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Research Article

Production of powder yeast starter culture for fermentation of cocoa beans

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Abstract - The unique flavor and aroma of cocoa beans are derived from the spontaneous fermentation with natural microorganisms, where yeast is the primary microorganism found during the initial period of fermentation. However, the natural fermentation process may cause variations in the quality of cocoa beans. The powdered starter culture of *Saccharomyces* yeast was produced for cocoa fermentation. The inoculums were prepared and dried at 40 °C for 14, 15, and 16 hours, and 45 °C and 50 °C for 7, 8, and 9 hours, respectively. Liquid starter culture fermentation was used as a control. The powder yeast starter culture was dried at 40 °C for 15 hours with a moisture content of < 8.5% (w/w) and the maximum survival of the yeast population ($P < 0.05$) was used for small-scale cocoa fermentation. The cut test results showed that the powdered starter culture could complete cocoa fermentation with a consistent quality of cocoa beans. To improve the quality of powdered starter cultures and better understand the role of powdered yeast inoculum on the quality of cocoa beans, the shelf life of powdered starter cultures and the chemical composition of cacao beans after fermentation with powdered starter cultures were further investigated.

Keywords: Cocoa, fermentation, inoculum, starter culture

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1. Introduction

Cocoa (*Theobroma cacao* L.) is a significant industrial crop with high potential for value-added manufacturing in Thailand (Chumphon Horticultural Research Centre, Department of Agriculture, 2021). Fermentation is one of the most important steps in cocoa processing, as it significantly influences the final quality of cocoa beans (Díaz-Muñoz & De Vuyst, 2022). After cocoa pod harvesting, the cocoa beans are separated from the pods and fermented in heaps, wooden boxes, plastic boxes, or plastic baskets with banana leaves (Schwan et al., 2023). Cocoa fermentation is a biologically complex process driven by a succession of specific microbial communities, primarily yeasts, lactic acid bacteria (LAB), and acetic acid bacteria (AAB), which the spontaneous fermentation of cocoa beans usually takes 2 – 10 days (Campos et al., 2025; De Vuyst & Weckx, 2016). The dynamic interaction of these microorganisms is necessary to produce high-quality final products, as each species contributes uniquely to the sensory attributes. However, their activity and composition depend on several environmental and process-related factors, including temperature, pH, substrate availability, and microbial inoculum (Afoakwa et al., 2013). Typically, cocoa fermentation proceeds naturally, leading to variability in microbial populations; consequently, this results in inconsistencies in cocoa bean quality and flavor profiles across batches. (De Vuyst & Leroy, 2020).

To initiate and control fermentation, specific strains are intentionally added using starter cultures. Starter cultures enhance fermentation efficiency, reduce fermentation time, and improve the predictability and consistency of final products. Additionally, using particular strains can produce desired flavor attributes, such as strengthened fruity or flowery notes and less bitterness (Martin & Lindner, 2022). Liquid starter cultures are commonly used because they can be evaluated and adjusted before application. However, they have limitations in terms of storage stability and transportation. The use of dry starter cultures could not only support decentralized cocoa processing by facilitating improved quality control and minimizing the risks of contamination, such as mold growth during cocoa bean fermentation, but also offer an extended shelf life and logistical advantages of starter cultures, particularly for small farmers. Thus, this study aimed to produce a powdered starter culture (PSC) and evaluate its potential for small-scale fermentation of cocoa beans.

2. Materials and methods

2.1 Microorganism and starter cultures preparation

A commercial yeast strain, *Saccharomyces cerevisiae* var. boulardii, was obtained from the Department of Food Technology, Faculty of Science, Chulalongkorn University. Yeasts were cultivated on YPD agar at 30 °C for 48 hours. For yeast inoculum preparation, the yeast was cultivated in 250 mL of YPD broth at 200 rpm for 24-48 hours. The suspension was centrifuged at 6,000 rpm for 15 minutes, and the cell pellet was washed three times and resuspended in 0.85% (w/v) NaCl. All experiments were performed aseptically.

2.2 Powdered starter culture preparation

The powdered inoculum was prepared as described by Phuapaiboon (2016), with some modifications. The yeast strain suspension in 2.1 was mixed with 150 g of sterilized rice flour and 100 mL of sterilized distilled water. After thoroughly mixing, the inoculums were dried as indicated in Table 1, and the PSC was collected in a tightly sealed plastic bag to determine the yield (%) (He et al., 2025) and moisture content (%) (Moisture analyzer MA 35, Sartorius, Germany). All experiments were performed in triplicate.

Table 1. Drying conditions of the yeast inoculum.

Temperature (°C)	Time (hours)
45	14
	15
	16
50	7
	8
	9
55	7
	8
	9

2.3 Yeast survival determination

The powdered starter cultures in section 2.2 were used to determine the yeast population by counting viable cells on Yeast Peptone Dextrose (YPD) agar. Each powdered starter culture (1 g) was serially diluted in 9 ml of 0.1% (w/v) sterile peptone water.The 0.1 mL of suspension was inoculatedon YPD agar, and then incubated at 30 °Cfor 48 hours. The percentage of yeast survival (He et al., 2025) and log reduction (Payne et al., 2025) were calculated.

2.4 Small-scale fermentation of cocoa beans

Cocoa pods were purchased from the Small and Micro Community Enterprise of Cocoa, Klaeng, Rayong Province, Thailand. They were cut, and then the raw cocoa beans were separated from their pods and placenta. Small-scale fermentation of raw cocoa beans was conducted in a laboratory. The PSC with a moisture content < 8.5% (w/w) and the maximum survival of yeast population in section 2.3 was inoculated into 1 kg of raw cocoa beans at the initial yeast population of 6 log cfu/mL. Fermentation of raw cocoa beans with liquid starter culture (LSC; preparation method of LSC as described in 2.1) and spontaneous fermentation with banana leaves (SFB) were also conducted. All experiments were performed in duplicate. The inoculated raw cocoa beans were fermented in a plastic basket at ambient temperature (28 - 30 °C). The temperature of the cocoa beans was monitored daily. Fermented cocoa beans were collected for a cut test after fermentation was completed.

2.5 Cut test

The 10 fermented cocoa beans from each experiment were randomly collected for a cut test to visually assess the internal color and texture of the beans (Oliveira et al., 2021). Each bean was carefully cut in half, lengthwise. Both halves of each surface were inspected for color under artificial light and divided into four categories: fully brown (FB), partly brown (PB), partly purple (PP), fully purple (FP), and slaty. The equivalent percent fully brown (EB) score was determined to compare the degree of fermentation (Mamot, 1989) using the following equation:

$$EB = [(1 \times \% FB) + (0.7 \times \% (PB + PP)) + (0.5 \times \% FP) + 0.3 \times \% slaty)]$$

2.6 Statistical analysis

One-way single-factor analysis of variance and Tukey's range test were used to determine significant differences between means using Minitab statistical software version 18 (Minitab, LLC., USA). Significant differences were considered at $P < 0.05$.

3. Results and discussion

3.1 Powdered starter culture production

The properties of all PSC were listed in Table 2. The yield and moisture content of PSC after drying were between 86 and 92 % and 4 - 10 %, respectively. Owing to its protective capabilities, moisture retention, cost-effectiveness, and availability, rice flour was used as a carrier material for the production of PSC. Its protein and starch could serve as protectors, promoting microbial survival

during drying and storage (Yunilas & Mirwandhono, 2018). The survival of the yeast population in PSC after drying at 40 °C for 14 and 15 hours was approximately 81%, which was significantly higher than that of other PSC, since the log reduction of the yeast population was 1.7 – 1.8, which was significantly lower than that of another PSC ($P < 0.05$). After drying at 45 °C and 50 °C for 7 – 9 hours, the survival yeast populations of PSC ranged from 67% to 70% and 51% to 57%, respectively. The increase in drying temperature led to significantly lower yeast population survival ($P < 0.05$). Similarly, longer exposure times at each temperature increased the reduction in the yeast population. The degree of heat exposure has a major effect on the particular mechanisms of yeast cells (Rikhvanov et al., 2014). At higher temperatures, yeast cells decrease because of various processes, including the generation of reactive oxygen species (ROS), membrane deterioration, and protein denaturation. More ROS generation and yeast cell death may result from moderate heat shock at 45 °C, however, distinct pathways may be involved in severe heat shock at 50 °C. The yeast cell membrane's integrity can be weakened by heat stress, which could result in intracellular contents leaking out and eventually cell death. (Rikhvanov et al., 2014; Verghese et al., 2012). Furthermore, proteins may lose their structure and function due to the higher temperature, which could result in cellular malfunction and death (Singh et al., 2024). The stability and shelf life of PSC are mainly influenced by its moisture content. The decrease in moisture content prevents microbial degradation during storage and enhances preservation (Alp & Bulantekin, 2021). Although the drying temperature at 40 °C for 14 hours provided PSC with the highest survival yeast population, its moisture content exceeded 8.5%, which could increase the risk of microbial contamination (Phuapaiboon, 2016). Therefore, the PSC of drying temperature at 40 °C for 15 hours was applied as a starter culture for further study.

Table 2. Properties of yeast starter culture powder of different drying conditions.

Temperature (°C)	Time (hours)	Yield (%)	Moisture content (%)	Yeast survival (%)	Yeast log reduction
40	14	$2.49^{ab} \pm 91.28$	9.20 ± 0.62^{a}	80.85 ± 0.75^{a}	1.76 ± 0.07^{e}
	15	88.25 ± 3.89^{ab}	8.08 ± 0.13^{b}	81.07 ± 2.26^{a}	1.74 ± 0.21^{e}
	16	89.10 ± 1.52^{ab}	7.85 ± 0.25^{c}	67.14 ± 1.65^{b}	3.01 ± 0.15^{c}
45	7	91.18 ± 0.96^{ab}	8.17 ± 0.28^{b}	69.59 ± 1.16^{b}	2.79 ± 0.11^{d}
	8	89.40 ± 3.07^{ab}	6.68 ± 0.48^{d}	67.88 ± 0.71^{b}	2.95 ± 0.07^{c}
	9	92.83 ± 1.03^{a}	6.45 ± 0.11^{de}	67.33 ± 0.86^{b}	3.00 ± 0.08^{c}
50	7	86.61 ± 2.85^{b}	5.91 ± 0.26^{e}	56.48 ± 2.19^{c}	3.99 ± 0.20^{b}
	8	88.63 ± 2.20^{ab}	5.84 ± 0.14^{e}	54.00 ± 0.26^{cd}	4.22 ± 0.02^{ab}
	9	88.14 ± 0.65^{ab}	4.95 ± 0.09^{f}	51.34 ± 0.29^{d}	4.46 ± 0.03^{a}

Note: Different alphabet in the same column indicates statistically significant differences ($P < 0.05$).

3.2 Small-scale fermentation of cocoa beans

The temperature profile of fermentation is a key factor to be monitored and controlled to achieve high-quality cocoa beans (Calvo et al., 2021). The change in temperature during the small-scale fermentation of cocoa beans was investigated, as shown in Figure 1. In all batches, the initial temperature of cocoa beans was roughly 24 °C, before rising by over 30 °C on the first day of fermentation. The temperature of cocoa beans fermented with PSC was higher than that of LSC and SFB fermentation on day 1. Then, the temperature slowly increased to the highest temperature of 38.0 °C on day 5 of fermentation, slightly decreased on day 6, and remained relatively steady between 35°C and 36°C until fermentation was completed. For the LSC and SFB fermentations, the temperatures of both batches slowly rose to their highest temperatures of 39.1 and 40.8 °C, respectively, on day 6 of fermentation, then rapidly decreased on day 7 and likely remained steady at about 32 °C until the end of fermentation.

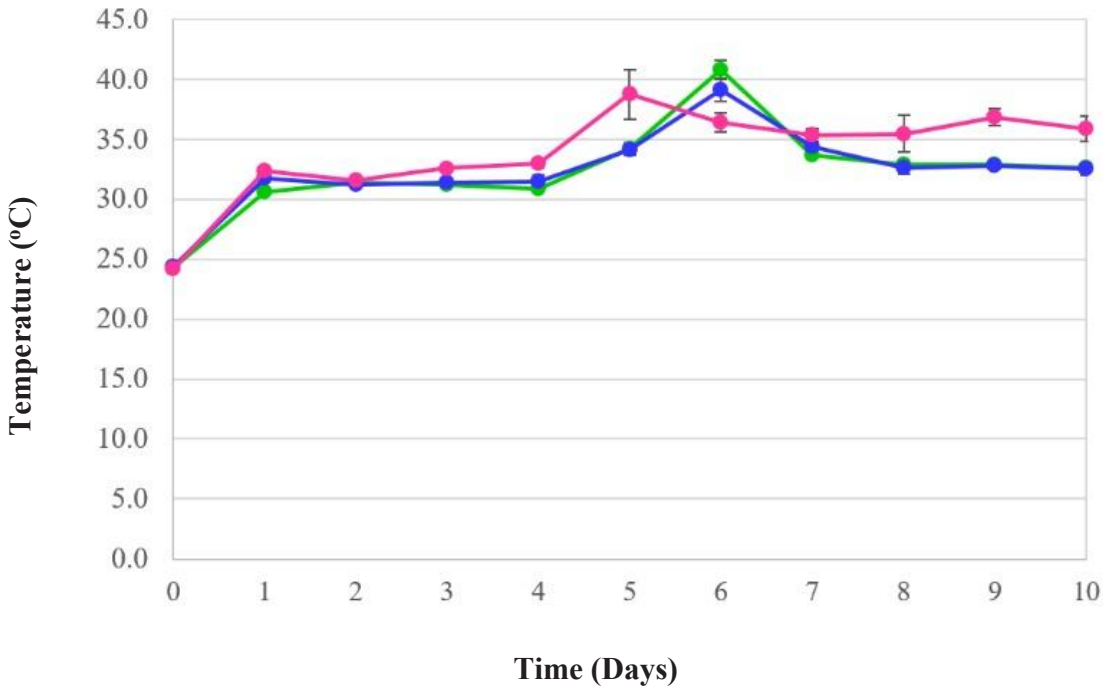

Figure 1. Temperature profiles of cocoa beans during fermentation: spontaneous fermentation with banana leaf (SFB ●), liquid starter culture (LSC ●), powdered starter culture (PSC ●).

The main cause of temperature variations during cocoa fermentation is the heat released by microbial metabolism (Reyes De Corcuera et al., 2020). During the initial stage of cocoa bean fermentation, the temperature increased due to the anaerobic fermentation of the high sugar content in the cocoa pulp. This process is largely carried out by yeasts, and optimal fermentation temperatures are usually between 32 °C and 60 °C (Campos et al., 2025). Following the initial yeast activity, lactic acid bacteria undergo multiplication and convert carbohydrates into lactic acid.

This process further decreases the pH and enhances the development of the flavor. acetic acid bacteria take over and convert the ethanol produced by yeasts into acetic acid, which is essential for seed viability and flavor development (De Vuyst & Weckx, 2016). The temperature profile revealed that cocoa beans fermented by PSC had a greater temperature than the other batches. PSC seems to ferment cocoa beans more effectively and reliably than both LSC and SFB, possibly because of its thorough dispersion and adherence to the cocoa pulp throughout the fermentation process. The rice flour in PSC acts as a dispersion matrix and a carrier for microorganisms, providing a stable and protective environment (Kavitake et al., 2018) that facilitates a steady fermentation process, resulting in a consistent quality of dried cocoa beans.

3.3 Cut test

The most widely used method for visually assessing the quality of a random sample of beans from a batch is a cut test (Kongor et al., 2013). The degree of fermentation is determined by the color and texture changes that occur in cocoa beans during the fermentation (Oliveira et al., 2021). After cutting the beans in half lengthwise, the beans of each experiment were divided into four different categories as mentioned in 2.5. Approximately 70% of the SFB cocoa beans showed partly purple with small fissures, whereas over 80% of the beans fermented with LSC and PSC were partly or entirely brown with larger fissures (Figures 2b and 2c). The EB scores of SFB, LSC, and PSC fermentation of cocoa beans were 72 ± 0 ± 82 ,4, and 82 ± 0, respectively ($P < 0.05$).

The degree of fermentation of cocoa beans can be determined by the EB score. The obtained EB scores of cocoa beans from LSC and PSC fermentation were higher than those of the SFB batch. Purple-colored cocoa beans were identified as under-fermented, but brown beans with larger fissures on the cotyledons indicate a successful fermentation process (Sulaiman & Yang, 2015). The inoculated yeast cells in LSC and PSC rapidly consumed the substrate in cocoa pulp and produced a range of metabolites early on in the fermentation process (Díaz-Muñoz et al., 2021), consistent with their temperature profile shown in Figure 2. Polyphenols like anthocyanidins can leak out of the beans as a result of their metabolites, but other polyphenols undergo oxidation and polymerization to form insoluble high-molecular-weight compounds known as tannins. As a result, during the fermentation process, the internal color of the cocoa bean changes from purple to brown, while the embryo's surface becomes more deeply and broadly ridged. (Castro-Alayo et al., 2019; Afoakwa et al., 2013). The results demonstrate that PSC achieved the same level of quality in the cocoa bean fermentation process as LSC, but with a more consistent quality of cocoa beans than SFB. Further investigation into the large-scale fermentation of cocoa beans is therefore required.

(a)

(b)

(c)

Figure 2. The cut test of cocoa beans after fermentation for 10 days: spontaneous fermentation with banana leaf (a), fermentation of liquid starter culture (b), and powdered starter culture (c).

4. Conclusions

This study showed that PSC can be used to improve the fermentation of cocoa beans reliably, producing high-quality dry cocoa beans. The low-temperature process with a prolonged drying time of 40°C for 15 hours was found to be suitable for the production of PSC. The quality of the cocoa beans obtained from fermentation using PSC was comparable to that of LSC. To improve the quality of PSC and better understand its role in the quality of cocoa beans, the shelf life of PSC and the chemical composition of cacao beans after fermentation with PSC were further investigated.

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References

Afoakwa, E. O., Kongor, J. E., Takrama, J. F., & Budu, A. S. (2013). Changes in acidification, sugars, and mineral composition of cocoa pulp during fermentation of pulp pre-conditioned cocoa *(Theobroma cacao) beans. International Food Research Journal, 20*(3), 1215-1222.

Alp, D., & Bulantekin, Ö. (2021). The microbiological quality of various foods dried by applying different drying methods: A review. *European Food Research and Technology, 247*(6), 1333–1343. https://doi.org/10.1007/s00217-021-03731-z

Calvo, A. M., Botina, B. L., García, M. C., Cardona, W. A., Montenegro, A. C., & Criollo, J. (2021). Dynamics of cocoa fermentation and its effect on quality. *Scientific Reports, 11*, Article 16746. https://doi.org/10.1038/s41598-021-95703-2

Campos, S. d. M., Martínez-Burgos, W. J., dos Reis, G. A., Ocán-Torres, D. Y., dos Santos Costa, G., Rosas Vega, F., Alvarez Badel, B., Sotelo Coronado, L., Lima Serra, J., & Soccol, C. R. (2025). The role of microbial dynamics, sensorial compounds, and producing regions in cocoa fermentation. *Microbiology Research, 16*(4), 75. https://doi.org/10.3390/microbiolres16040075

Castro-Alayo, E. M., Idrogo-Vásquez, G., Siche, R., & Cardenas-Toro, F. P. (2019). Formation of aromatic compounds precursors during fermentation of Criollo and Forastero cocoa. *Heliyon, 5*(1), e01157. https://doi.org/10.1016/j.heliyon.2019.e01157

Chumphon Horticultural Research Centre, Department of Agriculture. (2021). *Manual of Chumphon 1 hybrid cocoa farming* (In Thai). Retrieved June 6, 2025, from https://www.doa.go.th/hort/

De Vuyst, L., & Leroy, F. (2020). Functional role of yeasts, lactic acid bacteria and acetic acid bacteria in cocoa fermentation processes. *FEMS Microbiology Reviews, 44*(4), 432–453. https://doi.org/10.1093/femsre/fuaa014

De Vuyst, L., & Weckx, S. (2016). The cocoa bean fermentation process: From ecosystem analysis to starter culture development. *Journal of Applied Microbiology, 121*(1), 5-17. https://doi.org/10.1111/jam.13045

Díaz-Muñoz, C., & De Vuyst, L. (2022). Functional yeast starter cultures for cocoa fermentation. *Journal of Applied Microbiology, 133*(1), 39–66. https://doi.org/10.1111/jam.15312

Díaz-Muñoz, C., Van de Voorde, D., Comasio, A., Verce, M., Hernandez, C. E., Weckx, S., & De Vuyst, L. (2020). Curing of cocoa beans: Fine-scale monitoring of the starter cultures applied and metabolomics of the fermentation and drying steps. *Frontiers in Microbiology, 11*, Article 616875. https://doi.org/10.3389/fmicb.2020.616875

He, B.-L., Cui, R., Hu, T.-G., & Wu, H. (2025). Improved viability of probiotics by co-encapsulation of wheat germ oil under storage and gastrointestinal conditions: Effects of drying methods and wall composition. Food *Hydrocolloids, 158*, Article 110592. https://doi.org/10.1016/j.foodhyd.2024.110592

Kavitake, D., Kandasamy, S., Devi, P. B., & Shetty, P. H. (2018). Recent developments on encapsulation of lactic acid bacteria as potential starter culture in fermented foods — A review. *Food Bioscience, 21*, 34–44. https://doi.org/10.1016/j.fbio.2017.11.003

Kongor, J. E., Takrama, J. F., Budu, A. S., Mensah-Brown, H., & Afoakwa, E. O. (2013). Effects of fermentation and drying on the fermentation index and cut test of pulp pre-conditioned Ghanaian cocoa (Theobroma cacao) *beans. Journal of Food Science and Engineering, 3*(11), 625–634.

Mamot, S. (1989). Factors affecting the quality of cocoa beans during processing. In *Prosiding Seminar Teknologi Koko Masa Kini ke-3* (pp. 13–14). [Malay].

Martin, J. P. C., & Lindner, J. D. (2022). Culturas microbianas para aplicação em alimentos fermentados. In J. G. P. Martin & J. D. Lindner (Eds.), *Microbiologia de alimentos fermentados*. São Paulo: Blucher.

Oliveira, M. M., Cerqueira, B. V., Barbon, S. Jr., & Barbin, D. F. (2021). Classification of fermented cocoa beans (cut test) using computer vision. *Journal of Food Composition and Analysis, 97*, 103771. https://doi.org/10.1016/j.jfca.2020.103771

Payne, C. J., Phuong, V. H., Phuoc, N. N., Dung, T. T., Phuoc, L. H., & Crumlish, M. (2025). Genomic diversity and evolutionary patterns of Edwardsiella ictaluri affecting farmed striped catfish (Pangasianodon hypophthalmus) in Vietnam over 20 years. *Microbial Genomics, 11*(2), Article 001368. https://doi.org/10.1099/mgen.0.001368

Phuapaiboon, P. (2016). Immobilization of probiotic bacteria with banana flour and effect on quality of synbiotic ice cream and survival under simulated

gastrointestinal conditions.*Carpathian Journal of Food Science & Technology, 8*(4), 33–46.

Reyes-De-Corcuera, J., Hernández, A., Gabriela, M., Juan, M., & Cevallos, M. (2020). Temperature profiles during the fermentation of cacao 'Nacional' to understand chocolate quality. In *Agricultural, forestry and bioindustry biotechnology and biodiscovery* (pp. 319–328). Springer.

Rikhvanov, E. G., Fedoseyeva, I. V., Varakina, N. N., Rusaleva, T. M., & Fedyaeva, A. V. (2014). Mechanism of Saccharomyces cerevisiae yeast cell death induced by heat shock. Effect of cycloheximide on thermotolerance. *Biochemistry (Moscow), 79*(1), 16–24. https://doi.org/10.1134/S0006297914010039

Schwan, R. F., Bressani, A. P. P., Martinez, S. J., Batista, N. N., & Dias, D. R. (2023). The essential role of spontaneous and starter yeasts in cocoa and coffee fermentation. *FEMS Yeast Research, 23*(1), 1–13. https://doi.org/10.1093/femsyr/foad019

Singh, M. K., Shin, Y., Ju, S., Han, S., Choe, W., Yoon, K.-S., Kim, S. S., & Kang, I. (2024). Heat shock response and heat shock proteins: Current understanding and future opportunities in human diseases. *International Journal of Molecular Sciences, 25*(8), 4209. https://doi.org/10.3390/ijms25084209

Sulaiman, K. B., & Yang, T. A. (2015). Color characteristics of dried cocoa using shallow box fermentation technique. *World Academy of Science, Engineering and Technology, 9*(12), 1281–1285.

Verghese, J., Abrams, J., Wang, Y., & Morano, K. A. (2012). Biology of the heat shock response and protein chaperones: Budding yeast *(Saccharomyces cerevisiae) as a model system. Microbiology and Molecular Biology Reviews, 76*(2), 115–158. https://doi.org/10.1128/MMBR.05018-11

Yunilas, M. P., & Mirwandhono, E. (2018). The role of lactic acid bacteria (*Lactobacillus* sp. yel133) from beef in inhibiting microbial contaminants on various fillers of starter culture. IOP *Conference Series: Earth and Environmental Science, 122*(1), Article 012113. https://doi.org/10. 1088/1755-1315/122/1/012113