



Evaluation of Functional Characteristics and Reconstitution Behavior of Formulated Ready to Reconstitute Enteral Formula for Nutrition Therapy

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This work was carried out in collaboration among all authors. Author PLB performed the experiment, statistical analysis and wrote the first draft of the manuscript. Author RB helped in designing the study, Authors MG and MT managed the analyses of the study and the literature searches. Author MSB designed the study and supervised the work done. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present investigation was undertaken to evaluate the functional properties and reconstitution behavior of enteral formulae i.e. Balanced Enteral Formula, High Protein Enteral Formula and High Energy Enteral Formula formulated using locally available natural ingredients.

Place and Duration of Study: The study was conducted in the Department of Food Science and Nutrition, College of Community Science, Assam Agricultural University during the period of August 2017 to January 2018.

Methodology: Three enteral formulae namely Balanced Enteral Formula, High Protein Enteral Formula and High Energy Enteral Formula were formulated using naturally available low cost ingredients. Standard protocols were followed to evaluate the quality of ingredients used and formulated enteral formulae.

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Results: Both ingredients and enteral formulae had good functional properties with bulk density, tapped density, particle density, porosity, cohesiveness, flowability, water absorption index and water solubility index ranging from 0.33 to 0.50 g/ml, 0.38 to 0.56 g/ml, 0.59 to 1.25 g/ml, 35.00 to 60.00 per cent, 0.33 to 1.18, 0.00 to 17.77 per cent, 1.23 to 7.59 g/g and 23.66 to 87.62 per cent soluble solids respectively for the ingredients used and 0.44 to 0.47 g/ml; 0.50 to 0.53 g/ml; 0.89 to 0.90 g/ml; 41.00 to 44.44 per cent; 1.12 to 1.14; 11.30 to 12.00 per cent; 3.39 to 4.59 g/g and 42.66 to 51.00 per cent respectively for the formulated enteral formulae. Color analysis of the developed formulae using Hunter Lab showed desirable results with L^* , a^* and b^* value in the range of 82.43 to 87.10; 1.35 to 2.38 and 13.75 to 13.95 respectively. The reconstitution of formulated enteral formulae with warm water revealed a reconstitution time varying from 5 to 6 minutes. Further the reconstituted formula feed on passage through Ryles tube showed superior flow rate in formulae prepared with 20 per cent solid concentration (W/V).

Conclusion: The developed enteral formulae had excellent functional characteristics and reconstitution behavior indicating quality product suitable for enteral feeding.

Keywords: Enteral formula; functional; reconstitution; ready to reconstitute; nutrition therapy; bulk density; tapped density; flow ability.

1. INTRODUCTION

Nutrition support has become an important therapeutic intervention in maintaining the health and well being of individuals, more importantly hospitalized patients. Globally a large group of population admitted to critical care unit are deprived of essential nutrients and are at greater risk of malnutrition with a prevalence rate as high as 78.10 per cent and 50.80 per cent in developing and developed countries respectively [1]. Nutritional risk and malnutrition are greatly related to increased length of stay in the hospital which in turn increases the risk of mortality and morbidity [2,3]. The challenge of nutrient deficiency of patients during hospital stay could therefore be mitigated by provision of nutrition support in form of enteral nutrition. Enteral nutrition is an efficient artificial feeding method for patients suffering from chronic or acute diseases with adequate functional gut who are unable to eat sufficiently via oral route [4].

Traditionally enteral nutrition along with parenteral nutrition has been named artificial feeding, however in present time they are referred as medical nutrition therapy [5]. Studies have documented that a wide variety of commercial formula are available in market and they are preferred for enteral nutrition support since last 20 years. However, being expensive not everyone can afford them more specifically in Countries like India where a large section of population are from low socio-economic group. This necessitates the requirement for development of nutritious low cost enteral formulae which could be beneficial for enteral feeding in lieu of commercial formula which

tends to be expensive. One important factors along with the nutrient composition of the developed enteral formula is the functional characteristics which are important in food formulations more particularly to enteral foods as they are used for tube feeding. Functional properties of foods denote any physico-chemical property, which affects the processing of ingredients in a food systems, as judged by the quality attributes of the final product [6]. Low bulk density and higher water absorption capacities are some of the desirable functional properties of ingredients used in the formulations reflected in the functional characteristics of the final products. The density of ingredients indicates the amount of air spaces. The grains that have higher density contain a larger amount of reserve substances (nutrients), this condition being the most desirable [7].

Several properties of a powder are dependent on the preparation method, treatment and storage of the sample [8] like bulk density and have great importance in evaluating the product quality, since it is possible to verify whether the raw material used after the processing possesses suitable structure and composition for use as a food ingredient [9]. Bulking properties of the powder is generally influenced by the inter-particle interaction and it interfere the flow behavior. Developed formula characteristics are also depended on the particle density which is affected by the particle size and is important for determining packaging requirements, material handling and application in wet processing in the formulation of enteral feeding for the patients. Interactions among the different attributes in a given powder are often used as an index of the

ability of the flour to flow. The flow ability characteristics which is generally governed by the interactions between the compressibility index and Hausner ratio and also there are frequently greater inter-particulate interactions, and a greater difference between the bulk and tapped densities. Water Absorption Index (WAI) and water solubility are also important characteristic of flour because physicochemical properties such as viscosity and gelation are dependent on them and give valuable information on the behavior of formulated product. Water Absorption Index (WAI) measures the amount of water absorbed by starch granules after swelling in excess water and it is used as an index of gelatinization [10]. Overall reconstitution characteristic is influenced by a number of properties of the powder [11]. The chemical and physical properties of each ingredient, such as bulk density, tapped density, particle density, porosity, water solubility, water absorption index etc., often dictate the most suitable type for formulation. When mixing products, the final enteral feed achieved may sometimes be unsuitable for one or more of the components. Consequently, it may result in poor suspension or emulsion characteristics. This, in turn, may manifest as layering, settling or clogging the tube. Thus, the manner in which ingredients interact with each other when added to water should reach to a stable emulsion or suspension. The study provides the information about a functional characteristics of increasing possibility of using homemade enteral formulas and also these can be solve the problem of malnutrition and other essential macro and micro nutrients deficiency among the patients .Therefore, considering the following points, the present study was undertaken to develop three different enteral formulae i.e. Balanced Enteral Formula (BEF), High Protein Enteral Formula (HPEF) and High Energy Enteral Formula (HEEF) from natural food ingredients to meet specific purpose by following adequate techniques in accordance with all essential criteria of American Society of Parenteral and Enteral Nutrition (ASPEN). The objective of the present study was to evaluate the functional properties and reconstitution behavior as they greatly impact the free flow of enteral feeds which is necessary to avoid mechanical complications such as tube clogging Little work has been reported on the study of functional properties of homemade enteral formulae. Thus, the present study was conducted to assess the functional properties and reconstitution behavior of the developed enteral formulae.

2. MATERIALS AND METHODS

The experiment was conducted in the Food Processing Laboratory and Food Analysis Laboratory of Department of Food Science and Nutrition of College of Community Science, Assam Agricultural University, Jorhat, Assam. Raw ingredients such as rice, green gram, amaranth, flaxseed, skimmed milk powder, whey protein powder and coconut oil selected for developing enteral formulae were procured from Jorhat city, Assam. Selection of ingredients were done based on their nutritional potential, adaptability to processing, keeping quality and availability of the ingredients.

2.1 Formulation of Enteral Formula

The selected ingredients were subjected to processing to enhance their quality in terms of nutritional and functional attributes. The selected rice grains (*Masuri*) were steeped, germinated, kilned and milled as per the method given by Adebowale et al. [12] to produce malted rice flour. Uniform sized whole green gram was processed to malted green gram flour following the procedure of Mallashi and Deshikachar [13]. Processing of amaranth to popped amaranth flour was done according to method described by Lara et al. [14] with slight modifications. Amaranth seeds were dried to 12 per cent moisture level and popped in Softcl, Smart Snacker for 15- 20 sec and powdered to produce popped amaranth flour. Flaxseed flour were prepared by cleaning, drying and roasting flaxseed in microwave oven with 480W output, under the operating frequency of 450 Hz for 2.5 minutes and grounded to fine flour [15]. Finally, the processed flours and other ingredients were dry blended in a mixer (kenster) homogeneously at definite proportions as shown in Table 1 for formulation of ready to reconstitute enteral formulae following the recommended criteria of ASPEN, ISPEN, ESPEN and criteria adopted by Heimburger and Weinsier [16]. The developed enteral formulae were stored in airtight glass container at 4°C for further evaluation.

2.2 Functional Characterization of Ingredients and Developed Enteral Formula

The functional properties such as bulk density (g/ml), tapped density (ρ_t) (g/ml), particle density (ρ_p) (g/ml), porosity (ϵ)(%), cohesiveness (Hausner ratio), flowability (%), Water

Table 1. Proportion of ingredients used for formulation of enteral formulae

Ingredients	Enteral formulae		
	BEF	HPEF	HEEF
Malted rice flour (g)	40	20	40
Malted green gram flour (g)	25	30	20
Popped amaranth flour (g)	15	20	10
Flaxseed flour (g)	5	5	10
Skimmed milk powder (g)	5	10	5
Whey protein powder (g)	----	10	10
Coconut oil (ml)	10	5	5

BEF= Balanced Enteral Formula; HPEF= High Protein Enteral Formula; HEEF= High Energy Enteral Formula

Absorption Index (WAI)(g/g), Water Solubility Index (WSI) (%) and colour of the ingredients used and developed formulae were analyzed following standard protocol.

2.2.1 Bulk density

The bulk density was determined by the method described by Jangam and Thorat, [17]. One gram of sample was loaded into a 10 ml graduated measuring cylinder and the volume occupied was recorded. The recorded volume was used to calculate bulk density (ρ_B) in terms of weight per volume.

2.2.2 Tapped density

Tapped density was also determined following the method of Jangam and Thorat [17]. One gram of sample was weighed into a 10 ml graduated measuring cylinder and the volume obtained after tapping for 5 minutes (32 taps per minute) on a bench or firm surface was recorded to calculate tapped density using formula:

$$\text{Tapped density} = \frac{\text{Mass(g)}}{\text{Volume after tapping (ml)}}$$

2.2.3 Particle density

The particle density (ρ_p) was measured using the method suggested by Jinapong et al. [18]. One gram of sample was transferred into a 10 ml measuring cylinder with a glass stopper. Five ml of petroleum ether was added to it and shaken for some time so that all the particles get suspended. Finally, the wall of the cylinder was rinsed with another 1 ml of petroleum ether. The total volume of the petroleum ether and suspended particles were read and particle density was calculated using formula:

$$\text{Particle density} = \frac{\text{Weight of the powder(g)}}{\text{Total volume of petroleum ether and suspended particles (mL)}}$$

2.2.4 Porosity

The porosity (ε) was calculated using particle density (ρ_p) and tapped density (ρ_r) [18].

$$\text{Porosity} = \frac{\rho_p - \rho_r}{\rho_p} \times 100$$

2.2.5 Cohesiveness

The cohesiveness was calculated in terms of Hausner ratio (HR) from the bulk density (ρ_B) and tapped density (ρ_r) [18] using formula:

$$\text{HR} = \frac{\rho_r}{\rho_B}$$

2.2.6 Flowability

The flowability of ingredients used and developed formulae was expressed as Carr Index (CI) in terms of tapped density (ρ_r) and bulk density (ρ_B) as described by Jinapong et al. [18] using formula:

$$\text{CI} = \frac{\rho_r - \rho_B}{\rho_r} \times 100$$

2.2.7 Water absorption index and water solubility index

Water Absorption Index (WAI) and Water Solubility Index (WSI) were determined using the method described by Gomez [19]. A total of 2.5 g of dry powders was added to 30 ml of water at 30°C in a 50 ml centrifuge tube, stirred intermittently for 30 min and then centrifuged for 10 min at 5100 rpm. The supernatant was carefully poured off into a petri dish and oven-dried overnight. The amount of solid in the dried supernatant as a percentage of the total dry

solids in the original 2.5 g sample gave an indication of the WSI. Wet solid remaining after centrifugation was dried in an oven overnight which gave an indication of WAI. WAI was calculated as the weight of dry solid divided by the amount of dry sample.

2.2.8 Colour

Instrumental surface colour (CIE L*a*b*) of samples were evaluated using a Hunter Lab Mini Scan XE Plus Colour Meter (Illuminant D65, 2.5 cm diameter aperture, 10° standard observer; Hunter Associate Laboratory, Inc., Reston, VA). Calibration was performed by using standard black and white tiles prior to the colour measurement. CIE L* a* b* values were used to calculate saturation index/ chroma $[(a^2+b^2)^{1/2}]$ and hue angle $[\tan^{-1}(b^*/a^*)]$. The colour of the samples was measured after putting the samples in front of smallest aperture [20].

2.2.9 Viscosity

The viscosity of developed enteral formulae at 20%, 25% and 30% solid concentrations was determined. The 20-30 per cent w/v solid concentration was dispersed in warm water and mixed into fine paste. The slurry was heated slowly on water bath to boiling. The cooked slurry was cooled to room temperature, and the viscosity was measured in Brookfield viscometer using appropriate spindles depending on the slurry consistency as per the formulation techniques.

2.2.10 Osmolality

The osmolality of the developed formulae was determined using an osmolalometer by determining number of particles of solute present per unit weight of water and is expressed in milliosmoles per kg of water.

2.3 Reconstitution Behavior of Developed Enteral Formulae

The developed enteral formulae before feeding via enteral route needs to be reconstituted. In the present investigation, formulated enteral formulae were reconstituted with warm water at 20, 25 and 30 per cent solid concentration (W/V). After allowing the reconstituted foods to stand for an hour, homogeneity and phase separation characteristics of the foods were observed at hourly interval up to 6 hour. The reconstituted

samples were then subjected to evaluation of reconstitution characteristic like reconstitution time, flow rate and viscosity were determined by adopting standard protocols.

2.3.1 Reconstitution time

Reconstitution time (in minutes) was determined by the method described by Nwanekezi et al. [21]. Two gram of flour samples were spread on the surface of 50 mL distilled water at room temperature 28°C in 150 mL measuring cylinder. The time taken by flour to completely disperse in distilled water was recorded as reconstitution time.

2.3.2 Flow behavior

Flow behavior evaluation was carried out by means of drip test, and gravitational method using 200 ml of reconstituted enteral foods (20%, 25% and 30% solid concentration) taken in delivery sterilized bag and syringe fitted with 12, 14 and 16-Fr Ryle's tubes. It was allowed to flow from a height of about 3 ft from the working table under gravity. A stop watch was used to measure the time for 200 ml to pass through nasogastric tubes of various gauges into the measuring cylinder. Each solution was run through three times and the flow rates were recorded up to the fraction of a minute.

2.4 Nutrient Composition of Developed Enteral Formula Feed

The protein, fat and fiber contents of the developed enteral formulae feeds were analysed employing the method of AOAC (2005). Total carbohydrate contents were determined by difference method (Nitisewojo, 1995) and energy was calculated using the formula given by James (1990).

2.5 Statistical Analysis

Statistical analysis were performed using Microsoft office excel 2007 and Statistical Package for Social Science version 20.0 software. The significant difference among the ingredients used and developed formulae were determined by employing one way analysis of variance followed by post hoc analysis using Duncan test at 95 per cent level of significance. Pearson correlation and principal component analysis was performed to test the relationship between functional attributes of the developed enteral formulae.

3. RESULTS AND DISCUSSION

3.1 Functional Properties of Ingredients and Developed Formulae

Functional properties are the intrinsic physico-chemical properties that reflect the complex interaction between the composition, structure, confirmation and physicochemical properties of protein and other food components and the nature of environment in which these are associated and measured [22]. They are important characteristics of food more importantly for enteral foods as they are used for tube feeding. The quality and composition of the final product is greatly influence by the individual ingredients used and the basic components are responsible for the tolerance and nutrition efficacy. Therefore, it is necessary to have knowledge on functional characteristics of ingredients along with the final products. The functional properties of the ingredients and the developed enteral formulae are presented in Table 2 and 3 respectively.

The bulk density of flour is the density measured without influence of any compression. The bulk densities of the ingredients used ranged from 0.33 g/ml (skimmed milk powder) to 0.50 g/ml (malted rice flour) which is similar to the findings of Amandikwa et al. [23]. Among the developed enteral formulae, the bulk density was in the range of 0.44 g/g (HPEF) to 0.47 g/g (HEEF). The low bulk densities of the ingredients and the developed enteral formula may be due to small particle size of the sample as bulk densities of a product is greatly affected by particle size [24]. The bulk is also affected by protein fractions as protein fractions has lower density than starch granules [25] which could have been a reason for the lowest bulk density of HPEF due to presence of malted green gram flour in higher proportion which is a rich source of protein.

Data revealed a proportional increased in tapped density of the flour to the bulk density. There was increased in tapped density in all the ingredients proportionally to the bulk density. Highest tapped density was seen in malted rice flour (0.56 g/ml) while whey protein showed the lowest (0.42 g/ml) among the ingredients used for developing the enteral formulae. The results for tapped density of enteral formulae revealed tapped density of 0.52 0.50 and 0.53 g/g in BEF, HPEF and HEEF respectively. Like bulk density, the tapped density of a product is also influenced by particle

size [26]. This is because of the fact that particles with small size end to pack more loosely due to their irregular shape than larger granules [27].

The data on particle density of the ingredients showed that flaxseed had the highest particle density (0.59 g/ml) while skimmed milk had the lowest particle density (0.59 g/ml). Among the developed enteral formulae highest particle density was seen in HPEF while lowest in BEF which might have been affected by particle density of contributing ingredients.

Porosity is an important functional property of flour which depends on its bulk density and particle density. The porosity of the ingredients used for formulation of enteral formulae was varying from 35 per cent in whey protein to 60 per cent in flax seed flour while in developed enteral formulae it ranged between 41.00 (BEF) to 44.44 (HPEF) per cent. The discrepancies in the porosity of the developed enteral formulae in the present investigation could be due to difference in the values of bulk density and tapped density of the processed ingredients [28].

The cohesiveness in terms of Hausner ratio (HR) of a food is a number that measures the flowability of a food material. It is a good indicator of compactness mechanism which is takes place during processing of ingredients (Malave et al., 1985). The HR of the ingredients used and developed enteral formulae ranged from 0.33 (skimmed milk) to 1.18 (popped amaranth) and 1.12 (HEEF) to 1.14 (HPEF) respectively which were lower than 1.25 indicating a good flow ability of the ingredients and the developed enteral formulae [29].

Carr index is a frequently used attribute of flowability in powdered products. The carr index is lesser in case of a any free flowing product because of very minimal difference between bulk density and tapped density while in a poor flowing powder where there is greater inter-particle interaction, the car index would be larger. The ingredients used in formulation of enteral formulae and the developed enteral formulae had low Carr index i.e. below 25 indicating a superior flow ability of the developed enteral formulae [29].

Among the ingredients lowest WAI was found in malted green gram flour (2.25 ml/g), followed by malted rice flour (2.33 ml/g), whey protein (3.82 ml/g), popped amaranth flour (3.92 ml/g) and

Table 2. Functional properties of the ingredients used for formulation of enteral formula

Attributes	Ingredients					
	Malted rice flour	Malted green gram flour	Popped amaranth flour	Flaxseed flour	Skimmed milk powder	Whey protein
Bulk density (g/ml)	0.50± 0.05 ^a	0.45±0.04 ^{a,b}	0.38±0.11 ^{a,b}	0.42± 0.03 ^{a,b}	0.33± 0.10 ^b	0.38± 0.04 ^{a,b}
Tapped density (g/ml)	0.56± 0.03 ^a	0.52±0.02 ^a	0.45±0.03 ^b	0.50±0.05 ^{a,b}	0.45±0.01 ^b	0.38± 0.05 ^c
Particle density (g/ml)	1.11± 0.10 ^b	1.00±0.02 ^b	1.00±0.03 ^{b,c}	1.25±0.06 ^a	0.59±0.03 ^e	0.90± 0.02 ^d
Porosity (%)	49.54± 2.10 ^a	48.00±0.15 ^a	55.00±0.23 ^b	60.00±1.06 ^c	41.32±0.03 ^d	35.00±1.02 ^e
Cohesiveness (HR)	1.12± 2.10 ^a	1.15±0.01 ^{a,b}	1.18±0.04 ^{a,b}	0.42± 0.03 ^b	0.33± 0.10 ^b	0.38± 0.04 ^b
Flow ability (CI) (%)	11.54±0.07 ^a	13±0.10 ^b	15.55±0.05 ^c	16.00±0.28 ^d	17.77±0.30 ^e	0.00 ^f
WAI (g/g)	2.33±0.08 ^c	2.25±0.40 ^c	3.92±0.01 ^b	7.59±0.20 ^a	1.23±0.00 ^d	3.92±0.05 ^b
WSI (% soluble solids)	36.60±1.77 ^d	39.38±2.60 ^d	48.62±1.01 ^c	23.66±1.77 ^e	83.00±2.20 ^b	87.62±3.01 ^a

Note. Values are mean ± standard deviation of triplicates. Different superscript in a row represents significant difference ($p < 0.05$). HR: Hausner ratio; CI: Carr Index, WAI: Water Absorption Index; WSI: Water Solubility Index

highest was in flax seed flour (7.59 ml/g). Among the developed enteral formulae, a narrow range of WAI was found. HEEF being highest (4.59ml/g), followed by HPEF (3.59 ml/g) and Balanced Energy Formula (3.39 ml/g) were observed. It was observed that the water absorption capacities of the developed enteral formulae were higher than the individual ingredients which might be due to the combined effect of individual ingredients on final product.

The WSI of various processed ingredients was highest among the high protein ingredients like whey protein (87.62±0.01%) and skimmed milk powder (83.00±0.20%) followed by popped amaranth flour (48.62±0.01%), malted green gram (39.38±0.60%) while least per cent water solubility was observed in flaxseed (23.66±0.77%). This was because the solubility in water is contributed by protein structure in protein rich product [30]. Developed enteral formulae were observed to have 62.66±0.20 per cent, 51.00±0.12 per cent and 49.66±0.23 per cent in HPEF, BEF and HEEF respectively. Thus, result clearly suggested that samples having higher malt fraction had a higher WSI as compared to those having lesser malt. Increase in WSI due to malting was also reported by Pelembe et al. [31].

The porosity of the ingredients used for formulation of enteral formulae was varying from 35 per cent in whey protein to 60 per cent in flax seed flour while in developed enteral formulae it ranged between 4 to 44.44 per cent. Porosity is an important functional property of flour which depends on its bulk density and particle density. The porosity is a measure of voids between solid particles of a food material. The discrepancies in the porosity of the developed enteral formulae in the present investigation could be due to

difference in the values of bulk density and tapped density of the processed ingredients [28].

The cohesiveness in terms of Hausner ratio (HR) of a food is a number that measures the flowability of a food material. It is a good indicator of compactness mechanism which is takes place during processing of ingredients [32]. The HR of the ingredients used and developed enteral formulae ranged from 0.33 to 1.18 and 1.12 to 1.14 respectively which were lower than 1.25 indicating a good flowability of the ingredients and the developed enteral formulae [29].

Carr index is a frequently used attribute of flowability in powdered products. The carr index is lesser in case of a any free flowing product because of very minimal difference between bulk density and tapped density while in a poor flowing powder where there is greater inter-particle interaction, the car index would be larger. The ingredients used in formulation of enteral formulae and the developed enteral formulae had low Carr index i.e. below 25 indicating a superior flowability of the developed enteral formulae [29].

Among the ingredients lowest WAI was found in malted green gram flour (2.25 ml/g), followed by malted rice flour (2.33 ml/g), whey protein (3.82 ml/g), popped amaranth flour (3.92 ml/g) and highest was in flaxseed flour (7.59 ml/g). Among the developed enteral formulae, a narrow range of WAI was found. HEEF being highest (4.59ml/g), followed by HPEF (3.59 ml/g) and Balanced Energy Formula (3.39 ml/g) were observed .It was observed that the water absorption capacities of the developed enteral formulae were higher than the individual ingredients which might be due to the combined effect of individual ingredients on final product.

Table 3. Functional properties of the developed enteral formulae

Attributes	Formulae		
	BEF	HPEF	HEEF
Bulk density (g/ml)	0.46± 0.02 ^a	0.44±0.01 ^a	0.47±0.20 ^a
Tapped density (g/ml)	0.52±0.04 ^a	0.50±0.11 ^a	0.53± 0.03 ^a
Particle density (g/ml)	0.89±0.01 ^a	0.90±0.10 ^a	0.90± 0.05 ^a
Porosity (%)	41.00±0.11 ^a	44.44±0.14 ^b	41.11±2.05 ^a
Cohesiveness (HR)	1.13± 2.10 ^a	1.14±0.04 ^a	1.12±0.10
Carr Index (%)	11.54±0.07 ^a	12.00±0.07 ^a	11.30±0.11 ^a
WAI (g/g)	3.39±0.20	3.59±0.20	4.59±0.20 ^a
WSI (% soluble solids)	51.00±0.71 ^a	42.66±0.07 ^b	49.66±0.23 ^a

Note. Values are mean ± standard deviation of triplicates. Different superscript in a row represents significant difference ($p < 0.05$). HR: Hausner ratio; WAI: Water Absorption Index; WSI: Water Solubility Index; BEF: Balanced Enteral Formula; HPEF: High Protein Enteral Formula; HEEF: High Energy Enteral Formula

The WSI of various processed ingredients was highest among the high protein ingredients like whey protein (87.62±0.01%) and skimmed milk powder (83.00±0.20%) followed by popped amaranth flour (48.62±0.01%), malted green gram (39.38±0.60%) while least per cent water solubility was observed in flaxseed (23.66±0.77%). This was because the solubility in water is contributed by protein structure in protein rich product. Developed enteral formulae were observed to have 62.66±0.20 per cent, 51.00±0.12 per cent and 49.66±0.23 per cent in HPEF, BEF and HEEF respectively. Thus, result clearly suggested that samples having higher malt fraction had a higher WSI as compared to those having lesser malt. Increase in WSI due to malting was also reported by Pelembe et al. [31].

3.2 Color of the Ingredients and Developed Enteral Formulae

Color is an important quality attribute of both raw and processed foods due to its significant impact on consumer perception. The color values of the ingredients used and developed enteral formula are presented in Tables 4 and 5 respectively. The color data indicates that malted rice flour, malted green gram flour, popped amaranth flour and skimmed milk powder had higher L^* values moving towards 100 while hue values moving towards toward 90° and chroma value shifting towards 0° which indicates that these ingredients had slightly yellow color with lesser intensity. The color analyses of flaxseed and

whey protein showed that they have a lower L^* values indicating darker colour with comparatively lesser hue values. The chroma values of these ingredients were also lesser indicating dark reddish color with lesser intensity. The characteristic colour of different ingredients may be due to original colour of the ingredients or may also have been affected by several reactions occurring during drying and roasting process where lysine and other amino acids present in the raw material reacts with reducing sugar thereby leading to formation of a dark brown colour [33].

Among the developed enteral formulae, highest L^* value was of BEF (87.10) followed by HPEF (83.54) and HEEF (82.43). A decreasing trend of lightness was observed on addition of ingredients with high L^* value such as whey protein and flaxseed flour. The developed formulae had higher H^* value heading towards 90°, positive b^* value shifting towards yellowness and a lower chroma value in all the developed formulae indicating a light yellow colour of the product with lesser intensity. The colour of the developed enteral formulae shows that the final colour of the formulae has been contributed greatly by the colour of ingredients such as malted rice flour, malted green gram flour, popped amaranth flour and skimmed milk powder. However a little impact of flaxseed flour and whey protein was seen on the colour of final product probably due to their usage in smaller quantities during preparation of enteral formulae.

Table 4. Colour measurements in term of hunter lab values of ingredients

Ingredients	L	a*	b*	Hue	Chroma
Malted rice flour	91.70	0.02	7.10	89.84	7.10
Malted green gram flour	91.87	0.20	14.71	89.92	14.71
Popped amaranth flour	82.58	3.43	17.35	78.82	17.69
Flaxseed flour	50.37	5.97	11.34	62.24	12.82
Skimmed milk powder	92.92	2.83	17.73	80.93	17.95
Whey protein powder	70.50	5.99	15.33	68.66	16.46

In Hunter Colour Lab L^ indicates lightness or darkness (0= black, 100 = white), a^* indicates the hue on the green-to-red axis. (negative value = greenness, positive value = redness), b^* indicates the hue on the blue –to-yellow axis (negative value = blueness, positive value = yellowness). C^* is the intensity of the hue [$C^*=(a^2 + b^2)^{1/2}$]; and hue angle (H°) is the angle in the colour wheel of 360° ($H^\circ = \tan^{-1} b^*/a^*$)*

Table 5. Colour measurements in term of Hunter Lab values of developed enteral formulae

Formula	L	a*	b*	Hue	Chroma
BEF	87.10	1.35	13.75	84.18	13.32
HPEF	83.54	2.38	13.95	80.32	14.15
HEEF	82.43	2.01	13.75	81.66	13.86

In Hunter Colour Lab L^ indicates lightness or darkness (0= black, 100 = white), a^* indicates the hue on the green-to-red axis. (negative value = greenness, positive value = redness), b^* indicates the hue on the blue –to-yellow axis (negative value = blueness, positive value = yellowness), C^* is the intensity of the hue [$C^*=(a^2 + b^2)^{1/2}$]; and hue angle (H°) is the angle in the colour wheel of 360° ($H^\circ = \tan^{-1} b^*/a^*$)*

3.3 Viscosity of the Developed Enteral Formulae

Viscosity is referred to the magnitude of internal friction in a fluid which is one of the important properties of enteral formula. Enteral formula of higher viscosity may lead to tube clogging [34]. The viscosities of the developed enteral formula feed reconstituted with warm water at different concentrations are presented in Fig 1. The data in figure reveals that BEF had lowest viscosity level among the three developed formulae and HEEF has highest viscosity followed by HPEF. An increase in viscosity was noted with increased solute content. The study of [35] also showed somewhat similar viscosity of 250 cP in the developed formulae.

3.4 Osmolality of the Developed Enteral Formula

Osmolality is one of the most important characteristics of an enteral formula. All nutrients and dietary components except water has an influence on osmolality of a solution. It is the overall function of size and quantity of ionic and molecular particles within a given volume. Osmolality of enteral formula is an important parameter to be determined prior to administration into patients to prevent any intolerance. A hypertonic formula may cause gastric retention, nausea and vomiting [36].

As per Matresse, [37] Osmolality of enteral formula ranges from 270-700 mOsmol/kg. The

osmolality of the developed enteral formula presented in Table 6 ranges from 260 to 580 mOsmol/kg. The osmolality of BEF was 260.00 ± 9.59 mOsmol/kg which according to Henriques and Rosado [38] classified isomolar solution with less than 400 mOsmol/kg of solvent. Agarwal et al. [39] in their study observed an osmolality of 339 mOsmol/kg in an easy to reconstitute nutrient dense mix used for enteral feeding. The osmolality of HPEF and HEEF were 430.00 ± 12.56 and 580.00 ± 12.56 mOsmol/kg solvent which were classified isomolar and hyperisomolar respectively which are mainly meant for patients with fluid restriction. The higher osmolality may be contributed from the higher protein and energy content of formulae feed. However enteral diets above 600 mOsmol/kg demand special care with regard to their administration, following parameters by Brazilian Health Surviellane Agency.

3.5 Reconstitution Behavior of the Developed Enteral Formulae

The reconstitution behavior of any flour is affected by a number of properties [11]. The functional properties of ingredients such as bulk density, tapped density, particle density, porosity, water solubility etc influences the reconstitution behavior of developed enteral formulae to a greater extent. Poor reconstitution behavior of formulae may influence the flow of the developed enteral formulae feed resulting in tube clogging.

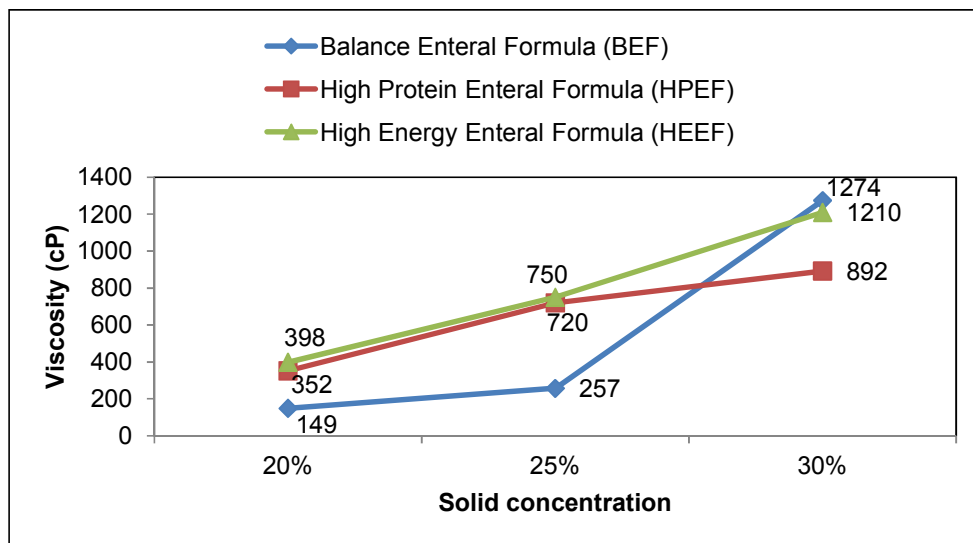


Fig. 1. Viscosity of the developed enteral formulae at different solid concentration

Table 6. Osmolality of the developed enteral formula

Formula	Osmolality (mOsmol / Kg solvent) 20% solid concentration	Classification
Balanced Enteral Formula (BEF)	260.00±9.59	Isomolar / Isotonic
High Protein Enteral Formula (HPEF)	430.00±12.56	Slightly hyperosmolar / Slightly hypertonic
High Energy Enteral Formula (HEEF)	580.00±12.56	Hyperosmolar / Hypertonic

Note: Values are mean ± standard deviation of triplicates

Table 7. Reconstitution time of the developed formulae

Type of Enteral feed	Reconstitution Time (minutes)
Balanced Enteral Formula (BEF)	5.0 ± 0.00
High Protein Enteral Formula (HPEF)	5.0 ± 0.00
High Energy Enteral Formula (HEEF)	6.0 ± 0.01

Note: Values are mean ± standard deviation of triplicates. BEF: Balanced Enteral Formula; HPEF: High Protein Enteral Formula; HEEF: High Energy Enteral Formula

3.5.1 Reconstitution time

The reconstitution time of the developed enteral formulae presented in Table 7 shows that the reconstitution time of the developed ranged from 5 to 6 minutes with reconstitution time of 5, 5 and 6 minutes in BEF, HPEF and HEEF respectively. A higher reconstitution time of 8 minutes in enteral feed formulated using malted maize and malted ground nut was reported in the study of [40]. Similar reconstitution time was also seen in the study of Jain and Joshi [41].

3.5.2 Flow rate of the developed enteral formula

Flow behavior is one of the most important criteria for selection of enteral formula. Enteral feeding can be delivered via a range of feeding tubes through feeding method such as continuous, bolus and gravity feeding [42]. According to Seres et al. [39] bolus feeding method require administration of formula feed after every 4-6 hour with a flow rate of 300 to 400 ml in 5-20 minutes where as intermittent feeding requires administration of formulae feed at the rate of 200-350 ml within 10-30 minutes. On the other hand the in continuous feeding, the flow rate of formulae feed varies depending on caloric density of formula, ranging from minimum of 10-40 ml per hour maximum of 50-100 ml per hour.

The flow rate of the developed enteral formulae reconstituted with warm water at 20, 25 and 30 per cent solid concentration is presented in Table 8. The feeding tubes comes in various sizes and

the size of ryles tube often used for enteral feeding have 10-16 Fr. The flow rate of all the developed formula feed was maximum for feed with 20 per cent solid concentration. The flow rate of the developed formula feed of 20 per cent solid concentration on passing through 12, 14 and 16 Fr Ryles tube was 20,22,29 ml/min respectively for BEF, 20,22 and 25 ml/min respectively for HPEF and 17, 20 and 25 ml/min respectively for HEEF. The flow of the enteral formulae feed recorded in the present investigation was appropriate and meets the recommendation of ASPEN [43]. Jain and Joshi [44] and Ramamurthi et al. [45] have tested the flow behavior of developed enteral feeds reconstituted with water at a ratio of 1:2 and 1:3. They observed a flow rate ranging from 15 ml/min to 38 ml/min on passing through 12 and 14 Fr Ryle's tube.

3.6 Nutrient Composition of the Formulated Enteral Formula Feed

The nutrient composition of the developed enteral formulae feed is presented in Table 9. The three enteral formulae developed from locally available ingredients were prepared to suite specific purposes. The developed BEF, HPEF and HEEF had an energy content of 100 Kcal, 100 Kcal and 200 Kcal respectively; protein content of 3.82, 5.46 and 7.72 g/100ml respectively and fat content of 2.7, 2.7 and 9 g/100ml respectively. The nutrient composition per 100 ml of the developed enteral formula feeds were as per the recommendation of ASPEN [44]; DAA [46] and Nilesh et al. [47].

3.7 Pearson Correlation between Functional Attributes of the Developed Enteral Formulae

The Pearson correlation coefficient between functional attributes of the developed enteral formulae is presented in Table 10. The data reveals a very strong positive correlation between bulk densities and tapped densities of the developed enteral formulae while significantly ($p < 0.01$) was seen between WSI and porosity. The table also depicts a significant correlation between ($p < 0.05$) viscosity and WAI. However the correlation shown between other attributes were not statistically significant.

3.8 Principal Component Analysis of Attributes of Developed Enteral Formulae

The principal component analyses of the functional attributes of developed enteral formulae is presented in Table 11 and Fig. 2. In PCA, the first six principal components were explaining over 99 per cent of the variances for

the functional attributes of developed enteral formulae, of which the first two principal components accounted for 6.75 per cent of the variance observed in the functional attributes of developed enteral formulae. The principal component 1 accounted for 38.42 per cent of the variances and the principal component two accounted for 28.33 per cent of variances. The Table 9 summarizes the loadings of two extracted component accounting for maximum variances. It showed that the most important variable of the PC-1 were bulk density, tapped density and WSI while in the PC-2 the most important attributes were porosity, particle density and viscosity. It can be seen in the figure that porosity, viscosity and particle density are on the right top quadrant of the biplot indicating maximum contribution to the sample scores. The attributes farther from the axes indicates maximum contribution to the principal components while the attributes nearer to axes had lower contribution to the principal components. The attributes clustered together are positively correlated to one while attributes opposite to one another tends to have a negative correlation between them.

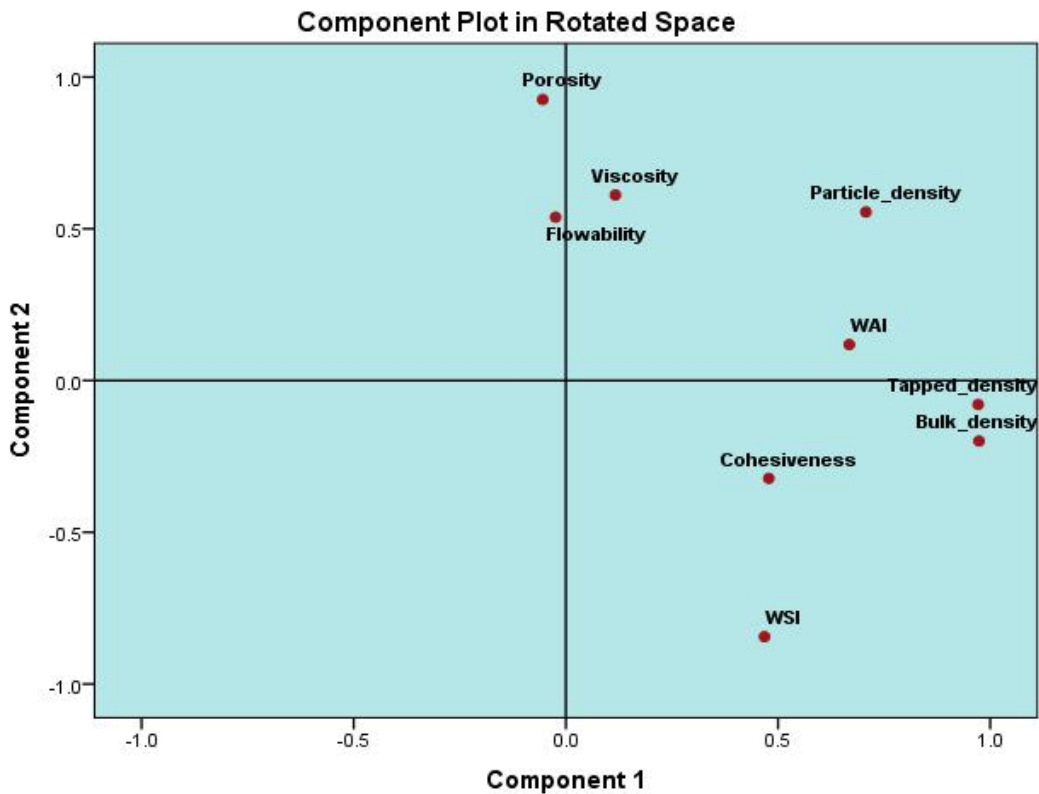


Fig. 2. Principal component analysis of functional attributes of developed enteral formulae

Table 8. Flow rate of reconstituted developed enteral formulae of different solid concentrations pass through 12, 14 and 16 Fr. Ryle's tube size

Solid concentration	Flow rate of reconstituted enteral formula feeds (ml/min)								
	20 % solid concentration (w/v)			25 % solid concentration (w/v)			30 % solid concentration (w/v)		
	Formula / Ryle's Tube size	RT*12 Fr.size	RT 14 Fr.size	RT 16 Fr.size	RT 12 Fr.size	RT 14 Fr.size	RT16 Fr.size	RT 12 Fr.size	RT14 Fr.size
BEF	20	22	29	17	20	25	13	18	22
HPEF	20	22	25	15	18	22	12	13	17
HPEF	17	20	25	12.5	14	17	10	11	13

Note. BEF: Balanced Enteral Formula; HPEF: High Protein Enteral Formula; HEEF: High Energy Enteral Formula; RT - Ryle's tube

Table 9. Nutritional composition of the developed Enteral Formula (BEF) feed in comparison to the suggested guidelines (per 100 ml)

Nutrients	Balanced enteral formula (BEF)	High protein enteral formula (HPEF)	High energy enteral formula (HEEF)
Energy (Kcal)	100.00	100.00	200.00
Crude protein (g)	3.82	5.46	7.72
Crude fat (g)	2.7	2.70	9.00
Total carbohydrates (g)	14.85	13.46	21.77
Crude fiber (g)	0.70	0.57	1.32

Table 10. Person correlation coefficient between attributes of the developed enteral formulae

	Bulk density (g/ml)	Tapped density (g/ml)	Particle density (g/ml)	Porosity (%)	Cohesiveness (HR)	Flow ability (%)	WAI (g/g)	WSI (%Soluble solids)	Viscosity (cp)
Bulk density (ρ_b) (g/ml)	1.000								
Tapped density (ρ_t) (g/ml)	0.957**	1.000							
Particle density (ρ_p) (g/ml)	0.591 ^{NS}	0.642 ^{NS}	1.000						
Porosity (ϵ) (%)	0.247 ^{NS}	-0.072 ^{NS}	0.586	1.000					
Cohesiveness (Hausner ratio)	0.489 ^{NS}	0.629 ^{NS}	0.136	-0.138 ^{NS}	1.000				
Flowability (%)	0.083 ^{NS}	0.010 ^{NS}	0.309	0.555 ^{NS}	0.135 ^{NS}	1.000			
WAI (g/g)	0.622 ^{NS}	0.522 ^{NS}	0.340	-0.178 ^{NS}	-0.129 ^{NS}	-0.302 ^{NS}	1.000		
WSI (% soluble solids)	0.645 ^{NS}	0.486 ^{NS}	-0.071	-0.824**	0.348 ^{NS}	-0.443 ^{NS}	0.227 ^{NS}	1.000	
Viscosity(cp)	-0.037 ^{NS}	-0.030 ^{NS}	0.178	0.325 ^{NS}	-0.454 ^{NS}	-0.113 ^{NS}	0.699*	-0.503 ^{NS}	1.000

Note. NS- correlation is not significant; * Correlation is significant at 95% level of significance; ** Correlation is significant at 99 per cent level of significance

Table 11. Loadings of PC-1 and PC-2 for different functional attributes

Attributes	Component	
	PC-1	PC-2
Bulk density	.969	.221
Tapped density	.917	.330
Water Solubility Index	-.775	-.575
Cohesiveness	.569	-.096
Water Absorption Index	.559	.384
Porosity	-.433	.820
Particle density	.414	.798
Viscosity	-.147	.605
Flowability	-.245	.480

Extraction Method: Principal Component Analysis. PC: Principal components

4. CONCLUSION

It is evident from the investigation that the developed Balanced Enteral Formula, High Protein Enteral Formula and High Energy Enteral Formula had superior functional characteristics which was mainly due to utilization of ingredients with good functional characteristics. The developed formulae also showed excellent reconstitution behavior and flow rate indicating their potentialities to be used as a quality enteral formula for administration during acute or chronic illness.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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