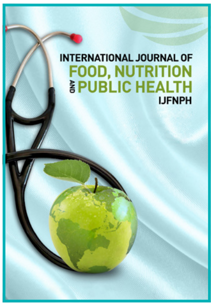

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RESEARCH PAPER

Physicochemical, Sensory and Quality Attributes of Fermented *Vigna Mungo* Soup Fortified with *Moringa Oleifera* for Leveraging Affordable Nutritional Benefits

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ABSTRACT:

PURPOSE: To develop a low cost/affordable nutritionally enhanced soup premix with adequate physicochemical and sensory attributes using a regionally available plant *Moringa oleifera* and the under-utilised legume, *Vigna mungo* (black gram).

DESIGN: The fermented *Vigna mungo* flour was fortified with *Moringa oleifera* leaf powder and formulated as a dry reconstitutable soup. A compositional analysis and sensory evaluation (Nine-Point Hedonic scale) was carried out, and shelf-life studies were carried out for six months at ambient conditions (32±2°C, 56±2% RH).

FINDINGS: Compositional analysis of the soup revealed low moisture (3.16%), ash (16.38%), protein (11.36%), fat (1.62%), fibre (29.70%), carbohydrate (57.62%) and energy (337.61Kcal/100g) respectively. There was no significant ($p>0.05$) changes in pH (5.30-5.48), sensory acceptance (8.5±0.02), gamma amino butyric acid (2.22mg/g), and microbial load (2.3×10^3 CFU g^{-1}) and sensory evaluation.

VALUE OF PAPER: Results of this study suggest that fermented *Vigna mungo* soup fortified with *Moringa oleifera* powder can serve as a sustainable, nutritionally enriched, affordable and acceptable soup suitable for all.

KEYWORDS: *Black Gram (Vigna mungo); Dry Leaf; Moringa Powder; Gamma-amino butyric acid; Soup; Sensory; Nutrition*

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INTRODUCTION

Consumption of nutrient-deficient foods ultimately leads to malnutrition and related diseases. In fact, the World Health Organization (WHO) estimated in a recent report that there are 178 million under-nourished children in the world, 20 million of whom suffer from severe malnutrition; under-nutrition contributes to 3.5 to 5 million deaths among children 5 years of age annually. Additionally, the recent post-pandemic scenario has accelerated malnutrition problems across the globe, the brunt of which is being increasingly felt in developing countries. Affordable, easy to cook foods fortified with micro nutrients are crucial at this juncture to fulfil nutritional inadequacies. Plant-based traditional foods represent an important part of the culture, tradition, history, identity and heritage of a region or country, and are key elements in dietary patterns (Kapelari *et al*., 2020). Foods utilising available plant resources rich in bioactives can be instrumental in fulfilling present and future social consumer requirements (Straksys *et al*., 2016), and promote sustainability in food production and consumption. Soups have a special relevance for delivering both sensory appeal and nutrition, and are an integral part of a diet. In dried form, soups have advantages of increased shelf life due to protection from enzymatic and oxidative spoilage, and flavour stability at room temperature over long periods of time. In addition, they can be readily reconstituted in a short time (Osman, 1991).

Legumes (Fabaceae/Leguminosae) are considered the second most important human food crop, and have a history of consumption as soups. Black gram (*Vigna mungo*) is mainly a staple food; the de-hulled and split seeds are a common dish in South Asia. In addition to being an important source of protein and carbohydrate, recent scientific evidence indicates that black gram has several beneficial health effects and prevents chronic disorders. For instance, animal studies have revealed that polysaccharide and protein fractions have a hypolipidemic action; glycosaminoglycan metabolism is altered upon inclusion of these fractions in the diet. Moreover, neutral detergent fibres of black gram possess significant hypolipidemic and hypoglycaemic effects as well as anti-colon cancer activity (Batra *et al*., 2019).

Moringa oleiefera has drawn widespread attention for its nutritional and therapeutic benefits. Its seeds, leaves and bark are used for the preparation of various foods, such as salads, juices, soups and medicines (Patel and Goyal, 2012). As a miracle tree of life, *Moringa* leaves are an excellent source of proteins, vitamins, βcarotenes, tocopherols and essential sulphur, containing amino acids rarely found in daily diets (Moyo *et al*., 2011). Antioxidants (Sreelatha and Padma, 2009; Verma *et al*., 2009) possess anti-inflammatory properties (Sulaiman *et al*., 2008), actively reduce serum cholesterol and blood pressure (Chumark *et al*., 2008). In addition, *Moringa* leaves contain Vitamin C (seven times higher than that of oranges), vitamin A (four times that of carrots), calcium and potassium (three times higher than that of bananas). Recent studies indicate that the dried powders of *Moringa* leaves retain nutritional quality upon storage at ambient temperature for at least six months (Ansari *et al*., 2020), making it convenient to develop *Moringa*-based formulations. In an earlier study, it was demonstrated that fermentation reduces non-nutrients in *Vigna mungo* and increases its nutritional qualities. Since legume soups are popular culinary practice in India and serve as an essential part of regular diets, it was envisaged that designing fermented *Vigna mungo* soup fortified with *Moringa* leaf can additionally enhance the overall intake of essential nutrients. The attributes of both *Vigna mungo* and *Moringa* leaves in terms of availability, economics, and nutritional values present viable prospects for developing innovative dietary options. Therefore, in the present study a fermented dry soup (using fermented *Vigna mungo* flour) fortified with *Moringa* leaf powder was developed. The physiochemical, sensory and nutritional attributes of the soup is reported.

MATERIALS AND METHODS

Preparation of Raw Material

Black gram (*Vigna mungo*) was collected from the Empire store (Baddi, Himachal Pradesh). Fresh *Moringa oleifera* leaves were collected from the medicinal tree garden of Vrindavan ayurvedic hospital (Baddi, Himachal Pradesh).

Processing of *Moringa* Leaf Powder

The processing of the *Moringa* leaf powder was done using the protocol described by Gernah and Sengev (2011). Briefly, destalking and washing of the leaves was followed by boiling with 0.1% (v/v) sodium meta-bi-sulphite for 5 minutes, spreading on racks for 10-15 minutes. The leaves were then re-spread thinly on mesh, and sun dried for about 4 hours (35°C-55°C). Dried leaves were then ground into powder and packed in airtight food grade containers at room temperature for further use.

Processing of Black Gram (*Vigna Mungo*) Powder

Fermentation of *Vigna mungo* was carried out as described (Batra *et al*., 2019). Fermented *Vigna mungo* flour was then dehydrated in an industrial food dehydrator (Model ST-60 MA, Leader Food Dryer, Kuroda Industry Co. Ltd, Japan) at 55°C for 6-8 hours and ground into powders separately in a mixer grinder (Phillips HL 3294/C). The powdered ingredients were immediately packed and sealed in Kraft food grade airtight containers for further use as the soup premix.

Preparation and Formulation of *Vigna Mungo-Moringa* Fortified Soup Powder

Mungo-Moringa soup powder was prepared by the addition and homogeneous mixing of weighed ingredients (corn starch, salt, flavours) (Table 1). The prepared soup powder was sealed in food grade self-sealable packets and used for chemical and sensory evaluation. For reconstitution, 10g of soup powder was dissolved in 150ml of boiled potable water with stirring that resulted in one cup (approximately 140ml) of soup.

Table 1: Formulation of *Vigna Mungo-Moringa* Leaf Dry Soup Powder

No.	Ingredients Used	Quantity (%)
1	Corn Starch	50.5
2	*Vigna mungo* powder	8.5
3	*Moringa* leaf powder	4.0
4	Salt	16.0

Source: Constructed by author

Sensory Evaluation

A sensory evaluation test was conducted to determine consumer's degree of liking for the prepared soup (Farzana *et al*., 2017). The sensory attributes includes flavour, taste, texture, consistency, colour and overall acceptability, and consumer intent of purchasing were evaluated among 10 panellists selected from the staff members of the Food Innovation Unit, Suryan Enterprises, Baddi. Each attribute was scored based on its intensity scaled on 9-point hedonic scale (9=liked very extremely, 8=liked very much, 7=like moderately, 6=like slightly, 5=neither like or dislike, 4=disliked slightly, 3=disliked moderately, 2=disliked very much, and 1=disliked extremely) for colour, flavour, taste and texture.

Compositional Analysis of *Mungo-Moringa* Soup Powder

The proximate composition (moisture, ash, protein, fat, fibre) of the *Mungo-Moringa* soup was estimated according to the standard analytical methods (AOAC, 2000).

Determination of Moisture

Moisture content was determined by drying a sample in an oven at 105°C for 5 hours. The weight loss incurred was calculated as:

$$\text{Moisture content (\%)} = (\text{weight loss on drying/weight of the sample}) \times 100$$

Determination of Crude Protein

Protein content of the samples was determined using the Kjeldahl method. The method consists of three basic steps:

1. digestion of the samples in sulphuric acid with a catalyst, which results in conversion of nitrogen to ammonia;
2. distillation of ammonia into the trapping solution; and
3. quantification of the ammonia by titration with a standard solution.

$$\text{Protein content of the sample (\%)} = \text{\% nitrogen} \times 6.25$$

Determination of Total Ash

To determine ash content, a dried and ground sample was ignited in a furnace at 600°C for 4 hours to oxidise all organic matter. Crucibles were first dried for about 2 hours at 100°C in an oven and placed in a desiccator. They were cooled and 2.0g of the sample was weighed in the crucible. The samples were further placed in a furnace at 600°C for 4 hours. Percentage ash content was determined by weighing the resulting inorganic residue.

$$\text{Weight of ash, g} = [\{(\text{weight of crucible} + \text{ash}) - (\text{weight of the crucible})\}/\text{weight of the sample}] \times 100$$

Determination of Fat

Fat content was determined using the Soxhlet extraction method. Fat was determined by extracting the dried materials with a light petroleum fraction in a continuous extraction apparatus. The solvent was distilled off and the extract was dried and weighed.

Determination of Crude Fibre

A moisture and fat free sample was boiled with 0.255N H_2SO_4 and 0.313N NaOH consecutively for 30 minutes under a reflux condenser; each time the sample was washed with boiling water properly to remove acid and alkali residue. The sample was then transferred to a crucible, dried overnight at 100°C and weighed in an analytical balance (W1). The crucible was heated in a muffle furnace at 600°C for 20 minutes, cooled and weighed again (W2). The difference in the weights (W1 − W2) represents the weight of crude fibre.

$$\text{Crude Fibre (g/100g)} = [(W1 - W2) \times 100]/\text{Weight of the dried sample.}$$

Determination of Carbohydrate Content

The carbohydrate content was determined by Eneche (1999). The content of the carbohydrate was determined by the equation:

$$\text{Total Carbohydrate (g/100g)} = 100 - \{(\text{moisture} + \text{fat} + \text{protein} + \text{ash} + \text{crude fibre})\}$$

$$\text{Total Energy content: Energy (kcal/100g)} = (\text{carbohydrate in g} \times 4) + (\text{protein in g} \times 4) + (\text{Fat in g} \times 9).$$

Determination of Gamma Aminobutyric Acid (GABA) and Trace Elements

Sodium and potassium content was determined by the flame photometric method (Jahan *et al*., 2011). Iron, manganese, and zinc were determined by the flame photometric method (AOAC, 2005). Gamma amino butyric acid content was analysed as described by Bhanwar *et al*. (2012).

Microbial Quality Analysis

Microbial analysis (especially total viable count, Coliforms and *E.coli*, yeast and moulds) were carried out according to the procedure in the *Bacteriological Analytical Manual* (Feng *et al*., 2013).

All results were expressed as per international methods (Maturin and Peeler, 2001).

Shelf-Life Evaluation

Samples were stored under ambient temperature (32±2°C) and 56±2% RH for further analysis. The pH, moisture, reconstitutability, microbial content and sensory characters were determined at 30 day intervals for a period of up to 6 months.

Statistical Analysis

Data analysis was carried out using Statistical Package for the Social Sciences (SPSS version 15.0 SPSS Inc. Chicago, Illinois and USA). Values were expressed as percentage and mean±SD (three replicates). The significance and non-significance of results was determined using a one-way ANOVA and Duncan Test.

RESULTS AND DISCUSSION

Previous studies explicitly demonstrated that non-nutrients in *Vigna mungo*, namely trypsin and amylase inhibitors, saponin, phytic acid, cyanides, tannin and oligosaccharides of raffinose series, are significantly ($P < 0.05$) reduced following fermentation by *L. lactis*. These results were instrumental for designing/developing traditional food(s) with nutritional sustainability. Moreover, these and other legumes are usually consumed after seeping, washing and boiling; therefore, a ready soup premix with acceptable sensory and nutritional quality can substantially promote consumption and penetrability amongst masses. *Moringa* leaf powder is easily available and has established nutritional properties (Moyo *et al*., 2011). Therefore, fortification of fermented *Vigna mungo* soup with *Moringa* should offer an ideal choice for fortification and extend the benefits of consumption.

Compositional Analysis of *Vigna Mungo* and *Moringa* Leaf Powder (On Dry Basis)

The moisture, protein, fat, ash, fibre and total carbohydrate of *Vigna mungo* powder were found to be 10.06%, 10.02%, 0.42%, 4.76%, 5.11% and 52.12% respectively (Table 2). The high carbohydrate content indicates its potential use as a prime source of energy and especially useful for children's development. These results are in partial agreement with those reported earlier for other legumes

(Zia-Ul-Haq *et al*., 2008). The moisture, protein, fat, ash, fibre and total carbohydrate content for *Moringa* leaf powder were 4.6%, 32.07%, 6.48%, 9.37%, 10.92%, and 39.20% respectively (Table 2), in line with other reports (Gernah and Sengev, 2011).

Table 2: Compositional Analysis of *Vigna Mungo* and *Moringa* Leaves (Dry Basis)

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Fibre (%)	Carbohydrate (%)
Vigna-mungo flour	10.06±0.96	10.02±0.79	0.42±0.05	4.76±0.53	5.11±0.32	52.12±0.97
Moringa-oliefera leaves	4.6±0.08	32.07±1.21	6.48±0.34	9.37±0.76	10.92±0.54	39.20±1.12

Source: Constructed by author

Compositional Analysis of *Vigna Mungo-Moringa* Soup (On Dry Basis)

Moisture

Moisture content is an important factor in maintaining food quality; this is because increased moisture facilitates the growth of the microbes that ultimately destroy the quality of food. Dried food formulations with a moisture content less than 10% is considered appropriate for keeping quality (El Wakeel, 2007). The moisture content of the *Vigna-Moringa* soup was found to be 3.16%; this was lower than reported in other studies (Rubilar *et al*., 2012). The low moisture is important for the product's stability and thus shelf storability. The incorporation of *Moringa* leaf powder is helpful in terms of the texture and microbiological quality of the soup.

Ash Content

The ash content of the *Vigna-Moringa* soup was 16.38% (Table 3) and was higher than that of other studies (Rubilar *et al*., 2012). Both *Moringa* leaf powder and *Vigna mungo* are good sources of minerals (Dachana *et al*., 2010; Farzana *et al*., 2017). Therefore, fortification further enhances the higher ash content of the *Vigna mungo*.

Table 3: Compositional Analysis of *Vigna Mungo-Moringa* Leaf Dry Soup Powder

Sample	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fibre (%)	Carbohydrate (%)	Energy (Kcal/100g)
Vigna-Moringa soup	3.16± 0.06	16.38± 0.04	11.36± 0.05	1.62± 0.07	29.70± 0.03	57.62± 0.02	337.61± 0.06

Values are means of three replicates±standard deviation
Source: Constructed by author

Protein

The protein content of the *Vigna-Moringa* soup powder was 11.36% (Table 3) and is higher than that of other studies (Rubilar *et al*., 2012). *Vigna mungo* is an excellent source of protein (20-25%)

and *Moringa* leaves are also a good source of proteins (26.2%). A complementation of both enables a fair proportion for daily intake of proteins.

Fat Content

The fat content of 1.62% (Table 3) observed may be attributed to the low fat content in both *Vigna mungo* and *Moringa* leaves (5.13%). The low fat level permits regular intake and alleviates the risk of heart disease, cholesterol, diabetes, etc.

Fibre Content

It has been suggested that dietary fibre has potential health benefits, including mitigation of cardiovascular diseases, obesity and diabetes. High fibre content (29.70%) was observed in the *Vigna mungo*-*Moringa* fortified soup. The increased fibre content may be explained as a contributory effect of the *Moringa* leaves that contain 21-23% fibre. On average, unfermented *Vigna mungo* contains 18% dietary fibre; however, there is a reduction in this following fermentation. The results of this study indicate that the developed soup meets the recommended daily allowance (RDA) (FAO/WHO, 2010) for dietary fibre (Park *et al*., 2011).

Carbohydrate Content

The carbohydrate content in *Vigna*-*Moringa* soup was found to be 57.62% (Table 3). The lower carbohydrate content of the developed soup powder was due to lower carbohydrate content of *Vigna mungo* (27.25%) and *Moringa* leaf (38.20%).

Energy Value

In the present study, the energy value of *Vigna*-*Moringa* soup was found to be 337.61kcal/100g (Table 3). The lower energy value noted is due to the low fat and carbohydrate content.

Gamma Aminobutyric Acid (GABA) and Trace Elements

GABA is known to effectively reduce hypertension and heart rate, decrease anxiety, and induce calm (Fang *et al*., 2014). Consequently, the possibility of enriching foods with GABA is an active area of research and is being explored in many food products such as brown rice (0.0001g/l), yogurt (0.000425g/l), cheese (0.000383g/g), GABA tea (0.019g/l), and kimchi (26.8g/l) with use of lactic acid bacteria (Bhanwar *et al*., 2012). The levels of GABA in the soup (2.22mg/g) are adequate for exhibiting therapeutic effects. Trace element deficiencies are associated with a reduced anti-oxidant potential in organisms, accelerated aging, developmental retardation in children, increased incidence of abnormal pregnancies, immunological abnormalities, and lifestyle-related diseases, The fermented *Vigna mungo*-*Moringa* fortified soup contained high levels of trace elements (Table 4).

Table 4: GABA Content and Trace Elements in *Vigna Mungo-Moringa* Soup Powder (Dry Basis)

Sample	Na (mg/100g)	K (mg/100g)	Mn (mg/100g)	Zn (mg/100g)	Fe (mg/100g)	GABA (mg/g)
Vigna-Moringa soup	57±0.02	743±0.01	0.107±0.03	2.19±0.02	5.26±0.03	2.22±0.07

Values are means of three replicates±standard deviation

Source: Constructed by author

Vigna-mungo soup powder was enriched with reasonable amounts of the trace elements sodium (57mg/100g), potassium (743mg/100g), Manganese (0.107mg/100g), zinc (2.19mg/110g) and iron (1.3mg/100g). The combination of important trace elements present in the soup adequately meets the RDA (Abbas *et al*., 2018).

Sensory and Microbiological Quality Evaluation

The fermented *Vigna mungo*/*Moringa* fortified soup possessed excellent acceptability in terms of sensory properties. The bitter taste associated with *Moringa oleifera* leaves (due to the presence of glucosinolates) poses a key barrier to its acceptance as food (Chan *et al*., 2020). Therefore, masking the bitterness is an essential prerequisite for *Moringa*-based food formulations. Bitterness was not perceived by the assessors in the *Vigna* soup; it is possible that the slight sour taste of the fermented *Vigna mungo* flour used as a base material (Chan *et al*., 2020) was helpful in masking the bitterness. The total viable microbial counts were below recommended limits and no increase was observed following storage; *E.coli* yeasts/moulds were not detected (Table 5).

Table 5: Microbial Quality Analysis of *Vigna-Moringa* Soup Powder

S.No	Parameters Evaluated:	0 days	60 days
1	Total aerobic bacteria (Cfu/g)	3.2×10^2	2.2×10^3
2	Total Coiliforms, MPN/g	<0.2[2]	<0.2[2]
3	*E.coli*, MPN/g	<0.2[2]	<0.2[2]
4	Total yeasts and Molds (Cfu/g) Salmonella sp Shigella sp	<10[1] ND ND	<10[1] ND ND

[1]<10 indicates absence of test organisms in1.0 g of soup sample

[2]<Most Probable number (MPN)<0.3 indicates absence of test organism in1.0 g of sample

ND: Not detected

Source: Constructed by author

Shelf-life studies indicated no significant ($p>0.05$) alteration in the quality attributes (Table 6), suggesting that the soup retained functionality and stability, important for production and consumption.

Table 6: Sensory Attributes of *Vigna Mungo-Moringa* Soup Powder

Sample	Colour	Texture	Flavor	Taste	Consistency	Overall Acceptability
Vigna-Moringa soup	8.3±0.03	8.5±0.05	8.7±0.02	8.2±0.04	8.6±0.03	8.5±0.02

Values are means of three replicates±standard deviation
Source: Constructed by author

CONCLUSIONS

Overall, this study suggests a refreshing new alternative to the traditional process of consumption of *Vigna mungo*. The use of fermented *Vigna mungo* with significantly ($p<0.05$) reduced non-nutrients offers safety and nutritional quality as a base material for the soup. Additionally, the presence of GABA in the fermented flours can be therapeutically beneficial. It has been suggested that fermented underutilised pulses possess high bioactivity and are sustainable and excellent options for leveraging public health and nutrition. In this study, the nutritional qualities of the fermented *Vigna mungo* were further elevated by fortification with *Moringa* leaf powder. Since both the major raw materials are widely available, the developed soup can offer a cost effective and nutritionally replete choice for enhanced health benefits. However, clinical studies are mandatory prior to commercialisation.

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CONFLICTS OF INTEREST

There were no conflicts of interest.

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