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THE ACIDITY LEVEL OF FERMENTED EGG FLOUR WITH VARIOUS DRYING TECHNOLOGIES

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ABSTRACT: Commonly, egg fermentation technology is carried out using *Lactobacillus plantarum* bacteria. Eggs that have undergone the fermentation process can be stored for a long time using the drying method. The results of fermentation in the drying process are expected to reduce the Maillard reaction. The purpose of this study was to identify the application of the right drying technology in fermented eggs on the stability of the acidity level of egg flour. This study was conducted experimentally using a Completely Randomized Design (CRD) factorial pattern of 3 × 4 treatments with 5 replications each. The treatment was carried out using the spray drying method, pan drying, freeze drying with a drying time of 6,12,18 and 24 hours. The acidity level can be measured by testing parameters for pH values and lactic acid levels using the titration method. The results showed that increasing drying time and different drying methods in fermented egg flour could maintain the pH value of eggs and the optimum at a drying time of 6 hours with the freeze drying method. Lactic acid levels tend to be stable because the fermentation product uses freeze drying which can maintain the lactic acid levels of fermented eggs. The acidity level of fermented egg flour can be seen from the stability of the pH value and lactic acid levels from the drying process. The best pH value and lactic acid content were obtained using the freeze drying method with a drying time of 24 hours.

Keywords: Eggs, Fermentation, Drying, Flour, Lactic acid.

INTRODUCTION

One of poultry product that is full of easily digestible nutrients is eggs. Although eggs provide necessary lipids, balanced amino acids, and a number of minerals and vitamins, they are a food item that is susceptible to contamination by bacteria and breakage (Vitkova et al., 2024). Drying eggs into flour offers many advantages, including ease of storage and distribution, protection against microbial growth (low water activity), reduced weight per volume of whole eggs, and a longer shelf life. Using egg flour as an additive to other food products is easier than using fresh eggs (Abreha et al., 2021).

One of the most popular techniques for preserving food is drying, which lowers the water content to decrease damage from reactions and microorganisms (Casella et al., 2020) which is documented in egg drying (Alp and Bulantekin, 2021; Ekpo et al., 2022). There are multiple methods for drying different egg products, such as spray drying, pan drying, and freeze drying. (Amelia et al., 2013) found that for drying small particles, the freeze-drying method works better than spray drying and is advised for both particle integrity and the amount of solids produced.

The primary issue with producing egg flour, particularly when using the drying method, is the product's brownish color shift, which is known as the Maillard Reaction (Martins et al., 2000). This issue affects how egg flour goods look in terms of color and odor as well as the deterioration of their functional qualities. The reaction between the aldehyde group of carbohydrates and the amino group of the protein that makes up the egg is what gives it its brown hue (Baynes et al., 2007). Products made using fermentation technology can lessen the Maillard process.

Food safety studies must be conducted on fermented chicken eggs before they are used as health food products. Identifying food items that are safe to eat, particularly fermented eggs, and recognizing antioxidants as a feature of food quality (Mangalisu et al., 2022). Given the numerous advantages of antioxidant content, it is intriguing to investigate the presence of anthocyanin molecules as a natural antioxidant source in fermented egg flour. The advantages of using egg flour in industry include a longer shelf life, ease of storage and transportation due to its floured form, and hygiene and safety. The use of egg flour offers significant flexibility and economic advantages on an industrial scale, especially for food manufacturers who prioritize efficiency without sacrificing product quality (Uhlmann and Hertel, 2016).

Assessing lactic acid content and viable lactic acid bacteria counts to ensure microbiological safety and functional quality of dried egg products. Determining lactic acid and total lactic acid bacteria levels is important for evaluating the microbiological stability and safety of fermented food products, including those in dry or floured form. Food products with low water content still allow certain microorganisms to survive during storage, making microbiological analysis and total titratable acidity important parameters in food quality assurance (Odu et al., 2017; Säde et al., 2016).

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Measurements of the egg flour's total bacteria, acidity, functional properties, and antioxidant quality are made while it is being stored at room temperature. A study on the identification of fermented egg flour antioxidants using different drying procedures was carried out in light of the aforementioned studies.

MATERIALS AND METHODS

Materials

The equipment used in this study were sample tubes, test tubes, petri dishes, erlenmeyers, micropipettes, tips, syringes, analytical scales, beakers, incubators, biuret, spatulas, autoclaves, colony counters, vortexes, pH meters, magnetic stirrers, PCR Hoods, tube racks, tube shakers, vortex mixers, measuring flasks, water bath shakers, spray dryers, ovens, and freeze dryers. The materials used were fresh chicken eggs, MRS (Man Rogosa Sharpe) agar, aluminum foil, tomato juice, distilled water, alcohol, 0.89% NaCl, 0.1 N NaOH, PP indicator (phenolphthalein), pH 4 and pH 7 buffer solutions.

Experimental design

This research was conducted experimentally using a Completely Randomized Design (CRD) with a factorial of 3 x 4 with 5 replications each. This research uses *Lactobacillus plantarum* in the fermentation process and then carries out drying treatment. The treatments consisted of Factor A is the drying method treatment A1: spray drying, A2: pan drying, A3: freeze drying. Factor B is the drying time treatment B1: 6 hours, B2: 12 hours, B3: 18 hours, B4: 24 hours.

Culture propagation

Lactobacillus plantarum FNCC 0027 was stored on De Man Ragosa Sharpe (MRS) agar. Culture propagation was performed by creating subcultures. Subcultures were prepared by transferring the culture stock to MRS broth (OXOID CM0359) with 20% tomato extract added and incubated for 24 hours (Pramono et al., 2003). The culture stored in MRS broth was inoculated at 10% concentration into egg white containing 20% tomato extract to produce a working culture (Legowo et al., 2013). Gram staining was performed on the starter culture to determine the morphology of *Lactobacillus plantarum*.

Making fermented chicken eggs

Egg samples were cleaned using clean water. The eggs were then fumigated using Calcium Permanganate (KP) flour and formalin in a closed room for 5 minutes and successively cleaned using a wet cloth, chlorine solution and wiped with alcohol using cotton. The eggs were wrapped in aluminum foil and pasteurized at 60 °C for 3.5 minutes (Froning et al., 2002) then separated from the shell and placed into sample bottles. The sample bottles were first cleaned using warm water and sterilized. A 200 ml sample was homogenized and then sterilized using ultraviolet by placing it in a PCR Hood for 15 minutes. The sterilized sample was added with 10% working culture Lactobacillus plantarum and then homogenized with a tube shaker, the sample was then fermented at 37 °C for 24 hours (Nahariah et al., 2015).

Making fermented egg flour

Fermented chicken eggs were dried using three drying methods. Fermented eggs were dried using a co-current spray dryer (A1). Pan drying was performed at 45°C (A2). Freeze drying was performed by drying to a thickness of 3.2 mm at -40°C, with the pressure on the freeze dryer set at 0.036 psi or 0.0025 bar (A3). The dried eggs were then ground. Grinding was carried out using a blender for 2 minutes. The resulting egg flour was then sieved using an 80-mesh sieve. The purpose of this sieving was to produce a uniform egg flour (Koswara, 2009). The flour samples were then tested for acidity level (as measured by a pH meter) and lactic acid level (as determined by the titration method) are the parameters that are tested.

Statistical analysis

Using the SPSS 23 data processing program, the acquired data were examined using Analysis of Variance based on Completely Randomized Design (CRD) (3 × 4) with a factorial design. Additionally, proceed with the Least Significant Difference (LSD) test if the treatment exhibits a significant effect.

RESULTS AND DISCUSSION

Tables 1 (pH value) and 2 (lactic acid content) show the degree of acidity of fermented chicken egg flour with various drying techniques and drying durations. Different drying times and techniques have a substantial ($P < 0.05$) impact on the pH value of fermented egg flour, and there is an interaction between the two, according to analysis of variance. The pH values in each treatment of the freeze drying method and various drying durations at 24 hours were substantially different

(P < 0.05), according to the results of the Least Significance Different (LSD) additional test. Because the fermentation product involves freeze drying, which may keep the pH value of fermented eggs, the pH value tends to be stable.

Table 1 demonstrated how varying drying times and techniques on fermented egg flour can preserve the pH level of the eggs, with the freeze drying method working best at a drying duration of six hours. After drying, the pH level of fermented egg flour is comparatively stable because spray drying inhibits microbial and enzyme activity. The pH value is nearly identical to what it was before to drying. The pH stays constant between 6 and 12 hours of drying since there is little humidity and little microbial activity. Because fermented eggs are exposed to moisture or oxidation, there are significant pH changes that take place between 18 and 24 hours of drying. This situation is thought to be caused by a decrease in the pH value of the product with citric acid before the fermentation stage and, in addition, an increase in the amount of acid in the product during the fermentation stage. It has also been reported that Lactobacillus converts glucose into organic acids (mainly lactic acid) and lowers the pH during fermentation (Doblado et al., 2003).

Table 1 - pH value of egg flour with different drying methods and drying times

Drying time (hours) \ Drying method	Spray drying	Pan drying	Freeze drying
6	4.80 ± 0.06^{a}	4.74 ± 0.02^{b}	4.84 ± 0.02^{a}
12	4.80 ± 0.02^{a}	4.70 ± 0.05^{b}	4.84 ± 0.10^{a}
18	4.75 ± 0.03^{b}	4.62 ± 0.03^{c}	4.82 ± 0.04^{a}
24	4.67 ± 0.03^{b}	4.52 ± 0.03^{c}	4.80 ± 0.03^{a}

Noted: Different superscripts in the same row indicate significant differences (P<0.05).

Table 2 - Lactic acid content (%) of egg flour with different drying methods and drying times

Drying time (hours) \ Drying method	Spray drying	Pan drying	Freeze drying
6	1.268 ± 0.02^{b}	1.277 ± 0.03^{a}	1.260 ± 0.04^{b}
12	1.268 ± 0.04^{b}	1.280 ± 0.02^{a}	1.260 ± 0.02^{b}
18	1.274 ± 0.03^{b}	1.305 ± 0.06^{a}	1.264 ± 0.07^{c}
24	1.295 ± 0.02^{b}	1.314 ± 0.04^{a}	1.268 ± 0.04^{c}

Noted: Different superscripts in the same row indicate significant differences (P<0.05).

Pan drying is the process of applying low to medium temperatures to a pan or flat surface. Compared to spray drying, this procedure takes longer. The slower procedure and lower drying temperature result in partial deactivation of certain bacteria and enzymes. After drying, this results in pH shifts. During drying, pH variations are more likely to happen, particularly if drying is not flawless. Because of moisture or slower chemical activity, the pH may alter somewhat over the course of 18 to 24 hours.

Freeze-drying is the technique of freezing fermented eggs and then using a sublimation process (a straight transition from solid to gas without going through the liquid phase) to extract the water. Because the drying process occurs at a low temperature and no major chemical changes occur, freeze drying preserves the pH value of the fermented eggs quite well. Due to the extremely low water content that is produced by freeze drying, the pH is extremely stable for 6 to 24 hours. Fermentation causes the pH to drop. This happens as the pH value drops due to an increase in the number and metabolism of lactic acid-producing bacteria brought on by longer incubation times. The pH of the fermentation medium may drop as a result of the buildup of lactic acid created by lactic acid bacteria. The concentration of H+ emitted throughout the fermentation process is the computed pH value (Legowo et al., 2013).

Because of the metabolic process that is aided by the availability of nutrients, the growth rate of bacteria is correlated with temperature. The supply of nutrients declines at a particular temperature due to the fast growth rate. Bacterial growth rate is directly influenced by temperature because temperature determines enzymatic activity and cellular metabolism. At optimum temperatures, metabolic activity increases, resulting in faster nutrient consumption, which can ultimately lead to substrate limitations at certain growth stages (Zwietering et al., 1991; Nedwell, 1999). Increasing the temperature to the optimum point increases the rate of bacterial growth and metabolic activity, so that nutrient consumption occurs more quickly and can cause substrate limitations at certain growth phases (Membré et al., 2005).

Different drying times and techniques had a substantial ($P < 0.05$) impact on the amount of lactic acid in fermented egg flour, and there was an interaction between the two, according to analysis of variance. Each treatment of the freeze drying method and different drying times at 24 hours had substantially different amounts of lactic acid ($P<0.05$), according to the results of the Least Significance Different (LSD) further test. Because the fermentation product involves freeze drying, which can preserve the lactic acid levels of fermented eggs, the lactic acid levels are generally constant.

Spray drying is a widely used drying method for fermented products because it can rapidly convert liquid ingredients into flour through contact with hot air. In this process, fermented egg flour containing lactic acid is atomized into small droplets and dried in a very short time. Although the inlet air temperature is relatively high, the short thermal contact time minimizes the degradation of organic compounds, including lactic acid. Several studies have reported that fermented organic acids are relatively stable during the spray drying process due to the short residence time of the particles in the drying chamber. Consequently, the lactic acid content in the final product does not change significantly compared to before drying (Gharsallaoui et al., 2007).

Furthermore, spray-dried products have low moisture content and water activity, which suppresses microbial activity and chemical reactions during storage. These conditions contribute to the stability of lactic acid and other fermentation metabolites in fermented egg flour. Changes in lactic acid levels after drying are generally more influenced by the initial fermentation conditions than the drying process itself. Therefore, the stability of lactic acid in fermented egg flour tends to be maintained during storage, although slow chemical degradation can still occur over a longer period of time due to oxidative reactions and interactions between food components (Barbosa-Cánovas et al., 2007; Mujumdar, 2014).

Drying using a pan (pandrying) or drying with a tray (tray drying) is a conventional drying method that takes place on a flat surface at low to moderate temperatures for a relatively long time. Compared to spray drying, this method has a slower rate of heat and mass movement so that the material is exposed to heat for a longer time. These conditions can increase the opportunity for physical and chemical changes to occur, such as protein denaturation, lipid oxidation, and changes in fermentation components. In addition, the stability of fermented compounds is influenced by temperature and heating time; the longer the exposure to heat, the greater the possibility of chemical changes and loss of heat-sensitive components (Santivarangkna et al., 2008; Mujumdar, 2014).

In addition to temperature, drying time and humidity conditions also affect lactic acid stability. In conventional drying, lactic acid retention tends to be lower than in spray drying because longer exposure to heat allows oxidation and other chemical changes to occur. Once the product's moisture content decreases, microbial activity is inhibited, thereby increasing lactic acid stability. However, in high humidity conditions or poor packaging systems, lactic acid degradation can still occur during storage due to residual chemical reactions and absorption of water vapor from the environment (Barbosa-Cánovas et al., 2007).

Freeze-drying (or lyophilization) is a drying method that involves first freezing the material, then removing the water through sublimation, a process where ice changes phase to water vapor without passing through the liquid phase. This process occurs at low temperatures and vacuum pressure, minimizing thermal damage to chemical and biological components (Rey, 2010). Compared to thermal drying methods, freeze-drying is more effective in maintaining the stability of bioactive compounds, including lactic acid and fermentation metabolites, because it does not involve intense heat exposure that can trigger chemical degradation (Ratti, 2001).

The stability of lactic acid in freeze-dried products generally remains high due to low water activity and minimal oxidative reactions during the drying process. Dry, low-temperature environmental conditions inhibit microbial activity, enzymes, and other chemical reactions, so that lactic acid content remains relatively unchanged after the drying process (Barbosa-Cánovas et al., 2007). Generally, the method of drying, as well as the method of pretreatment, has a strong effect on the final product. However, thermal processing before drying differs the samples in comparison to non-thermal processing. Furthermore, even the sonication and pulsed electric field cause a similar effect as cell-wall rupturing, and structure changes, the mechanism is different, and this resulted in different properties for the samples (Raybak et al., 2022).

CONCLUSIONS

Drying technologies have different effects on the acidity level of fermented flour, particularly regarding lactic acid stability and total titratable acid values. Freeze-drying methods demonstrate the best ability to maintain product moisture because the process takes place at low temperatures and vacuum pressure, thus minimizing thermal degradation of lactic acid. Consequently, the characteristics of the lactic acid content and acidity of the product remain relatively stable after drying. Future studies should investigate the combined effects of fermentation conditions and drying technologies on the physicochemical, microbiological, and functional properties of fermented egg flour. Particular attention should be given to optimizing fermentation parameters, including fermentation duration, and drying conditions to maximize lactic acid retention while preserving protein functionality. Research should assess the shelf life during storage under different packaging conditions. Finally, pilot-scale production and techno-economic analyses should be conducted to support the commercial development of fermented egg flour as a functional food ingredient.

DECLARATIONS

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The corresponding author is responsible for communications during the submission, review, and publication process. All correspondence regarding this article should be addressed to Azmi Mangalisu at Universitas Muhammadiyah Sinjai (email: azmimangalisu@gmail.com).

Data availability

The data used and/or analyzed in this study are available from the corresponding author on reasonable request through the research results.

Author contributions

All authors contributed substantially to this research. Contributions included study design, experimental execution, data analysis, interpretation of results, drafting the manuscript, and final revision of the manuscript. All authors have read and approved the final version of the manuscript for publication.

Consent for publication

All authors have approved the final version of the manuscript and agreed to the publication of this article.

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Ethical considerations

This research did not involve human subjects or laboratory animals directly, requiring specific ethical approval. All research procedures were conducted in accordance with applicable laboratory standards and research guidelines.

Competing interests

The authors declare that they have no competing interests in the research or publication of this article.

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