

---

## ABSTRACT

---

*"यदि वयं प्रत्येकं व्यक्तिं सम्यक् पोषणं व्यायामं च दातुं शक्नुमः, न तु अल्पं न च अधिकं, तर्हि अस्माकं स्वास्थ्यस्य सुरक्षिततमः मार्गः स्यात्।"*

"If we could give every individual the right amount of nourishment and exercise, not too little and not too much, we would have the safest way to health."

-Hippocrates (Gazettedoi, 2019).

The increasing trend of physical activity and dedicated lifestyles has sparked a keen interest in foods that can positively impact the health and fitness routines of health-conscious, active individuals. This group, with its specific nutritional needs, emphasizes the importance of consuming enough protein and energy. Due to their increased activity levels, these individuals are at risk of lacking essential nutrients, potentially leading to decreased physical performance and overall health decline. Consequently, people are seeking convenient on-the-go food options that offer substantial nutrient levels in a compact and cost-effective manner. To bridge the gap between optimizing nutrient intake and exercise routines, protein emerges as a pivotal element. As muscles endure stress and damage during exercise, protein intake becomes essential for repair and rebuilding, fostering muscle growth and enhancing overall physical function. However, recognizing the importance of protein is just the beginning; the real challenge lies in providing this vital nutrient in a convenient and enjoyable form that meets the diverse needs and preferences of athletes and fitness enthusiasts. Protein bars have become increasingly popular as a solution, offering a portable and easy-to-consume source of essential nutrients. These bars cater to the needs of individuals with limited time for meal preparation or consumption, providing a practical option rich in proteins, carbohydrates and fats.

The International Olympic Committee (IOC), the American Council of Sports Medicine (ACSM) and the International Society for Sports Nutrition (ISSN) provide general dietary guidelines for athletes. These guidelines are dependent on the kind, level of intensity and duration of physical activity and are not specific to any one sport. On

the other hand, performance-based dietary guidelines tailored to different sports are created by the Sports Authority of India (SAI), National Institute of dietary (NIN) and International Life Sciences Institute (ILSI). As to the IOC's summary of common sports food, protein-rich bars are generally low in carbs and provide 20–50 g of protein per serving, either from premium animal sources like whey, casein, milk and eggs, or vegetable sources like soy.

Maximizing the nutritional regimen for athletes requires the development of sports-specific, convenient and cost-effective foods that excel in visual appeal, taste, texture and shelf life, ensuring high acceptability. Athletes are more likely to adhere to their dietary plans when they find the taste and texture of food products enjoyable. Crafting protein bars entails a delicate balance between nutritional quality and sensory appeal, necessitating innovative approaches for formulation optimization. Response Surface Methodology (RSM) provides a structured framework to explore the synergistic effects of ingredients, concentrations and processing parameters on the final product. By leveraging RSM, this study aims to refine the protein bar development process, harmonizing nutritional content with consumer preferences. The primary aim of this study is to utilize RSM to create protein-energy bars that address the macronutrient needs of individuals involved in fitness activities. This entails optimizing the bars to meet both nutritional standards and customer expectations in terms of sensory appeal, affordability and shelf life.

With this objective in mind, the current study titled "Development, Optimization and Characterization of Protein-Energy Bars Using Response Surface Methodology" unfolded in four phases. Initially in Phase I, an online market survey was conducted to evaluate the availability of diverse food bars tailored for Indian consumers. This survey aimed to identify various brands offering food bars and examine their essential attributes, including protein content, serving size and average cost. Phase II focused on Procuring the raw materials from local markets, online platforms and suppliers, followed by pre-treatment processes like extrusion, cleaning, roasting, dehusking and milling to prepare them for analysis. The nutritional composition of the raw materials was assessed according to the Proximate principles outlined in the AOAC standard protocol. Subsequently, ingredient standardization was achieved using the IFCT (Indian Food Composition Tables) 2017, NIN (National Institute of Nutrition) food

composition database and ingredient optimization was performed using Response Surface Methodology (RSM) software. In Phase III, sensory evaluation was carried out by a semi-trained panel using Composite Score Card (CSC) and 9-Point Hedonic Scale (9-PHS). Proximate analysis, adhering to AOAC standards, determined protein, crude fat, crude fiber, moisture and ash content, while carbohydrate content was calculated using the difference method and energy values were obtained from FDA-regulated labelling requirements. Physicochemical properties were assessed based on AOAC standards and texture analysis was conducted using a Texture Analyser. Microbial analysis, following IS standards, tested for yeast, mold, total coliforms and total bacteria. Additionally, an accelerated shelf-life study was conducted to evaluate product stability and quality under elevated temperature conditions. Phase IV involved an acceptability trial with 120 physically active individuals recruited from the Physical Education Department of The Maharaja Sayajirao University of Baroda and various gymnasiums in the Vadodara city, utilizing a questionnaire and a 9-point Hedonic scale to gauge the food bars' acceptability among participants.

The survey analyzed 250 food bars from 32 brands, grouped into eight categories based on their suggested usage. High-protein bars, comprising 38.2% of the sample, offered over 20g of protein per serving with an average size of 63.82g and were priced highest at Rs 118.71 per bar. Whey Protein Concentrate (WPC) emerged as the primary protein source (39.3%), followed by Soy Protein Isolate (SPI) (29.8%) and Whey Protein Isolate (WPI) (19.9%), showing varied preferences in protein sources among consumers. Utilizing Response Surface Methodology (RSM), various combinations of Crispies, WPC-80 and SPI were examined for formula development. Crispies, developed through extrusion technology, contained approximately 68.36g carbohydrates, 23.22g protein and 2.05g fat per 100g. WPC-80 had 79.04g protein per 100g, while SPI had 89.29g protein per 100g. RSM analysis using CCRD suggested an optimal combination of 7.3% Crispies, 14.5% WPC-80 and 13.7% SPI, achieving a desirability of '1'. Predicted values closely matched actual values, indicating successful optimization of sensory attributes and nutritional composition. Statistical validation via the 't' test confirmed the efficacy of the suggested solution in optimizing sensory responses for Protein-Energy Bar production. Comprehensive sensory analysis using CSC and 9-PHS revealed significant effects of ingredients on appearance, taste, flavor, texture and mouthfeel. Process optimization aimed to identify the ideal combination of

Crispies, WPC-80 and SPI levels, with statistical analysis demonstrating significant effects on sensory attributes, aligning predicted and actual values. The proximate composition analysis of the P-E Bars at a 70g serving size revealed favorable characteristics: low moisture content of 4.43g ensuring shelf stability, high energy content of 354 Kcal (5 Kcal/g) suitable for physical activity, significant protein content at 25.07% promoting muscle recovery, carbohydrate content of 33.35% providing essential energy, crude fat of 12.45% contributing to energy density and satiety, crude fiber of 4.13% and ash content of 1.60%. Nitrogen content was 3.63%, indicating the protein-rich nature of the bars. The Acid Value as 0.63, Peroxide Value as 0.60 mEq of O<sub>2</sub>/kg fat, Free Fatty Acid as 0.32% oleic acid and Water Activity as 0.46 aw, indicates freshness, low oxidation, desirable freshness levels and low microbial growth risk, respectively. The production cost for 10 kg of P-E Bars, translated to cost per piece (70g), was determined to be INR Rs. 36.41.

During storage under accelerated condition of  $35 \pm 2$  °C and 70 % Relative Humidity for 70 days, Peroxide Value increased to 2.17 mEq of O<sub>2</sub>/kg fat, FFA rose to 1.61% oleic acid and Acid Value increased to 3.19, while Water Activity rose to 0.60, all remaining within acceptable limits. Despite compositional changes, microbial stability was maintained, ensuring product safety. The bars exhibited desirable texture with a hardness increasing from 23.23 N to 116.87 N and fracturability decreasing from 22.10 mm to 0.30 mm over the storage period, indicating increased resistance to deformation and reduced susceptibility to fracturing. Organoleptic analysis showed significant ( $p \leq 0.05$ ) declines in visual appeal, taste, flavor, texture, mouthfeel and overall sensory scores for P-E Bars. Visual appeal scores decreased from  $8.11 \pm 0.32$  to  $7.22 \pm 0.73$  on the CSC and from  $7.83 \pm 0.51$  to  $5.67 \pm 0.77$  on the 9-PHS. Though there was a significant decrease in mouthfeel perception, overall sensory scores remained above 5.0, indicating a moderate level of acceptability even after storage. The initial Total Bacterial Count (TBC) of fresh P-E Bars was  $1.5 \pm 0.083$  log<sub>10</sub> cfu/g, with low moisture levels (water activity of  $0.46 \pm 0.02$  and moisture content of  $6.33 \pm 0.07$  percent). Over the storage at (ASL), TBC increased to  $2.50 \pm 0.02$ , indicating microbial growth, though no E. coli, yeast, or mold were detected, suggesting sanitary manufacturing conditions and high-quality ingredients.

The acceptability trials with 120 participants comprised of physically active individuals, aged 18-25, primarily consisting of 60% males and 40% females engaged in regular exercise. Preferences for snacks leaned towards healthy options, with a high familiarity with sports bars. Participants valued taste, nutritional value and price, with most preferring a budget range of 80-100 Rupees for sports bars. Feedback showed high satisfaction levels with the study bar, with 98.33% intending to repurchase and 100% willing to recommend it. Ratings using a 9-point hedonic scale revealed positive perceptions of taste, flavor and serving size, though texture scored slightly lower, suggesting room for improvement. Overall, participants responded positively to the sensory attributes of the sports bar, with minor considerations for texture refinement.

Recommendations stemming from this study suggest integrating Protein Energy Bars into sports institutes' canteens and bars to meet macronutrient needs. Collaboration on intervention studies with physically active populations is advised to gauge effectiveness. Encouraging informed food choices can be achieved by offering bars in vending machines for convenience. Physically active consumers can include bars in breakfast or snacks to enhance protein intake, with availability ensured in retail and fitness centres. Support for patents and technology transfer is recommended for commercial production in industries. Researchers are encouraged to conduct long-term studies and collaborate to foster innovation in the food industry.

The study's findings indicate that Protein Energy Bars (P-E-Bars) can be formulated to meet acceptable sensory standards and maintain stability over an extended storage period of 150 days at a controlled temperature of  $15 \pm 2^{\circ}\text{C}$ . This suggests that the bars can retain their quality and remain suitable for consumption for a considerable duration under these storage conditions. Additionally, the study highlights the feasibility of producing P-E-Bars that are both cost-effective and of high quality. By using ingredients such as crispies, WPC 80 and SPI as primary components, manufacturers can create bars that not only meet sensory expectations but also offer nutritional benefits. This underscores the potential for developing P-E-Bars as a practical and accessible option for individuals seeking convenient sources of energy and nutrition, particularly those engaged in physically demanding activities or adhering to specific dietary requirements.