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FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

Development and quality evaluation of hypoallergic complementary foods from rice incorporated with sprouted green gram flour

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Abstract: Rice flour and green gram flour (sprouted as well as unsprouted) were blended in different proportions with apple pulp, sugar, milk and water for formulation of 12 hypoallergic diets. The formulations were studied for physico-chemical properties, antinutrient content (phytate and oxalate) and in vitro protein digestibility. Blending and sprouting significantly affected all the studied parameters. Sprouting significantly ($p \leq 0.05$) improved protein content and in vitro protein digestibility, while carbohydrate content and antinutrients decreased significantly. Nutrition profile of water-based diets met RDA guidelines only after addition of sprouted green gram flour and were comparable to control (commercial weaning food). Water-based formulation showed lower sensory score than milk-based diets; however, addition of sprouted green gram flour significantly ($p \leq 0.05$) improved their overall acceptability. It could be concluded that by incorporating sprouted green gram flour, milk could be replaced with water for production of hypoallergic weaning foods without affecting the nutritional and sensory attributes of formulations and thereby making the production more economical, especially for underdeveloped countries.

Subjects: Bioscience; Environment & Agriculture; Food Science & Technology

Keywords: weaning; hypoallergic; in vitro-protein digestibility; sprouting; quality evaluation

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PUBLIC INTEREST STATEMENT

Complementary foods consumed by the infants in many parts of world are deficient in essential macronutrients and micronutrients leading to malnutrition, which is one of the serious problems in developing countries. Milk and cereals are nutrient rich sources that are commonly used in complementary foods, but are associated with different medical conditions like allergies and lactose and gluten intolerance. A specific combination of gluten-free cereals and appropriate processing technique might improve the nutritional profile to the extent that milk could be replaced with water resulting in production of a hypoallergic gluten-free diet that can not only combat PEM but also provide an alternative against complications arising from use of milk and wheat.

1. Introduction

Complementary foods play a vital role on child growth and development since it complements for both nutritional and developmental needs of the infant when breast milk alone is no longer sufficient (Temesgen, 2013). According to WHO (2003), good quality weaning food must have high nutrient density, low viscosity, bulk density and appropriate texture along with high energy, protein and micronutrient contents and should have a consistency that allows easy consumption (Balasubramanian, Kaur, & Singh, 2014). Several studies have reported that most of the complementary foods consumed by the infants in many parts of world are deficient in essential macronutrients and micronutrients leading to malnutrition, which is one of the serious problems in developing countries. According to UNICEF, every year one million children below the age of five years die due to malnutrition-related causes in India alone. Most lethal forms of malnutrition include protein energy malnutrition, “the silent emergency of the world” which may have hunted mankind since the dawn of history. Protein energy malnutrition (PEM) generally occurs during the crucial transitional phase when children are weaned from liquid (i.e. breast milk) to semi-solid or fully adult (family) foods. Milk and cereals are nutrient rich sources that are commonly used in complementary foods (Aderonke, Fashakin, & Ibrinke, 2014), but are associated with different medical conditions like allergies and lactose and gluten intolerance. Food allergy is an increasing health care concern. According to National Institute of Allergy and Infectious Diseases (NIAID) expert panel, food allergy is an adverse health effect arising from a specific immune response that occurs reproducibly following consumption of a given food (Sampson et al., 2014). The “Big 8” food allergens cause more than 90% of all food allergies (American Dietetic Association (ADA), 2000). They include: eggs, milk, peanut, tree nuts (such as walnuts, almonds, cashews, hazelnuts and pistachio nuts), soy (primarily in infants), wheat, fish and crustacean shellfish (such as lobster, crab and shrimp). Overall, the typical allergens of infancy and early childhood are eggs, milk, peanuts, fish, tree nuts, wheat and soy, while allergens in older children and adults are caused primarily by peanuts, tree nuts, fish and crustacean shellfish (Sampson, 2004; Sicherer and Sampson, Munoz-Furlong, & Sicherer, 2006). Food allergies generally develop early in life, but can develop at any age. For example, milk allergy tends to develop early in life, whereas shrimp allergy generally develops later in life. Children usually outgrow their egg, milk and soy allergies, but people who develop allergies as adults usually have their allergies for life.

Certain infants are known to be allergic to milk due to presence of several proteins present in milk principally alpha s1-casein (Anonyms, 1997). The main allergens of cow’s milk are distributed among the whey and casein fractions. The whey allergens include: (a) alpha-lactalbumin, (b) beta-lactoglobulin, (c) bovine serum albumin and (d) bovine immunoglobulins. Milk allergy can lead to gastrointestinal, dermatological, respiratory disorders and even anaphylaxis, a life-threatening allergic response that can be prevented by completely avoiding consumption of milk proteins. In developing countries, about 2–3% of infants are affected by milk allergy (Høst, 2002) and avoiding milk proteins in complementary diets would further increase prevalence of PEM. Although various milk substitute formulas have been devised that include soy-based diets and partially or extensively hydrolyzed proteins. However, infants allergic to milk are usually allergic to soy proteins also (milk soy protein intolerance), affecting 85,000 infants every year in USA (Wade, 2011). Also hydrolyzed food formulations are not suitable for treating acute cases of milk allergy.

In view of these nutritional problems, several strategies have been used to formulate hypoallergic complementary foods, through a combination of locally available food sources that complement each other in such a way as to create a new pattern of amino acids that provide the recommended daily allowance for infant (Ijarotimi & Ashipa, 2006). Cereals are deficient in lysine, but have sufficient sulphur containing amino acids which are limiting factors in legumes, whereas legumes are rich in lysine (Mishra, Mishra, & Shukla, 2014). Processing techniques used for formulating complementary foods such as soaking, sprouting, fermentation and roasting enhance the bioavailability of micronutrients by decreasing the antinutritional factors and improving overall digestibility and absorption of nutrients. Such processing techniques also reduce the high bulk of complementary food by reducing the viscosity (Rasane, Jha, & Kumar, 2015). Therefore, a specific combination of gluten-free cereals and appropriate processing technique might improve the nutritional profile to the

extent that milk could be replaced with water resulting in production of a hypoallergic gluten-free diet that can not only combat PEM, but also provide an alternative against complications arising from use of milk and wheat. A study is therefore designed to develop nutrient-dense, safe, low-cost complementary food from the combination of rice and apple incorporated with sprouted green gram.

2. Materials and methods

2.1. Procurement of raw materials

Raw materials: rice (*Oryza sativa*), green gram (*Vigna radiata*) and apple (*Malus domestica*) were used to formulate 12 composite blends (diets) and procured from local market. The diets were prepared by blending all the ingredients in different proportions. Rice was used as major ingredients of the mixture. Protein source was derived from green gram. Other raw materials used include sugar, milk and potable water.

2.2. Preparation of raw material

2.2.1. Preparation of rice and green gram flour

Rice (*O. sativa*) grains and green gram (*V. radiata*) were thoroughly cleaned to remove extraneous material. The grains were separately washed, drained and dried at ambient temperature followed by milling in the electric grinder (Sujata powermatic plus) to make fine flour and sieved by 80–100 mesh sieves and then packaged separately in airtight plastic container till further use.

2.2.2. Preparation of sprouted green gram flour

The cleaned green gram seeds were washed three times in excess distilled water. Then, the cleaned and washed green gram seeds were soaked in a volume of water three times the weight of seeds (3:1) for 12 h in a container at ambient temperature. The steeping water was drained off and the soaked green gram seeds were washed twice using distilled water to prevent the growth of microorganisms during sprouting. The soaked seeds were wrapped in damp muslin cloth to stimulate sprouting. The green gram seeds were allowed to sprout for 24 h. The content was left in ambient conditions and watered 2–3 times a day to enhance the sprouting process. The sprouts were dried overnight at room temperature by keeping under a fan. The sprouted grains were ground in an electric grinder (Sujata powermatic plus) to make fine powder and sieved by 80–100 mesh sieve. The milled sample was then packed in airtight plastic container. The container was stored at room temperature until further use.

2.2.3. Preparation of apple pulp

Fresh, mature and healthy apple was harvested from local gardens. Red delicious variety of apples was used for the pulp. Apple pulp was prepared according to method of Nisar, Baba, and Masoodi (2015) without any thermal or chemical treatment. Freshly prepared apple pulp was added to diet formulations and added at a constant level of blending (10%) to each diet formulation.

2.2.4. Formulation of complementary foods

Three different formulations of complementary food were developed in which quantities of rice and green gram flour was varied. Apple and sugar were also added. Both milk and water were used thus 12 different combinations (Diet 1–12) were prepared. The composition of formulations is shown in Table 1. The schematic diagram for development of complementary foods is shown in Figure 1.

2.3. Physico-chemical analysis

2.3.1. Proximate composition

Proximate composition (Protein, ash and fat) of formulated weaning formulations was determined by Association of Official Analytical Chemists (2000) method. Total percentage carbohydrate was

Table 1. Formulation of rice-based complementary foods

Weaning mixes	Ingredients						
	Water	Milk	Rice	UGG	SGG	Apple	Sugar
DIET 1	1,060	-	70	10	0	10	10
DIET 2	960	-	60	20	0	10	10
DIET 3	880	-	50	30	0	10	10
DIET 4	1,060	-	70	0	10	10	10
DIET 5	960	-	60	0	20	10	10
DIET 6	880	-	50	0	30	10	10
DIET 7	-	1,060	70	10	0	10	10
DIET 8	-	960	60	20	0	10	10
DIET 9	-	880	50	30	0	10	10
DIET 10	-	1,060	70	0	10	10	10
DIET 11	-	960	60	0	20	10	10
DIET 12	-	880	50	0	30	10	10

determined by the difference method as reported by Omemu (2011). The formula for the calculation of percentage carbohydrate is shown as:

$$\text{Carbohydrate (\%)} = 100 - (\text{fat\%} + \text{protein\%} + \text{ash\%})$$

The energy values of the weaning food formulations was determined by computation and expressed in calories. It was calculated from protein, fat and carbohydrate contents using the Atwater's conversion factors:

$$1 \text{ kcal}/100 \text{ g} = [(4 \times \text{carbohydrate}) + (4 \times \text{protein}) + (9 \times \text{fat})]$$

2.3.2. Determination of pH and titrable acidity

Physico-chemical analysis was carried out by Association of Official Analytical Chemists (2000) method. The pH of formulated diets was determined using digital pH metre (Inolab WTW Series 720). The pH metre was first calibrated using buffer of pH 4.0 and 7.0 at room temperature. The sample was then taken in a 100 mL beaker, stirred and electrode of pH metre put in it and direct reading from pH metre was taken when the reading stabilized.

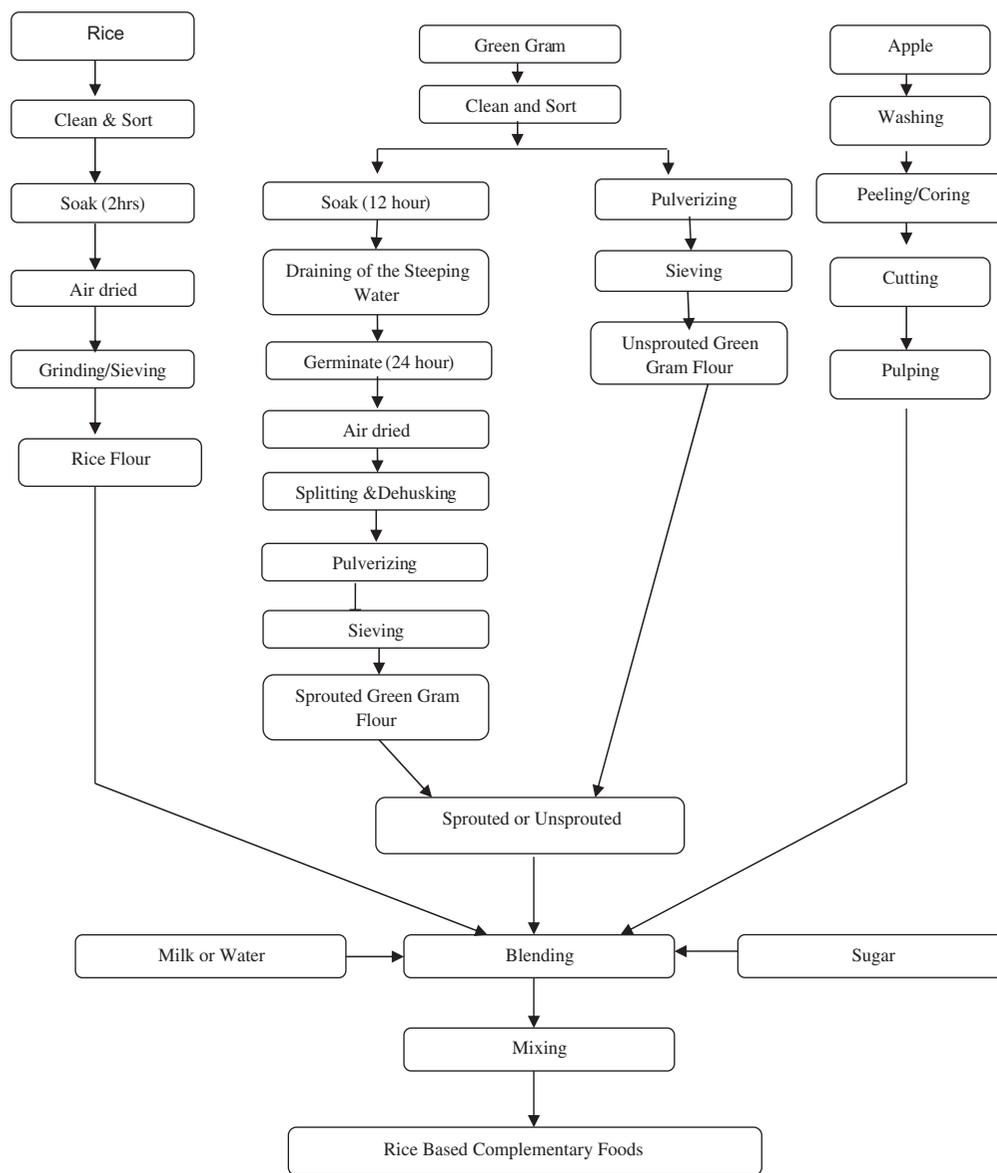
Titrable acidity was done using the method of (Association of Official Analytical Chemists, 2000). Ten millilitres of sample was taken and homogenized with distilled water in a home scale blender. The whole mass was then transferred to a 100 mL volumetric flask and the volume was made up to the mark with distilled water. Ten millilitres of this sample solution was taken in a conical flask. Two to three drops of phenolphthalein indicator were added and then flask was shaken vigorously. It was then titrated immediately with 0.1-N NaOH solutions from a burette till pink colour appeared. The volume of NaOH solution required for titration was noted and per cent of titrable acidity was calculated as below:

$$\text{Acidity\%} = \frac{\text{Titre} \times \text{Normality of alkali} \times \text{Volume made} \times \text{equivalent wt. of acid} \times 100}{\text{Volume of sample (taken for estimation)} \times \text{Wt. or Volume of sample} \times 1000} \times 100$$

2.3.3. Determination of specific gravity and viscosity

Specific gravity of the samples was determined according to AOCS method (1993). The specific gravity bottle was placed in a water bath maintained at 25°C and filled with distilled water. It was removed, wiped dry (outside the bottle) and weighed. The bottle was emptied, dried and again placed in water bath at 25°C. The bottle was refilled with the sample and made to stay in the water bath for

Figure 1. Flow diagram of complementary food formulation prepared from rice, green gram, apple, milk and sugar.



30 min. It was then removed, cleaned and wiped (outside the bottle) completely dry and weighed. The specific gravity at 25°C was calculated using the equation:

$$\text{Specific gravity at } 25/25^{\circ}\text{C} = \frac{\text{Weight of bottle an sample at } 25^{\circ}\text{C} - \text{Weight of bottle at } 25^{\circ}\text{C}}{\text{Weight of water at } 25^{\circ}\text{C}}$$

Viscosity was measured using a Brookfield Viscometer (Model LV DV-E 230; Middleboro, Massachusetts, USA). The cooked gruel was poured into the viscometer beaker, cooled to 40°C and viscosity was measured in centipoises, cP, using spindle number 62 at a shear rate of 30 revolution per minute (RPM). Within 3 min, the average of the maximum and minimum viscosity reading was recorded according to the speed.

2.3.4. Determination of colour

The colour of the diets was measured using a Hunter's Lab colour analyser (MiniScan XE Plus, Model 45/0-S, Hunter Associates Laboratory Inc., Reston, VA, USA). The equipment was calibrated using

white and black standard ceramic tiles. In the Hunter's lab colorimeter, the colour of a sample is denoted in three dimension, L*, a* and b*. Where L* refers to lightness of the colour of the diets and ranges from black = 0 to white = 100. A negative value of a* indicates a green colour where the positive value indicates red-purple colour. A positive value of b* indicates a yellow colour and the negative value a blue colour. ΔE^* is a parameter that quantifies the overall colour difference of a given sample compared to a reference sample. It was calculated using the equation:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where $\Delta L^* = (L^*_1 - L^*_0)$; $\Delta a^* = (a^*_1 - a^*_0)$; and $\Delta b^* = (b^*_1 - b^*_0)$.

Subscript 'o' depicts the colour value for reference sample (untreated sample) and subscript '1' depicts the colour value for the sample being analysed (Caivano, 2012). C* coordinate is the chroma [Equation (1) and H* coordinate the hue (Equation (2))] (Wrolstad & Smith, 2010). The L*a*b* values from samples were used for the determination of whiteness and yellowness index.

$$\text{Chroma } C^* = \sqrt{(a^*)^2 + (b^*)^2}$$

$$\text{Hue angle } H^\circ = \tan^{-1} \{b^* / a^*\}$$

2.3. In vitro protein digestibility

The in vitro protein digestibility of the diets was done by the modified procedure of Saunders, Connor, Booth, Bickoff, and Kohler (1973) with some modifications.

2.4. Antinutritional factors

The titration method described by Day and Underwood (1986) was used to determine the oxalate content. The phytic acid content was measured by the method of Thompson and Erdman (1982).

2.5. Sensory analysis

Weaning formulations were evaluated for sensory quality characteristics using Nine Point Hedonic scale by a semi-trained panel of 15 lactating mothers.

2.6. Statistical design

Results were expressed as mean of triplicate analyses. A one-way analysis of variance and Duncan's test were used to establish the significance of differences among the mean values at the 0.05 significance level. The statistical analyses were performed using SPSS software (Systat statistical program version 21 (SPSS Inc., USA).

3. Results and discussion

3.1. Physico-chemical analysis

3.1.1. Proximate composition

Different proportions of rice flour were used in Diet 1–12 for the production of rice-based weaning foods. The moisture content of diets (1–3) decreased non-significantly ($p \leq 0.05$) with the decrease in content of rice flour (Table 2). A similar trend was seen in diets (7–9) in which water was replaced with milk for improving the nutritional quality of the formulated foods. The decrease in moisture content may be either due to the decrease in rice flour or an increase in green gram flour. These results were similar to those of reported in weaning food formulated from rice flour and groundnut by Adebayo-Oyetero et al. (2011). From the Table 2, it is found that the moisture content of diets (4–6) and (10–12) decreased with the increase in sprouted green gram flour. However, the moisture content was found to increase significantly ($p \leq 0.05$) in diets where unsprouted green gram flour was replaced by sprouted green gram flour. This finding is in harmony with that of Khatoun and Prakash (2006). Seeds of legumes and cereals take up water when sufficient moisture is available

which in turn results in their sprouting. Hydrolysis and solubilization of complex carbohydrates and protein takes place during their sprouting. This results in variation in their water binding properties. Variation in the moisture content of formulations containing sprouted green gram from those containing unsprouted green gram may be the result of their different moisture holding capacities. Water which is held tightly is not estimated while drying food samples for moisture estimation.

Ash content is an important nutritional indicator of mineral content and a quality parameter that determines contamination with foreign matters (Mishra et al., 2014). The ash content of diets (1–3) increased non-significantly ($p \leq 0.05$) with an increase in the content of unsprouted green gram flour (Table 2). A similar trend was seen as green gram content increased in diet (7–9) in which water was replaced with milk (Table 2). The increase in the ash content could be due to the higher mineral content of green gram (Mubarak, 2005). Similar increase in ash content have been reported by Yaseen et al. (2014) in green gram, Munasinghe, Jayarathne, Sarananda, and Silva (2012) in lentil and Martin, Laswai, and Kulwa (2010) in cowpea. In diets 4–6 and diets 10–12 a non-significant ($p \leq 0.05$) decrease in the ash content was seen with incorporation of sprouted green gram flour (Table 2). With increase in the proportion of sprouted green gram flour there was decrease in ash content suggesting sprouting of green gram leads to decrease in ash content of weaning foods. The reduction in ash content after sprouting was in agreement with those reported by Megat-Rusydi, Noraliza, Azrina, and Zulkhairi (2011) for sprouted green gram and Masood, Shah, and Zeb (2014) for chick pea. This decrease in ash content after sprouting might be due to the leaching out of minerals into the soaking and cooking water (Kavitha & Parimalavalli, 2014).

Protein is one of the most important nutrients required in complementary foods and is required for speedy growth and development of a child. The protein content of diets (1–3) increased significantly ($p \leq 0.05$) with an increase in the content of green gram flour (Table 2). A similar trend was seen in diets (7–9) in which water was replaced with milk for improving the nutritional quality of the formulated foods (Table 2). It was observed that increased level of substitution led to significant increase

Table 2. Proximate composition of formulated complementary foods from rice flour, unsprouted/sprouted green gram flour and apple pulp

Ingredients/ blends	Moisture	Ash	Crude protein	Crude fat	Carbohydrate	Energy
Rice	9.75 ^a ± 0.3	0.41 ^a ± 0.02	7.27 ^a ± 0.84	0.80 ^a ± 0.27	81.77 ^a ± 0.7	363.36 ^a ± 1.60
GG	14.71 ^b ± 0.01	3.3 ^b ± 0.17	24.33 ^b ± 1.13	1.11 ^b ± 0.33	56.55 ^b ± 1.12	333.51 ^b ± 1.49
Apple	83.91 ^c ± 0.1	0.2 ^c ± 0.05	0.2 ^c ± 0.05	0.48 ^c ± 0.02	15.21 ^c ± 0.15	65.96 ^c ± 0.74
Diet 1	91.63 ^d ± 0.01	0.52 ^d ± 0.01	9.84 ^d ± 0.18	3.21 ^d ± 0.01	86.43 ^d ± 0.19	413.97 ^d ± 0.09
Diet 2	91.24 ^d ± 0.01	0.53 ^d ± 0.01	11.60 ^e ± 0.12	3.87 ^e ± 0.01	83.99 ^e ± 0.11	417.19 ^e ± 0.02
Diet 3	90.81 ^d ± 0.03	0.55 ^d ± 0.01	13.37 ^f ± 0.05	4.52 ^f ± 0.01	81.56 ^f ± 0.07	420.36 ^f ± 0.04
Diet 4	93.57 ^e ± 1.59	0.50 ^e ± 0.01	11.32 ^g ± 0.06	4.90 ^g ± 0.01	83.27 ^g ± 0.02	422.46 ^g ± 0.01
Diet 5	92.42 ^d ± 0.52	0.46 ^e ± 0.01	12.69 ^h ± 0.12	5.05 ^h ± 0.01	81.80 ^h ± 0.11	423.41 ^h ± 0.08
Diet 6	91.30 ^f ± 0.32	0.43 ^e ± 0.03	14.56 ⁱ ± 0.09	5.15 ⁱ ± 0.03	79.86 ⁱ ± 0.11	424.03 ⁱ ± 0.01
Diet 7	82.8 ^g ± 0.03	0.61 ^f ± 0.01	14.50 ⁱ ± 0.08	6.28 ^j ± 0.10	78.59 ^j ± 0.11	428.88 ^j ± 0.57
Diet 8	82.32 ^h ± 0.01	0.63 ^f ± 0.01	17.49 ^k ± 0.19	6.89 ^k ± 0.04	74.97 ^k ± 0.23	431.85 ^k ± 0.20
Diet 9	81.20 ⁱ ± 0.04	0.65 ^f ± 0.01	19.69 ^l ± 0.07	7.23 ^l ± 0.02	72.42 ^l ± 0.08	433.51 ^l ± 0.08
Diet 10	83.98 ^j ± 0.17	0.56 ^g ± 0.03	16.04 ^m ± 0.54	7.17 ^m ± 0.02	76.23 ^m ± 0.64	433.61 ^m ± 0.57
Diet 11	83.40 ^k ± 0.08	0.51 ^g ± 0.03	18.86 ⁿ ± 0.33	8.03 ⁿ ± 0.01	72.60 ⁿ ± 0.30	438.11 ⁿ ± 0.20
Diet 12	82.78 ^l ± 0.12	0.48 ^g ± 0.01	20.78 ^o ± 0.46	8.50 ^o ± 0.01	70.24 ^o ± 0.27	440.58 ^o ± 0.08
Control (g/100 g)	-	-	15	9	67.5	411 kcal
RDA*		-	11–13 (g/100 g)	12 (g/day)	-	≈450 (kcal/100 g)

Notes: Values are mean ± standard deviations of three ($n = 3$) replications with different superscripts in a column vary significantly ($p \leq 0.05$).

in the protein content. The increase in protein content could be attributed to the higher levels of green gram. Increase in protein content of weaning foods with increase in green gram fraction has also been reported earlier (Ukey, Diamond, Raheem, & Karande, 2014). In diets 4–6 and diets 10–12 a significant ($p \leq 0.05$) increase in the protein content with increase in proportion of sprouted green gram flour (Table 2) was observed. The increase in protein content in sprouted green gram incorporated diets was in agreement with those reported by Khatun, Haque, Hosain, and Amin (2013) for weaning foods formulated from sprouted wheat and lentil flour, Ikujenlola and Adurotoye (2014) for weaning foods from mixtures of malted quality maize and steamed cowpea, Mubarak (2005) and Ghavidel and Prakash (2007) for sprouted green gram seeds and Chaudhary and Vyas (2014) for millet (Ragi)-based premixes. This increase in protein content after sprouting might be due to enzymatic changes (Bau, Villaume, Nicolas, & Méjean, 1997), hormonal changes or a compositional change following the degradation of other constituents (D'souza, 2013). The enzymes produced during sprouting lead to the hydrolysis of starch and proteins with release of sugar and amino acids. Proteolytic enzymes improves amino acid availability mainly lysine, methionine and tryptophan (Bolanle et al., 2012). The protein contents of weaning food for infants recommended by ICMR are 11–13 g per day. Rice-based weaning formulations in which water was used instead of milk meets the Recommended Dietary Allowance for infants only when higher proportion of unsprouted green gram flour was added. However, the protein content is adequate in formulations, where water is used instead of milk and sprouted green gram is used as protein source instead of unsprouted green gram (Table 2). Thus if sprouting is practised, green gram-based diets could be made more economical by cutting out the use of milk with water and still meeting the RDA recommended by ICMR for infants. In developing countries of Asia where PEM is a major threat, sprouting can be used as an economically viable technique to improve nutritional security.

Fat is another important component of infant diet because it provides essential fatty acids, facilitates absorption of fat soluble vitamins, and enhances dietary energy density and sensory qualities (Solomon, 2005). The fat content of diets (1–3) increased significantly ($p \leq 0.05$) with an increase in the content of green gram flour. A similar trend was seen in diets (7–9) in which water was replaced with milk (Table 2). Increase in fat content of weaning foods with increase in legume fraction has also been reported earlier by Hussain and Uddin (2011) for mung bean and Ojinnaka, Ebinyasi, Ihemeje, and Okorie (2013) for soybean. In diets 4–6 and diets 10–12 a significant ($p \leq 0.05$) increase in the fat content was seen with the increase in proportion of sprouted green gram flour in the diets and the values obtained were higher than corresponding diets of same composition in which unsprouted green gram flour was used (Table 2). The increase in fat content in sprouted green gram incorporated diets was in agreement with those reported by Onyango, Noetzold, Bley, and Henle (2004) for weaning food formulated from maize and fermented finger millet and Khattak, Zeb, and Bibi (2008) for sprouted chick pea. This increase in fat content after sprouting of green gram might be due to the biosynthesis of new compounds during sprouting (Kim et al., 2012) and also removal of soluble carbohydrates during sprouting could concentrate the fat content proportionately (Bekele, 2011). These results are in harmony with earlier results for soybean and sprouted brown rice (Anuchita & Nattawat, 2010). However, the fat content of a food sample can affect its shelf stability. This is because fat can undergo oxidative deterioration, which leads to rancidification and spoilage than one with a lower fat content. Hence, a food sample with high fat content is more liable to spoilage than one with a lower fat content. Moreover, the fat content of the formulated weaning foods slightly fulfils the Recommended Dietary Allowance (RDA) for infants.

The carbohydrate content of diets (1–3) decreased significantly ($p \leq 0.05$) with a decrease in the content of rice flour (Table 2). A similar trend was seen in diets (7–9) in which water was replaced with milk for improving the nutritional quality of the formulated foods (Table 2). The decrease in carbohydrate is principally due to decrease in rice flour. These results were similar to those reported by Adebayo-Oyetoro et al. (2011). In diets 4–6 and diets 10–12 a significant decrease in the carbohydrate content was seen with increase in proportion of sprouted green gram flour in the diets (Table 2). The carbohydrate content was found to decrease in sprouted green gram diets. This reduction in carbohydrate content after sprouting can be attributed to the utilization of carbohydrate as a

source of energy for embryonic growth during sprouting (Vidal-Valverde et al., 2003) and D'souza (2013). Additionally alpha-amylase activity increased that breaks down complex carbohydrates to simpler and more absorbable sugars which are utilized by the growing seedlings during the early stages of sprouting (Megat-Rusydi et al., 2011). Similar results were reported by Kakati, Dekka, Kotaki, and Saikia (2010) and Mubarak (2005).

The FAO/WHO (1998) and ICMR (2010) have recommended that foods fed to infants and children should be energy rich and should provide ≈450 kcal/day. According to the recommendation, this is necessary because low-energy foods tend to limit total energy intake and utilization of other nutrients. The energy values (kcal/100 g) of the rice-based diets (1–6) and (7–12) ranged from 413.98–424.02 kcal and 428.95–440.58 kcal, respectively, highest being in sprouted diets suggesting sprouting leads to a significant increase in the energy content of the diets. The energy content of weaning formulations having sprouted green gram flour and water fulfils the RDA of energy requirement for infants even at lower levels of blending in comparison to diets containing unsprouted green gram flour. Thus, if sprouting was practised, green gram-based diets could be made more economical by cutting out the use of milk with water and still meeting the RDA of infants. Hence, in developing countries of Asia, where PEM is a major threat, sprouting can be used as an economical tool in combating the debacle of food security without incurring high costs.

3.1.2. pH and titrable acidity

Incorporation of varying proportions of unsprouted or sprouted green gram non-significantly affected acidity and pH of weaning formulations (Table 3). However, the variation in the two parameters was significant because of sprouting. Acidity and pH are interdependent and lower the pH, higher is the acidity. The pH of diets (1–3) decreased non-significantly with an increase in the proportion of green gram flour (Table 3). A similar trend was seen in diets (7–9) in which water was replaced with milk (Table 3). Sprouting lead to further decrease in pH in both milk (Diets 10–12) as well as water (4–6) containing diets. Decrease in pH value by sprouting has also been reported by Ariahu, Ingbian, and Ojo (2009) in maize/mushroom-based formulation. The titrable acidity of diets (1–3) increased non-significantly with an increase in the content of green gram flour (Table 3). A similar trend was seen in diets (7–9) in which water was replaced with milk (Table 3). An increase in acidity has also been reported by Sefa-Dedeh, Kluitse, and Afoakwa (2001) in the production of weaning food from maize-cowpea blends. Diets with sprouted green gram (4–6 and 10–12) had higher acidity values than those containing unsprouted green gram that increased with an increase in its

Table 3. Physico-chemical properties of formulated complementary foods from rice flour, unsprouted sprouted green gram flour and apple pulp

	pH	Titrable acidity (%)	Specific gravity	Viscosity (cp)	Colour			Hue	Chroma
					L*	a*	b		
Diet 1	7.42 ^a ± 0.03	0.06 ^a ± 0.06	1.048 ^a ± 0.01	250.13 ^a ± 0.12	75.37 ^a ± 0.52	-1.98 ^a ± 0.06	13.39 ^a ± 0.15	81.57 ^a ± 0.18	13.53 ^a ± 0.16
Diet 2	7.38 ^a ± 0.02	0.08 ^a ± 0.01	1.043 ^a ± 0.01	207.32 ^b ± 0.10	74.37 ^a ± 2.17	-3.13 ^a ± 1.36	20.95 ^b ± 1.07	82.95 ^a ± 4.67	21.20 ^b ± 1.27
Diet 3	7.36 ^a ± 0.02	0.10 ^a ± 0.04	1.038 ^a ± 0.01	185.49 ^c ± 0.81	70.77 ^b ± 1.06	-3.71 ^a ± 1.71	24.95 ^c ± 0.80	81.51 ^a ± 3.96	25.26 ^c ± 0.72
Diet 4	7.01 ^b ± 0.01	0.17 ^b ± 0.01	1.027 ^b ± 0.01	208.30 ^d ± 0.06	72.23 ^c ± 0.53	-0.30 ^b ± 0.03	12.21 ^d ± 0.04	88.56 ^b ± 0.14	12.21 ^d ± 0.03
Diet 5	6.99 ^b ± 0.01	0.19 ^b ± 0.01	1.024 ^b ± 0.05	167.46 ^e ± 0.07	69.54 ^c ± 0.49	-0.68 ^b ± 0.03	15.92 ^e ± 1.24	87.01 ^b ± 0.29	16.68 ^e ± 1.24
Diet 6	6.96 ^b ± 0.01	0.21 ^b ± 0.02	1.020 ^b ± 0.01	128.95 ^f ± 0.01	67.51 ^d ± 0.46	-0.86 ^b ± 0.07	16.66 ^e ± 1.32	87.53 ^b ± 0.21	15.93 ^e ± 1.32
Diet 7	6.67 ^c ± 0.01	0.29 ^c ± 0.04	1.078 ^c ± 0.01	268.24 ^g ± 0.16	87.89 ^e ± 1.11	-0.44 ^c ± 0.14	19.86 ^f ± 0.33	88.72 ^c ± 4.79	19.86 ^f ± 0.44
Diet 8	6.65 ^c ± 0.04	0.31 ^c ± 0.01	1.073 ^c ± 0.01	213.13 ^h ± 1.74	83.67 ^f ± 0.29	-0.09 ^c ± 0.07	23.71 ^g ± 0.16	89.76 ^c ± 0.13	23.71 ^f ± 0.16
Diet 9	6.62 ^c ± 0.03	0.33 ^c ± 0.01	1.069 ^c ± 0.01	162.71 ⁱ ± 0.01	77.37 ^g ± 1.46	-1.043 ^d ± 1.70	25.09 ^g ± 0.08	87.61 ^c ± 0.33	25.11 ^f ± 0.08
Diet 10	6.58 ^d ± 0.03	0.47 ^d ± 0.01	1.065 ^c ± 0.01	221.73 ^j ± 0.07	85.17 ^h ± 1.04	-0.51 ^c ± 0.12	13.01 ^h ± 1.22	87.75 ^c ± 0.43	13.02 ^g ± 1.22
Diet 11	6.54 ^d ± 0.01	0.49 ^d ± 0.03	1.061 ^c ± 0.05	175.61 ^k ± 0.01	80.36 ⁱ ± 1.06	-0.69 ^c ± 0.81	14.02 ^h ± 0.25	87.15 ^c ± 3.24	14.05 ^g ± 0.31
Diet 12	6.50 ^d ± 0.02	0.50 ^d ± 0.01	1.057 ^c ± 0.01	131.22 ^l ± 0.03	75.81 ^j ± 1.05	-1.11 ^d ± 0.41	15.99 ⁱ ± 0.26	86.01 ^c ± 1.54	16.03 ^h ± 0.23

Notes: Values are mean ± standard deviations of three (n = 3) replications with different superscripts in a column vary significantly (p ≤ 0.05).

proportion. These results are in conformity with those obtained by Kure and Wyasu (2013) and Jood, Khetarpaul, and Goyal (2012). Sprouting lead to an increase in acidity that enhances the keeping quality of weaning foods, by inhibiting microbial growth and also contributes to the flavour development. An increase in acidity and decrease in pH may be due to the hydrolysis of fats to produce fatty acids and production of ascorbic acid during sprouting process (Desai, Kulkarni, Sahoo, Ranveer, & Dandge, 2010).

3.1.3. Specific gravity & viscosity

Specific gravity of rice-based complementary foods is shown in Table 3. The specific gravity of diets (1–6) and (7–12) ranged from 1.020 to 1.048 and 1.057–1.078, respectively. The effect of proportion of green gram showed a non-significant decrease in specific gravity. Sprouting leads to a decrease in specific gravity which is directly proportional to density. Victor (2014) reported a decrease in bulk density with sprouting that is due to the activity of alpha amylase enzyme which was activated during malting process and dextrinifies starch to its constituent's sub-units.

Weaning food of high viscosity and high bulk density is usually unacceptable to infants as it makes feeding taskful and causes choking. Infants can easily consume sufficient quantity of food if it is low in viscosity/bulk density because it allows incorporation of more solids in mixture leading to an increase in nutrient density of the gruel. Low viscosity and low bulk density weaning food with a high nutrient content is a desirable characteristic in complementary foods (Onweluzo & Nwabugwu, 2009). Sprouting as well as level of green gram had a significant ($p \leq 0.05$) effect on viscosity of weaning foods (Table 3). The viscosity of diets (1–3) decreased significantly with an increase in green gram flour. A similar trend was seen in diets (7–9) in which water was replaced with milk (Table 3). Adebayo-Oyetero et al. (2011) have reported decrease in viscosity in complementary food from fermented sorghum, walnut and ginger. Variation in the content of rice and green gram had a significant effect of viscosity of formulations. Increase in green gram or decrease in rice content significantly decreased the viscosity of formulation irrespective of the fact whether milk or water was used in the formulations. The decrease was more prominent when sprouted green gram was used. These results are in agreement with the findings of Gernah, Ariahu, and Ingbian (2012) and Victor (2014), who reported that processing methods like sprouting and fermentation are valuable in reducing the viscosity of infant gruels, increase total solids and nutrient density of weaning food. This decrease in viscosity due to sprouting might be due to the enzymatic breakdown of macromolecules such as polysaccharides and polypeptides to smaller units, such as dextrans and peptides, respectively (Gernah et al., 2012).

3.1.4. Colour

Colour characteristic is a major criterion that affects the quality of the final product. Blending and sprouting significantly affected colour parameters. Blending with sprouted as well as unsprouted green gram flour significantly ($p \leq 0.05$) decreased L^* value of diets (1–3) & (4–6) (Table 3). A similar trend was seen in diets (7–9; 10–12) in which water was replaced with milk (Table 3). Similar change in L^* value was reported earlier by Frost, Adhikari, and Lewis (2011) in barley cookies. Decrease in L^* value with an increase in sprouted as well as unsprouted green gram flour can be due to higher protein content of green gram. It has been previously reported that with an increase in protein content there is a decrease in L^* value (Bhise & Kaur, 2013). The decrease in L^* value due to sprouting can be due to Maillard reaction (Barnwal, Kore, & Sharma, 2013). Sprouting results in greater release of sugars and proteins that act as raw material for maillard reaction resulting in an increase in formation of brown pigment (melanoidins) that leads to darkening of the diets.

Blending significantly ($p \leq 0.05$) increased b^* value of diets (1–3; 4–6) with an increase in the content of green gram flour (Table 3). A similar trend was seen in diets (7–9; 10–12) in which water was replaced with milk (Table 3). Similar change in L^* value was reported earlier by Frost et al. (2011) in barley cookies with increase in barley flour. Increase in b^* value with increase in content of green gram flour can be due to pigments such as xanthophylls and carotenoids present in green gram. Although blending with green gram (sprouted and unsprouted) resulted in an increase in b^* value of

diets however, incorporation of sprouted green gram resulted in decrease in b^* value in comparison to diets in which unsprouted green gram was incorporated. Decrease in b^* value in sprouted diets can be due to degradation of browning pigments (Eissa, Hassanane, & Sharaf, 2014). The chroma value describes its brightness while the hue angle represents a coordinate in a standardized colour space. The chroma and hue value did not show any consistent trends for the rice-based diets.

3.2. In vitro protein digestibility of rice-based weaning formulations

Different proportions of rice and green gram flour both sprouted and unsprouted were blended with water (Diet 1–6) and milk (Diet 7–12) separately for the production of rice-based weaning foods. Incorporation of varying proportions of unsprouted or sprouted green gram significantly affected in vitro protein digestibility of food samples. The in vitro protein digestibility of diets (1–3) decreased significantly with an increase in the proportion of unsprouted green gram flour (Figure 2). A similar trend was seen in diets (7–9) in which water was replaced with milk (Figure 2). Diets with the least protein content had highest IVPD, while diets with the highest protein content had the lowest IVPD. This result showed that high protein content does not necessarily imply high protein digestibility. Green gram is reported to contain various antinutritional factors such as tannins and phytic acid that adversely affects protein digestibility (Mubarak, 2005) and thus decreasing IVPD with an increase in green gram proportion. The decrease in IVPD can also be due to non-enzymic browning reactions, which involve interactions between inherent proteins and added sugar, resulting in non-reversible formation of compounds causing a decrease in the availability of protein for digestion (Okpala & Chinyelu, 2011). Similar decrease in in vitro protein digestibility has also been reported by Okpala and Chinyelu (2011) with an increase in the proportion of pigeon pea flour in cookies. Diets with sprouted green gram (4–6; 10–12) had significantly ($p \leq 0.05$) higher IVPD than those containing unsprouted green gram. These results are in conformity with those obtained by Ghavidel and Prakash (2007) for green gram and Pérez-Conesa, Ros, and Periago (2002) for infant cereals. This increase in in vitro protein digestibility after sprouting might be due to either reduction of antinutritional factors (tannins, trypsin inhibitors and phytic acid) and/or denaturation of proteins making them more vulnerable to proteolytic enzyme activity (Viswanathan & Ho, 2014).

Figure 2. In vitro protein digestibility of complementary food formulations prepared from rice flour, green gram flour and apple pulp.

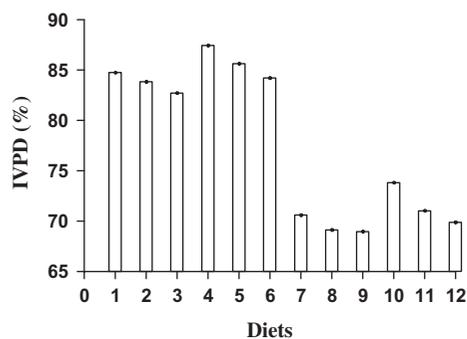
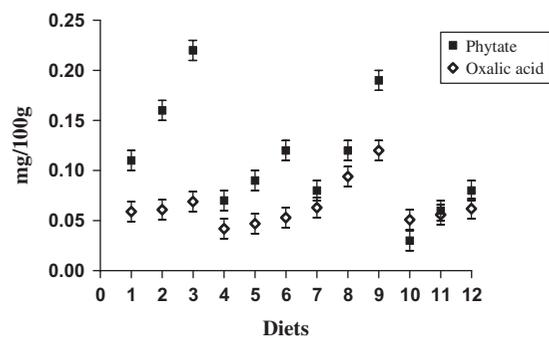


Figure 3. Antinutrient content of complementary food formulations constituted from rice, green gram and apple.



3.3. Antinutrient composition

Cereals and legumes contain numerous antinutrients that hinder the bioavailability of other nutrients in the GI tract. Phytic acid and oxalic acid are two prominent antinutrients found in both green gram and rice. Phytic acid in legumes binds to mineral elements such as calcium, zinc, manganese, iron and magnesium to form complexes that are indigestible, thereby decreasing the bioavailability of the element for absorption (Dahouenon-Ahoussi et al., 2012). Incorporation of varying proportions of unsprouted or sprouted green gram non-significantly ($p \leq 0.05$) affected antinutrient factors of rice-based weaning formulations (Figure 3). However, sprouting lead to a significant variation in the antinutrient content of food formulations. The oxalic acid and phytic acid of diets (1–3) increased non-significantly with an increase in the proportion of unsprouted green gram flour (Figure 3). A similar trend was seen in diets (7–9) in which water was replaced with milk (Figure 3). An increase in oxalic acid and phytic acid has been reported by Steve and Ayobami (2006) in the production of weaning food from sorghum and pigeon pea blends. Sprouting significantly ($p \leq 0.05$) decreased the antinutrient compounds in both milk (Diets 10–12) as well as water (4–6) containing diets. The decrease in oxalic acid and phytic acid as a result of sprouting was in agreement with findings of Desalegn, Abegaz, and Kinfe (2015) in quality protein maize-based complementary foods and Ghavidel and Prakash (2007) for green gram. This decrease in antinutrient components during sprouting might be due to leaching of antinutrients out into the soaking water under the concentration gradient (Vadivel, Stuetz, Scherbaum, & Biesalski, 2011). Decrease in the level of phytic acid during sprouting may also be attributed to hydrolytic activity of phytase enzyme in the sprouting grains (Osman, 2007). Since phytic acid is responsible for reducing minerals bioavailability, its reduction during sprouting may have enhanced the nutritional quality (Shimelis & Rakshit, 2007). Thus, sprouting increases bioavailability of nutrients by reducing the content of antinutrient factors in diets.

3.4. Sensory analysis

The sensory characteristics of weaning formulations prepared from rice flour, green gram flour and apple pulp evaluated in terms of different sensory attributes namely; colour/appearance, flavour, odour, mouthfeel, consistency and overall acceptability are presented in Table 4. Appearance is important attribute in food choice and acceptance. There was significant difference ($p \leq 0.05$) in the colour/appearance of the diets containing unsprouted green gram and sprouted green gram. The colour/appearance score of diets (1–6) increased with an increase in the content of green gram (Table 4). A similar trend was seen in diets (7–12) in which water was replaced with milk (Table 4). Diets with 60% green gram flour both milk as well as water-based got the highest score for colour.

Table 4. Sensory evaluation of formulated complementary foods from rice, unsprouted/sprouted green gram and apple

	Appearance	Taste	Flavour	Odour	Mouth feel	Consistency	Overall Acceptability
Diet 1	5.26 ^a ± 0.88	3.73 ^a ± 0.59	3.40 ^a ± 0.82	5.26 ^a ± 0.79	3.66 ^a ± 0.97	8.26 ^a ± 0.51	4.97 ^a ± 1.33
Diet 2	5.67 ^a ± 1.22	4.06 ^a ± 1.16	3.53 ^a ± 0.74	5.36 ^a ± 0.91	3.73 ^a ± 0.79	8.33 ^a ± 0.50	5.01 ^a ± 1.09
Diet 3	5.98 ^a ± 0.89	3.13 ^a ± 0.63	3.20 ^a ± 0.77	5.06 ^a ± 1.27	3.53 ^a ± 0.48	8.43 ^a ± 0.41	4.64 ^a ± 0.91
Diet 4	8.33 ^b ± 0.63	4.80 ^b ± 0.94	4.20 ^b ± 0.56	5.60 ^b ± 0.63	4.26 ^b ± 1.16	8.86 ^b ± 0.35	5.97 ^b ± 1.03
Diet 5	8.45 ^b ± 0.25	5.60 ^c ± 1.12	4.93 ^c ± 1.03	6.03 ^b ± 0.91	4.80 ^c ± 0.94	8.93 ^b ± 0.25	6.55 ^c ± 1.04
Diet 6	8.86 ^c ± 0.74	4.26 ^b ± 0.70	3.93 ^b ± 0.79	6.53 ^c ± 0.99	4.06 ^b ± 0.88	9.0 ^c ± 0.13	5.94 ^b ± 0.79
Diet 7	8.16 ^d ± 0.79	7.66 ^d ± 0.81	7.73 ^d ± 0.70	7.86 ^d ± 1.18	7.53 ^d ± 1.06	8.20 ^d ± 0.67	7.86 ^d ± 0.63
Diet 8	8.28 ^d ± 0.50	8.51 ^e ± 0.70	8.58 ^e ± 0.81	8.35 ^e ± 0.77	8.39 ^d ± 0.84	8.46 ^d ± 0.51	8.42 ^e ± 0.72
Diet 9	8.32 ^d ± 0.74	7.33 ^d ± 0.81	7.60 ^d ± 0.91	7.53 ^d ± 0.63	7.33 ^d ± 1.11	8.73 ^e ± 0.45	7.80 ^d ± 0.94
Diet 10	8.29 ^e ± 0.51	8.45 ^e ± 0.61	8.30 ^f ± 0.70	8.29 ^f ± 0.51	8.16 ^e ± 0.50	8.5 ^f ± 0.72	8.34 ^f ± 0.89
Diet 11	8.86 ^e ± 0.73	8.76 ^e ± 0.67	9.00 ^g ± 0.78	8.57 ^f ± 0.83	8.70 ^f ± 0.89	8.68 ^f ± 0.54	8.83 ^g ± 0.99
Diet 12	8.93 ^e ± 0.83	9.00 ^f ± 0.98	8.56 ^f ± 0.76	9.00 ^g ± 0.76	8.35 ^e ± 0.74	8.80 ^g ± 0.66	9.00 ^h ± 1.12

Notes: Values are mean ± standard deviations of three ($n = 3$) replications with different superscripts in a column vary significantly ($p \leq 0.05$).

Table 5. Cost evaluation of formulated complementary foods from rice, green gram and apple

Blends	Rice (`/100 gm)	Green gram (`/100 gm)	Apple (`/100 gm)	Sugar (`/100 gm)	Water (/100 gm)	Milk (`/100 gm)	Utilities (`/100 gm)	Total cost (`/100 gm)
Diet 1	0.77	0.8	0.2	0.15	0	0	4	5.92
Diet 2	0.66	1.6	0.2	0.15	0	0	4	6.61
Diet 3	0.55	2.4	0.2	0.15	0	0	4	7.3
Diet 4	0.77	0.8	0.2	0.15	0	0	4	5.92
Diet 5	0.66	1.6	0.2	0.15	0	0	4	6.61
Diet 6	0.55	2.4	0.2	0.15	0	0	4	7.3
Diet 7	0.77	0.8	0.2	0.15	0	26.5	4	28.42
Diet 8	0.66	1.6	0.2	0.15	0	24.0	4	26.61
Diet 9	0.55	2.4	0.2	0.15	0	22.0	4	25.3
Diet 10	0.77	0.8	0.2	0.15	0	26.5	4	28.42
Diet 11	0.66	1.6	0.2	0.15	0	24.0	4	26.61
Diet 12	0.55	2.4	0.2	0.15	0	22.0	4	25.3
Control 1	-	-	-	-	-	-	-	49.0
Control 2	-	-	-	-	-	-	-	61.6
Control 3	-	-	-	-	-	-	-	50.0

An increase in colour score might be attributed to the colour pigments found in green gram. Similar results were obtained by Muhimbula, Zachari, and Kinabo (2011) in complementary foods from cereals and legumes.

Taste and flavour were important attributes in sensory evaluation of food. The product might be appealing and having high energy density, but without good taste and flavour, such a product is likely to be unaccepted. The taste and flavour attributes of diets with sprouted green gram was significantly ($p \leq 0.05$) higher than those containing unsprouted green gram. The taste and flavour score of the diet 5 (5.60) and diet 12 (9) rated highest as compared to rest of rice-based diets. The increase in taste score of the rice-based diets with increasing the level of green gram flour may be attributed to the typical taste of green gram. Mouth feel is very important in complementary food as it will determine the amount of food an infant would consume since they can only swallow a smooth gruel not a course one. Likewise, odour is an integral part of taste and general acceptance of the food before it is put in the mouth. It is therefore important parameters when testing acceptability of formulated diets. Odour and mouthfeel of diets containing sprouted green gram were significantly ($p \leq 0.05$) higher than that of unsprouted diets. The mean score of mouthfeel and odour for diet 11 and diet 12 were higher than rest of rice-based diets.

The consistency and overall acceptability score exhibited highest for diet 12 (9.00) in sprouted diets, whereas in unsprouted diets the consistency and overall acceptability was highest in diet 8 (8.42). The overall acceptability score by mothers was more for milk-based diets than that of water-based diets (Table 4).

3.5. Cost estimation of rice-based weaning formulations

The estimated cost of approximately 100 g of each formulated diets based on the current prices of foodstuffs in the market, and the cost of control diets (commercial weaning food) are presented in Table 5. The estimates indicate that the formulated diets would cost in range of `5.92–28.42 while commercial weaning foods cost `49–61. The formulated weaning foods cost less by 20–30% and therefore more cost-effective and affordable. Since low-income families cannot afford these

commercial weaning foods, formulation of low-cost weaning foods can prove an important approach to alleviate protein energy malnutrition.

4. Conclusions

Rice can be an economical source for production of gluten-free weaning foods if blended with green gram. Sprouting significantly ($p \leq 0.05$) improves the nutritional value of the weaning formulations by improving their protein content. Incorporation of sprouted green gram can replace milk in weaning formulations without adversely affecting the nutritional profile of food formulations and thereby cut down cost of production. Sprouting reduced the viscosity and the specific gravity of the diets that is advantageous to the infant and can make feeding easier. This can ensure availability of quality weaning foods to children of underdeveloped nations. Sprouting also enhanced *in vitro* protein digestibility of these formulations probably due to reduction of antinutrient contents of samples and thus increase the food intake and bioavailability of nutrients. Hence, sprouting can be used as a promising tool for improving the nutritional profile and enhancing bioavailability of weaning formulation. Sprouting of legumes and their incorporation thus can help in countering protein energy malnutrition in developing countries. Water containing diets although had lower acceptability than diets containing milk, but sprouting could significantly improve the sensory characteristics of the formulations.

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