International Journal of Agriculture and Food Science 2025; 7(3): 134-139

ISSN Print: 2664-844X
ISSN Online: 2664-8458
NAAS Rating: 4.97
IJAFS 2025; 7(3): 134-139
www.agriculturaljournals.com
Received: 07-01-2025
Accepted: 13-02-2025

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Development of a healthy whey protein-based pumpkin seed bar

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DOI: https://www.doi.org/10.33545/2664844X.2025.v7.i3b.324

Abstract
This study explores the development of a nutritious whey protein-based pumpkin seed bar, formulated to enhance protein intake through natural, functional ingredients. Various formulations incorporating different proportions of pumpkin seeds and dates, along with whey protein, were evaluated for their physico-chemical properties, sensory attributes, and nutritional content. The optimized formulation (T_4), comprising 120 g of pumpkin seeds and 80 g of dates, demonstrated superior sensory performance with an overall acceptability score of 8.6 on a 9-point hedonic scale. Additionally, proximate analysis revealed that the optimized bar contained 32.51% protein, 23.75% fat, and 26.33% carbohydrates, with the protein content significantly higher than that of many conventional snack bars. Statistical analysis confirmed that the variations in ingredient ratios had a significant impact on both sensory and nutritional properties ($p< 0.05$). Furthermore, a cost estimation study indicated that the developed product is economically feasible for large-scale production. Overall, the whey protein-based pumpkin seed bar offers a balanced nutritional profile and appealing sensory characteristics, making it a promising functional snack for health-conscious consumers.

Keywords: Pumpkin seeds, whey protein, nutritional evaluation, sensory analysis, functional foods

Introduction
Nutrient-dense snack bars are gaining popularity due to their convenience and health benefits. Whey protein is a high-quality protein source, widely recognized for its biological and functional properties (Gangurde *et al*., 2011) [5]. It contains essential amino acids and bioactive peptides that offer various health benefits, including muscle synthesis, immune enhancement, and antioxidative effects (Patel, 2015) [6]. In addition, whey protein has been associated with reducing inflammation and improving metabolic health (Solak *et al*., 2012) [7].

Pumpkin seeds (*Cucurbita pepo*) are another valuable ingredient, offering high levels of protein, healthy fats, and essential micronutrients such as magnesium, zinc, and iron (Ahmad and Khan, 2019) [1]. The seeds are known for their bioactive properties, including antioxidant, anti-inflammatory, and cardioprotective effects (Dar *et al*., 2017) [3]. Due to their nutritional profile, pumpkin seeds have been increasingly utilized in functional food formulations (Syed *et al*., 2019) [8]. Incorporating these seeds into snack bars enhances their health benefits while maintaining good sensory attributes.

One of the critical concerns in modern diets is protein deficiency, particularly in developing nations, where access to high-protein foods is limited (Wang *et al*., 2021) [9]. The development of whey protein-based snack bars offers a viable solution to this issue, providing a portable, protein-rich alternative. In recent years, there has been a growing trend toward replacing refined sugars with natural sweeteners like dates, which not only enhance the taste but also offer additional fiber, vitamins, and minerals (Farooqui *et al*., 2022) [4]. Dates provide natural sugars that are easily digestible and serve as an excellent binding agent in protein bars (Al-Farsi *et al*., 2008) [2].

This study aims to develop a healthy whey protein-based pumpkin seed bar and evaluate its physico-chemical properties, sensory acceptability, and nutritional composition. By optimizing ingredient combinations and assessing the feasibility of large-scale production, this research contributes to the advancement of functional food products that promote overall health and well-being.

Materials and Methods
This section details the materials, equipment, and methodologies employed in the formulation, preparation, and evaluation of the whey protein-based pumpkin seed bar. The process involved multiple stages, including ingredient selection, bar preparation, experimental trials, and subsequent analysis of sensory and nutritional properties.

Raw Materials
The key ingredients used in this study were selected based on their nutritional properties and functional benefits:

1. **Pumpkin Seeds (*Cucurbita pepo*):** Rich in protein, fiber, and essential minerals such as magnesium, zinc, and iron. The seeds were procured from local markets and were manually cleaned and roasted before use.
2. **Whey Protein:** A high-quality protein source obtained from dairy, known for its high biological value and essential amino acid content. Commercial whey protein concentrate was used in the formulations.
3. **Dates (*Phoenix dactylifera*):** A natural sweetener, providing fiber, vitamins, and minerals. Medjool dates were selected for their high sugar content and binding properties.
4. **Dry Fruits (Almonds and Cashews):** Incorporated to enhance the texture and provide additional micronutrients.
5. **Ghee (Clarified Butter):** Used to improve the binding of ingredients and enhance flavor.

The primary ingredients used in this study include pumpkin seeds, whey protein, and dates. Pumpkin seeds were selected for their high protein content and essential micronutrients. Whey protein was used as the primary protein source, enhancing the bar's nutritional profile. Dates were incorporated as a natural sweetener and binding agent. Additional ingredients such as dry fruits and ghee were included to improve texture and taste.

Equipment Used
The following equipment was employed to process ingredients and develop the pumpkin seed bar:

1. **Weighing Balance:** Used for accurate measurement of ingredients to ensure consistency across formulations.
2. **Food Processor:** Utilized for grinding dry ingredients, ensuring uniform particle size for improved mixing and texture.
3. **LPG Gas Stove:** Used for roasting pumpkin seeds and dry fruits, enhancing their flavor and reducing moisture content.
4. **Pan and Spatula:** Employed for mixing ingredients under controlled heating conditions to facilitate binding.
5. **Molding Tray and Knife:** Used for shaping and cutting the bars into uniform sizes after cooling.

Preparation of Whey Protein
The whey protein used in this study was obtained from standardized milk through a coagulation and separation process. The preparation steps was shown in Figure 1 and describes as follows:

1. Fresh cow's milk was heated to 90°C and maintained for 10 minutes.
2. Coagulation was induced using 5 ml Vinegar, leading to the separation of curds and whey.
3. The whey was collected and filtered using 125µ ISI sieves to remove residual solids.
4. The filtered whey was dried at 60°C in a hot air oven to obtain whey protein powder.

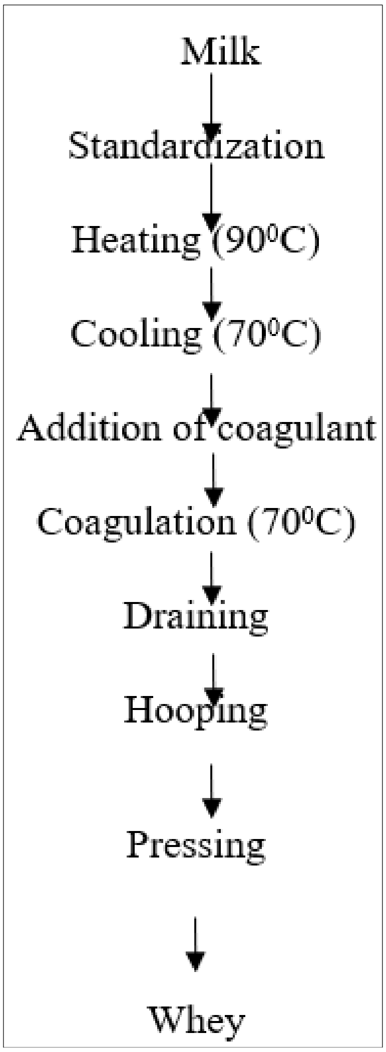

Fig 1: Flowchart of Whey Preparation

Formulation of Pumpkin Seed Bar
The pumpkin seed bar was formulated through a systematic blending of ingredients, ensuring proper binding and texture. The stepwise preparation discussed in Figure 2 and follows below steps:

1. **Roasting:** Pumpkin seeds and dry fruits were roasted separately at 50 °C for 10 minutes to enhance flavor and reduce moisture.
2. **Grinding:** Roasted pumpkin seeds were coarsely ground using a food processor.
3. **Date Paste Preparation:** Dates were soaked in warm water for **10 minutes**, blended into a paste, and mixed with whey protein.
4. **Mixing and Heating:** The dry ingredients were combined with the date paste and ghee in a pan, heated at **50°C**, and stirred continuously until a uniform dough-like consistency was achieved.
5. **Molding and Cooling:** The mixture was transferred to a molding tray, pressed evenly, and cooled at room temperature for 2 hours before being cut into bars.

Pumpkin seeds and dry fruits were roasted, ground, and mixed with dates to form a dough-like consistency. Whey protein was added, and the mixture was molded, cooled, and cut into uniform bars.

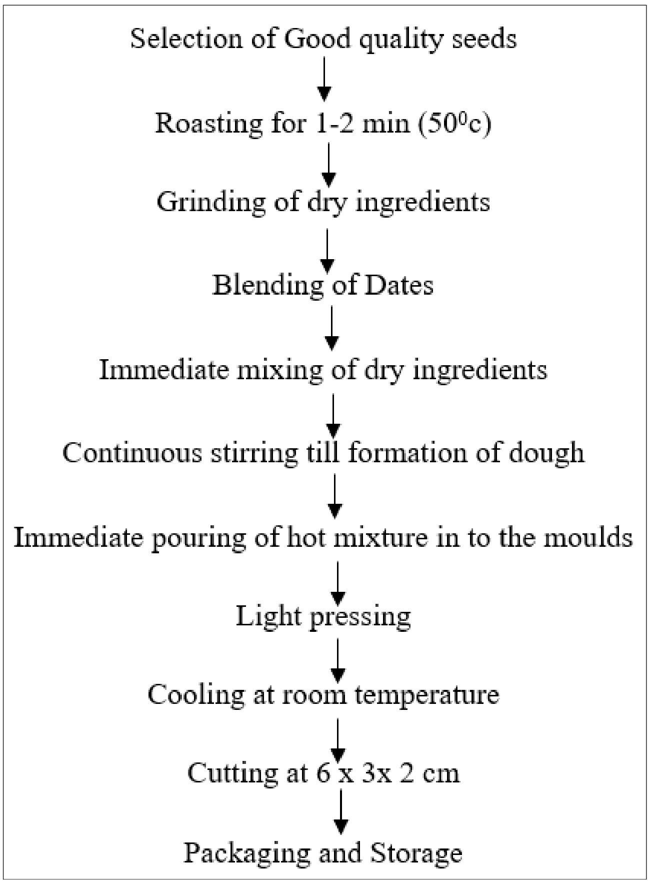

Fig 2: Flowchart of Pumpkin Seed Protein Bar Preparation

Experimental Trials

Six formulations (T_1-T_6) with varying proportions of pumpkin seeds and dates were prepared to determine the optimal blend. The key variations of all formulations were given in Table 1.

Table 1: Combination of Pumpkin Seeds and Dates

Treatments	Replications	Pumpkin seed (g)	Dates (g)	Whey protein (g)	Dry fruits (g)
T_1	R1	150	50	40	50
	R2	150	30	40	50
	R3	150	10	40	50
T_4	R1	120	40	40	50
	R2	120	60	40	50
	R3	120	80	40	50
T_6	R1	90	70	40	50
	R2	90	90	40	50
	R3	90	110	40	50

The formulated bars were then subjected to sensory evaluation by a trained panel using a 9-point hedonic scale, where scores ranged from 1 (dislike extremely) to 9 (like extremely) described in Table 2. The evaluation criteria included:

- Appearance
- Texture
- Taste
- Mouthfeel
- Overall acceptability

Table 2: Sensory Evaluation Scale

Scale	Score
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 Extreme	1

Analytical Methods

To ensure a comprehensive assessment of the developed bars, various analytical methods were employed:

1. **Proximate Analysis:** The bars were analyzed for moisture, ash, fat, protein, carbohydrate, fiber, and sugar content using standard AOAC (Association of Official Analytical Chemists) methods.
2. **Sensory Evaluation:** A panel of 10 trained individuals assessed the sensory characteristics, and data were recorded for statistical analysis.
3. **Statistical Analysis:** Data collected from sensory and nutritional evaluation were subjected to ANOVA (Analysis of Variance) to identify statistically significant differences among formulations. Duncan's multiple range test was applied to determine the optimal formulation with the best sensory attributes.

Results and Discussion

This section presents a comprehensive analysis of the data collected from the experimental trials, including sensory evaluation, nutritional composition, statistical analysis, and cost estimation. The discussion integrates the findings with literature insights and highlights the potential of the developed product.

Sensory Evaluation

Sensory evaluation was conducted using a 9-point hedonic scale where 1 indicated "dislike extremely" and 9 "like extremely." A trained panel of 10 individuals evaluated the six formulations (T_1-T_6) based on several parameters, including appearance, texture, taste, mouthfeel, and overall acceptability.

Appearance and Texture

The visual appeal of the bars was a critical factor in consumer acceptability. Formulations with a higher proportion of dates tended to be overly glossy and sticky, while those with lower dates content appeared drier. Notably, formulation T_4 (120g pumpkin seeds and 80g dates) exhibited an optimal balance, resulting in a bar with a pleasant color and an even, cohesive texture. Panellists reported that T_4 had a crisp exterior combined with a soft interior, which contributed significantly to its favourable ratings.

Taste and Mouthfeel

Taste tests revealed that the natural sweetness of dates complemented the nutty flavor of roasted pumpkin seeds. Formulation T_4 scored highest in taste (average score of 8.8) and mouthfeel (8.6), as its balanced composition avoided the pitfalls of being too sweet or too dry. In contrast, formulations with either an excess of pumpkin seeds (resulting in a bitter aftertaste) or dates (overly sweet and sticky texture) scored lower.

Overall Acceptability

When considering all sensory parameters, T_4 achieved an average overall acceptability score of 8.6. This suggests that the 60:40 ratio of pumpkin seeds to dates is ideal for maintaining the nutritional benefits of the ingredients while ensuring a product that is both palatable and appealing.

Table 3 summarizes the scores for all formulations, and Figure 3 visually depicts the superior performance of T_4 compared to the other formulations.

Table 3: Sensory Evaluation Results

Sensory attributes							
Sr. No	**Treatment**	**Appearance**	**Taste/ Flavor**	**Texture**	**Mouth Feel**	**Colour**	**Overall acceptability**
1.	T_1	7.4	7	7.5	7.3	7	7.7
2.	T_2	6.7	6.6	7	7.1	6.9	7.2
3.	T_3	7.2	8.1	7.4	7	6.7	7.1
4.	T_4	8.8	8.9	8.6	8.1	8.4	8.6
5.	T_5	6.7	7.3	6.7	6.9	6.3	6.5
6.	T_6	6.8	7.1	6.1	6.7	6.2	6.6

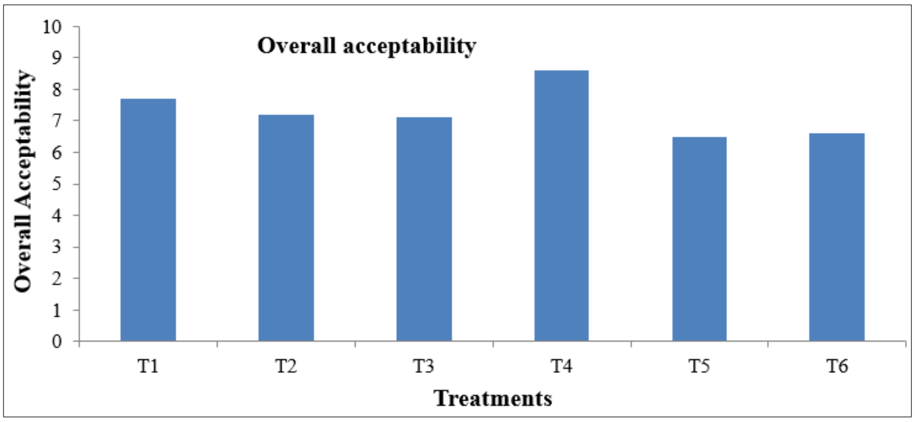

Fig 3: Sensory Parameter Comparisons

Nutritional Composition

The proximate analysis of the optimized formulation (T_4) was performed to assess its nutritional quality. The results are indicated the Table 4.

Table 4: Proximate analysis of optimized formulation of the pumpkin seed bar (100 g)

Sr. No	**Test parameters**	**T_4**		
		R1	**R2**	**R3**
1	Moisture (%)	10.33	10.32	10.34
2	Ash (%)	1.63	1.62	1.64
3	Protein (%)	32.51	32.48	32.54
4	Total Carbohydrates (%)	26.33	25.31	26.35
5	Energy (Kcal)	923.33	923.27	923.39
6	Fiber (%)	7.6	7.59	7.61
7	Fat (%)	23.75	23.76	23.74
8	Total Sugar (%)	19.3	19.28	19.32

Protein Content

Formulation T_4 contained 32.51% protein per 100g, which is significantly higher than many conventional snack bars. This high protein content is largely attributed to the whey protein addition as well as the intrinsic protein in pumpkin seeds. Such a profile is particularly beneficial for athletes and individuals seeking to increase their protein intake for muscle repair and growth.

Fat and Carbohydrates

The fat content was measured at 23.75%, primarily deriving from the natural oils in pumpkin seeds. These fats include essential fatty acids that are beneficial for cardiovascular health. Carbohydrate content was recorded at 26.33%, with dates providing a natural source of sugars that supply a sustained energy release. The balance between fats and carbohydrates ensures that the bar is not only nutritious but also provides an immediate energy boost without a rapid spike in blood sugar.

Fiber and Moisture

A total fiber content of 7.6% was observed, which supports digestive health and promotes satiety. The moisture content of 10.33% helps in maintaining the shelf-stability of the product without compromising the texture.

Statistical Analysis

Statistical validation of the sensory and nutritional data was performed using Analysis of Variance (ANOVA). The key findings include:

Significant Differences: The ANOVA results revealed statistically significant differences ($p < 0.05$) among the different formulations regarding both sensory attributes and nutritional composition. This indicates that the variations in pumpkin seed and date ratios have a pronounced effect on the overall quality of the bar.

Optimal Formulation Identification

Duncan's multiple range test was applied to further compare the formulations, confirming that formulation T_4 is statistically superior in terms of taste, texture, and overall acceptability

Table 5 summarizes these statistical findings, reinforcing the selection of T_4 as the optimal formulation.

Table 5: ANOVA Results

Sr. No.	Test Parameters	T_1	T_4	T_6	Control	SE	CD
1.	Moisture (%)	8.250	10.33	12.33	8.7	0.0208	0.0987
2.	Total Ash (%)	1.9667	1.63	2.53	8.38	0.0066	0.0316
3.	Total Fat (%)	33.966	23.75	30.21	23.49	0.0174	0.0825
4.	Total Carbohydrates (%)	21.09	26.33	25.33	13.47	0.16447	0.78048
5.	Protein (%)	32.326	32.51	29.66	23.94	0.3351	1. 590
6.	Energy (Kcal)	882.9	923.3	986.65	387.89	0.0611	0.2903
7.	Total Fiber (%)	5.89	7.6	6.9866	8.4	0.0206	0.0981
8.	Total Sugar (%)	16.3	19.3	28.63	17.89	0.0301	0.1430

Cost Estimation

An economic analysis was conducted to evaluate the production cost of the developed bar, ensuring its commercial viability:

Ingredient and Production Costs

The cost estimation considered the prices of pumpkin seeds, whey protein, dates, and other ingredients, as well as processing and packaging expenses. The analysis revealed that the production cost per bar is significantly lower than that of many commercial protein bars, largely due to the local sourcing of raw materials and efficient processing techniques.

Scalability and Market Feasibility

Bulk procurement and economies of scale can further reduce costs, making the product competitive in the market. The cost analysis supports the notion that the developed bar is not only nutritionally superior but also economically feasible for large-scale production.
Table 6 provides a detailed cost breakdown.

Table 6: Cost Estimation of Developed Product

Sr. No.	Ingredients	Quantity	Cost
1	Pumpkin seeds@570/kg	120 g	69
2	Whey protein @800/kg	50 g	40
3	Dates @180/kg	80 g	15
4	Dry fruits @Rs. 500/kg	50 g	25
5	Labour charges @Rs.250/8hrs	2 hrs	63
6	Packaging @ 20 Rs 100 pouch	20	5
Total cost			217

Functional Benefits

The integration of whey protein and pumpkin seeds results in a product that offers multiple health benefits:

Health Implications

High protein intake from the developed bar can aid in muscle repair and growth, which is particularly beneficial for athletes and active individuals. Moreover, the presence of bioactive peptides in whey protein has been linked to improved immune function and antioxidative properties (Patel, 2015; Solak *et al.*, 2012) [6, 7].

Nutritional synergy

Pumpkin seeds contribute not only proteins but also essential micronutrients and healthy fats, which together support cardiovascular health, cognitive function, and overall well-being (Ahmad and Khan, 2019; Dar *et al.*, 2017) [1, 3]. The natural sugars and fiber from dates further enhance the bar's nutritional profile, making it a balanced snack that provides both immediate energy and sustained nutritional benefits.

Consumer Appeal

The absence of artificial additives and preservatives, combined with the use of natural ingredients, enhances the product's appeal among health-conscious consumers. Additionally, the sensory attributes such as taste, texture, and appearance align with consumer expectations for high-quality, functional foods.
In summary, the results and discussion clearly indicate that formulation T_4 not only meets but exceeds expectations in terms of sensory acceptability and nutritional value. The statistical and cost analyses further validate its potential for commercialization as a health-promoting, protein-rich snack.

Conclusion

The present study successfully developed a whey protein-based pumpkin seed bar that meets both nutritional and sensory expectations. The product combines high-quality whey protein and nutrient-dense pumpkin seeds with natural sweetening from dates, resulting in a functional food item that offers significant health benefits. Detailed analyses revealed that the optimized formulation (T_4), consisting of 120g pumpkin seeds and 80g dates, exhibited superior sensory attributes, including an appealing appearance, balanced taste, desirable texture, and overall high acceptability among the panelists.
Nutritional analysis demonstrated that the optimized bar is rich in protein (32.51%), making it an excellent option for individuals seeking to enhance muscle repair and growth. The inclusion of pumpkin seeds also contributes beneficial fats, essential micronutrients, and dietary fiber, while the natural sugars from dates provide a sustained energy release. Together, these components create a balanced macronutrient profile that can help address common nutritional deficiencies, particularly in populations with limited access to high-protein foods.
Statistical evaluations, including ANOVA confirmed that the formulation variations significantly impacted both sensory and nutritional qualities. The data consistently indicated that formulation T_4 is optimal, with significant improvements in taste, texture, and overall acceptability. Furthermore, the cost analysis suggested that the product is economically viable for large-scale production, as the production cost per unit is competitive compared to many commercial protein bars.
In summary, the developed whey protein-based pumpkin seed bar not only fulfills the nutritional requirements but also satisfies consumer preferences in terms of taste and texture. Its production using locally sourced ingredients and natural additives underscores its potential for commercial

success and widespread consumer acceptance. Future research should focus on extended shelf-life studies, larger-scale consumer testing, and exploring additional functional ingredients to further enhance the product's health benefits. This study lays a solid foundation for the development of functional, nutrient-rich snack bars that can contribute positively to public health and nutrition.

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