Original Research Article

Effect of Whey Protein Concentrate and Little Millet on the sensory attributes of Low-fat Greek yoghurt spread

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ABSTRACT

Aims:
To develop and evaluate a low-fat functional Greek yoghurt spread by incorporating whey protein concentrate (WPC) and little millet flour, assessing their impact on sensory, rheological, and overall acceptability characteristics.

Study Design:
A completely randomized design (CRD) was employed to evaluate the effect of different concentrations of WPC (1%, 2%, 3%) and little millet flour (1%, 2%, 3%) on the sensory attributes of Greek yoghurt spread.

Place and Duration of Study:
The study was carried out at the Department of Dairy Technology, Dairy Science College, Hebbal, Bengaluru, Karnataka, India, during the period from January 2024 to October 2024.

Methodology:
Fresh cow milk (4.0% fat) was standardized and pasteurized to 90ºC before incorporating varying concentrations of WPC (@1, 2, and 3%). Greek yoghurt was prepared by fermentation using commercial starter cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*). The fermented yoghurt was drained to achieve a concentrated Greek yoghurt, which was emulsified with 45% butter and little millet (@1, 2, and 3%). Sensory evaluations were performed using a 9-point hedonic scale by trained panelists. Statistical analysis of data, including ANOVA and critical difference (CD), was carried out using R software (version 4.1.2).

Results:

- **Effect of WPC:** Greek yoghurt with 2% WPC demonstrated superior sensory attributes, including flavor (8.66), color and appearance (8.45), texture (8.41), spreadability (8.62), and overall acceptability (8.41). Higher WPC concentrations resulted in a bitter taste and yellowish hue.
- **Effect of Little Millet:** Greek yoghurt spread with 2% little millet achieved the highest sensory scores for flavor (8.71), color and appearance (8.54), texture (8.60), spreadability (8.53), and overall acceptability (8.51). Higher concentrations of millet resulted in a thicker texture, reducing spreadability and overall scores.

Conclusion:
The incorporation of 2% WPC and 2% little millet flour significantly enhanced the sensory attributes and overall acceptability of the low-fat functional Greek yoghurt spread. This formulation offers a nutritious and functional alternative to traditional spreads, meeting consumer demand for healthier options. The incorporation of WPC and little millet flour into the low-fat Greek yoghurt spread enhances its protein content and offers several health benefits. WPC supports muscle repair, immune function, and overall tissue health, while little millet flour, rich in fiber and antioxidants, aids digestion, regulates blood sugar, and reduces the risk of chronic diseases. This formulation provides a nutritious, heart-healthy, and weight-management-friendly alternative to traditional spreads.

Keywords:
Whey Protein Concentrate, Little Millet, Sensory Attributes, Greek Yoghurt Spread

1. INTRODUCTION

Spreads are versatile food products with a ‘plastic’ consistency, making them easy to apply as a thin layer on items such as bread slices, pizza bases, chapatis, and more. Traditionally, options like margarine, butter, and other fat-based blends have been popular. However, modern consumers prioritize taste, convenience, and health-promoting properties, leading to the rise of ready-to-eat spreads such as cheese spreads and mayonnaise. These consumer-friendly options cater to convenience and serve as versatile toppings for bread, burgers, rotis, pizzas, and other dishes. Additionally, healthier alternatives are now gaining traction to meet the growing demand for nutritious and functional spreads. (Rao and Devaraju, 2021).

According to CODEX (2011) a dairy spread is described as a milk product that is relatively high in fat in the form of a malleable emulsion, primarily water-in-oil that solidifies at 20°C. However, other necessary ingredients required in their production may be incorporated providing these substances complete or partial substitute milk components. According to Young and Wassell (2019), spreads have a more uncertain definition because they may include a wide range of fat contents, thus encouraging the low-fat and reduced-fat spread concepts. This normally refers to anything with 25 to 70% fat per centage. However, there is an increasing demand for low-fat spreads. Furthermore, there is an increasing preference for spreads fortified with functional ingredients, reflecting a shift toward healthier and more purposeful food choices.

List 1 : Types and classification of food spreads

	WHO/IDF	**FAO**
Dairy spread (% fat)	62-80	NLT 60
Reduced fat spread (% fat)	41-61	60-70
Low-fat spread (% fat)	<41	40-60

(Rao and Devaraja, 2021)

Little millet (*Panicum sumatrense*), a resilient and highly nutritious grain, is gaining popularity in food and dairy products due to its significant health benefits. Rich in essential nutrients such as protein, dietary fiber, vitamins, and minerals, little millet is particularly valuable in addressing nutritional needs, especially in developing regions. Its low glycemic index and high antioxidant content make it an excellent option for improving health outcomes, particularly for individuals with diabetes (Upadhyay *et al.,* 2022). Incorporating little millet into dairy products enhances their nutritional value, offering a healthier alternative to traditional dairy products by reducing concerns over high saturated fat content. Fortifying various dairy items, including fermented and composite foods, with little millet not only boosts their acceptability but also amplifies their health benefits (Srivastava & Kumar, 2024).

Whey is a coproduct of cheese-making and casein manufacture in the dairy industry and is rich in lactose, proteins, and fats, and this organic matter. Caseins and whey proteins are the two types of proteins found in milk. The group of milk proteins referred to as whey proteins are those that, following the precipitation of caseins during processing, arestill soluble in milk serum or whey. The major proteins in whey are β-lactoglobulin (β-lg), α- lactalbumin (α-la), bovine serum albumin (BSA), immunoglobulins (Ig), lactoferrin, and protease-peptone fractions (Kadam *et al.,* 2018). WPC's most important technological functions in products with reduced fat content are water binding, emulsification, gelation, and increased viscosity. Besides these nutritional aspects, whey proteins possess ideal rheological and functional properties for the extrusion process, as they have high solubility, gel and foam-forming ability (Kristensen *et al.,* 2021). Whey protein has long been considered the "Gold Standard" of proteins for athletes becauseit is a rich source of BCAAs. BCAAs are important for athletes as they metabolize directly into muscle tissue. Whey protein provides the body with BCAAs to replenish depleted levels and start repairing and rebuilding lean muscle tissue (Kanhed *et al.,* 2023).

2. MATERIAL AND METHODS

2.1 MATERIALS

Fresh cow milk, containing 4.0% fat, 8.8% solids-not-fat (SNF), 0.103% acidity (as lactic acid), and a pH of 6.62, was obtained from the Students Experimental Dairy Plant, Dairy Science College, Hebbal, Bengaluru. This milk served as the primary ingredient in the preparation of the Greek yoghurt spread.

The freeze-dried yoghurt cultures used for fermentation were sourced from Delvo DSL Pvt. Ltd., Netherlands, and stored at -40°C to ensure viability. Before use, the cultures were activated by incubating them in MRS broth at 37°C for 24 hours. The resulting pellets were reconstituted in a 10% skimmed milk suspension and incubated at 37–38°C for 18 hours to prepare an active starter culture for Greek yoghurt production.

Unsalted butter from Amul, Bangalore, was utilized as the oil phase during the emulsification of the Greek yoghurt spread. Whey protein concentrate (80% protein) was procured from Nutrilac, DKSH India Pvt. Ltd., Mumbai. Additionally, high-quality little millet flour was sourced from the Gaanagam brand, based in Karur district, Tamil Nadu, India.

2.2 METHOD

The preparation of Greek yoghurt spread began with standardized cow milk. Whey protein concentrate was incorporated at concentrations of 1%, 2%, and 3%, and the mixture was pasteurized by heating to 95°C for 5 minutes. The pasteurized milk was then rapidly cooled to 45°C. A commercial starter culture comprising *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* was added at a concentration of 2% (w/v). The inoculated milk was incubated at 42°C until it reached a pH of 4.5, signifying the completion of fermentation.

The coagulated milk was then transferred to a cloth bag, allowing the whey to drain naturally. This step was carried out overnight at a controlled temperature of 4±1°C to produce thick and concentrated Greek yoghurt.

To create the Greek yoghurt spread, the yoghurt was emulsified with 45% butter and blended with little millet at concentrations of 1%, 2%, and 3%. Sensory evaluations were conducted first with varying levels of whey protein concentrate to identify the optimal sensory properties. Once the ideal whey protein concentrate level was determined, the process was repeated with the inclusion of little millet.

A detailed process flowchart (Fig. 1) outlines the production steps and experimental framework employed in this study.

2.3 SENSORY EVALUATION

A sensory evaluation was carried out to analyze the attributes of the study samples, including color and appearance, body and texture, flavor, and overall acceptability. The samples were assessed by a panel of highly trained judges selected for their expertise in sensory analysis. A 9-point hedonic scale was employed, and each sample was assigned a random three-digit code to ensure blind testing. The evaluation was conducted in a controlled environment to minimize external factors. The process adhered to ethical guidelines for sensory studies, prioritizing the health and safety of the panelists. The use of trained judges and a standardized protocol highlighted the scientific rigor and ethical integrity of the sensory evaluation.

2.4 STATISTICAL ANALYSIS

The data were analyzed using R software (version 4.1.2) for statistical computations, utilizing the dplyr and agricolae packages for data organization and processing. Response variables were obtained from three trial replications, and ANOVA tables were generated to examine the effects of the variables on the response measures. When the F value indicated significance, the critical difference at a 5% probability level (P = 0.05) was calculated to identify significant differences. Significant differences among treatment means were indicated 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

FLOW DIAGRAM

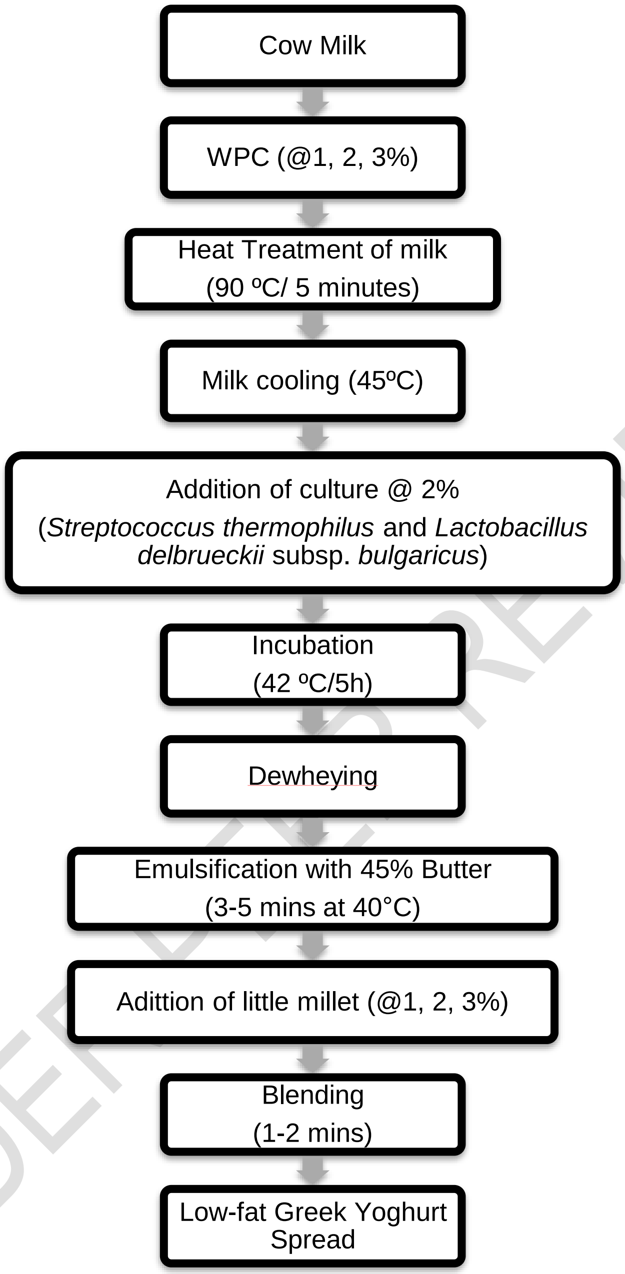

Fig1. Process Flow Chart for Production of Low-fat Greek Yoghurt Spread

3. RESULTS AND DISCUSSION

3.1 Effect of different levels of whey protein concentrate (WPC) on the sensory attributes of Greek yoghurt

The sensory evaluation of Greek yoghurt spread samples with varying concentrations of whey protein concentrate (WPC) revealed significant differences across several sensory attributes, particularly flavour, colour, appearance, and texture. The yoghurt incorporated with 2% WPC consistently outperformed the other samples, achieving the highest flavour score of 8.66, which was significantly different from the other samples ($p \leq 0.05$). This concentration appeared to strike an optimal balance, whereas higher levels of WPC led to a bitter taste, likely due to interactions between nutrients and starter cultures that increased acetaldehyde production, affecting flavour perception (Ramakrishnan et al., 2024).

Similarly, in terms of colour and appearance, the 2% WPC sample received the highest score of 8.45, while the 3% WPC sample showed a more pronounced yellowish colour, reducing its score to 8.06. This observation is consistent with the findings of Delikanli and Ozcan (2014), who noted that increased WPC concentrations lead to a more intense yellow hue in yoghurt. Regarding body and texture, spreadability and overall acceptability, the 2% WPC yoghurt achieved the highest score of 8.41, 8.62, and 8.41 respectively, indicating an ideal balance between firmness and creaminess. Lower concentrations, such as the 1% WPC sample, resulted in a softer gel formation and higher whey syneresis, reflected in a lower score of 7.13. These results also align with Brodziak *et al.* (2020) reported that Yoghurt incorporated with 2% WPC had a sensory score of 4.5, 4.1. 3.8 & 4.3 for colour, body, flavour and overall acceptance. Theses research indicated that whey protein additives in yoghurt manufacturing create a more compact structure with stiffer casein particles, larger aggregates, and increased hardness and adhesiveness.

Table 1. Effect of different levels of whey protein concentrate (WPC) on the sensory attributes of Greek yoghurt

Whey protein concentrate (%)	Flavour	Colour and appearance	Body and Texture	Spreadability	Overall Acceptability
Control	7.17^{c}	7.06^{c}	7.06^{c}	7.05^{c}	7.03^{d}
1	7.22^{c}	8.05^{b}	7.13^{b}	7.44^{c}	7.36^{c}
2	**8.66^{a}**	**8.45^{a}**	**8.41^{a}**	**8.62^{a}**	**8.41^{a}**
3	8.04^{b}	8.06^{b}	7.68^{b}	8.03^{b}	7.95^{b}
CD(P=0.05)	**0.40**	**0.13**	**0.37**	**0.40**	**0.21**

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

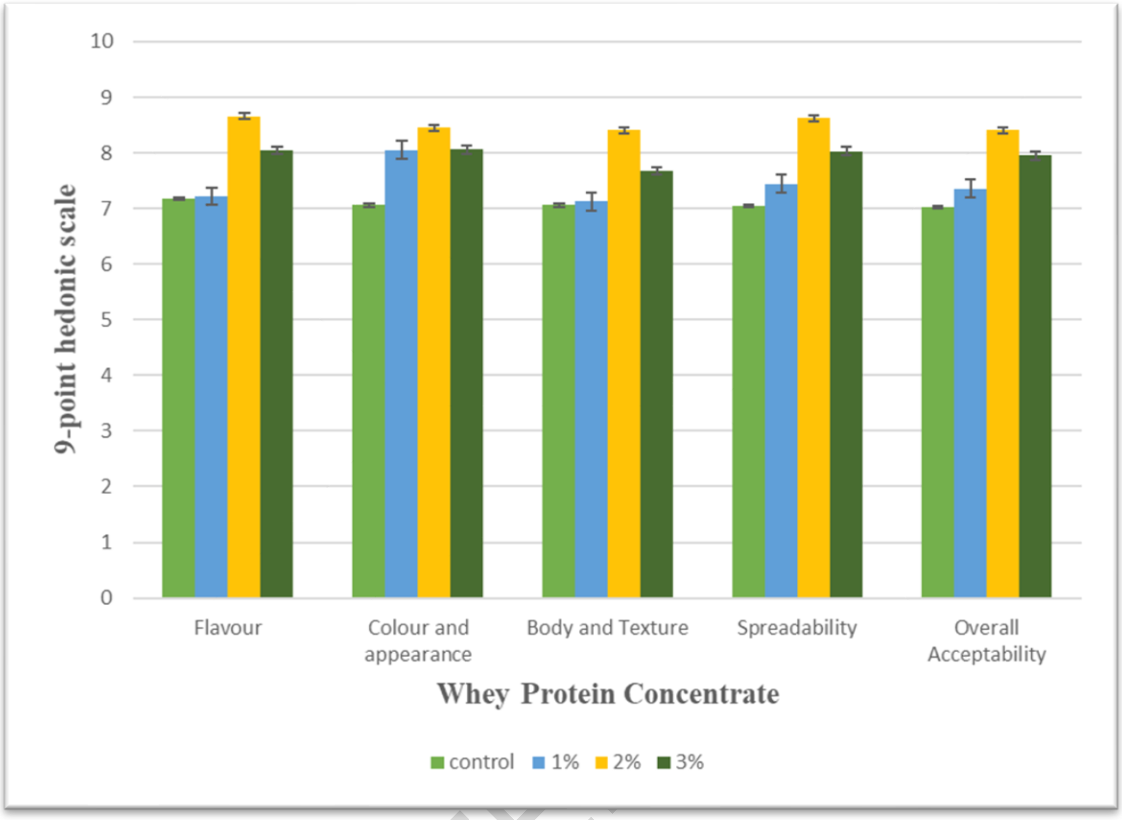

Fig 2: **Effect of little millet on the sensory attributes of Low-fat Greek yoghurt spread.**

3.2 Effect of little millet on the sensory attributes of Low-fat functional Greek yoghurt *spread.*

The addition of little millet significantly enhanced the sensory attributes of the product, with the 2% millet sample consistently achieving the highest scores across all characteristics. Flavour scores showed a marked improvement from the control (7.33) to the 2% sample (8.71), with the 1% (8.01) and 3% (8.36) millet samples falling between these values. Similarly, for colour and appearance, the control (7.22) and 1% (7.80) samples were significantly lower than the 2% sample (8.54), while the 3% sample (8.20) was intermediate. Mor *et al.* (2017) observed that the best average scores for colour and appearance, consistency, flavour, sweetness, and overall acceptability were achieved with a combination of 4.0% little millet and a 2.0 concentration ratio. However, increasing the millet content beyond this level resulted in a slightly dull and dark appearance.

Body and texture scores followed the same trend, with the control (7.20) being significantly lower than the 2% sample (8.60). Spreadability also improved with millet content, with the control sample (7.18) scoring lowest and the 2% sample (8.53) highest, though higher millet concentrations resulted in a thicker texture that slightly reduced spreadability. Sandey *et al.* (2009) reported that dairy products made with 10, and 20% little millet flour had similar flavour scores. The product with a 10% millet addition developed a smooth, soft texture, while higher levels of millet created a thicker, more viscous texture with increased sogginess, leading to lower scores. Overall acceptability mirrored these trends, with the 2% sample (8.51) significantly outperforming both the control (7.22) and the 1% (7.70) and 3% (8.00) samples.

Similarly, Jambukiya *et al.* (2022) showed that the addition of little millet @ 7.09% in gluten-free chhana cake had an overall acceptability of 8.20.

Table 2. Effect of little millet on the sensory attributes of Low-fat functional Greek yoghurt *spread.*

Little millet (%)	Flavour	Colour and appearance	Body and Texture	Spreadability	Overall Acceptability
Control	7.33[c]	7.22[c]	7.20[c]	7.18[b]	7.22[c]
1	8.01[b]	7.80[b]	7.77[b]	8.24[a]	7.70[b]
2	**8.71[a]**	**8.54[a]**	**8.60[a]**	**8.53[a]**	**8.51[a]**
3	8.36[ab]	8.20[ab]	8.07[b]	7.92[b]	8.00[b]
CD(P=0.05)	**0.38**	**0.40**	**0.25**	**0.56**	**0.33**

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

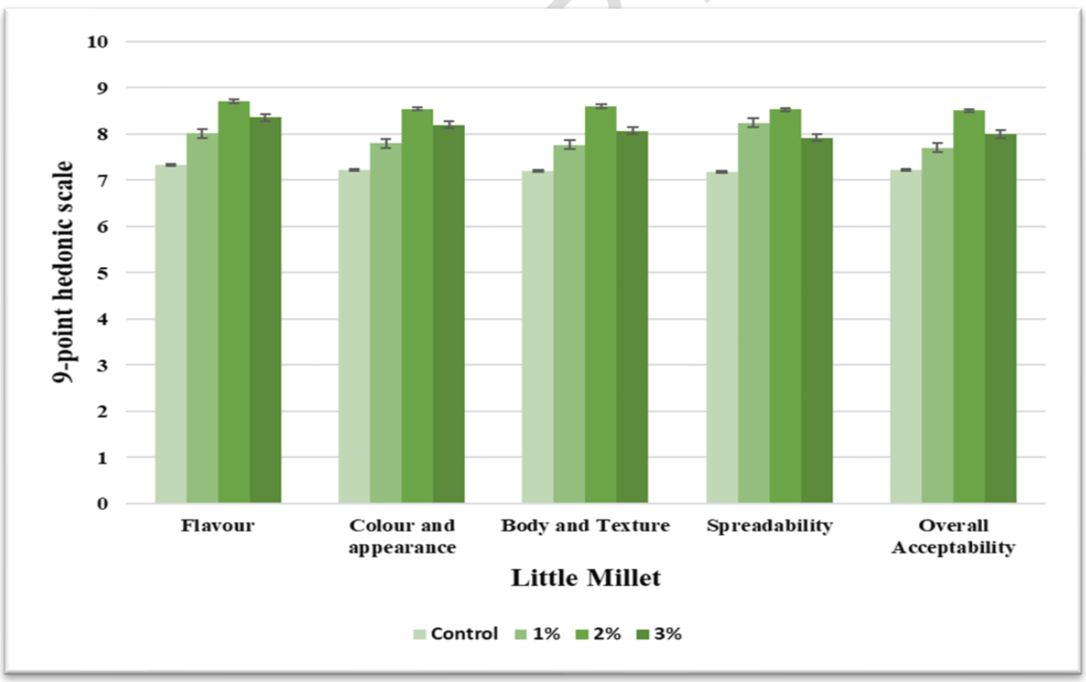

Fig 3: **Effect of little millet on the sensory attributes of Low-fat Greek yoghurt spread.**

4. CONCLUSION

In conclusion, the incorporation of whey protein concentrate (WPC) and little millet significantly improved the sensory attributes of low-fat functional Greek yoghurt spread. The 2% WPC sample demonstrated optimal flavor, texture, and overall acceptability, with a notable balance between firmness and creaminess. Similarly, the addition of 2% little millet enhanced flavor, color, texture, and spreadability, with the highest overall acceptability observed in this formulation. These findings suggest that both WPC and little millet contribute positively to the sensory qualities of Greek yoghurt spread, offering a promising approach for developing healthier, functional dairy spreads with improved nutritional and sensory profiles.

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COMPETING INTERESTS

The authors have declared that no competing interests exist. 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 the writing or editing of this manuscript.

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ABBREVIATIONS

ANOVA: Analysis of Variance
WHO: World Health Organization
FAO: Food And Agriculture Organization
WPC: Whey Protein Concentrate
IDF: Indian Dairy Federation