



# Effect of Concentration of Starter Culture and Incubation Time on Sensory Attributes of RTE Functional Greek Yoghurt

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

**Aims:** The aim of the current study was to optimize starter culture concentration and incubation time for the preparation of Ready-To- Eat functional Greek yoghurt.

**Study Design:** Optimization of culture concentration and incubation time for RTE functional Greek yoghurt.

**Place and Duration of Study:** Department of Dairy Technology, Dairy Science College, Hebbal, Bengaluru, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India. Between June 2023 and April 2024.

**Methodology:** Ready-To-eat functional Greek yoghurt was prepared by different levels of starter culture concentration and incubation time period and optimized based on sensory analysis using 9-point hedonic scale.

**Results:** The addition of various levels of yoghurt culture (0.15, 0.20, 0.25, and 0.30%), the yoghurt was evaluated by sensory characteristics. According to trial results, 0.20% yoghurt culture scored higher than the control sample (7.14, 7.16, 7.15, and 7.16) in each category for colour and appearance, body and texture, flavour, and overall acceptability (8.06, 8.44, 8.60, and 8.65 out of 9.00). Effect of varying incubation period was analyzed after inoculation of yoghurt culture for different incubation hours (5, 6, 7 and 8 h). While judging sensory attributes for different parameters such as colour and appearance, body and texture, flavour, and overall acceptability, experimental sample at 6 hours of incubation scored maximum (8.27, 8.55, 8.72 and 8.67) as opposed to the control (7.15, 7.16, 7.17 and 7.16) respectively.

**Conclusion:** The optimized levels of starter culture concentration and incubation time improved the overall sensory quality of the Ready-To-Eat Greek yoghurt.

*Keywords: Starter culture; incubation time; Greek yoghurt; sensory attributes.*

## 1. INTRODUCTION

Yoghurt has the ability to introduce probiotic microorganisms into human intestine, as well as the high nutritional value of yoghurt constitute growing importance of Greek yoghurt as a potent functional food in food industry. Beyond their nutritional worth, fermented foods have been shown to provide health benefits. Among the popular exotic fermented milk products, the consumption of yoghurt has surged rapidly in recent decade due to its remarkable nutritional as well as therapeutic properties [1].

Greek yogurt is traditionally obtained by straining normal yogurt, which gives it a thicker texture. It is a concentrated fermented milk as defined by Codex Alimentarius [2], which specifies that the protein content must be at least 5.6 g per 100 g. During the draining process, the aqueous part of the milk – the whey or lactoserum – is removed, which explains the low lactose content of Greek yogurt [3,4].

Greek yoghurt is a highly nutritious fermented product characterized by its higher protein, low sodium and low carbohydrate contents, and has gained immense popularity compared to traditional yoghurt. It has a unique semi-solid texture and rich mouthfeel due to the particular process during manufacturing. Moreover, Greek

yogurt has a creamy white color, a soft and smooth body, good spreadability and the product is slightly acidic. Since it is a concentrated form of yogurt, it possesses sensory attributes that are different from regular yogurt as well as superior nutritional properties make it a good choice to be included in daily diet [5].

The production of yogurt is a basic ecology that depends on the interactions of two bacteria, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. The ecological term proto-cooperation describes the relationship between *S. thermophilus* and *Lb. bulgaricus* in a yogurt starter culture. Lactic acid bacteria (LAB) are picky microorganisms, and lack of vital nutrients in milk that frequently limits their ability to develop. Thus, the cooperation of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* is often critical to the success of milk fermentation. Proto-cooperation is the term for this indirect positive connection that occurs when both bacteria are able to develop independently in milk. This positive relationship often has a beneficial effect on bacterial growth and on the production of lactic acid and aroma compounds like acetaldehyde [6].

So, in order to maximize process efficiency and consumer acceptance, the current study was

done to investigate the impact of culture concentration and incubation time on various characteristics of Greek yoghurt produced.

## 2. MATERIALS AND METHODS

For the preparation of RTE functional Greek yoghurt, Fresh Cow milk was procured from Students Experimental Dairy Plant (SEDP) of Dairy Science College, Hebbal, Bengaluru and Freeze dried starter culture was procured from Delvo DSL pvt Ltd, Netherlands. The milk was heated to 90°C/5 minutes and cooled to 42°C followed by addition of freeze-dried DVS Yoghurt culture of selected microflora of Mixed culture used which is specified Inoculation of freeze dried DVS starter culture at 0.1% for control to get  $10^7$ - $10^{10}$  cfu/ml (Streptococcus thermophilus and Lactobacillus bulgaricus) of concentrated form so as to be added at lower concentrations of 0.15, 0.20, 0.25, and 0.30 percent. Product inoculated at standard conditions according to chemical and microbiological parameter. Then further incubation at 42°C for 5, 6, 7 and 8 hours and then subjected for de-wheyng.

The panel of trained judges that was chosen assessed the generated study sample's sensory attributes, such as colour and appearance, body and texture, flavour, and overall acceptability, using a 9-point hedonic scale. Statistical analysis was conducted using the output with the highest score as per Peryam and Pilgrim [7]. Using R software (R. version 4.0.3), the data collected for the research investigations was examined to evaluate the significant or non-significant effects of various treatments and trials obtained for the current study. The mean and critical difference was calculated.

## 3. RESULTS AND DISCUSSION

### 3.1 Effect of concentration of Starter Culture on Sensory Attributes of RTE Functional Greek Yoghurt

The colour and appearance scores of the control and other treated samples showed a significant difference ( $P < 0.05$ ). Highest score of 8.06 was obtained for RTE functional Greek yoghurt made with 0.20% yoghurt culture and lowest score of 7.03 was obtained for Greek yoghurt made with 0.30% yoghurt culture respectively. The colour and appearance may have got affected in sample with 0.20% culture addition as the surface was smooth and uniform. The study

conducted by Gamage et al. [8] observed the similar effects in drinking yoghurt developed from freeze dried yoghurt culture at 0.2% culture level (4.4, 3.9, 4.0 & 4.0 out of 5 for colour, flavour, texture and acceptability, respectively) against 0.3 % and 0.4%.

Body and texture of yoghurt were significantly affected by yoghurt culture concentration ( $P < 0.05$ ) as the Lactic Acid Bacteria has the ability to produce exopolysaccharides and bioactive peptide which could act as the stabilizer, thickener and emulsifier like other polysaccharides. As 0.20% yoghurt culture forming firm gel-like body distributed throughout the product it received the highest sensory score of 8.44, whereas 0.15 % gave weak body. 0.3% yoghurt culture addition resulted in non-uniform body due to free whey separation and obtained least score (7.00) for sensory. Highest texture scores were recorded for yoghurt samples prepared with 2.00 and 2.50 % sub-cultured inoculum in the research findings of Anjum et al. [9]. The ability of proteins to retain water and milk fat cells in the structure of yogurt is the main factor influencing syneresis which weakens the body and texture. As considered by many researchers, syneresis is one of the most important parameters indicating the quality of yogurt increased with increase in culture concentration and storage time in light of consumer satisfaction according to Shakerian et al. [10].

Flavour score for yoghurt culture addition was highest (8.60) for 0.20% with natural yoghurt flavour with significant differences ( $P < 0.05$ ) whereas 0.30% culture addition resulted acidic flavour scored lowest sensory score (7.10) as starter culture has symbiotic interaction which responsible for aroma compound acetaldehyde. The judges recorded similar level of scores for yoghurts for flavour and taste for the yoghurt culture after sub-culturing concentration of 1% showed higher acceptability as opposed to 0.9% and 1.1% culture level for buffalo milk yoghurt from the research of Iftikhar et al. [11].

Overall acceptability rating was significantly higher ( $P < 0.05$ ) for yoghurt with 0.2% culture addition compared to control and other experimental samples and scored 8.65. The least scored sample was 0.3% yoghurt culture addition with a score of 7.03. The highest overall acceptability was recorded in drinking yoghurt developed from regular yoghurt culture at 0.2% freeze dried yoghurt culture level out of 0.2%,

0.3% and 0.4% culture level in the research study by Gamage et al. 2016. Dabija et al. [12] studied the influence of inoculation doses for yoghurt from cow milk on rheological properties and observed that the optimum freeze-dried culture dose is 0.2 %, while at 0.3 % the rheological parameters decrease. The decrease of the rheological parameters at high inoculation doses is because of the powerful proteolysis activity of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*.

The effect of different levels of yoghurt culture on sensory characteristics of RTE functional Greek yoghurt based on 9-point hedonic scale has been mentioned in Table 1.

### 3.2 Effect of Incubation Time on Sensory Attributes of RTE Functional Greek Yoghurt

There was a substantial difference ( $P < 0.05$ ) between the control and other treated samples' colour and appearance evaluations. The Greek yoghurt that made with 6 hours of incubation had the highest score of 8.27 due to smooth and even surface, while the 4 hours of incubation resulted in getting lowest score (7.15) of control. Eze et al. [13] got the highest score for the set yoghurt made of 5 hours of incubation time (7.35, 6.8, 6.9 & 7.35 for colour, flavour, texture and acceptability, respectively) against 3 and 4 hours.

There is a significant difference ( $P < 0.05$ ) between the body and texture scores of the control and experimental samples. The sample's body and texture ratings raised to 8.55 with 6 hours of incubation and lowest score of 7.00 for 8 hours of incubation. This may be due to longer incubation time tends to weaken the structure of yoghurt due to over-fermentation. The findings are correlated with Aman et al. [14] who got soft

texture for yoghurt after 8 hours of incubation time. The protein content increased with the escalation in the concentration of starter culture. However, increase in the incubation time protein content decreases as it could be denatured in acidic state which adversely affect the final body [15].

The research revealed a significant difference ( $P < 0.05$ ) in the flavour ratings between the experimental and control samples of Greek yoghurt. The flavour score increased to 8.72 with 6 hours of incubation time for yoghurt due to fully developed flavour and sample with 8 hours of incubation had the lowest score of 7.16. Shorter incubation time leads to milder and incomplete flavour while incubating for longer time can cause high-acid flavour. The results aligned with the research conducted by Eze et al. (2021), which indicated that scores intensified when set yoghurt made with 5 hours of incubation time. For short set yoghurt, all the sensory parameters, especially flavour improved as fermentation time progressed (3–5 hr) and this could be attributed to the acidity level developed during fermentation according to Alakali et al. [16].

When compared to the control and other experimental samples, the yoghurt with 6 hours of incubation time had the greatest overall acceptance rating ( $P < 0.05$ ), scoring 8.67. With a score of 7.05, the sample with the lowest score was the experimental sample with 8 hours of incubation time. In Aman et al.'s [14] research, the yoghurt that was developed with 8 hours of incubation time scored more for overall acceptability as opposed to 6 and 10 hours with different processing condition.

Table 2 discuss the impact of varying incubation period on the sensory attributes of Greek yoghurt.

**Table 1. Effect of yoghurt culture addition percentage on the sensory characteristics of RTE functional Greek yoghurt**

Culture addition (%)	Colour and appearance	Body and texture	Flavour	Overall acceptability
Control(0.1%)	7.14 <sup>b</sup>	7.16 <sup>bc</sup>	7.15 <sup>b</sup>	7.16 <sup>cd</sup>
0.15	7.16 <sup>b</sup>	7.57 <sup>bc</sup>	7.82 <sup>ab</sup>	8.21 <sup>b</sup>
0.2	8.06 <sup>a</sup>	8.44 <sup>a</sup>	8.60 <sup>a</sup>	8.65 <sup>a</sup>
0.25	7.23 <sup>b</sup>	7.60 <sup>b</sup>	7.60 <sup>b</sup>	7.40 <sup>c</sup>
0.30	7.03 <sup>b</sup>	7.00 <sup>c</sup>	7.10 <sup>b</sup>	7.03 <sup>d</sup>
CD (P=0.05)	0.26	0.40	0.61	0.19

\* Note: Inoculation of freeze dried DVS starter culture at 0.1% for control to get 107-1010 cfu/ml of bacterial cell concentration and incubated at 42 °C for 4 hours; All the values are average of three trials; Similar superscripts indicate non - significance at the corresponding critical difference; Sensory analysis – 9-point hedonic scale

**Table 2. Effect of incubation time on sensory attributes of RTE functional Greek yoghurt**

Incubation hours at 42°C	Colour and appearance	Body and texture	Flavour	Overall acceptability
Control (4 hr.)	7.15 <sup>c</sup>	7.16 <sup>c</sup>	7.17 <sup>c</sup>	7.16 <sup>c</sup>
5	7.70 <sup>b</sup>	7.47 <sup>b</sup>	8.10 <sup>b</sup>	8.07 <sup>b</sup>
6	8.27 <sup>a</sup>	8.55 <sup>a</sup>	8.72 <sup>a</sup>	8.67 <sup>a</sup>
7	7.40 <sup>bc</sup>	7.32 <sup>bc</sup>	7.30 <sup>c</sup>	7.33 <sup>c</sup>
8	7.20 <sup>c</sup>	7.00 <sup>c</sup>	7.16 <sup>c</sup>	7.05 <sup>c</sup>
CD (P=0.05)	0.29	0.24	0.31	0.36

\* Note: All the values are average of three trials; Similar superscripts indicate non - significance at the corresponding critical difference; Sensory analysis – 9-point hedonic scale

#### 4. CONCLUSION

The addition of different levels of yoghurt culture the RTE functional Greek yoghurt was evaluated by sensory characteristics. According to research trial results, 0.2% yoghurt culture scored higher than the control for colour and appearance, body and texture, flavour, and overall acceptability as higher concentration causes the bitterness due proteolysis. Effect of varying incubation period was analysed after inoculation of yoghurt culture for different incubation hours. While subjected for judging sensory attributes for different parameters such as colour and appearance, body and texture, flavour, and overall acceptability, experimental sample at 6 hours of incubation scored maximum as opposed to the control and as time prolongs the product results stale flavour that reduces overall sensory quality. The metabolic processes of the starter cultures are the source of some of the beneficial effects such as rise in acidity and typical flavour and production of exopolysaccharide which helps in suitable body formation.

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Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

- Vijay L, Karthikeyan N, Kumaresan G and Kathirvelan C. Sensory properties of functional Greek yoghurt incorporated with Moringa oleifera leaf powder. J. Pharm. Innov. 2022;(4):251-254.
- Codex Alimentarius. Codex Standard for Fermented Milks. Codex stan. 2003;243-2013.
- Aktar T. Physicochemical and sensory characterisation of different yoghurt production methods. International Dairy Journal. 2022;125:105245.
- Bayarri S, Carbonell I, Barrios EX, Costell E. Impact of sensory differences on consumer acceptability of yoghurt and yoghurt-like products. International Dairy Journal. 2011;21(2): 111-8.
- Mani F. and Hannachi C. Quality mentoring of Greek yoghurt fortified with pomegranate juice and arils (*Punica granatum* L.) during storage. J. New Sci. 2017;44 (5):2430-2437.
- Mihail A, Kostov G, Simova E, Beshkova D, Koprinkova-Hristova P. Proto-cooperation factors in yoghurt starter cultures. Revue de Génie Industriel. 2009; 3:4-12.
- Peryam D R and Pilgrim F J. Hedonic scale method of measuring food preferences. Food Technol. 1957;15(6):9-14.
- Gamage GGAP, Adikari AMJB, Nayananjali W, Prasanna P, Jayawardena NWIA, Wathsala R HGR. Physicochemical, microbiological and sensory properties of probiotic drinking yoghurt developed with goat milk. Int. J. Sci. Res. Publ. 2016;6(6):203-208.
- Anjum RR, Zahoor T and Akhtar S. Comparative study of yoghurt prepared by using local isolated and commercial

- imported starter culture. J. Res. Sci. 2007; 18(1):35-41.
10. Shakerian MSH., Razavi SA, Ziai F, Khodaiyan MS, Yarmand, Moayedi A. Proteolytic and ACE inhibitory activities of probiotic yogurt containing non-viable bacteria as affected by different levels of fat, inulin and starter culture. Journal of Food Science and Technology. 2015;52: 2428-2433.
  11. Iftikhar MA, Pasha TN, Inayat S, Javed K, Ullah R. Effect of concentration of culture on incubation time, storage and sensory characteristics of set yoghurt. Pak. J. Agric. Sci. 2023;60(1):149-161.
  12. Dabija A, Oroian M, Codină GG, Rusu L. Assessment the influence of the main technological factors on yogurt quality. Scientific Study & Research. Chemistry & Chemical Engineering, Biotechnology, Food Industry. 2020;21(1): 83-94.
  13. Eze CM, Aremu KO, Alamu EO, Okonkwo TM. Impact of type and level of stabilizers and fermentation period on the nutritional, microbiological, and sensory properties of short-set Yoghurt. Food Sci. Nutr. 2021;9(10):5477-5492.
  14. Aman, Santos R, Rini P, Vina W, Anugrah A, Tiara S. The effect of starter concentration and incubation time on yogurt characteristics. AIP Conference Proceedings. 2021;2353(1):1-6
  15. Beshkova DM, Simova ED, Frengova GI, Simov ZI, Spasov ZN. World J. Production of flavour compounds by yogurt starter cultures. Microbiol. And Technol. 2002;18: 365
  16. Alakali JS, Okonkwo TM, and lordye EM. Effect of stabilizers on the physio-chemical and sensory attributes of thermized yoghurt. African Journal of Biotechnology. 2007;2:158–163.

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