**Development and Physicochemical Analysis of Protein Fortified Bead Based Functional Beverage**

**ABSTRACT:**

The encapsulation technology enhances stability and bioavailability of bioactive compounds in functional beverages. Appealing sensory attributes with enhanced nutritional value was focused as a key feature for developing the beverage. Sodium chloride and calcium chloride were used for encapsulation technology. The gum based encapsulation of whey protein with fruit flavour was developed as a ready to serve protein fortified bead beverage. The different samples such as beads of blue curacao (lime orange) in blueberry RTS, rose beads in watermelon RTS and green apple beads in Kiwi RTS were developed. The sensory evaluation of the samples demonstrated the highest preference for blueberry RTS and blue curacao protein bead beverage. The proximate analysis of the beverage revealed 5% protein, 7% total carbohydrates, 4% of crude dietary fiber, and 0.3 % crude fat. Protein beads stay stable almost for 14 days under refrigeration condition. The beverage showed potential acceptability in the functional beverage category as step towards reducing protein-energy malnutrition.

KEY WORDS: Development and Physicochemical Analysis , Protein Fortified ,Functional Beverage, RTS

**1.Introduction:**

Proteins are basic macronutrients that are necessary for the growth, development, and upkeep of the human body. Made up of long chains of amino acids, proteins are used as building blocks for body tissues, enzymes, hormones, and other essential molecules. In contrast to carbohydrates and fats, which primarily act as sources of energy, proteins play a more extensive biological function that involves muscle synthesis, immune protection, cellular repair, and metabolic control. The human body holds more than 100,000 distinct proteins, all of which are produced from only 20 amino acids, nine of which are indispensable and have to be consumed through the diet (Wu, 2016). Since proteins are involved in virtually all physiological functions, adequate and quality intake is required to promote optimal health throughout the life cycle. Protein inadequacy is associated with a number of conditions such as suboptimal growth in children, muscle wasting, impaired immune status, fatigue, and impaired wound healing (Phillips et al., 2016). The Recommended Dietary Allowance (RDA) for protein is 0.8 grams per kilogram of body weight per day for healthy adults. Nevertheless, increasing evidence points towards the use of higher intakes, especially from high-quality sources, to be advantageous for active individuals, the elderly, and those who are recovering from illness (Bauer et al., 2013).Contemporary dietary practices, frequently dominated by processed food and carbohydrate-based meals, have led to protein being under-consumed in certain population groups. Additionally, busy lifestyles of most consumers pose difficulties in adhering to nutritional requirements regularly through regular meals. This has generated the need and demand for functional foods—foods that not only provide fundamental nutrition but also have other beneficial effects on health. Functional ingredients-enriched beverages have found popularity as an easy means to fill nutritional gaps, mainly among younger generations and working-age adults (Granato et al., 2020). Unlike typical protein shakes or powders, the developed product presents a visually interesting, texturally rich, and nutritionally robust alternative for consumers looking for a convenient yet efficient way to augment their protein intake. Use of fortified beads makes targeted release and enhanced bioavailability possible, while also providing the potential to package several nutrients within one matrix. These innovations correspond with recent moves towards personalized nutrition and the demand for food products that are healthy but also easy to eat and enjoyable (Saris et al., 2019).This research paper discusses the nutritional justification, formulation, and functional advantages of this new protein-fortified drink. Through the integration of scientific concepts of protein metabolism and food science, the study hopes to contribute to the increasing body of knowledge in favor of novel delivery systems for essential nutrients.

**2. Materials and methods:**

**2.1. Material**

Whey Protein Concentrate (WPC): Pure whey protein concentrate (80% protein content) was procured from H20 Nutrition (Hadapsar, Pune), Sodium Alginate: Natural polysaccharide, sodium alginate (low viscosity) bought from Gandhi chemical corporation (Moti Chowk, Pune). Calcium Chloride: Analytical grade calcium chloride (CaCl₂) bought from Gandhi chemical corporation (Moti Chowk, Pune). Syrup was procured from Jay Foods (Market Yard, Pune). All reagents and chemical used were analytical grade.

**2.2.1. Protein Bead Preparation**

**2.2.1.1. Preparation of Sodium Alginate Solution**

Concentration: A solution of 4% (w/v) sodium alginate was prepared by dissolving sodium alginate in sterile distilled water.

Mixing Conditions: The solution was stirred at room temperature under 1200 rpm for 10 minutes to facilitate complete dissolution and prevent clumping.

Refrigeration: After dissolution, the solution was chilled in the refrigerator for 2 hours to promote viscosity and stability and enable it to produce more uniform beads upon gelling.

Sodium alginate is a natural polysaccharide derived from brown seaweed. It has guluronic (G) and mannuronic (M) acid residues. Cooling prevents degradation and enhances consistency in viscosity.

**2.2.1.2. Preparation of Whey Protein Concentrate (WPC) Suspension**

The powder was dissolved in sterile distilled water to prepare a 5% (w/v) WPC solution. The mixture was blended until homogenized, resulting in a uniform protein matrix.

**2.2.1.3. Mixing Alginate with Protein Suspension**

The 4% alginate and 5% WPC solutions were combined in 1:1 proportion with continuous stirring in order to evenly distribute. This homogeneous suspension was utilized for bead formation.Even encapsulation of protein in the alginate gel is ensured at this step. The mixing should be uniform so that there is no heterogeneity in bead composition, which influences morphology, release kinetics, and stability.

**2.2.1.4. Bead Washing**

The beads were washed extensively with sterile distilled water after gelation to remove free Ca²⁺.This process also minimizes surface contamination and enhances bead purity.

Preparation of sodium alginate solution

↓

Preparation of whey protein concentrate

↓

Mixing alginate and protein suspension

↓

Bead formation

↓

Bead Washing

***Flowchart 1: Process of protein encapsulation bead***

**Table 1: Formulation of protein beads**

|  |  |
| --- | --- |
| **Ingredients** | **Quantity (per 100 g)** |
| Whey protein Concentrate (80%) | 6.25g |
| Sodium alginate | 4g |
| Sodium benzoate | 0.1g |
| Water | 59.74g |
| Blue Curacao syrup | 30g |
| Total | 100g |

**2.2.2. Sensory Evaluation**

Beads were assessed for their colour, visual appeal, and behaviour in the beverage medium. Coloured and flavoured beads (e.g., Blue Curacao, Rose, and Green Apple) remained stable in Ready-to-Serve (RTS) drinks without disintegration or clouding. The beads' visual clarity and floating behaviour enhanced the beverage's aesthetic appeal.

**2.2.3. Nutritional analysis-**

The biochemical estimation of the sodium alginate bead-encapsulated protein-fortified drink was done to determine its nutritional profile. Major parameters measured were moisture content, protein, fat, ash, dietary fibre, carbohydrates, and energy value.

**2.2.4. Characterization of the Beads**

**Physical properties**

The shape and other surface characteristics were determined by visual evaluation.

**2.2.5. Stability**

Preliminary stability was monitored briefly to observe changes in bead structure and appearance. Beads retained their shape and colour when stored under refrigeration, indicating good short-term stability and resistance to breakdown in the beverage's aqueous environment.

**2.2.6. Beverage Preparation**

Protein beads were incorporated into a base drink made from water and taste and mouth feel agents to enhance flavour and palate. The end drink was properly mixed to maintain settled bead distribution.

Sensory Analysis: A group of 10 panellists assessed the texture, taste, and acceptability of the protein-fortified drink using a 9-point hedonic scale. The parameters tested were taste, texture, mouth feel, and aftertaste.

**2.2.7. Shelf-Life Study:**

 The protein-fortified drinks were stored at 4°C for 4 weeks. Physical appearance, texture, and protein content were checked at intervals to assess the stability of the protein beads in the drink.

**2.2.8. Statistical analysis**

All experiments were conducted in set of three, and the results were presented as the mean ± standard deviation (SD). To assess significant levels of differences between the mean values, one-way ANOVA followed by Duncan's multiple range test was applied. A difference was considered statistically significant at p < 0.05.

**3. Result and Discussions:**

**3.1. Sensory analysis**

During the sensory evaluation, three fortified Ready-To-Serve (RTS) beverages were analysed:

1. Blueberry RTS with plain protein beads

2. Kiwi RTS with green apple-flavoured protein beads

3. Watermelon RTS with rose-flavoured protein beads

Based on the sensory scores across six parameters — appearance, color, taste, flavor, consistency, and overall acceptability — the Blueberry RTS beverage emerged as the most preferred sample. The blueberry beverage exhibited a rich, deep purple colour which is visually appealing and often associated with antioxidant-rich products. This natural colour created an impression of health and freshness. The protein beads remained suspended evenly without settling, indicating good stability and visual harmony. In contrast, the Kiwi RTS had a duller green hue, and the green apple beads offered limited contrast, leading to lower visual excitement. The Watermelon RTS was vibrant red, but the rose-flavoured beads showed a slight cloudiness over time, which may have affected clarity. Blueberry, being mildly sweet with subtle acidity, paired well with the neutral protein beads, allowing the fruit flavour to dominate without being masked or altered. Additionally, blueberry is less prone to oxidation, which helps maintain a stable flavour profile over time. The Kiwi-green apple combination resulted in a more acidic and tangy profile, which may not appeal to all consumers. The Watermelon-rose combination introduced floral notes that may have clashed with the base flavour, leading to mixed palatability responses. Blueberry RTS maintained a smoother, uniform mouth feel with the beads adding a pleasant textural contrast. The protein beads did not disrupt flow or cause excessive thickening. In the Kiwi variant, some tasters reported a slight graininess — possibly due to the interaction between kiwi enzymes and protein matrix. The Watermelon variant showed minor phase separation, possibly due to pH-induced protein aggregation. The sensory data indicated that the Blueberry RTS scored highest in overall acceptability, combining desirable taste, visual appeal, and mouth feel. Its familiar and palatable profile, free from extreme flavours or off-notes, made it a safer and more widely acceptable option for a functional beverage prototype.

***Figure 1: Sensory evaluation of protein beads beverage***

Sensory testing identified that the Blueberry RTS containing Blue Curacao-flavoured protein beads was ranked highest for overall acceptability (8.5 ± 0.3) on the 9-point hedonic scale. Panellists liked the visual appeal, texture, and harmonious flavour profile. The use of beads not only improved the look but also helped in mouth feel, giving rise to a new sensory experience. Relative to this, Watermelon RTS with Rose beads and Kiwi RTS with Green Apple beads had scores of 7.4 ± 0.5 and 7.0 ± 0.6, respectively, representing moderate acceptability.

**3.2. Proximate Composition of fortified beverage**

The progressive incorporation of whey protein concentrate into the beverage formulation led to notable alterations in its proximate composition. As protein content increased significantly from 0.98% in Sample 1 to 5.21% in Sample 6, corresponding trends were observed across other nutritional parameters. Moisture content exhibited a slight but consistent decline (from 82.60% to 81.51%), which can be attributed to the displacement effect caused by the increased addition of dry matter. Similarly, fat content showed a gradual rise (from 0.321% to 0.417%), reflecting the intrinsic lipid fraction present in whey protein concentrate. The ash content also increased marginally (from 0.127% to 0.138%), likely due to the mineral constituents associated with the protein source. A marked decrease in carbohydrate content (from 15.94% to 12.72%) was observed, possibly resulting from a reduction in carbohydrate-rich components, such as syrup, to accommodate the additional protein while maintaining a constant 100 g formulation. Energy content rose progressively from 70.57 to 74.32 kcal/100 g, consistent with the increase in protein and fat, both of which contribute significantly to caloric density. Dietary fibre content remained relatively stable across all samples, indicating no substantial variation in fibre-containing ingredients. These findings underscore the nutritional shifts that occur when fortifying beverages with protein, offering insights relevant to the development of

**Table 2: Proximate content of fortified beverage**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | **Sample 1** | **Sample 2** | **Sample 3** | **Sample 4** | **Sample 5** | **Sample 6** |
| Moisture | 82.60 ± 0.26a | 82.36 ± 0.11a | 81.98 ± 0.36a | 81.90 ± 0.77a | 81.58 ± 0.12a | 81.51 ± 0.33a |
| Ash | 0.127 ± 0.12a | 0.129 ± 0.32a | 0.134 ± 0.06a | 0.134 ± 0.36a | 0.137 ± 0.22a | 0.138± 0.11a |
| Fat content | 0.321 ± 0.21a | 0.336 ± 0.24ab | 0.347 ± 0.04bc | 0.403 ± 0.24de | 0.411 ± 0.32de | 0.417 ± 0.26ef |
| Protein | 0.98 ± 0.31a | 2.08 ± 0.01b | 3.33 ± 1.36c | 4.078 ± 0.14d | 4.26 ± 0.36de | 5.214 ± 1.01f |
| Carbohydrates | 15.94 ± 0.04a | 15.095 ± 0.24ab | 14.20 ± 0.3c | 13.48 ± 0.44cd | 13.214 ± 0.14ef | 12.72 ± 0.32ef |
| Energy | 70.569 ± 0.31a | 71.724 ± 0.11a | 73.243 ± 0.14a | 73.739 ± 0.17a | 74.0 ± 014a | 74.321±0.47a |
| Dietary Fibre | 3.01 ± 0.88a | 3.21± 0.14a | 3.11 ± 0.21a | 3.06 ± 0.15a | 3.27 ± 0.24a | 3.14 ± 0.07a |

All results are depicted as mean ± SD. The results within the same row state a significant difference (*p* < 0.05) from each other. Superscript letters (a,b, ab, etc) indicate multiple comparisons conducted using Ducan’s test after one way ANOVA.

**3.3 Storage Stability of Alginate-Encapsulated Protein Beads in Functional Beverages**

The stability of protein-enriched functional beverages containing alginate-encapsulated protein beads can be described through distinct shelf-life phases. In the initial phase, immediately post-production, the beads remain fully hydrated and structurally intact, with optimal protein bioavailability and no evidence of physical or sensory degradation. During the stabilization phase (7–14 days), the beverage retains its clarity, protein content, and organoleptic properties, with only minor textural changes in the beads. The degradation phase (14–21 days) is characterized by early signs of protein denaturation, bead softening, and slight sensory changes, though the overall integrity remains acceptable under refrigeration. Beyond 21 days, in the advanced degradation phase, significant structural breakdown of the beads and protein loss occur, along with microbial spoilage risks and perceptible quality deterioration. The size of beads play important role in how well the protein is encapsulated and released, with smaller beads often giving better protection and more controlled release because they reduce surface area and limit leakage (Sood et al., 2023). These findings highlight the importance of storage conditions and support the protective role of alginate encapsulation against cold-induced degradation, consistent with the observations of Narin et al. (2020).

**Table 3: Shelf-life stability of protein beads**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Storage period (days) | TPC (CFU/mL) | TSS(Bx) | Titratable Acidity (%citric Acid) | pH |
| 0 | 8.0×10^2 ± 0.09a | 11.0 ± 0.32**b** | 0.15 ± 0.65c | 7.3 ± 0.14d |
| 7 | 9.0×10^2 ± 0.32a | 11.5 ± 0.17**b** | 0.25 ± 0.47c | 7.2 ± 0.34d |
| 14 | 5.0×10^3 ±0.57a | 10.5 ± 0.36**b** | 0.35 ± 0.34c | 6.9 ± 0.11d |
| 21 | 5.0×10^3 ± 0.29a | 10.5 ± 0.07**b** | 0.35 ± 0.24c | 6.9 ± 0.46d |
| 28 | 2.0×10^4 ± 1.07a | 9.5 ± 0.98**b** | 0.50 ± 0.18c | 6.6 ± 0.26d |

All results are depicted as mean ± SD. The results within the same row state a significant difference (*p* < 0.05) from each other.

Table 3 represents the CFU count increases steadily over time, with a dramatic rise after 14 days. This indicates that microbial contamination is progressively worsening as the beads age, potentially rendering the product unsafe for consumption or use. The microbial growth could be due to the breakdown of the protein matrix, which becomes more accessible for microbial colonization as the structure of the protein beads degrades over time. The small increase in TSS from Day 0 to Day 7 likely results from leaching of soluble solids. However, the gradual decline after Day 7 suggests that the microbial population is consuming the available nutrients, decreasing the TSS. The reduction in TSS at Day 28 indicates the depletion of soluble nutrients due to microbial activity. Microbial consumption of nutrients (especially carbohydrates and proteins) is a well-known cause of decreased TSS in microbial cultures (Scotto di Uccio et al.2023).

The steady increase in acidity over time, particularly from Day 7 to Day 28, reflects microbial fermentation or protein breakdown, leading to the accumulation of organic acids. This is a clear indication of spoilage due to microbial metabolic activity. The increase in acidity is consistent with the fermentation of sugars or the breakdown of protein molecules into amino acids and organic acids. The slight decrease in pH from Day 0 to Day 7 reflects early-stage microbial metabolism. By Day 14, the pH continues to decline, indicating increasing microbial activity. The further decline by Day 28 suggests that the product has become more acidic, likely due to the accumulation of fermentation by-products. The pH decrease is consistent with the increased acidity in the sample and is a direct result of microbial fermentation processes. From the microbial growth (TPC) to the increase in acidity and decrease in pH, the product is progressively spoiled due to microbial activity. The TSS decrease further supports the idea that nutrients are being consumed by microorganisms.Microbial growth becomes the most significant factor in spoilage after Day 14, leading to increased acidity and decreased pH. The shelf life of these beads is likely limited to the first 7-14 days, after which spoilage becomes more severe, rendering the product unsafe and less desirable for consumption.

**Conclusion:**

The present research effectively proves the viability of sodium alginate and calcium chloride-based encapsulation technology in the production of protein-fortified drinks, specifically in terms of enhancing the stability, texture, and sensory acceptability. The encapsulation process using whey protein concentrate (WPC) produced spherical, smooth-surfaced protein beads that were structurally intact and stable throughout the preparation and storage periods. The sensory test with panellists established the Blueberry RTS drink with Blue Curacao-flavoured protein beads as the most acceptable, which shows the significance of visual appearance, texture, and taste in consumer acceptance. ghting protein-energy malnutrition.

The higher acceptability rating of Blueberry RTS indicates that not only are consumers influenced by the health advantages of the product, but also by the sensory qualities and uniqueness of the encapsulated protein beads, which create an enhanced appearance and mouth feel for the beverage. Future research should aim at optimizing the encapsulation process, investigating the effect of various protein types and flavour formulations, and performing long-term stability tests under different environmental conditions. This will eventually lead to the production of functional beverages that not only address the nutritional requirements of consumers but also offer a pleasant and convenient means of fighting protein-energy malnutrition.

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1.

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