***Original Research Article***

**"Effect of Horse Gram Fortification on the Sensory and Functional Properties of Greek Yoghurt"**

**Abstract:**

This study aimed to develop a functional Greek yogurt fortified with horse gram *(Macrotyloma uniflorum)* and evaluate its sensory and functional properties. Greek yogurt was prepared using fresh cow milk, heated to 90°C for 5 minutes, followed by inoculation with 0.30% freeze-dried DVS yogurt culture and incubation at 45°C for 4 hours. After de-wheying, horse gram flour was incorporated at 2%, 4%, and 6% levels. Sensory analysis was conducted using a 9-point hedonic scale by a trained panel (n=6). Results showed that the sample fortified with 4% horse gram had the highest overall acceptability (8.27), flavor (8.28), and body and texture (8.40) scores, with no significant difference in color and appearance compared to the control (p > 0.05). Higher horse gram levels (6%) negatively impacted texture and flavor scores. The findings suggest that fortifying Greek yogurt with 4% horse gram enhances its nutritional value while maintaining desirable sensory attributes. This study demonstrates the potential for utilizing underutilized legumes in functional dairy product development.

**Keywords**: Greek yogurt, Horse gram, Sensory analysis, Functional dairy products.

**Introduction**

Legumes as good sources of proteins, carbohydrates, several water-soluble vitamins, and minerals, in general they make major contribution to human nutrition. Horse gram (*Macrotyloma uniflorum*) has been recognised as potential sources of protein and other nutrients. Horse gram is largely cultivated, especially in dry areas of Australia, Burma, India and Srilanka, mainly for animal feed. It is also used as a vegetable in India and is known as the poor man’s pulse crop in southern India. Horse gram belongs to the Macrotyloma genus, Phaseolinae sub-tribe, Phaseoleae tribe, Faboideae subfamily, Fabales order, Class of dicotyledons or Magnoliopsida, and subclass Rosi-dae (Ingle *et al.,* 2021). Locally, Horse gram is known as muthira, kulthi, hurali, madras gram, and gaheth. It is a member of the Fabaceae family (Sharma *et al.,* 2019). Horse gram has been applied as a tra-   
conventional medication to treat conditions such as kidney stones, urinary issues, jaundice, choleterol, fever, common cold, diabetes, throat infections, and piles (Kumar *et al.,* 2019; Prasad & Singh, 2015). Additionally, in Uttarakhand, seeds are utilized in a variety of ethnic recipes after being soaked to increase their digestibility prior to cooking (Bhartiya *et al.,* 2015). But like other green vegetables, Horse gram leaves are used in a variety of recipes and contain important components including vitamins, minerals, antioxidants, etc. (Bhartiya *et al.,* 2015).

Horse gram wheat has a good amount of antioxidants, including 1.2 mg/g ferric acid, 0.78 mg/g total phenol content, and 0.91 mg/g total flavonoid concentration.   
Proteins, fats, carbs, and dietary fiber are only a few of the many nutrients that are abundant in Horse gram seeds . Blood sugar regulation, which helps regulate diabetes, depends on specific dietary components, like as the carbohydrates in Horse gram. A healthy brain system also requires fats. In a similar vein, linoleic acid and other lipids are used to treat diabetes and heart disease.

Calcium, fiber, and phenolic compounds included in Horse gram seed coat aid with bone strength, digestive regulation, and the prevention of heart disease. Similarly, seeds   
  
are a good source of vitamins, minerals, lipids, proteins, carbs, amino acids, and calcium and potassium in particular (Sudha & Saral, 2023). Aspartic acid and glutamic acid are two of the most prevalent amino acids, and their combination has been shown to prevent the growth of tumor cells (Yamaguchi et al., 2016)

Recently, health-promoting and disease-preventing properties have been attributed to these photochemical with antinutrient effects, thus attracting more and more interest from both researchers and food manufacturer’s. Horse gram has been reported to have a lot of medicinal value. The rich fibre content of horse gram helps in reducing the body fat in fast mode. It is believed that consuming horse gram makes our body strong and is also good in treating kidney stones, menstrual problems, obesity, and curing cough and cold (Joshi and Awasthi, 2020; Haripriya et al., 2017; Jayapriya and Parameshwari, 2017).

The seeds and sprouts of horse gram are excellent examples of ‘functional food’ as it has role in lowering the risk of various diseases and exerting health promoting effects in addition to its nutritive value. Horse gram seed contains carbohydrate (57.2%), protein (22%), dietary fibre (5.3%), fat (0.50%), calcium (287mg), phosphorous (311mg), iron (6.77mg) and calories (321 Kcal) as well as vitamins like thiamine (0.4mg), riboflavin (0.2mg) and niacin (1.5mg) per 100g of dry matter .Horse gram has excellent therapeutic properties and traditionally used to cure kidney stones, asthma, bronchitis, leucoderma, urinary discharges, heart diseases, piles etc. Besides, it also possesses anti-diabetic, anti-ulcer activity and also helps in dietary management of obesity due to the presence of beneficial bioactive compounds (Bhartiya *et al*., 2015).

Greek Yoghurt 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. The industrial methods for Greek Yoghurt production involve whey removal by mechanical procedures to achieve the desired level of solidity. As outlined in the Greek Codex Alimentarius, strained yoghurt is produced from full-fat yoghurt by partially removing whey. When made from cow or goat milk, it must contain at least 5.6% protein, while sheep milk- based yoghurt requires a minimum of 8%. The production of Greek Yoghurt builds on the conventional yoghurt-making is notable for its white appearance, smooth texture, and mildly tangy taste. It is predominantly made from cow milk and involves bacterial fermentation *(Lactobacillus bulgaricus and Streptococcus thermophilus)* followed by whey removal from regular yoghurt to produce a thicker, creamier product with high solids content and a pronounced tangy flavour. In contrast, traditional greek yoghurt, a set-style variety, thickens naturally during fermentation without whey removal and can be made with cow, goat, or sheep milk. (Terpiłowski *et al*.,2023).

Greek yogurt has been chosen as a medium for fortifying horse gram due to its high protein content, probiotic benefits, and ability to enhance nutrient bioavailability. The fermentation process in yogurt reduces anti-nutritional factors in horse gram, improving mineral absorption. Its thick and creamy texture ensures better incorporation of horse gram without compromising sensory appeal. Additionally, Greek yogurt extends shelf life and provides a stable matrix for fortification. This combination results in a nutritious, functional food that supports gut health and meets consumer demand for protein- and fiber-rich dairy products.

**Materials and Methodology**

The following materials were used in this research study for the preparation of functional Greek yoghurt. Fresh Cow milk was procured from Students Experimental Dairy Plant (SEDP) of Dairy Science College, Hebbal, Bengaluru. Good quality Whey protein concentrate was procured from Nutrilac,DKSH India pvt ltd..Good quality of freeze dried starter culture was procured from Delvo DSL pvt Ltd, Netherlands. Greek yoghurt was made and it was added with horse gram ,blended and packaged using PET Cups The panel of 6 trained judges that was chosen assessed the generated study sample's sensory attributes, such as color and appearance, body and texture, flavor, and overall acceptability, using a 9-point hedonic scale. Statistical analysis was conducted using R software (R. version 4.0.3), (ANOVA). 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

Fresh cow milk

(Fat-4.5 % & SNF- 9.0 %)

Heat treatment (90 ̊C/no hold)

Cooling to 45 ̊C

Addition of Whey Protein Concentrate (**5%**)

Addition of **0.30**% freeze dried DVS culture at 1:1

(*Streptococcus thermophilus* and *Lactobacillus bulgaricus*)

Incubation (45°C/ 4h)

De-wheying (cloth bag filtration at 4°C/overnight)

Addition of Horse gram Flour (2, 4 and **6%**)

Blending and Packaging in PET cups(100ml)

Cooling and storage (7±1°C)

Flow chart 1 : Study protocol

**Results and discussion**

**Effect of supplementation of Horse gram** (*Macrotyloma uniflorum*) **on sensory characteristics of Functional Greek Yoghurt**

The functional Greek Yoghurt was prepared which was further added with horse gram with supplementation rate at 2, 4, and 6 per cent. The effects of horse gram on sensory attributes of RTE food were evaluated and the results are reflected in Table 1. As the incorporation level of horse gram in the product increased, the product exhibited non-significant effect on colour appearance which ranged from 8.37 to 7.17 for 2-6 per cent levels of horse gram whereas the control possessed colour and appearance score of 8.33. The body and texture scores were also significantly decreased upon addition of horse gram and the scores awarded for 4 % were 8.40, and the control had 8.30 scores. The best body and texture score was awarded highest for 4 % horse gram which exhibited non-significant scores. The flavour scores too had an increasing score for formulated product. The scores allotted 8.28 for 4 per cent levels of horse gram, respectively. Thus, the results of the study inferred that the best product could be obtained by supplementing horse gram at 4 per cent.

As per the observations, there was no statistical difference for colour and appearance when horse gram was blended from 2 to 4 per cent product. However, body and texture, flavour and overall acceptability had the significant influence. Similar trend has also observed for flavour attribute as well. Highest score for flavour was observed for 4 per cent horse gram incorporated product which had a pleasant nutty flavour. The overall acceptability score for the formulated product was 8.27 which reflect that this suggests that horse gram which is neglected or under-utilized can be exploited in development of various food products formulations that could result in acceptable form. The findings correlates with Niharika and Verma, 2016 and Sawant *et al*., 2015 for developing value added products from horse gram which received overall acceptability scores of 8.5 and 8.10 respectively. Kanhed *et al* 2023 emphasized the same.

**Table 1: Effect of supplementation of Horse gram** (*Macrotyloma uniflorum*) **on sensory characteristics of Functional Greek Yoghurt**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Levels of Horse Gram (%)** | **Colour & Appearance** | **Body & Texture** | **Flavour** | **Overall  Acceptability** |
|  | **Scores on 9-point hedonic scale** | | | |
| Control | 8.33a | 8.30a | 8.32a | 8.33a |
| 2 | 8.37a | 8.30a | 8.31a | 8.31a |
| 4 | 8.80a | 8.40a | 8.28a | 8.27a |
| 6 | 7.17a | 7.67b | 7.09b | 7.90b |
| CD (*P =* .05) | 0.25 | 0.35 | 0.27 | 0.30 |

Note:

\* All values are average of three trails

Similar super scripts indicate non-significance at the corresponding critical difference (CD)

**Figure 1: Effect of supplementation of Horse gram** (*Macrotyloma uniflorum*) **on sensory characteristics of Functional Greek Yoghurt**

**Conclusion:** The present study demonstrated that Greek yogurt fortified with horse gram (Macrotyloma uniflorum) at 4% exhibited the best sensory acceptability, with an overall score of 8.27, making it a viable option for functional dairy product development. The incorporation of horse gram significantly enhanced the nutritional profile of Greek yogurt while maintaining desirable sensory attributes, particularly in terms of flavor (8.28) and body and texture (8.40). Higher incorporation levels (6%) negatively impacted sensory characteristics, indicating that 4% is the optimal fortification level. The results highlight the potential of utilizing underutilized legumes like horse gram in dairy applications to improve protein and fiber intake. This study provides a foundation for further research into the functional and health benefits of horse gram-fortified dairy products, with potential implications for addressing nutritional deficiencies and promoting sustainable food innovations.

**Disclaimer (Artificial intelligence)**

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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 the writing or editing of this manuscript.

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Details of the AI usage are given below:

1.

2.

3.

**References:**

1. Bhartiya, J. P., Aditya, & Kanth, L. (2015). Nutritional and remedial potential of an under-utilized food legume horse gram—A review. *The Journal of Animal and Plant Science, 25*(4), 908-920.
2. Haripriya, A., & Radhika, V., & Kavitha, K. (2017). Impact of pre-treatments on the selected nutrient and anti-nutrient profile of horse gram-based traditional recipes. *International Journal of Home Science, 3*(1), 415-419.
3. Ingle, K. P., Al-Khayri, J. M., Chakraborty, P., Narkhede, G. W., & Suprasanna, P. (2021). Bioactive compounds of horse gram (*Macrotyloma uniflorum* Lam. [Verdc.]). In H. N. Murthy & K. Y. Paek (Eds.), *Bioactive Compounds in Underutilized Vegetables and Legumes* (pp. 583–621).
4. Jayapriya, M., & Parameshwari, S. (2020). Physicochemical, nutritive and sensory attributes of value-added horse gram incorporated cookies and chapati. *Indian Journal of Public Health Research & Development, 11*(5).
5. Joshi, H., & Awasthi, P. (2020). Evaluation of physical properties and sensory attributes of biscuits developed from whole wheat flour supplemented with horse gram flour. *Journal of Pharmacognosy and Phytochemistry, 9*(5), 1652-1656.
6. Kanhed, M. I., Harinivenugopal, Arunkumar, H., Manjunatha, H., & BG, S. (2023). Formulation of WPC-enriched ready-to-serve yogurt smoothie. *International Research Journal of Modernization in Engineering Technology and Science, 5*(5), 2582-5208.
7. Kumar, D. S., Prashanthi, G., Avasarala, H., & Banji, D. (2013). Antihypercholesterolemic effect of *Macrotyloma uniflorum* (Lam.) Verdc (Fabaceae) extract on high-fat diet-induced hypercholesterolemia in Sprague-Dawley rats. *Journal of Dietary Supplements, 10*(2), 116–128.
8. Niharika, & Verma. (2016). Development and sensory evaluation of value-added products incorporating germinated horse gram powder. *International Journal of Multidisciplinary Research and Development, 3*(5), 55-58.
9. Prasad, S. K., & Singh, M. K. (2015). Horse gram—An underutilized nutraceutical pulse crop: A review. *Journal of Food Science and Technology, 52*, 2489–2499.
10. Sharma, N., Bisht, S. S., Gupta, S., Rana, M., & Kumar, A. (2019). Nutraceutical evaluation of horse gram (*Macrotyloma uniflorum*) cultivated in high altitudes of Uttarakhand Himalaya, India. *Indian Journal of Pure and Applied Biosciences, 7*, 190–202.
11. Singh, B., Kumar, A., Singh, H., Kaur, S., Arora, S., & Singh, B. (2022). Protective effect of vanillic acid against diabetes and diabetic nephropathy by attenuating oxidative stress and upregulation of NF-κB, TNF-α, and COX-2 proteins in rats. *Phytotherapy Research, 36*(3), 1338–1350.
12. Sudha, S., & Saral, A. M. (2023). Studies on phytochemical, mineral content, in vitro anti-urolithiatic and anti-diabetic activities of horse gram flour extracts and its biosynthesized Ag nanoparticles. *Heliyon, 9*(6), e16572.
13. Terpiłowski, K., Lange, I., Kowalczyk, K., Tomczyńska-Mleko, M., Sapiga, V., & Wesołowska, A. (2023). Impact of storage conditions of yogurt dry ingredients on the physicochemical properties of the final product. *Applied Sciences, 13*(24), 13201.
14. Yamaguchi, Y., Yamamoto, K., Sato, Y., Inoue, S., Morinaga, T., & Hirano, E. (2016). Combination of aspartic acid and glutamic acid inhibits tumor cell proliferation. *Biomedical Research, 37*(2), 153–159.

**ANNEXURE**

**DAIRY SCIENCE COLLEGE, KVAFSU, BENGALURU-24**

**DEPARTMENT OF DAIRY TECHNOLOGY**

**Score card for Sensory Evaluation Using 9-Point Hedonic Scale**

**Name of the Judge: Date:**

You are requested to assess the product in terms of general acceptability on a 9-point hedonic scale score system.

**score system:**

Like extremely 9

Like very much 8

Like moderately 7

Like slightly 6

Neither like nor dislike 5

Dislike slightly 4

Dislike moderately 3

Dislike very much 2

Dislike extremely 1

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sensory Characteristics** | **Sample Code** | | | | | |
|  |  |  |  |  |  |
| Color and Appearance |  |  |  |  |  |  |
| Body and Texture |  |  |  |  |  |  |
| Flavour |  |  |  |  |  |  |
| Overall Acceptability |  |  |  |  |  |  |

**Comments: Signature**