

# SENSORY EVALUATION, NUTRITIONAL QUALITY AND ANTINUTRITIONAL FACTORS OF TRADITIONALLY PRODUCE CEREALS-LEGUMES WITH *MORINGA OLEIFERA* LEAVES POWDER TO OVERCOME MICRONUTRIENTS DEFICIENCY IN CHILDREN AFTER COVID CRISIS

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## Abstract

Childhood malnutrition is a household issue and urgent health challenge particularly after COVID crisis in underdeveloped countries including Pakistan. To address this challenge, present research is designed to evaluate the effect of optimized ratio of cereals (wheat, rice) and legume (chickpea) blended with *Moringa oleifera* leaves (MOLs) powder. The prepared weaning formula was analyzed for its nutritional parameters. The formula contained 3, 6, 9, 12 and 15% MOLs powder and limited up to 15% after many trails. It is noted that supplemented weaning formula is the nutritious source of macro and micro-minerals with significantly ( $P \geq 0.05$ ) increasing trend up to 15% supplementation. The phytochemicals as total flavonoid contents (TFC) ( $57.79 \pm 0.94$  mg/100g), total phenolic content (TPC) ( $542.83 \pm 1.00$  mg/100g) and antioxidants ( $56.52 \pm 0.45\%$ ) also followed the same trend. According to sensory point of view prepared weaning formula with 12% *Moringa oleifera* leaves powder supplementation gain more acceptability due to its taste and color, afterward it was not acceptable. In this study, it was concluded that functional properties of the product remained consistent with the addition MOLs powder. Furthermore in physiochemical properties it was observed that bulk density was significantly decreased ( $P \leq 0.05$ ) with the addition of MOLs powder ( $0.71 \pm 0.01$  to  $0.65 \pm 0.04$  g/mL), reconstitute index increased ( $4.23 \pm 0.25$  to  $5.23 \pm 0.25$  mL) and viscosity was decreased ( $1.46 \pm 0.41$  to  $1.19 \pm 0.01$  Pa.s). The prepared weaning formula could be very effective in combating nutritional deficiencies in malnourished children especially after COVID crisis. According to WHO/ FAO nutritional standards the 12% of MOLs powder is more than enough to overcome the recommended daily allowance of children.

**Keywords:** *Moringa Oleifera*, Cereals, Legume, Micro-Nutrients, Macro-Nutrients, TPC, TFC, Malnutrition, Weaning Formula.

## 1. INTRODUCTION

The COVID 19 pandemic repercussions are much more extensive than that of any of the other viral illnesses which had infected the global community and it poses a risk to undermine the years of painstaking human development. Prior to the COVID 19 outbreak, Pakistan already had serious issues with food insecurity and malnutrition. The pandemic worsened the situation and posed a significant challenge to the food and health systems, threatening the public nutrition. Emphasis has been made on dietary modifications and the need to overcome micronutrient malnutrition which negatively impacted poor households, in varied contexts (FAO, IFAD & WFP, 2015). When crisis emerges, epidemic or economic, the first type of malnutrition is hidden hunger as the more expensive and nutritious foods will be entirely removed from household meal plans (Ezinne et al., 2023).

Fortifying local food crops can be an important approach to provide added nutrients to those at risk of nutritional deficiencies (Boateng, Norley, Ohemeng, Asante & Steiner-Asiedu, 2019). In order to tackle micronutrient deficiencies and to minimize their occurrences in human population, food fortification is supposed as crucial intervention strategy (Rowe, 2020). Recently, the food and agricultural organization has been facilitating to utilize the crops which are cost effective and readily accessible to prevent and control micronutrient deficiencies. However, the use and incorporation of the underutilized plants into our daily diet remains a challenge. Hence there is need to focus more on the implementation of these food-based approaches to achieve a healthy living (Ezinne et al., 2023).

*Moringa oleifera* leaves (MOLs) serve as nutritive vegetables in various countries. These provide a good extent of vitamins (C and A), protein and minerals including calcium, potassium and iron when compared with carrot, milk, banana and spinach. Iron content of MOLs is higher than that of spinach (Gopalakrishnan et al., 2016; Rockwood et al., 2013). MOLs are used by the African countries in their daily diets either in fresh and dried form to overcome nutritional deficiencies (Sahay et al., 2017). Different studies have revealed that different parts of moringa plant are actively used in food applications, making numerous types of complementary weaning foods products (Olusanya et al., 2020). MOLs powder is utilized to fight against malnutrition particularly among infants, children, pregnant women and feeding mothers (Anwar et al., 2007). It is also used globally as an additive in food fortification programs to increase nutritional contents of other foods (Govender and Siwela, 2020; Hekmat et al., 2015).

Cereals (wheat and rice) are major staple foods, specifically in Asian countries and commonly used in preparation of complementary foods. Wheat flour is considered as the most essential dietary staple in Pakistan (Sztupecki et al., 2023). It is a vital source of protein, carbohydrates in the form of starch and dietary fiber, vitamins, minerals and valuable phytochemicals which have substantial impact on human health. Rice must be considered in the first complementary diet for babies after 6 months of age due to good source of nutrients and higher digestibility (Chaudhari et al., 2023). But the cereals are deficient with some essential amino acids so its protein is not considered as good quality

protein. Similarly, legumes are important sources of macro- and micro-nutrients, especially the low-cost vegetable proteins in contrast to expensive animal origin products (Carbas et al., 2023; Ganzon-Naret, 2018). But alone the pulses or cereals cannot fulfill the all-body needs, their combination provides a healthy and nutritious product. This current study is designed to make a low-cost product using MOLs powder that could help in combating malnutrition. Although cereals (wheat and rice) made from indigenously grown crops have better nutritional quality to meet the dietary requirements, but inflation and skyrocketing prices are causing affordability problem for masses in low-income countries, contributing towards malnutrition.

## **2. MATERIALS AND METHOD**

### **2.1. Materials**

Fresh leaves of *Moringa oleifera* were obtained from “Forestry and Wildlife Management Department” of the University of Agriculture Faisalabad, Pakistan. Cereals (wheat and rice), legume (chickpea) and other ingredients were taken from the local market of Faisalabad. Analytical grade reagents were procured either from Sigma-Aldrich (Tokyo, Japan) and/or Merck KGaA (Darmstadt, Germany).

### **2.2. Powder Preparation of all Ingredients**

The cereals wheat and rice were prepared by using the procedure described by Saini et al., (2014). The cereals were separately cleaned, washed with tap water and placed in dehydrator (12 hours) for drying. The dried wheat and rice were roasted to improve the nutritional properties and reduce anti-nutritional components then ground to form a fine powder. The powders were separately packed in polythene bag for further preparation.

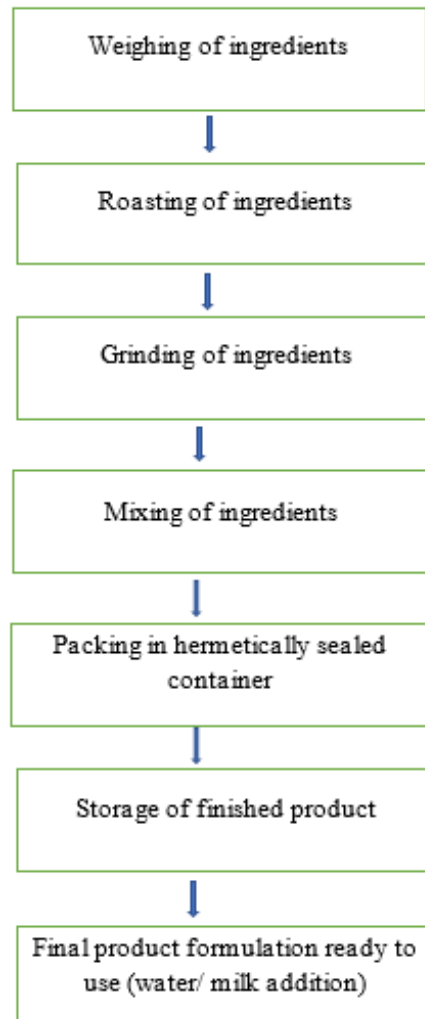
The chickpea powder was prepared by using the protocol described by Dida Bulbula and Urga, (2018). The chickpea was carefully cleaned to remove the broken seeds and extraneous matter. The seeds were soaked in water for 12 hrs. (1:2 w/v), then water was removed which was followed by the drying in the drying oven at  $45\pm 5^{\circ}\text{C}$  for 2 hr. The dried chickpeas were roasted and ground to form a fine powder, packed in polythene bag for further preparation.

Protocol as suggested by Gernah and Sengev, (2011) was followed for powder preparation of MOLs with some modifications. The leaves were washed with the water having 5% sodium chloride and dried at ambient temperature (covering it with cloth to prevent from contamination) for 4 days then ground and strained with a 500  $\mu\text{m}$  sieve to achieve the fine powder. The powder obtained was then further dried in a hot air oven at  $60^{\circ}\text{C}$  till no moisture remained. The prepared powder was packed and stored in airtight plastic jars at room temperature.

### **2.3. Moringa Leaves Powder Supplemented Weaning Product Formulation**

The nutritional enriched weaning formula was prepared based on the cereals-legume and MOLs powder. The composite flour (wheat, rice and chickpea) was supplemented with different levels of MOLs powder i.e., 3, 6, 9, 12 and 15 % with other ingredients like sugar,

milk powder and vegetable oil were used in different proportions in total of 100 g of sample. Finally, the selection of weaning formula was done on the basis of sensory evaluation.



**Figure 1: Flow Diagram for the Development of *Moringa Oleifera* Leaves Powder Supplemented Weaning Formula**

### 2.3.1. Proximate Analysis

The proximate analysis was carried out in triplicate following the accepted procedures outlined in the Association of Official Analytical Chemist (A.O.A.C, 2016).

### 2.3.2. Mineral Analysis

MOLs powder was analyzed for its mineral contents including Na, Ca, P, K, Fe, Mg and Zn through Atomic Absorption Spectrophotometer (AAS) (Varian AA240, Australia) and Flame Photometer (Sherwood Scientific Ltd., Cambridge, UK), as described in (AOAC, 2016). The 0.5 g of dried sample of weaning formula was taken in digestion flask including

5 mL HClO<sub>4</sub> and 10 mL of HNO<sub>3</sub> for digestion of sample. The sample was heated on hot plate till it became transparent. The volume of digested sample was made up to 100 mL in a volumetric flask using double distilled water following by filtration. The digested sample solutions were then calculated with the help of AAS and Flame Photometer against standard solutions of known concentration.

### **2.3.3. Phytochemical Screening of Weaning Formula**

#### **2.3.3.1. Total Phenolic Content**

Determination of total phenolic contents was done by applying the protocol as explained by William et al. (2018). Methanolic extracted weaning formula (50 µL) was dissolved in 1.0 mL of Na<sub>2</sub>CO<sub>3</sub> (20 %) followed by adding 50 µL of Folin-Ciocalteu reagent. The prepared combination was incubated at 25 °C for 40 min in a dark area. Folin-Ciocalteu reagent substance (phosphotungstic acids) changes the color of the mixture (colorless to blue) under alkaline environment and alteration in absorbance gives a quantity of total phenolic contents (TPCs). Absorbance of gallic acid (GAE) as standard and methanolic extract was measured by using atomic absorption spectrophotometer at 765 nm.

#### **2.3.3.2. Total Flavonoid Content**

These were determined by using the protocol described by William et al. (2018). Purposely, 100 µL of methanolic extract was added in the glass tube and quercetin (standard) was also added [300 µL (5% NaNO<sub>2</sub>)] in them. The sample was allowed to stay for 5 min, afterward 600 µL of (10% AlCl<sub>3</sub>) and 2 mL of (1.0 M NaOH) was added. The total volume was made by using distilled water up to 5 mL. Flavonoids in weaning formula extracts were measured after the appearance of pink colored mixture (flavonoid aluminum complex). Absorbance of the standard quercetin and methanolic extract was measured immediately at 510 nm by using spectrophotometer.

#### **2.3.3.3. Antioxidant Analysis**

The antioxidant potential of methanolic extracts of weaning formula was ascertained established on 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging impact following the procedure described by Ameer et al. (2023).

### **2.3.4. Anti-nutritional Components**

#### **2.3.4.1. Oxalate**

The oxalate content of the prepared sample of weaning formula was calculated by using a colorimeter (Gwer et al., 2020). The 1 g sample of weaning formula was taken in a conical flask and then add 75 mL of 3 M H<sub>2</sub>SO<sub>4</sub>, and stirred the solution carefully with a magnetic stirrer for 1 hour. The sample was filtered by using Whatman No 1: 11 µm filter paper. After filtration the filtered sample was titrated against 0.1 N KMnO<sub>4</sub> solution to get the concluding point indicated as pink color of solution that remained for minimum of 30 sec.

#### 2.3.4.2. Phytate

The phytate (phytic acid) content of weaning formula was calculated by using calorimeter method using wade's reagent as described by Bilal et al. (2017). The absorbance was measured at 490 nm through spectrophotometer (CE 7200-7000 series, Cecil, UK) by using sodium phytate as a standard.

#### 2.3.4.3. Tannins

The tannin components of weaning formula were analyzed by using vanillin reagent method as described by Shanmugavel et al. (2018). The absorbance at 415 nm was measured spectrophotometer (CE 7200-7000 series, Cecil, UK) by using tannic acid as a standard.

### 2.3.5. Functional Properties of Supplemented Weaning Formula

#### 2.3.5.1. Oil Absorption Capacity (OAC)

The capacity of oil absorption was evaluated by using the method given by Adewumi et al. (2022). The 0.5 g sample of weaning formula and 6 mL corn oil were taken in centrifuge tube. After 30 min. the tube was put into a centrifugation machine for 25 min at 3000 rpm. Subsequently, the oil was removed from the tube into 10 mL graduated cylinder, and tubes were reweighed to measure the oil absorption capacity of weaning formula.

$$\text{Oil absorption capacity (\%)} = \frac{\text{final wt} - \text{initial wt}}{\text{wt of sample (w)}} \times 100$$

#### 2.3.5.2. Water Holding Capacity (WHC)

The water holding capacity of the weaning food sample was examined by using the method given by Oyeyinka et al. (2019). Sample weighing 3 g of weaning formula was mixed with 25 mL distilled water in a centrifuge tube. The sample was then stirred and put into a centrifugation machine at 3000 rpm for 25 min. The supernatant water was drained out and tubes were reweighed to get the water holding capacity of the sample.

$$\text{Water holding capacity (\%)} = \frac{\text{final wt} - \text{initial wt}}{\text{wt of sample (w)}} \times 100$$

#### 2.3.5.3. Foaming Potential

The foaming potential was evaluated by using the procedure given by Bello et al. (2020). The foaming potential of the weaning formula was assessed by taking 1 g of blended flour by adding 250 mL of distilled water in a volumetric flask (250 mL). The formation of foam, foaming potential, was stated as mL/100 mL. Therefore, foaming potential was observed through time of retention (time of foam formation to its retention).

$$\text{Foaming potential (\%)} = \frac{\text{Foam vol after stay (vol after} - \text{vol before)}}{\text{vol befor foam formation}} \times 100$$

#### 2.3.5.4. Emulsion Characteristics (EC)

The emulsion characteristics was evaluated by using the procedure described by Adewumi et al. (2022). The sample of 0.5 g was added to 3 mL distilled water and 3 mL of oil mixture. The mixture was shaken with arbitrary shaker for 5 min and put into a centrifugation machine at 2000 rpm for 30 min. After that, the emulsion layer was separated from whole slurry to calculate emulsion capacity. The stability of layer was measured by heating at 80 °C and again slurry was centrifuged (2000 rpm for 30 min). The layers were divided from whole slurry and result was calculated in mL/100mL by measuring layer volume.

$$\text{Emulsion characteristic (\%)} = \frac{\text{height of emulsified layer after heating}}{\text{height of emulsified layer before heating}} \times 100$$

#### 2.3.6. Sensory Evaluation

The sensory assessment of MOLs based weaning formula was done by a panel of judges (based on mothers n= 10) from University of Agriculture Faisalabad, Pakistan following the procedure given by Yilmaz, (2020). Accordingly, 9- points hedonic score system; based on points (9=like extremely; 1= dislike extremely) was used to determine the sensory perceptions for color, taste, flavor, mouthfeel, appeal and overall acceptability of the product. All the treatments (*Moringa oleifera* leaves fortification with concentration of 3, 6, 9, 12 and 15%) including control were served in the paste form (like cerelac) under soft white light. To eradicate any biasness, the treatments were presented in transparent plates to the judges with random numbering. For effective results, panelists were offered with mineral water and unseasoned crackers to counterbalance their mouth receptors.

#### 2.3.7. Physiochemical Properties of Weaning Formula

##### 2.3.7.1. Bulk Density (BD)

Bulk density was investigated through the procedure as described by Bello et al. (2020). The 50 g of sample was poured into 100 mL graduated cylinder, whereas the concentration was determined by tapping the cylinder gently on laboratory shelve for numerous times. The result of bulk density was expressed in g/mL of sample volume before and after beating.

##### 2.3.7.2. Reconstitution Index

The reconstitute index was evaluated by using the procedure given by Yusuf et al. (2022). The weaning formula blend was mixed with boiling water for 90 secs and poured into volumetric cylinder (250 mL). The dumped material volume was measured after 10 min to calculate the reconstitution index of sample.

##### 2.3.7.3. Viscosity

The viscosity was investigated by using viscometer (LVDVE 230, Brookfield Viscometers Ltd., Harlow, UK) by following the procedure given by Bilal et al. (2017).

### 2.3.8. Statistical Analysis

The obtained data from each parameter was subjected to statistical design concluded by completely randomized design (CRD). Moreover, the level of significance was evaluated at ( $P < 0.05$ ) through the method (analysis of variance, ANOVA), for further comparison Tukey's HSD test was used for means comparison (Montgomery, 2017).

## 3. RESULTS AND DISCUSSION

### 3.1. Proximate Analysis

The results regarding proximate analysis of weaning formula supplemented with different levels of *Moringa oleifera* leaves powder are shown in Table 1. The measured values indicated the highly remarkable variations ( $P \geq 0.05$ ) in ash, crude protein, crude fat, crude fiber and nitrogen free extract, whereas the moisture content was found to be non-significantly ( $P \leq 0.05$ ) variable with the values ranging from  $4.38 \pm 0.18$  to  $4.12 \pm 0.07\%$ . The findings were compared with the results reported by William et al. (2018). It is observed that the moisture content of the *Moringa oleifera* leaves supplemented weaning formula were within the recommended norms ( $\leq 5\%$ ) prescribes for storage of non-perishable commodities (CAC, 1991). It is recognized that the complementary food with low moisture level increases the nutrient density of product (Ijarotimi and Keshinro, 2013), which hinders the biochemical actions of attacking microorganisms and protect food from spoilage during storage (Tiencheu et al., 2016).

The ash content increased significantly ( $P \geq 0.05$ ) with moringa leaves supplementation with the value range of  $6.34 \pm 0.05$  to  $6.73 \pm 0.06\%$ . It is noticed that 15% addition of *Moringa oleifera* leaves powder increased it up to the remarkable level to satisfy nutritional need of infants, it also indicated that the blend formula had high mineral contents. The increased ash ratio in the weaning formula seems to be associated with the addition of moringa leaves powder and also implies the bioavailability of higher minerals content in those formulations (Ahmed et al., 2023).

The protein content is also increased significantly from  $20.63 \pm 0.23$  to  $25 \pm 0.55\%$  in formula. The protein level of supplemented diets is within the suggested standards of weaning formulas (11-21%) (FAO/OMS, 2007). As proteins have a vital role in shielding mechanisms of the body and also important for the growth and development in children. It also contributes in proper functioning of enzymes and hormones (Ponka et al., 2016).

The fat content showed non-significant ( $P \leq 0.05$ ) variations among the treatment with moringa leaves addition. It was not increased or decreased with leaves addition. It is an important element of diet to give flavour and it is reported that moringa formulated diet have appropriate levels of fat to treat the moderate to acute stage of malnutrition in children. The fat is vital in children's diets to promote absorption of fat-soluble vitamins and it is also a high-energy nutrient (Amegovu et al., 2013).

The fiber content is also significantly increased from  $4.81 \pm 0.01$  to  $6.69 \pm 0.20\%$  with 15% moringa leaves addition. The results exhibited that the moringa fortification increases the



fiber content of formulated diet. So, its values are within the recommended range for infants ( $\leq 5\%$ ) (CAC, 1991). Fiber controls intestinal transportation of food and prevents storage of excess cholesterol in the body. It exerts positive consequences on the body in weight management and metabolic diseases. It also helpful in lowering blood sugar level and blood pressure level of human body (Ponka et al., 2016).

The carbohydrates are significantly decreased ( $P \geq 0.05$ ) in weaning formula as shown in Table 1. with the value range of ( $T_0$  to  $T_5$ )  $50.00 \pm 0.04$  to  $48.86 \pm 0.51$  Kcal/g, These results are in line, when compared with the findings of Awonegan et al. (2022). The prepared weaning formula is nutrient dense, although having less calories in experimental formulas than  $T_0$  (without moringa leaves addition). Carbohydrates give energy to the body directly; considered as a major source of energy and contribute to breakdown of body protein. Some are used in body fundamental tissues including cartilage, nucleic acid, mucous formation and some antigenic constituents (Ponka et al., 2016).

**Table 1: Mean Values for Proximate Composition (%) of Weaning Formula**

Treatments	Moisture	Ash	Crude protein	Crude fat	Crude fiber	NFE
$T_0$	$4.38 \pm 0.18^a$	$6.34 \pm 0.05^c$	$20.63 \pm 0.23^d$	$16.60 \pm 0.40^a$	$4.81 \pm 0.01^b$	$50.00 \pm 0.04^b$
$T_1$	$4.30 \pm 0.17^a$	$6.41 \pm 0.05^{bc}$	$22.95 \pm 0.05^c$	$16.80 \pm 0.63^a$	$5.08 \pm 0.67^b$	$50.17 \pm 0.26^a$
$T_2$	$4.55 \pm 0.08^a$	$6.53 \pm 0.16^{abc}$	$23.21 \pm 0.10^c$	$16.60 \pm 1.21^a$	$5.17 \pm 0.60^b$	$50.20 \pm 0.49^{ab}$
$T_3$	$4.56 \pm 0.89^a$	$6.57 \pm 0.07^{abc}$	$23.62 \pm 0.54^{bc}$	$16.46 \pm 1.15^a$	$5.24 \pm 0.56^b$	$50.53 \pm 0.54^{ab}$
$T_4$	$4.51 \pm 0.28^a$	$6.67 \pm 0.08^{ab}$	$24.85 \pm 0.16^a$	$16.53 \pm 0.65^a$	$5.85 \pm 0.11^{ab}$	$49.38 \pm 0.54^c$
$T_5$	$4.12 \pm 0.07^a$	$6.73 \pm 0.06^a$	$25.26 \pm 0.55^{ab}$	$16.25 \pm 0.85^a$	$6.69 \pm 0.20^a$	$48.86 \pm 0.51^d$

Values are means  $\pm$  SD.

Same superscripts within the same row are not significantly different ( $p > 0.05$ ).

Keys:  $T_0$ =control,  $T_1$ = 3% powder of moringa leaves,  $T_2$ = 6% powder of moringa leaves,  $T_3$ = 9% powder of moringa leaves,  $T_4$ = 12% powder of moringa leaves  $T_5$ = 15% powder of moringa leaves.

### 3.2. Mineral Analysis

The moringa based weaning formula is high in all minerals content as shown in the Table 2. This reveals the highly significant ( $P \geq 0.05$ ) variations when compared to the control treated formula. Moringa enriched formula is high in sodium content with the value range  $20.26 \pm 0.26$  to  $25.27 \pm 0.45$  (mg/100g) from control to treated samples, similar finding were reported by Mounika et al. (2021). *Moringa oleifera* leaves powder, being rich source of sodium contents, are important for development and growth of human body (Ezinne et al., 2023). In present results, the sodium (Na) is in the range of 20.26 to 25.27 mg/100g is significantly lower from Recommended daily allowance (RDA) of 200 mg/day for children and infants (WHO, 2000). So, the *Moringa oleifera* leaves powder with 20% substitution will help to fulfill up to 89% of the RDA value to accomplish body requirement.

The potassium content is also significantly increased from ( $T_0$  to  $T_5$ )  $12.53 \pm 0.53$  to  $124.43 \pm 0.37$  (mg/100g) with moringa leaves supplementation. The results were similar with the finding of Aderinola et al. (2020). Potassium plays a major role in sustaining

normal water equilibrium in the cells, nerve impulse transmission, keeping equilibrium in alkalis, acids and maintaining the normal fluid movements of the intestinal territory of body (Netshiheni et al., 2019). The Recommended daily allowance (RDA) is 800 mg/day for infants and children of age 1 to 3 years that is high than the current study results 120.53 to 125.42 mg/100g (SON, 2010). But it is noticed that potassium content increases with increasing moringa leaves concentration, therefore, 20% addition can fulfill up to 80% daily body requirements.

The calcium profile of current study is within the range of 333.51 to 850.36 mg/100g and the recommended daily allowance (RDA) for children (1-3) and infant is 400mg (SON, 2010). It is significantly increased in weaning formula as similar finding were reported by Aderinola et al. (2020). It is also important in high quality for the infants and children (0-5 years) for developing their teeth, bone, nerves and muscles performance, blood coagulation and boosting immunity (Gwer et al., 2020). So, the prepared weaning formula is rich with calcium content to fulfill body requirement of infant, in which up to 15% fortification was done but according to RDA value 9% fortification of *Moringa oleifera* leaves is more than enough to achieve desire goals (SON, 2010).

The iron, magnesium and zinc are significantly higher in prepared weaning formula with the value ranging  $19.53 \pm 0.12$  to  $25.82 \pm 0.28$ ,  $86.53 \pm 0.47$  to  $150.39 \pm 0.34$  and  $1.41 \pm 0.01$  to  $4.15 \pm 0.05$  (mg/g), as shown in Table 2. The current research findings are supported by the observations of Steve and Babatunde, (2013) and Awonegan et al. (2022), who also investigated that the mineral content increased with increasing *Moringa oleifera* leaves concentration.

Basically, it is assumed that the plants based weaning formula have non-heme form of iron, which have less bioavailability to the human body in comparison to the animal-based iron that have more bioavailability (Hurrell and Egli, 2010). It is also important to produce hemoglobin which transports oxygen in the blood (Abbaspour et al., 2014). High quantity of iron (28 mg) is found in moringa in comparison to beef, so it reduces iron deficiency in vulnerable groups (Salihu et al., 2024).

The recommended daily allowance (RDA) of iron is 11 mg/g for infants and children as demonstrated in present study, with high value of iron in control and treated samples (13.52 to 25.82 mg/100g) (SON, 2010). Although the magnesium value of current investigation is 86.53 to 150.39 mg/100g, significantly higher than RDA (75 mg/day) for infants and children (IOM, 1997). The *Moringa oleifera* leaf powder addition in cereals-legume based weaning formula improved its magnesium content that is most important for infant and children (WHO, 2000). The Zn is an important element of the enzyme that alters the provitamin A into retinol and its deficiency leads to restrict the metabolism of vitamin A (Christian and West Jr, 1998). The zinc value is higher with 12% and 15% moringa leaves fortification respectively in comparison with RDA 3.9 mg/day for the infant and children (SON, 2010). Moringa supplemented weaning formula has high value of zinc up to 12% addition which is enough to accomplish body requirements as per recommended daily allowance (RDA) of minerals to infant and children, major consumer of weaning formula. (SON, 2010).

**Table 2: Mean Values for Mineral Analysis (mg/100g) of Weaning Formula**

Treatments	Sodium	Potassium	Calcium	Iron	Magnesium	Zinc
T <sub>0</sub>	20.26±0.26 <sup>d</sup>	120.53±0.46 <sup>d</sup>	333.51±0.83 <sup>f</sup>	13.52±0.54 <sup>e</sup>	25.4±0.36 <sup>f</sup>	1.41±0.01 <sup>d</sup>
T <sub>1</sub>	22.33±0.29 <sup>c</sup>	122.59±0.51 <sup>c</sup>	564.92±1.23 <sup>e</sup>	19.53±0.12 <sup>d</sup>	86.53±0.47 <sup>e</sup>	1.98±0.05 <sup>cd</sup>
T <sub>2</sub>	23.51±0.46 <sup>b</sup>	122.96±0.95 <sup>bc</sup>	680.34±0.28 <sup>d</sup>	22.21±0.29 <sup>c</sup>	102.40±0.35 <sup>d</sup>	2.65±0.05 <sup>bc</sup>
T <sub>3</sub>	23.62±0.54 <sup>b</sup>	123.51±0.49 <sup>bc</sup>	799.34±0.28 <sup>c</sup>	23.56±0.50 <sup>b</sup>	122.38±0.33 <sup>c</sup>	3.28±0.61 <sup>b</sup>
T <sub>4</sub>	24.48±0.45 <sup>ab</sup>	124.43±0.37 <sup>ab</sup>	826.41±0.38 <sup>b</sup>	24.3±0.26 <sup>a</sup>	140.40±0.16 <sup>b</sup>	3.98±0.05 <sup>a</sup>
T <sub>5</sub>	25.27±0.45 <sup>a</sup>	125.42±0.38 <sup>a</sup>	850.36±0.32 <sup>a</sup>	25.82±0.28 <sup>a</sup>	150.39±0.34 <sup>a</sup>	4.15±0.05 <sup>a</sup>

Values are means ± SD.

Same superscripts within the same row are not significantly different ( $p > 0.05$ ).

Keys: T<sub>0</sub>=control, T<sub>1</sub>= 3% powder of moringa leaves, T<sub>2</sub>= 6% powder of moringa leaves, T<sub>3</sub>= 9% powder of moringa leaves, T<sub>4</sub>= 12% powder of moringa leaves T<sub>5</sub>= 15% powder of moringa leaf.

### 3.3. Phytochemical Screening of Supplemented Weaning Product

The polyphenol and antioxidant activity of weaning formula are significantly increased ( $P \geq 0.05$ ) with moringa leaves addition as shown in Table 3. It indicated that by increasing level of *Moringa oleifera* leaves, the concentration of polyphenol and antioxidants activity was also increased, conforming the direct relationship between the factors. The highest total phenolic content was recorded in T<sub>5</sub> (542.83±1.00 mg/100g) and the lowest was observed in T<sub>0</sub> (109.37±1.05 mg/100g). The current study results are in a line with the findings of William *et al.* (2018) and Bianchi *et al.* (2021). The increasing level of polyphenol were related with moringa leaves addition, so their consumption helps the human body as safeguard from oxidative stress related diseases (Duthie and Morrice, 2012).

Similar increasing trend is documented for flavonoids content, it increased from (T<sub>0</sub> to T<sub>5</sub>) 6.75±1.07 to 57.79±0.94 mg/100g in prepared weaning formula. The similar trend is reported in previous studies (Rainero *et al.*, 2022; Tolve *et al.*, 2020; Troilo *et al.*, 2022 ). So, the plants flavonoids are assumed as secondary metabolites and exhibit high antioxidant activities. These make a bound with free radicals to avert and prevent from causing damage to cell membranes and biological deterioration in body (Annegowda *et al.*, 2013). Furthermore, their significant acceptable limit in any formula can provide benefits to the consumer.

The values for antioxidant activity also follows the significantly ( $P \geq 0.05$ ) increasing trend in moringa based weaning formula with the value range (T<sub>0</sub> to T<sub>5</sub>) 20.26±0.46 to 56.52±0.45 mg/100g respectively. The results was compared with the finding of Bianchi *et al.* (2021) as reported for moringa fortified pasta. Similar findings was reported by Tolve *et al.* (2021). In the present study, increased levels of phenolic compounds and antioxidants provides health benefits to the consumers, proving that increased level in the product developed (weaning formulation) is due to antioxidant potential of moringa leaves. Additionally, it helps to prevent cancer, which mainly relies on moringa dual action (prooxidant and antioxidant) (Liu *et al.*, 2019). Moringa leaves are considered as natural

source of antioxidant, tend to be non-toxic and increase the body immunity against diseases.

**Table 3: Mean Values for Polyphenol and Antioxidants Activity of Weaning Formula**

Treatments	TPC mg/100g	TFC mg/100g	Antioxidant %
T <sub>0</sub>	109.37±1.05 <sup>f</sup>	6.75±1.07 <sup>f</sup>	20.26±0.46 <sup>f</sup>
T <sub>1</sub>	207.76±1.00 <sup>e</sup>	15.1±0.60 <sup>e</sup>	25.4±0.45 <sup>e</sup>
T <sub>2</sub>	298.86±1.00 <sup>d</sup>	21.99±0.56 <sup>d</sup>	31.57±0.48 <sup>d</sup>
T <sub>3</sub>	313.45±1.45 <sup>c</sup>	29.58±0.54 <sup>c</sup>	40.59 <sup>c</sup> ±0.52 <sup>c</sup>
T <sub>4</sub>	427.39±1.13 <sup>b</sup>	43.75±1.16 <sup>b</sup>	46.25 <sup>b</sup> ±1.08 <sup>b</sup>
T <sub>5</sub>	542.83±1.00 <sup>a</sup>	57.79±0.94 <sup>a</sup>	56.52±0.45 <sup>a</sup>

Values are means ± SD.

Same superscripts within the same row are not significantly different ( $p>0.05$ ).

Keys: T<sub>0</sub>=control, T<sub>1</sub>= 3% powder of moringa leaves, T<sub>2</sub>= 6% powder of moringa leaves, T<sub>3</sub>= 9% powder of moringa leaves, T<sub>4</sub>= 12% powder of moringa leaves T<sub>5</sub>= 15% powder of moringa leaves.

### 3.4. Anti-nutritional Components

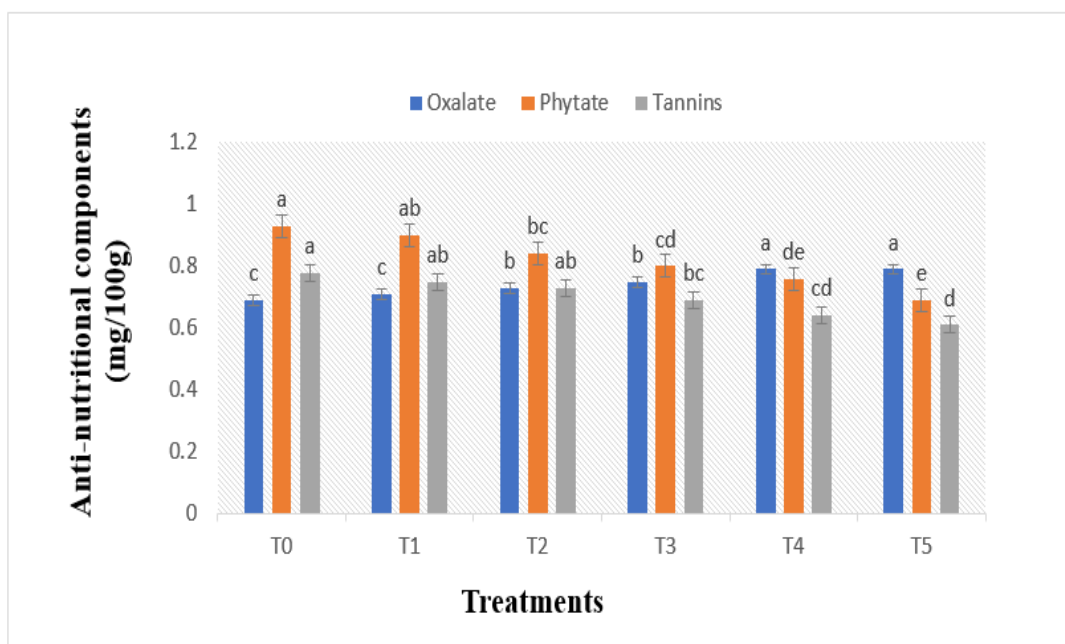
The anti-nutritional components hinder the bioavailability and absorption of nutrients in the body. In present study, the weaning formula has low level of oxalates (0.79±0.005 mg/100g) with 15% moringa leaves addition that is considered as a safe for the child consumption. The oxalates content shows a significantly increasing trend with *Moringa oleifera* leaves powder as presented in the Table 4. Current results are aligned to the outcomes described by Gwer et al. (2020). The oxalic acids and its salts are produced from metabolism of plants tissues as end-product. Therefore, consumption of these plants increases oxalate content of body, which binds the calcium and other minerals and prevent its absorption in the body (Holmes and Kennedy, 2000; Ladeji et al., 2004). It is reported that high concentration of oxalates is poisonous, but in the small quantity it is harmless (Chai and Liebman, 2004).

The phytate contents showed a significantly ( $P\leq 0.05$ ) decreasing trend by adding powder of *Moringa oleifera* leaves. The result shows that treated samples (T<sub>1</sub> to T<sub>5</sub>) have low phytic acid content with the range of 0.90±0.005 to 0.69±0.005 mg/100g respectively. The control sample (T<sub>0</sub>) has high phytic acid with the norms 0.93±0.02 mg/100g but the level of these values is underneath the acceptable maximum phytate dosage in the body. The low level of phytate was recorded due to pre-treatments (blanching, roasting). Comparative to the recent study results were described by Gwer et al. (2020), who evaluated that the phytate content (0.19 to 0.21 mg/100g) was remarkably reduced with increasing moringa leaves addition. Similar results reported by Kouevi, (2014) and Zula et al. (2021) in moringa fortified products. The low level of phytate is observed in current study, predictable to increase the bioavailability of proteins and other dietary minerals (zinc, iron, magnesium and calcium) of food (Osunbitan et al., 2015). Their presence in

low level has beneficial effect on health, perform as an anti-carcinogens, antioxidant and also have key role to control atherosclerosis and hypercholesterolemia (Omueti et al., 2009a).

The tannins content shows significant reduction ( $P \leq 0.05$ ) by adding the powder of *Moringa oleifera* leaves with the value range from ( $0.75 \pm 0.005$ ,  $0.61 \pm 0.01$  mg/100g) in moringa treated samples ( $T_1$  to  $T_5$ ) but it is recorded ( $0.78 \pm 0.02$  mg/100g) in sample without moringa leaves addition which is highly significant when it is statistically evaluated. The findings of the current research work were agreed with the Gwer et al. (2020), that the tannins content had inversely proportion relation with moringa leaf concentration with the range from 0.19 to 0.21 mg/100g.

Its low level was recorded due to soaking, cooking or blenching improves the quality of food nutrition and also decreases the anti-nutritional content up to a safe level (Obizoba and Atii, 1991; Paredes-López et al., 1988). The low level of tannins also has important role as an antioxidant, work as a vascular protective, anti-diarrheal and have therapeutic properties. They are also considered as anti-inflammatory, anti-carcinogenic and anti-mutagenic agents (Kumari and Jain, 2015). Overall, the characteristics to tannins are imperative and have valuable nutritional attention for children and the health of people at risk.



**Figure 2: Anti-nutritional Components of *Moringa Oleifera* Leaves Based Weaning Formula**

Keys: T<sub>0</sub>=control, T<sub>1</sub>= 3% powder of moringa leaves, T<sub>2</sub>= 6% powder of moringa leaves, T<sub>3</sub>= 9% powder of moringa leaves, T<sub>4</sub>= 12% powder of moringa leaves T<sub>5</sub>= 15% powder of moringa leaves.

### 3.5. Functional Properties of Supplemented Weaning Formula

The functional properties of cereals legume based weaning formula with moringa leaves addition and without leaves addition is presented in Table 4. The oil holding capacity (OHC) is significantly increased ( $P \geq 0.05$ ) with moringa leaves addition, the highest was recorded in  $T_4$  ( $192.67 \pm 0.731\%$ ) and lowest was recorded in  $T_0$  ( $162.5 \pm 2.64\%$ ). The results of this research are in line with the outcomes of William et al. (2018) and Adewumi et al. (2022), who reported the same trend (increasing) as given in present study. The oil holding capacity is an imperative property of weaning formulations, its presence enhanced the flavor and mouthfeel of product, particularly in weaning formulas and in bakery products (Ubbor and Akobundu, 2009). It also affects the storage stability of products through rancidity (Siddiq et al., 2010).

The property, water holding capacity (WHC) presented highly significant variation ( $P \geq 0.05$ ) with the value range of  $148.42 \pm 0.37\%$  to  $197.91 \pm 0.05\%$  in moringa enriched weaning formula. Present investigation is compared with the finding of Steve and Babatunde, (2013), Adewumi et al. (2022) and Ijarotimi, (2022).

According to present study results water holding capacity may also be attributed to protein concentration, denatured protein of flours absorbed more water. Therefore, descriptions by WHO, (2003) about complementary food for the infant, are that these foods should not thick or too thin in consistency, have suitable gruel form, nutritionally dens and be easily eatable. As thin consistency of complementary food reduces the nutrients density and also less water absorption is desired because it has high caloric density per unit volume.

The foaming capacity and stability of moringa supplemented weaning formula exhibited significant variations ( $P \geq 0.05$ ). The lowest change was recorded in  $T_0$  ( $6.47 \pm 0.41\%$ ) and highest in  $T_5$  ( $8.77 \pm 0.16\%$ ). The results are in line with the findings of Adewumi et al. (2022). Foam formation after shaking or stirring depends on the ability of constituent and play role in food uniformity, appearance and texture improvement (Kone et al., 2014). According to current study results, protein is denatured and accumulated by cooking process, its vigorous agitation increases the foaming ability (Tiencheu et al., 2016). Furthermore, it is noted that, if food have no are less foaming potential it tends to affect the solidity of product.

The emulsion characteristics shown highly significant variations ( $P \geq 0.05$ ) among the treatments. The lowest vale of emulsion capacity is noted in treated sample as  $T_5$  ( $63.77 \pm 0.16\%$ ) and highest value is demonstrated in control sample ( $78.51 \pm 0.44\%$ ). It is assumed that emulsion capacity decreased with increasing the *Moringa oleifera* leaves powder concentration. Outcomes of present study are in according with William et al. (2018) and Adewumi et al. (2022) that emulsion capacity had inversely proportion relationship with moringa leaves concentration.

Furthermore, increasing amounts of moringa decreased the dispersibility remarkably, as noted in present study. Dispersibility of flour indicates about the suspensibility and reconstitute ability of elements (diets) in water and it is valuable functional parameter in

development of many food product (Nandiolo-Anelone et al., 2014). It is reported in literature that lower value for dispersibility of diet depicts the superior quality of the intake.

**Table 4: Mean Values for Functional Properties (%) of Weaning Formula**

Treatment	OHC	WHC	Foaming ability	Emulsion capacity
T <sub>0</sub>	162.5±2.64 <sup>d</sup>	148.42±0.37 <sup>a</sup>	6.47±0.41 <sup>c</sup>	78.51±0.44 <sup>b</sup>
T <sub>1</sub>	177.21±1.94 <sup>c</sup>	153.51±0.49 <sup>b</sup>	7.39±0.45 <sup>b</sup>	71.39±0.44 <sup>a</sup>
T <sub>2</sub>	183.88±1.64 <sup>b</sup>	159.07±0.44 <sup>d</sup>	8.33±0.05 <sup>a</sup>	68.52±0.46 <sup>b</sup>
T <sub>3</sub>	188.38±0.34 <sup>a</sup>	161.59±0.35 <sup>c</sup>	8.25±0.26 <sup>a</sup>	67.33±0.55 <sup>b</sup>
T <sub>4</sub>	192.67±0.731 <sup>a</sup>	173.69±0.27 <sup>b</sup>	8.21±0.12 <sup>a</sup>	64.48±0.47 <sup>c</sup>
T <sub>5</sub>	191.68±0.79 <sup>a</sup>	197.91±0.05 <sup>a</sup>	8.77±0.16 <sup>a</sup>	63.41±0.50 <sup>c</sup>

Values are means ± SD.

Same superscripts within the same row are not significantly different ( $p>0.05$ ).

Keys: T<sub>0</sub>=control, T<sub>1</sub>= 3% powder of moringa leaves, T<sub>2</sub>= 6% powder of moringa leaves, T<sub>3</sub>= 9% powder of moringa leaves, T<sub>4</sub>= 12% powder of moringa leaves T<sub>5</sub>= 15% powder of moringa leaves.

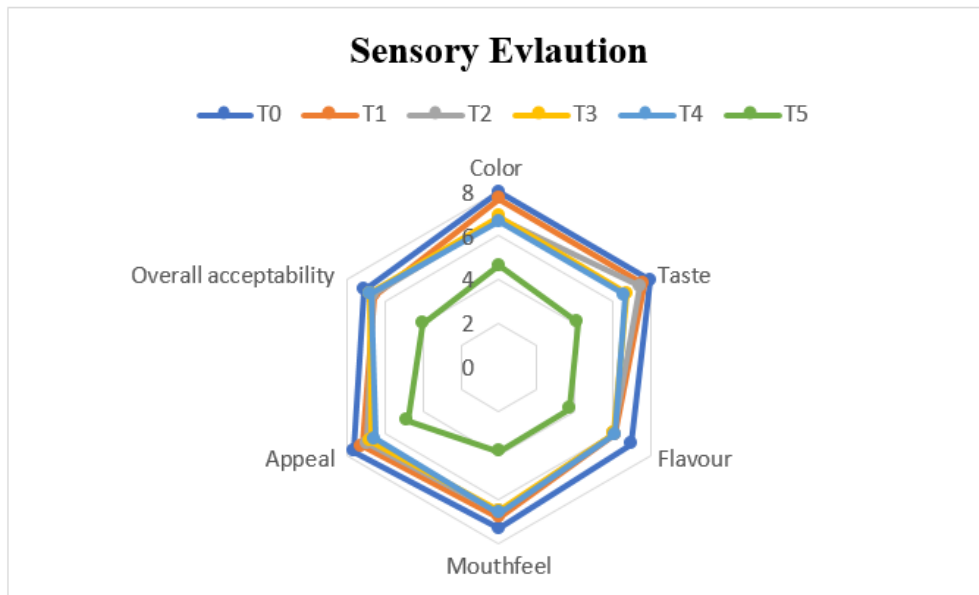
### 3.6. Sensory Evaluation

It is one of the major attributes of the product (moringa based weaning formula) to judge its market value and to take the opinion of panelists regarding its acceptance and rejection. In the current study, the hedonic scale of scoring for weaning formulas was used by trained panels (mothers).

Statistically, it is observed that significant variations ( $P\geq 0.05$ ) are not among the treatments for all the parameters (color, taste, flavor, mouthfeel, appeal and overall acceptability) as shown in Table 5. According to current investigation, supplemented products decreased the sensory attributes in comparison to the control one. The recommended range was up to 12% with moringa leaves powder addition after sensory evaluation by mother's panel.

The lowest was recorded in T<sub>5</sub> because increasing powder addition uplift the bitterness and decreases the consumer acceptability by its appearance, taste and flavor. The present findings are agreed with Roni et al. (2021) observed the sensory evaluation of moringa fortified cake. Oluwamukomi et al. (2021) investigated the sensory scores of moringa enriched "kokoro".

They reported that weaning formula fortified with *Moringa oleifera* leaves achieved lower score for taste, flavor and general acceptability, color and appeal in comparison to cereal-legumes based weaning formula which have the higher score. In spite of this, the nutritional profile of moringa based weaning formula increased its importance for mothers and suggested it as acceptable with recommended range as same practice is done.



**Figure 3: Sensory Evaluation of *Moringa Oleifera* Leaves Based Weaning Formula**

**Keys:** T<sub>0</sub>=control, T<sub>1</sub>= 3% powder of moringa leaves, T<sub>2</sub>= 6% powder of moringa leaves, T<sub>3</sub>= 9% powder of moringa leaves, T<sub>4</sub>= 12% powder of moringa leaves T<sub>5</sub>= 15% powder of moringa leaves.

### 3.7. Physicochemical Properties of Weaning Food

In the present study, non-significant variation ( $P \leq 0.05$ ) is observed with decreasing trend ( $0.68 \pm 0.03$  to  $0.65 \pm 0.04$ ) in treated samples as shown in Table 5. The bulk density agrees with the findings of Bello et al. (2020) and Reddy et al. (2020). The decrease in the bulk density would be advantageous in the preparation of infant formulas because high bulk density limits the caloric and nutrient intake per feed per child and infant sometimes are unable to consume enough to satisfy their energy and nutrient requirement (Omueti et al., 2009b). According to the current study results, weaning foods require low bulk density as its lower value keeps larger quantity of formula elements together which in result enhances the energy content of diets (Tiencheu et al., 2016). Resultantly, with small amount of water, more samples can be prepared simultaneously and fulfill the desired energy requirement of infants.

The reconstitute index basically provides the knowledge about weaning foods preparation. The ready to eat infant weaning formula are instant and preparation is easy through use of warm water instead of traditional cooking. The significant variation is recorded in the cereals- legume based weaning formula as shown in Table 5. The highest value was recorded in the T<sub>5</sub>  $5.23 \pm 0.25$  (mL) and lowest in the control formula T<sub>0</sub>  $4.23 \pm 0.25$  (mL). The reconstitute index of the current study prepared with the addition of *Moringa oleifera* leaves powder agrees with the results found by the Bello et al. (2020) and Ibrahim et al. (2021). So, protein structure breakdown during roasting responsible for increasing the reconstitute index of weaning formula (Hallén et al., 2004). So, the



ingredient structure and its functional property is also responsible for higher the reconstitute of weaning formula.

The viscosity of the final product is responsible for its acceptability. The no-significant variation ( $P \leq 0.05$ ) was observed in the present study results, viscosity of *Moringa oleifera* leaves supplemented weaning food was found in agreement with the results derived by the Bello et al. (2020) and Karim et al. (2015), who investigated that the viscosity decreased with increasing moringa leaves concentration. The mothers usually prefer the weaning formula that gives comfort in spoon feeding to their infants, and it is recommended between 1000 and 3000 centipoise (cP). The current study has low viscosity with moringa leaves powder addition, it may be due to the macromolecules breakdown into smaller sugars subunits and breakdown of amino acids by the enzyme's activity respectively (Hallén et al., 2004). Reduction in viscosity helps in the utilization of the food nutrients as accumulation of higher quantities of food solids to the gruels (Gernah et al., 2012).

**Table 5: Mean Table for Physiochemical Properties of Weaning Formula**

Treatments	Bulk density (g/mL)	Reconstitute index (mL)	Viscosity (Pa.s)
T <sub>0</sub>	0.71±0.01 <sup>a</sup>	4.23±0.25 <sup>c</sup>	1.46±0.41 <sup>a</sup>
T <sub>1</sub>	0.68±0.03 <sup>a</sup>	4.56±0.40 <sup>bc</sup>	1.34±0.03 <sup>a</sup>
T <sub>2</sub>	0.67±0.02 <sup>a</sup>	4.83±0.15 <sup>bc</sup>	1.29±0.01 <sup>a</sup>
T <sub>3</sub>	0.67±0.02 <sup>a</sup>	5.00±0.1 <sup>ab</sup>	1.25±0.05 <sup>a</sup>
T <sub>4</sub>	0.66±0.03 <sup>a</sup>	5.11±0.10 <sup>ab</sup>	1.21±0.01 <sup>a</sup>
T <sub>5</sub>	0.65±0.04 <sup>a</sup>	5.23±0.25 <sup>a</sup>	1.19±0.01 <sup>a</sup>

Values are means ± SD.

Same superscripts within the same row are not significantly different ( $p > 0.05$ ).

Keys: T<sub>0</sub>=control, T<sub>1</sub>= 3% powder of moringa leaves, T<sub>2</sub>= 6% powder of moringa leaves, T<sub>3</sub>= 9% powder of moringa leaves, T<sub>4</sub>= 12% powder of moringa leaves T<sub>5</sub>= % powder of moringa leaves.

## CONCLUSION

Micronutrient deficiency is a public health concern in various countries same as in Pakistan especially in the children under the age of 5 years. A food-based intervention such as fortification of *Moringa oleifera* leaves powder in staple food of our country could improve the nutritional status of individuals. The results presented in this study depicted that the cereals-legumes blend with moringa leaves powder had a higher level of ash content, fiber, crude protein and crude fat. The mineral content of the weaning formula had higher values in terms of sodium, calcium, potassium iron and zinc that is important for infants at their early stage for growth and development. The phytochemical constitute (polyphenol and antioxidant) are also important for the children to improve the immunity and prevent from diseases. The functional properties revealed that powder blends may find application in food formulations. This study has demonstrated that cereals and

legumes with moringa leaves powder boost the body's nutritional value and health advantages to prevent from malnutrition (PEM). However, further research needs to be conducted in order evaluate the nutritional and medicinal importance of this plant by consuming solely and in fortification with different food items.

### Acknowledgement

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