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# Quality Parameters of Protein-Enriched Beverage Mix

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#### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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#### **ABSTRACT**

The health or protein drink mix is a product containing vital nutrients designed to enhance protein levels. A nourishing protein beverage blend was created by incorporating cowpea protein isolate along with malted finger millet flour to elevate its nutritional value. The study aims to focus on developing a rich protein-rich beverage mix from the cowpea protein isolate, which could be convenient to use. The finger millet, jaggery, cardamom, chocolate and vanilla essence were procured from the local market in Dharwad. Malting of finger millet was performed by soaking, germination, drying, and roasting. The protein-enriched beverage mix was prepared by combining and blending all the ingredients as per the formulations. The present investigation focused on the development of a protein beverage mix by incorporating cowpea protein isolate to enhance the protein quality parameters. For organoleptic evaluation of protein-rich beverage mix and to check the significance between the variations, one-way ANOVA was used. The different variations of cowpea protein isolate are tested with levels of 0, 7.5, 10, 12.5per cent. The performed investigation resulted in the finding that all samples (T0, T1, T2 and T3) were organoleptically

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Keywords: Cowpea protein isolate; in vitro protein digestibility; malt; organoleptic; beverage.

#### 1. INTRODUCTION

Protein insufficiency is a significant concern globally, particularly in less developed and emerging nations such as India. Protein-energy malnutrition is marked by low plasma protein concentration. The use of serum protein measurement is widespread for the assessment of nutritional status (Oladibu et al., 2022; Kasula et al., 2023). Inadequacy of protein and energy consumption can result in various indications, including hindered physical and cognitive growth, elevated susceptibility to illnesses and infections. delayed recovery rate, decreased efficiency, and amplified mortality rates (Ritchie et al., 2018). Increasing consciousness regarding security, the adoption of health-conscious vegan dietary principles, elevated costs of animalderived proteins, and the extensive use of potent antibiotics in animal rearing have prompted consumers to shift from conventional meat-based proteins to natural plant-derived alternatives (Banerjee et al., 2022).

India, despite its abundant food production, continues to confront challenges related to food hunger and insecurity. Improving the nutritional status of the country requires a concerted effort to ensure a consistent supply of healthy and nourishing foods. Leveraging regional crops for the development of supplementary foods can significantly enhance nutritional security for the population. In India, malt-based food drinks are commonly referred to as health beverages and are extensively enjoyed by children, women, and adults to boost their nutritional intake. These beverages also act as flavour enhancers, augmenting overall health and increasing energy levels when incorporated into one's (Gaikwad et al., 2022).

The World Health Organisation's emphasis on the health benefits of functional foods and beverages has contributed to the rise in their popularity globally. Besides, these consumers have become more aware of the importance of their food composition and nutrition (Gupta et al., 2023; Scutaraşu & Trincă, 2023). Beverages are ready-to-consume products which offer several benefits. They are convenient and nourishing foods. High protein foods are also being recommended for the management of disease, including diabetes, etc, for convalescents. So this study focused on developing a protein-rich beverage mix from the cowpea protein isolate, which could be convenient to use.

#### 2. MATERIALS AND METHODOLOGY

#### 2.1 Procurement of Raw Materials

The finger millet, jaggery, cardamom, chocolate and vanilla essence were procured from the local market in Dharwad. Care was taken to purchase ingredients of the same brand and quality throughout the study.

## 2.2 Formulation and Optimisation of Protein-Rich Beverage Mix

- a. Malting: Malting of finger millet was performed by soaking, germination, drying, and roasting. The obtained malt was further ground/ milled to obtain finger millet malt flour.
- b. Preparation of protein-enriched beverage mix: The protein-enriched beverage mix was prepared by combining and blending all the ingredients as per the formulations.
  - Addition of cowpea protein: Trials were made using 7.5, 10 and 12.5 per cent of cowpea protein isolate.
  - Replacement of sugar with jaggery: Sugar was completely eliminated from the adopted formulation; instead, jaggery was incorporated at 45, 50 and 55a.
  - Optimisation of water: Water was added at 600, 800 and 1000 ml.

 Addition of flavour: To enhance the flavour and taste of the beverage, different flavours were used, like cardamom, vanilla and chocolate.

## 2.3 Sensory Evaluation of Protein Beverage Mix

Sensory evaluation of the beverage was carried out using a trained panel of 10 judges drawn from staff and students of the Department of Food Science and Nutrition, College of Community Science, UAS, Dharwad, on a ninepoint hedonic scale. The protein beverage mix was evaluated for sensory evaluation based on colour, taste, flavour, consistency and overall acceptability. Scores were given on a hedonic scale ranging from 9 to 1, representing like extremely to dislike extremely respectively.

### 2.4 Evaluation of the Protein Quality of the Protein Beverage

#### 2.4.1 Protein content

The total nitrogen content of the free sample was estimated in the Pelican model, Kelplus digestion and distillation units. Organic nitrogen, when digested with sulphuric acid in the presence of a catalyst (copper sulphate and potassium ammonium sulphate), was converted into sulphate and was further distilled with sodium hydroxide to convert ammonium sulphate to ammonium borate. Further, it was titrated against standard hydrochloric acid to obtain nitrogen. Nitrogen was multiplied by a constant 6.25 to get crude protein content (Anonymous, 2019).

(%) nitrogen=
$$\frac{(Titre\ value\ -\ Blank)\ \times Normality\ of\ HCl\ \times 14.007}{Sample\ weight\ (g)}\times 100$$

Protein (%) = Nitrogen (%)  $\times$  6.25

#### 2.4.2 In vitro protein digestibility

In vitro protein digestibility was estimated using a suitable enzymatic method (pepsin and pancreatic enzymes) described by Moulishwar et al. (1993). A sample containing 100mg of protein was treated with 0.1 N HCl containing 12.5mg of pepsin at 37 °C for three hours. The contents were neutralised with 0.5 M sodium hydroxide, then 25ml of phosphate buffer containing 6 mg of pancreatin was added and incubated for 24 hours at 37 °C. The volume was made up to 100 ml with distilled water, and 50 ml of the aliquot was treated with 10 per cent trichloroacetic acid and left overnight to precipitate the proteins.

Suspensions were centrifuged, and the residue was analysed for protein by micro-Kjeldahl method. The amount of protein digested was calculated as

Digested protein =  $\frac{Total \ protein - Undigested \ protein}{Total \ protein} \times 100$ 

#### 2.5 Statistical Analysis

For organoleptic evaluation of protein rich beverage mix and to check the significance between the variations, one-way ANOVA was used.

#### 3. RESULTS AND DISCUSSION

## 3.1 Optimisation and Organoleptic Score of Beverage with Variation in Cowpea Protein Isolate, Jaggery, Water & Flavours

Cowpea protein beverage mixes were formulated with varying ratios of cowpea protein isolate to malted finger millet flour (7.5:42.5, 10:40, and 12.5:37.5), while keeping other ingredients constant, providing diverse formulations for evaluation in Table 1. Organoleptic evaluation of cowpea protein beverages, with proportions of isolate using a nine-point hedonic scale, revealed consistent scores in appearance, colour, flavour, and consistency (8.10 to 8.70, 7.90 to 8.50, and 7.70 to 8.40, respectively). significant taste and However, acceptability differences (P≤ 0.01) were noted, with formulation T2 standing out with the highest acceptability index (93.89%) and selected for further trials. Cowpea protein isolate incorporated into the beverage enhances the protein content and essential amino acids, with finger millet providing complete food in the beverage. A ten per cent level of incorporation of cowpea protein isolate was acceptable in formulating a beverage mix, and similar results were found in a study conducted by Syeunda et al. (2019) in the development of cowpea with malted finger millet beverage.

The most accepted variation of cowpea protein isolate with malted finger millet flour in a 10:40 ratio was selected for further studies. Jaggery, being less refined, typically contains small quantities of additional nutrients such as minerals and antioxidants when compared to highly processed refined sugar. The variation in jaggery content (45g, 50g, 55g) showed a significant impact on flavour, taste, consistency, and overall acceptability, with 50g of jaggery yielding the highest acceptability index of 93.51per cent, as

Table 1. Organoleptic scores# of cowpea protein beverage with variation in cowpea protein isolate

Variations Cowpea protein isolate: Malted finger millet flour	Appearance	Colour	Flavour	Taste	Consistency	OA	Al
0:50	$8.50 \pm 0.71$	$8.20 \pm 0.79$	$8.50 \pm 0.71$	$8.00 \pm 0.47$	$7.80 \pm 0.92$	$8.40 \pm 0.69$	91.48±5.87
$T_1$	$8.20 \pm 0.79$	8.10 ± 1.19	$7.90 \pm 0.88$	$7.40 \pm 0.84$	$8.10 \pm 0.99$	$8.30 \pm 0.82$	88.89±8.64
7.5:42.5							
$T_2$	$8.70 \pm 0.48$	$8.70 \pm 0.48$	$8.30 \pm 0.82$	$8.20 \pm 0.63$	$8.40 \pm 0.52$	$8.40 \pm 0.52$	93.89±3.49
10:40							
$T_3$	8.10 ± 0.57	$8.20 \pm 0.63$	$7.90 \pm 1.10$	$6.80 \pm 0.92$	$7.70 \pm 0.82$	$7.30 \pm 0.95$	85.19±6.04
12.5:37.5							
F-value	1.89	1.09	1.14	7.35	1.44	4.91	3.51
S. Em	0.20	0.25	0.28	0.23	0.26	0.24	1.98
CD	0.58 <sup>NS</sup>	0.74 <sup>NS</sup>	0.80 <sup>NS</sup>	0.66**	0.75 <sup>NS</sup>	0.69**	5.69*

Values are # Mean ± SD of three replications, S.Em- Standard error mean, CD- Critical difference, \*significant at 5%, \*\*significant at 1%, NS- Non significant, OA-Overall Acceptability, Al- Acceptability index,CPI-Cowpea protein isolate; MFF- Malted finger millet flour; Control- Malted finger millet beverage,

T<sub>1</sub>- CPI: MFF= 7.5:42.5, T<sub>2</sub>-CPI: MFF= 10:40, T<sub>3</sub>-CPI: MFF= 12.5: 37.5

Table 2. Sensory scores of cowpea protein beverage with variation in jiggery

Sample	Appearance	Colour	Flavour	Taste	Consistency	OA	Al
45g	8.50 ± 0.53	8.40 ± 0.52	7.40 ± 0.84	7.00 ± 0.66	$7.80 \pm 0.63$	$7.30 \pm 0.82$	85.92±4.87
50g	$8.70 \pm 0.48$	$8.20 \pm 0.42$	$7.90 \pm 0.57$	$8.50 \pm 0.53$	$8.60 \pm 0.52$	$8.60 \pm 0.52$	93.51±2.92
55g	$8.20 \pm 0.42$	8.10 ± 0.57	$6.80 \pm 0.79$	$6.40 \pm 0.79$	$7.40 \pm 0.52$	$6.80 \pm 0.79$	80.92±5.09
F-value	2.76	0.91	5.49	35.49	12.00	16.53	20.68
S. Em	0.15	0.16	0.23	0.18	0.17	0.22	1.39
CD	0.44 <sup>NS</sup>	0.46 <sup>NS</sup>	0.68**	0.52**	0.51**	0.66**	4.04**

Values are # Mean ± SD of three replications, S.Em- Standard error mean, CD- Critical difference, \*\*significant at 1%, NS- Non significant

Table 3. Sensory scores of cowpea protein beverage with variation in water

Sample	Appearance	Colour	Flavour	Taste	Consistency	OA	Al
600ml	8.10 ± 0.32	$8.00 \pm 0.47$	$6.30 \pm 0.48$	6.40 ± 0.52	$8.20 \pm 0.42$	$6.60 \pm 0.69$	80.74±3.04
800ml	$8.50 \pm 0.53$	$8.20 \pm 0.42$	$7.40 \pm 0.84$	$7.10 \pm 0.74$	$7.90 \pm 0.32$	$7.50 \pm 0.53$	86.29±2.92
1000ml	$8.70 \pm 0.48$	$8.20 \pm 0.42$	$7.90 \pm 0.57$	$8.50 \pm 0.53$	$8.60 \pm 0.52$	$8.60 \pm 0.52$	93.51±2.92
F-value	4.58	0.69	15.87	31.50	6.79	29.13	46.61
S.Em	0.14	0.13	0.20	0.19	0.13	0.18	0.93
CD	0.41*	0.40 <sup>NS</sup>	0.59**	0.55**	0.39**	0.53**	2.72**

Values are # Mean ± SD of three replications, S.Em- Standard error mean, CD- Critical difference, \*significant at 5%, \*\*significant at 1%, NS- Non significant

presented in Table 2. Further exploration will concentrate on this selected quantity of jaggery for additional ingredient variations. These outcomes are consistent with the findings reported by Gaikwad et al. (2022).

Optimising water content (600ml, 800ml, 1000ml) in cowpea protein beverage revealed superior sensory scores, as presented in Table 3. Water plays a pivotal role as the primary ingredient essential for maintaining the desired consistency. The variant with 1000ml water exhibited significantly higher scores in flavour (7.90), taste (8.50).consistency (8.60),and acceptability (8.60), yielding an acceptability index of 93.51. Despite a slight difference in appearance (8.70), the colour remained consistent, making 1000ml the chosen water quantity for further beverage enhancement. These findings are in accordance with the results reported by Gaikwad et al. (2022).

Table 4 depicts the optimisation of flavours and sensory score of cowpea protein beverage. Different flavours like cardamom, vanilla and chocolate were used to add flavour. These flavouring agents are deliberately incorporated

into food products to enhance their sensory characteristics, including appearance, taste, and overall acceptability. A Beverage prepared with cardamom was found highly acceptable, with an acceptability index of 94.81 per cent. Trials made with chocolate were the least acceptable among all three variations, with low scores (80.74%) of acceptability index. These findings are consistent with results reported in a study on finger millet beverage conducted by Shelly et al. (2017).

### 3.2 Optimised Ingredients for Preparation of Cowpea Protein Beverage

After optimisation of each ingredient, the standardised recipe formulated had 10:40 per cent of cowpea protein isolate to malted finger millet flour, 49.5g jaggery, 0.5g cardamom and added water was 1000 ml. Fig.1 depicts sensory scores of optimised cowpea protein beverage. The sensory scores obtained through appearance (8.53), colour (8.40), flavour (8.26), taste (7.96), consistency (8.26) and overall acceptability (8.46) indicated that cowpea protein beverage was highly acceptable with an acceptability index score of 92.40 per cent.

Table 4. Sensory scores of protein rich beverage with different flavors

Sample	Appearance	Colour	Flavour	Taste	Consistency	OA	Al
Cardamom	8.70 ± 0.48	8.60 ± 0.52	$8.70 \pm 0.48$	$8.30 \pm 0.48$	$8.30 \pm 0.48$	8.60 ± 0.52	94.81±2.87
Vanilla	$8.50 \pm 0.53$	$8.20 \pm 0.42$	$7.40 \pm 0.84$	$7.40 \pm 0.32$	$7.90 \pm 0.32$	$7.50 \pm 0.53$	86.29±2.92
Chocolate	$8.10 \pm 0.32$	$8.00 \pm 0.47$	$6.30 \pm 0.48$	$6.30 \pm 0.52$	$8.20 \pm 0.42$	$6.60 \pm 0.69$	80.74±3.04
F-value	4.58	4.20	36.76	26.52	2.54	29.13	57.79
S. Em	0.14	0.14	0.19	0.18	0.13	0.18	0.93
CD	0.41*	0.43*	0.57**	0.54**	0.37 <sup>NS</sup>	0.53**	2.70**

Values are # Mean ± SD of three replications, S.Em- Standard error mean, CD- Critical difference, \*significant at 5%, \*\*significant at 1%, NS- Non significant

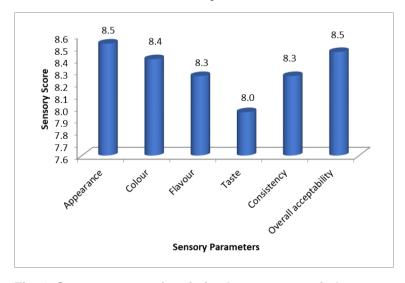


Fig. 1. Sensory score of optimized cowpea protein beverage

Values are # Mean of three replications

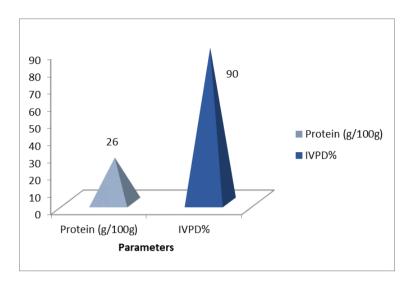


Fig. 2. Protein quality of cowpea protein beverage

Values indicate #Mean of three replications, IVPD- In vitro protein digestibility

#### 3.3 Protein Quality

Fig. 2 provides information about the protein characteristics of a cowpea protein-based beverage, including its protein content and *in vitro* protein digestibility. Specifically, the protein content of the beverage was recorded as 26g/100g, while its *in vitro* protein digestibility was 90.00 per cent. The results were similar to those of the study by Annor et al. (2017).

#### 4. CONCLUSION

The incorporation of 10 per cent cowpea protein isolate and 40 per cent malted finger millet had better sensory characteristics of the beverage. The nutritional analysis revealed that the cowpea protein beverage mix possesses a favourable nutritional profile, boasting elevated levels of approximately 26 g of protein content and in vitro protein digestibility is reported to be 90 per cent. Hence, protein beverage mix can be easily prepared; the incorporation of malt flour in the protein beverage mix is successfully performed with a good nutrient profile and enhanced protein quality. Thus, it can be concluded that protein beverage mix can contribute to the dietary status of consumers, leading to improvement in product acceptability.

#### **DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative Al technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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