Role of YoghurtCultures and IncubationTimein Enhancing Sensory Qualities of Yoghurt and Water Holding Capacityof Greek Yoghurt

ABSTRACT

Aims: The current research study aimed to investigate the impact of varying starter concentration, incubation temperature and temperature individually on sensory characteristics of yoghurt and water holding capacity of Greek yoghurt.
Study design: The study was experimental and was laboratory-based for optimizing the base for Greek yoghurt spread production.
Place and Duration of Study: The study was conducted at the Department of Dairy Technology, Dairy Science College, Hebbal, Bengaluru, Karnataka, India, Between January 2024 and October 2024.
Methodology: Yoghurt was prepared by different levels of starter culture concentration (1, 2 and 3%) and incubation period (4, 5 and 6 hours) with optimization based on sensory analysis using 9- point hedonic scale. Water holding capacity was analyzed using the centrifugation method.
Results: The Study elucidated that yoghurt prepared with inoculation of 2% starter culture concentration showcased the most favourable sensory characteristics obtaining a high score in flavour (8.4), colour and appearance (8.37), body and texture (8.44) and overall acceptability (8.49) on a 9-point hedonic scale. Among the different incubation hours at 42ºC, a 5-hour duration yielded the most promising results with sensory evaluations achieving 8.46, 8.42, 8.53 and 8.53 for flavour, colour and appearance, body and texture and overall acceptability, respectively. In addition, the combination of a 2% starter culture and 5-hour incubation at 42ºC resulted in highest water holding capacity, quantified as 76.11%.
Conclusion: The study showed that the sensory qualities of yoghurt and water-holding capacity of Greek yoghurt are greatly influenced by the optimal yoghurt culture concentration and incubation time, with the most favourable results obtained with a 2% culture addition and a 5-hour incubation period.

Keywords: Yoghurt, Sensory characteristics, Water Holding Capacity

1. INTRODUCTION

Dairy products areessential to human nutrition, where fermented milk is preferred for its several health benefits such as improved digestibility, support gut health and boost immunity. Fermented foods have been a part of the human diet for millennia, and the application of microbial starters can offer distinct flavour and texture characteristics to these products. In recent years, the global interest has grown regarding the health advantages and functional roles of bioactive compounds such as free amino acids, peptides, and vitamins generated during fermentation [1]. In the current market,yoghurt is among the most widely consumed fermented and functional milk-based beverages.

The word "yoghurt" suggested to be derived from the Turkish word "yoğurmak," which signifies to curdle, coagulate, or thicken. Yoghurt isthe result of beneficial bacterial cultures, lactic acid fermentation by *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* strains convert lactose into lactic acid. Lactose content in raw milk is 4.6% and is a primary carbohydrate in milk which is the main reason for lactose intolerance in people. However, yoghurt ferments lactose due to the action of lactic acid bacteria, reducing the lactose content of milk by 20-30%, thus rendering it more palatable for those individuals who are lactose intolerant. Hence it isa highly nutritious food, offering a substantial source of dairy proteins, calcium, magnesium, vitamin B_{12}, essential fatty acids, and other important compounds [2]. Several studies have demonstrated that yoghurt offers various health benefits, including antimicrobial and antioxidant properties. It is effective in combating gastrointestinal disorders, possesses anti-inflammatory qualities, and may help manage high blood pressure. Additionally, yoghurt supports the immune system, lowers the risk of osteoporosis, and contributes to the management of cardiovascular diseases and diabetes [3].

Greek yoghurt or lebnah is defined as a semi-solid product derived from regular yoghurt by draining away part of its water and water-soluble components, mainly lactose and salt. Traditional methods utilize cloth bags for whey drainage, industrial production often employs mechanical separators, membrane filtration techniques, or specific product formulations.Greek yoghurt is recognized as a concentrated fermented milk product by the Codex Alimentarius [4], requiring a minimum protein content of 5.6 g per 100 g. The straining process removes the liquid component of the milk, referred to as whey or lactoserum, which significantly lowers the lactose content in Greek yoghurt. This strained yoghurt is a high-protein dairy product with low lactose levels[5].

The quality of yoghurt is influenced by factors such as the rate of addition starter culture, incubation temperature, incubation time and various other parameters. Each parameter significantly impacts the properties of yoghurt and Greek yoghurt such as sensory attributes, textural properties and water holding capacity. Therefore, optimizing these parameters are crucial in the production process as the directly influence the quality of final product.

2. MATERIAL AND METHODS

2.1 Materials

For preparation of Greek yoghurt spread, fresh cow milk (4.0%, 8.8% SNF, acidity 0.103% lactic acid and pH 6.62) was procured from the Students Experimental Dairy Plant of Dairy Science College, Hebbal, Bengaluru. The freeze-dried yoghurt culture was procured from Delvo DSL pvt. Ltd., Netherlands. The cultures were in the form of freeze-dried powder and stored at -40°C until they were used. These cultures were first cultivated in MRS broth medium and incubated at 37°C for 24 hours. The resulting pellets were then reconstituted in a 10% skimmed milk suspension and incubated at 37-38°C for 18 hours which were further used in various concentrations to prepare yoghurt.

2.2 Methods

For the production ofGreekyoghurt cow milk with 4.0% fat, 8.8% SNF,acidity of 0.103% lactic acid and pH 6.62was used. The milk was pasteurized to 90°C for 5 minutes, followed by cooling to 42°C to prepare milk for culture addition. Starter culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus* were addedat concentrations of 1, 2, and 3% based on the milk volume. The inoculated milk was incubated at 42°C until pHreached 4.5.The optimal concentration of starter culture was determined based on sensory scores. Further optimization involved adjusting the incubation period, which was incubated for 4, 5, and 6 hours. Yoghurt prepared was assessed for sensory characteristics.

Additionally,water holding capacity was assessed by centrifugation with two parameters i.e., Incubation temperature and incubation hours. Based on trials and results obtained, the most suitable combination was subjected to de-wheying to obtain Greek yoghurt.The yield of Greek yoghurt obtained was42.8% on a milk weight basis.
Control yoghurt was prepared according to Anjum *et al*. (2007), a 2% starter culture was added to the milk and incubated at 42°C for 5 hours. Experimental samples were then prepared by varying the starter culture concentration, incubation temperature, and incubation duration to observe their individual effects on the sensory characteristics of the yoghurt and the water-holding capacity of the Greek yoghurt.

2.3Sensory Evaluation

A panel of trained judges evaluated the sensory attributes of the study samples, including colour and appearance, body and texture, flavour, and overall acceptability. Each sample was labeled with a code, and the panelists were instructed to score the sample using a 9-point hedonic scale. Following this, a hedonic test was conducted to assess the yoghurt preferences.

2.4 Water Holding Capacity

The water holding capacity was measured by following Krisnaningsih *et al.* (2019) [6].

$$\text{WHC= (\%). } = \frac{(W_2 - W_1) \times 100}{W_2}$$

Where,

W_1=Weight of whey after centrifugation,
W_2= Initial weight of yoghurt.

2.5 Statistical analysis

The data was analyzed using R software (version 4.1.2) for statistical computingwith the *dplyr*and*agricolae*packagesutilized to organize and process the data. Response variables were collected from three replications of the trials, and ANOVA tables were generated to assess the effects of the variables on the response measures for data analysis. When the F value was significant, the critical difference (*P*=.05) was calculated using a formula to determine significant differences. Significant differences among treatment means are marked in the tables using distinct superscripts.

$$\text{Critical difference (CD)} = \frac{\sqrt{2 \times MSS(E)} \times t\alpha}{r}$$

Where,

MSS (E) = Mean Sum of squares of the error
r = number of replications
tα = table t value of the α level of significance

3. RESULTS AND DISCUSSION

3.1 Effect of Yoghurt Culture on the Sensory CharacteristicsofYoghurt

The current study revealed significant differences in the flavour profile of Greek yoghurt samples based on varying levels of yoghurt culture. The 2% yoghurt culture sample

achieved the significantly highest flavour score of 8.42, indicating the most favorable flavour profile, while the lowest score of 7.33 was found in the 3% culture sample. This finding aligns with Ramakrishnan *et al*. (2024), who reported the increasing amount culture addition leads to higher acidity which contributes to a higher sour taste [7].

For colour and appearance, the 2% culture addition produced the highest score (8.37), significantly higher than the 3% addition (8.11), but not significantly different from the control (8.27) or 1% addition (8.04).This contrasts with Anjum *et al*. (2007), who reported non-significant differences in colour and appearance across varying culture concentrations, suggesting that formulation and processing variations may influence visual attributes, but does not contribute to significant differencesin the colour and appearance of the final product [8].

The control sample with 2% yoghurt culture had a mean sensory score of 8.25 for body and texture. In comparison, the experimental samples with 1, 2, and 3% yoghurt culture had mean scores of 7.53, 8.44, and 7.79, respectively. The highest score of 8.44 was observed in the 2% yoghurt culture sample, indicating superior body and texture characteristics at this concentration. Statistical analysis showed a significant relationship between culture concentration and product texture, with higher concentrations resulting in a thicker yoghurt. Anjum *et al.*(2007),Found that yoghurt prepared with 2.00% and 2.50% sub-cultured inoculum recorded the highest texture scores [8]. The ability of proteins to retain water and fat in yoghurt structure is a key factor affecting syneresis, which compromises body and texture. According to Arab *et al.* (2022), syneresis, an important quality indicator, tends to increase with higher culture concentrations and extended storage time, impacting consumer satisfaction [9].

The analysis of overall acceptability followed a similar trend, with the 2% culture addition achieving the highest score (8.49), significantly higher than both the 3% (7.51) and 1% (7.97) additions, although no significant difference was observed between the 2% addition and the control group (8.30). This is consistent with Ramakrishnan *et al*. (2024), who reported that a 0.2% culture level of freeze-dried culture yielded the highest overall acceptability score (8.60), reinforcing the idea that optimizing culture concentration is crucial for enhancing both sensory and overall acceptability in yoghurt products [7].

The effect of varying yoghurt culture percentages on the sensory characteristics of yoghurt, based on a 9-point hedonic scale, is presented in Table 1.

3.2 Effect of Incubation Period on Sensory Attributes of Yoghurt

The statistical analysis of the flavour attribute under varying incubation temperatures shows significant differences. The highest flavour score of 8.46 was observed in the yoghurt incubated for 5 hours, which was not significantly different from the control (8.35), both scoring higher than the samples incubated for 4 hours (7.44) and 6 hours (7.30). This is consistent with Eze *et al*. (2021) found that yoghurt incubated for 5 hours achieved the highest sensory scores across several attributes, including colour (7.35), flavour (6.8), texture (6.9), and overall acceptability (7.35). These scores were notably higher compared to yoghurt incubated for shorter periods of 3 and 4 hours. Similarly, the colour and appearance of yoghurt incubated for 5 hours (8.42) and the control (8.38) showed no significant differences, maintaining superior scores, while shorter (4 hours) and longer (6 hours) incubation periods yielded significantly lower scores (7.64 and 7.67, respectively), indicating that extreme incubation conditions adversely affect the visual appeal [10].

The body and texture of the samples followed the same trend, with the control and 5-hour incubated yoghurt receiving the highest scores (8.50 and 8.53, respectively), whereas the 4-hour (7.51) and 6-hour (7.50) samples were rated lower, suggesting that both insufficient and excessive incubation periods negatively impact texture. These results mirrored to study by Santoso*et al*. (2021) who investigated and reported the impact of varying culture concentrations (2, 3, 4, and 5%) and different incubation periods (6, 8, and 10 hours) on yoghurt's sensory characteristics. The study revealed that yoghurt produced under these conditions exhibited a soft texture, distinctive yoghurt aroma, very sour taste, and a white colour. The sourness resulted from bacterial fermentation of lactose into lactic acid, which also contributed to the thick texture due to the coagulation of milk proteins. As the pH of the yoghurt approached 4.6, the solubility of casein decreased, leading to hydrophobic interactions between casein micelles. This phenomenon played a crucial role in defining the yoghurt's physical properties, such as texture, viscosity, water-binding capacity, and syneresis [11,6].

Finally, the overall acceptability of the samples mirrored the trends in other sensory attributes, with the control and 5-hour incubated yoghurt achieving the highest scores (8.45 and 8.53, respectively), while 4-hour (7.43) and 6-hour (7.38) incubation periods resulted in lower acceptability. These findings align with Aman *et al.* (2021), who reported higher acceptability scores for yoghurt incubated for 8 hours compared to 6 and 10 hours, highlighting the importance of optimizing incubation time to achieve desirable sensory properties [12].

The results focus on the significance of the incubation period on sensory attributes of yoghurt which is presented in Table 2, illustrating the effect of varying incubation time (4, 5, and 6 hours) on the sensory characteristics of the yoghurt spread, at a constant incubation temperature of 42^{o}C**.**

3.3 Effect of Incubation Temperature and Time on Water Holding Capacity (WHC) of Greek Yoghurt

The impact of incubation temperature and time on the WHC of yoghurt is documented in Table 3. Higher incubation temperatures, such as 42°C, have been shown to enhance WHC significantly. Swelam *et al*. (2019) reported similar findings, with the optimal WHC observed at 42°C when applied for different incubation temperatures of (40°C, 42°C and 45°C). This is consistent with the data presented here, where yoghurt incubated at 42°C exhibited the highest WHC across all time points, with a peak value of 76.11% after 5 hours of incubation [13]. The prolonged incubation times further improved WHC, particularly at 42°C, aligning with Krisnaningsih *et al*. (2019), who indicated that extended incubation times enhance WHC and viscosity while reducing moisture content [6].

At 37°C, WHC was generally lower compared to the control and 42°C groups, with a maximum of 65.16% after 5 hours. This temperature also showed a decrease in WHC with prolonged incubation beyond 5 hours, indicating that optimal results at this temperature might be time-sensitive. Conversely, the lowest WHC was observed at 55°C across all incubation times, with values significantly lower than those at 42°C or even 37°C. The results further indicate that incubation time plays a crucial role in determining WHC. Gyawali and Ibrahim (2018) reported that the incorporation of whey protein concentrate (WPC) increases WHC by approximately 21% compared to control samples. The role of incubation conditions in WHC enhancement aligns with their findings [14].

Table 1. Effect of yoghurt culture on the sensory characteristics of yoghurt.

Culture Addition (%)	Flavour	Colour and appearance	Body and Texture	Overall Acceptability
Control	8.24^{ab}	8.27^{a}	8.25^{ab}	8.30^{ab}
1	8.12^{b}	8.04^{a}	7.53^{b}	7.97^{b}
2	8.42^{a}	8.37^{a}	8.44^{a}	8.49^{a}
3	7.33^{c}	8.11^{c}	7.79^{c}	7.51^{b}
CD(*P*= .05)	0.20	0.27	0.35	0.28

Note: The control sample is yoghurt with 2% culture addition, CD= Critical difference, all the results are average of three trials (n=3), and same superscript indicates non-significance while different, indicating statistically significant difference at *P*= .05.

Table 2. Effect of Incubation Period on Sensory Attributes ofYoghurt.

Incubation Period (Hours)	Flavour	Colour and appearance	Body and Texture	Overall Acceptability
Control	8.35^{a}	8.38^{a}	8.50^{a}	8.45^{a}
4	7.44^{b}	7.64^{b}	7.51^{b}	7.43^{b}
5	8.46^{a}	8.42^{a}	8.53^{a}	8.53^{a}
6	7.30^{b}	7.67^{b}	7.50^{b}	7.38^{b}
CD(*P*=.05)	0.27	0.20	0.17	0.16

Note: Temperature constant at 42ºC, CD= Critical difference, all the results are average of three trials (n=3) and same superscript indicates non-significance while different, indicating statistically significant difference at *P*= .05.

Table 3. Effect of Incubation Temperature and Time on Water Holding Capacity (WHC) Of Greek Yoghurt

Incubation Temperature (°C)	4 hours	5 hours	6 hours	CD (*P*=.05)
Control	72.16^{aAB}	75.68^{cA}	64.27^{aB}	8.43
37	61.31^{bAB}	65.16^{bA}	50.86^{bB}	10.30
42	71.69^{aAB}	76.11^{aA}	61.89^{aB}	9.92
55	46.80^{cAB}	43.30^{cA}	40.67^{cB}	4.93
CD (*P*=.05)	3.61	4.81	2.11	

Note: Culture is constant (2%), CD= Critical difference, all the results are average of three trials (n=3) and same superscript indicates non-significance while different, indicating statistically significant difference at *P*=.05. A, B = Indicate significant differences between different incubation times within the same treatment

4. CONCLUSION

The study's conclusions highlight the crucial effects of temperature, incubation time, and yoghurt culture concentration on the sensory qualities and water holding capacity (WHC) of

Greek yoghurt. The optimal concentration of 2% yoghurt culture yielded the most favourable flavour, body and texture, and overall acceptability scores, confirming the importance of balancing culture levels to enhance product quality. Additionally, a 5-hour incubation period at 42°C demonstrated superior sensory attributes, aligning with previous research that highlights the detrimental effects of both insufficient and excessive incubation times on yoghurt quality. The results also indicate that at 42ºC for 5 hours showed improved water holding capacity of yoghurt. Overall, this research provides valuable insights into optimizing production parameters for Greek yoghurt, suggesting that careful consideration of culture concentration and incubation conditions can lead to enhanced product quality and increased consumer appeal.

ACKNOWLEDGEMENTS

The authors would like to extend their heartfelt appreciation to the faculty, both teaching and non-teaching, from the Department of Dairy Technology, Dairy Microbiology, and Dairy Chemistry at Dairy Science College, Bengaluru, KVAFSU, for their continuous support throughout this research.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that no competing interests exist.Author(s) hereby declare that NO generative AI technologies such as Large LanguageModels (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during thewriting or editing of this manuscript.

REFERENCES

1. Lang, F., Wen, J., Wu, Z., Pan, D., & Wang, L. (2022). Evaluation of probiotic yoghurt by the mixed culture with Lactobacillus plantarum A3. *Food Science and Human Wellness*, *11*(2), 323-331.https://doi.org/10.1016/j.fshw.2021.11.006

2. Predescu, C., ȘTEFAN, G., & Papuc, C. (2022). Impact of the addition of different forest berry fruits on functional, physicochemical and sensory properties of yogurt shelf life. *Scientific Works. Series C, Veterinary Medicine*, *68*(1), 43-49.

3. Hoxha, R., Evstatieva, Y., & Nikolova, D. (2023). Physicochemical, rheological, and sensory characteristics of yogurt fermented by lactic acid bacteria with probiotic potential and bioprotective properties. *Foods*, *12*(13), 2552.https://doi.org/10.3390/foods12132552

4. Codex Alimentarius. Codex Standard for Fermented Milks. Codex stan. 2003;243-2013.

5. Pappa, E. C., Kondyli, E., Pappas, A. C., Kyriakaki, P., Zoidis, E., Papalamprou, L., ... & Georgiou, C. A. (2024). Physicochemical Characteristics of Commercially Available Greek Yoghurts. *Dairy*, *5*(3), 436-450.https://doi.org/10.3390/dairy5030034

6. Krisnaningsih, A. T., Radiati, L. E., Evanuarini, H., & Rosyidi, D. (2019). The Effect of Incubation Time to The Physicochemical and Microbial Properties of Yoghurt with Local Taro (Colocasia Esculenta (L.) Schott) Starch as Stabilizer. *Current Research in Nutrition & Food Science*, *7*(2), 547-

554.http://dx.doi.org/10.12944/CRNFSJ.7.2.23

7. Ramakrishnan, V., Arunkumar, H., Venkatesh, M., Manjunatha, H., & Pushpa, B. P. (2024). Effect of Concentration of Starter Culture and Incubation Time on Sensory Attributes of RTE Functional Greek Yoghurt. *Journal of Scientific Research and Reports*, *30*(7), 340-345.https://doi.org/10.9734/jsrr/2024/v30i72149

8. Anjum, R. R., Zahoor, T., & Akhtar, S. (2007). Comparative study of yoghurt prepared by using local isolated and commercial imported starter culture. *J. Res. Sci*, *18*, 35-41.

9. Arab, M., Yousefi, M., Khanniri, E., Azari, M., Ghasemzadeh-Mohammadi, V., & Mollakhalili-Meybodi, N. (2023). A comprehensive review on yogurt syneresis: Effect of processing conditions and added additives. *Journal of Food Science and Technology*, *60*(6), 1656-1665.https://doi.org/10.1007/s13197-022-05403-6

10. Eze, C. M., Aremu, K. O., Alamu, E. O., & Okonkwo, T. M. (2021). Impact of type and level of stabilizers and fermentation period on the nutritional, microbiological, and sensory properties of short-set Yoghurt. *Food science & nutrition*, *9*(10), 5477-5492. https://doi.org/10.1002/fsn3.2507

11. Santoso, A., Retnosari, R., Pramudita, V., Wijaya, A. R., Anisa, T. N., & Sanjaya, E. H. (2021, May). The effect of starter concentration and incubation time on yogurt characteristics. In *AIP Conference Proceedings* (Vol. 2353, No. 1). AIP Publishing.https://doi.org/10.1063/5.0052694
12. Aman, Santos R, Rini P, Vina W, Anugrah A & Tiara S. (2021). The effect of starter concentration and incubation time on yoghurt characteristics. AIP Conference Proceedings, 2353 (1),1-6.

13. Swelam, S., & Rashed, M. A. (2019). Properties of non-fat yoghurt as influenced by the incubation temperature of exopolysaccharide producing culture. *Journal of Food and Dairy Sciences*, *10*(12), 447-452.10.21608/jfds.2019.71333

14. Gyawali, R., & Ibrahim, S. A. (2018). Addition of pectin and whey protein concentrate minimises the generation of acid whey in Greek-style yogurt. *Journal of dairy research*, *85*(2), 238-242.https://doi.org/10.1017/S0022029918000109