow color in duck egg powder is also influenced by the natural pigments present in fresh eggs, specifically xanthophyll pigments. Xanthophyll pigments are derived from the feed given to ducks. According to [22], feeds rich in xanthophyll pigments and vitamin A will be deposited in the egg yolk. Feeds containing abundant xanthophyll pigments and vitamin A can come from yellow corn, sweet potato leaves, and water hyacinth [23].

Conclusion

The duration of fermentation had no significant effect on moisture content, protein content, redness, and yellowness values, but it did have a significant impact on the lightness value. The concentration of *Lactococcus lactis* nested within the fermentation duration did not significantly affect moisture content, protein content, and the color of duck egg powder. The research results indicate that with a fermentation duration of 12 hours, the optimal concentration of *Lactococcus lactis* is 6%, resulting in a moisture content of 8.25%, protein content of 18.16%, lightness value of 20.84, redness value of 33.15, and yellowness value of 13.28. In contrast, with a fermentation duration of 24 hours, the optimal concentration of *Lactococcus lactis* is 8%, resulting in a moisture content of 6.37%, protein content of 23.67%, lightness value of 22.73, redness value of 31.60, and yellowness value of 11.84.

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