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Formulation, nutritional and phytochemical analysis of ready to mix infant Food using Gorgon Nut, Samak Rice and Banana powder

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Abstract

The objective of the study was to develop nutritious, accessible and stable infant food formulation which would improve cognitive and behavior abilities of Indian children. Two formulations (F1 and F2) based on Gorgon Nut (*Euryale ferox*), Samak rice (*Echinochloa colonum*) and Banana Powder (*Musa cavendish*) were prepared in which milk powder and sugar were added to increase the solubility and taste of the product. The formulation with better taste was accepted and further testing procedures were performed on the best formulation (F1). The formulation was prepared according to daily nutritional requirements and composed to reach an equal energy value around 400 Kcal/100g. It was found that the formulations are rich in dietary fiber content with 18.94% dietary fiber in F1 and 17.33% dietary fiber in F2. The mineral analysis of the formulation (F1) showed that they are rich in Calcium (180 ppm), Magnesium (230 ppm), Phosphorus (765 ppm), Iron (23.75 ppm) and Zinc (12.50 ppm) content. Solvent extract of raw formulation has revealed that it has total phenolic content 16.71 µg GAE / mg extract and has total flavonoid content 85.26 µg CE / mg extract.

Keywords: Gorgon Nut (*Euryale ferox*), Samak Rice (*Echinochloa colonum*), Banana Powder (*Musa cavendish*), Antioxidant, Phytochemical, Phenol, Flavanol, Antibacterial

1. Introduction

Infant food can be described as an easily consumed food, other than breast milk that is designed specifically for infants, specifically between the ages of four to six months and two years [1]. Infant foods are supposed to be any easily chewed food, whether it is in solid, liquid or paste form. When the mother's milk or nursing formula is not suitable or sufficient for child's appetite, then there is a typical requirement of infant formula to feed the baby properly. Infant foods are available in various flavors and multiple varieties in the market or it can be any normal food that has been mashed or broken down into very small pieces. As babies do not have teeth to chew solid foods, so care should be taken as the food containing vegetables or fruits can cause a choking hazard. Babies can be given aqueous based food containing pureed vegetables and fruits which can be mixed with rice or other cereal formula, or breast milk. As the baby start growing and able to chew, small and soft pieces or lumps may be included in their food. Babies with teeth have the ability to break off pieces of food, but they do not have the back molars teeth to grind, so food should be carefully mashed or pre-mashed, or it can be broken into smaller pieces by the parents for their baby [1].

Infant food can be separated into various categories based on the different ingredients used; as cereal based foods, fruit/vegetable based infant foods or they can be categorized on the basis of taste i.e. sweet and salty foods, savory foods etc.

Nutritional need of Infants: Newborns need a diet of breast milk or infant formula. The main energy source in the milk comes from carbohydrates, mostly from a simple sugar called lactose, which is 40% in weightage. By the age of two, toddlers need a diet having a greater carbohydrate level, around 55% [14].

Several studies have indicated that the consumption of cereal based products or fruit and vegetables had decreased incidences of diseases like coronary heart disease, aging, cancer and Alzheimer's disease [2]. Studies indicate that a diet rich in antioxidant phytochemicals such as poly-phenolics, carotenoids, flavonoids and terpenoids protects against cellular damage due to its potency to scavenge oxygen-derived free radicals and serves lifelong terms [3, 4, 5].

Various researchers focused their study on developing nutritious; accessible and stable infants

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flour, which would improve cognitive and behavior abilities of children. Flours formulation stability was studied by storage at 20 °C and 5 relative humidities (0% to 95%) for 10 months. Flour dextrose equivalent; color and fat composition were followed and all formulated flours showed very good stability when stored at relative humidity below 75% [6]. A comprehensive study was required to analyze the actual quantity of antioxidants and their beneficial effects through cereal or fruit based infant foods available in the market. This study showed that the physicochemical properties like total carotenoids, ascorbic acid, total phenolic content, trolox equivalent antioxidant capacity (TEAC) and oxygen radical absorbance capacity (ORAC) of 23 different commercially available fruit-based baby foods were investigated in a study of scientist Carbonell-Capella [7]. Infant formula containing fruits and vegetables showed higher antioxidant activity. The preparation method also plays an important role in the quality and quantity of different bioactive compounds present in baby foods. It has been observed that gentle steam cooking process had high levels of bioactive compounds and antioxidant capacity and it was found to be beneficial in children's diet [7]. Cereals possess a large amount of the daily intake of our diet, along with certain substances that provide antioxidant properties, i.e. Vitamin C (ascorbic acid), carotenoids polyphenols etc. Cereals contain a wide range of bioactive molecule, which make them suitable to use in the formulation of different functional foods. Studies showed that biochemical functions of these phytochemicals for the prevention of health damage to human beings as well as newborns, are becoming more of an interest now a days [12].

Euryale ferox is commonly as Makhana in Hindi and Punjabi, foxnut or Gorgon nut. It is a flowering plant which comes under the water lily family, *Nymphaeaceae*, although it is occasionally considered to belong from a distinct family *Euryalaceae* [8]. It is cultivated in different parts of China [9]. It prefers tropical and sub-tropical climate, temperature between 20-35 (C, humidity between 50-90%, rainfall between 100-250 cm [10]. Gorgon nut is an aquatic weed which grows in the still, fresh water pools (1-5 m deep) of the north and northeastern states of India. In North Bihar, the seed is consumed in popped form, but in Manipur, other parts (leaves and stalks) are consumed as vegetables. Its cultivation and harvesting is also distributed in West Bengal, Manipur, Orissa, Assam and Kashmir. Wild populations of Gorgon nut are also found in Bangladesh, Nepal, China, Japan, Korea, Russia and North America [11]. Gorgon nut possesses significant antioxidant activity associated with medical applications such as inhibition of proteinuria or diabetic nephropathy. Gorgon nut (*Euryale ferox*) is used as a delicious food in India while being nutritionally rich. The plant produces starchy white seeds which are edible [12]. It is a storehouse of macro and micro nutrients and one of the most popularly consumed dry fruits owing to its low fat content and high nutritional value. Gorgon Nut, whether raw or fried contains appreciable amounts of essential amino acids and contains no cholesterol [13]. Various studies concluded the physical and hygroscopic properties of Makhana that make it suitable for use in formulating food for infants.

Echinochloa colonum commonly known as Samak rice, Samo rice, Varyacha tandul, Vari Tandul, Bhagaror Kodri, jungle

rice etc. It is generally considered as a fruit not cereal. It is the seed of a grass family, which grows along with the rice paddy. It provides the equal nourishment and energy that is equivalent to rice. It contains 169 Calories per 174g. Samak rice is found to have a high amount of digestible fiber. It has a similar taste and texture as that of rice. It is a rich source of minerals and vitamins, which are found to be beneficial during the growth stage of the children.

Banana powder (*Musa cavendish*) is usually prepared from processed bananas. It is widely used in the production of milk shakes and baby foods [1]. Banana powder is a rich source of carbohydrate and calories. Although it is a low source of protein, but the presence of other beneficial component of powder make it a better source of protein as compared to other fruits [15]. The powder is also found to be useful as a general treatment for dyspepsia (indigestion) [16]. Bananas are a very good source of vitamin B6 and contain moderate amounts of vitamin C, manganese and dietary fiber. Shinichi Someyaa and Yumiko Yoshikib studied the antioxidant properties of commercial bananas. One of the antioxidant gallic acid was isolated (using HPLC) from banana peel extract, which showed strong antioxidant activity. Gallic acid was more abundant in peel (158 mg/100 g dry wt.) than in pulp (29.6 mg/100 g dry wt.) [17]. the higher gallic acid content shows better antioxidant effects. Thus, gallic acid content mainly accounts for the antioxidant capacity of the bananas. Bananas are therefore considered as a good source of natural antioxidants in foods [17].

2. Materials and Methods

2.1 Raw Material Gorgon nut (Makhana), Samak, Banana Powder, Milk Powder and Sugar were taken as raw ingredients. Makhana and samak were grounded in a mixer grinder to get respective flours. These flours were then cleaned to sift out impurities like sand and stones. Banana Powder was bought from local dried fruit powder retailer named SV Agro Foods, Delhi. Milk Powder and sugar were purchased from local markets.

2.2 Preparation of Raw formulation

Gorgon Nut, Samak, Banana Powder, Milk Powder and Sugar were brought from the local market.

2.2.1 Preparation

Processing of Gorgon Nut Flour:

- Roasting of Gorgon Nut- Gorgon Nuts were roasted using a microwave oven for 8-10 minutes.
- Milling- The cleaned and roasted nuts were ground using a plate mill to obtain whole flour and then whole flour was sieved.

Processing of Samak Rice:

- Milling- The cleaned grains of samak were ground using a plate mill to obtain whole flour and the same process of sieving is repeated.

2.2.2 Preparing Raw Formulation

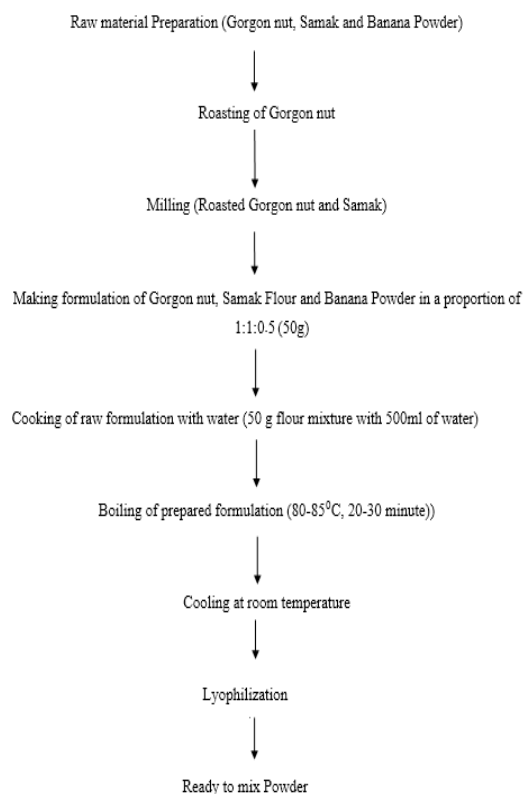
- Two Raw formulations for infants (age 6 months to 2years) were prepared using Gorgon nut flour, Samak flour, banana powder in different ratios.

1. F1- Gorgon nut flour + Samak Flour + Banana Powder (1:1:0.5).
2. F2- Gorgon nut flour + Samak Flour + Banana Powder (1:1:0.25).

2.3 Lyophilisation

Lyophilisation, Freeze-drying or cryodesiccation, is a drying method typically used to preserve a perishable eating goods or to make them easy to transport. Freeze-drying works primarily by freezing the material and then directly sublimates the frozen water present in the material in gaseous form by reducing the surrounding pressure.

Flow Chart of Preparation



2.4 Sample extraction and Extract Preparation

Solvent extraction with methanol as a solvent was performed. 50 g of sample was weighed on an electronic balance and 100 ml solvent (methanol) was added to the conical flask containing the sample. The mixture was mixed properly and was incubated in an incubator shaker at 40 °C and 140 rpm for 24 hours. The mixture was filtered through a whattman paper to obtain the filtrate. The filtrate obtained was transferred to a beaker, covered with aluminium foil and stored in refrigerator. Again 100 ml solvent was added to the conical flask containing residue from first extraction. The mixture was mixed properly and was incubated in an incubator shaker at 40 °C and 140 rpm for 24 hours. The mixture was filtered through a whattman paper. Filtrate from first extraction was mixed with filtrate from second extraction and the solvent was allowed to evaporate at room temperature. The extract obtained after double extraction with solvent was orange-brown in color. The extract was dissolved in DMSO to obtain a stock of 100 mg/ml and stored in the refrigerator at 4 °C.

2.5 Antioxidant activity

2.5.1 DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay

The antioxidant efficacy of the extract was measured by the DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging process [18]. DPPH is a nitrogen centered, stable free radical having maximum absorption at 517 nm in alcoholic solution. 1ml of the prepared extracts was added to 1 ml 0.3 mM DPPH (1.1829 mg in 10 ml methanol) and 1ml methanol and a blank was prepared by just adding DPPH, distilled water and methanol 1 ml each. All the solutions were kept in the dark for 10 mins. OD was measured at an absorbance of 517 nm. % inhibition was calculated by the following formulae:

$$\% \text{ inhibition} = [(B-A)/B] \times 100$$

Where B= Absorbance of blank solution,

A= Absorbance of sample

2.5.2 FRAP Assay

FRAP stands for ferric reducing antioxidant power [19]. FRAP comprises of 3 reagents A, B, C prepared. Reagent A comprises of 3.1 gm sodium acetate added to 16 ml glacial acetic acid and volume made up to 100 ml followed by freezing for 4-5 hrs. Reagent B is a TPTZ solution in HCl, 40 mM HCl was prepared followed by the addition of 0.0781 gm TPTZ in 25 ml HCl. Heat the solution at 50 °C. Reagent C is light sensitive, i.e. 20 mM FeCl₃.6H₂O. FRAP is made by adding reagent A, B, C in the ratio 1:1:10. 200 µl sample added to 1800 µl FRAP incubated for 4 mins at RT and absorbance measured at 593 nm.

2.6 Nutritional Analysis

All the nutritional analyses were executed under the methods given by AOAC (Association for Applied Chemists). The total moisture and ash content by the AOAC method Ref. 942.05 respectively [31]. The total protein content was calculated using the Kjeldahl method Ref. 976.05 (AOAC, 1990) [32]. A gravimetric method was used for determination of total dietary fiber after the enzymatic digestion of starch and protein in fat and moisture free sample (AOAC, 1990) [33]. Crude fat content was determined by extracting the sample in petroleum ether followed by evaporation and weighing. The total carbohydrate content was also measured. Minerals, trace elements and heavy metals in the examined material were determined by using Optima 2100 DV ICP-OES, after prior mineralization in a microwave digester.

2.6 Phytochemical Analysis

2.6.1 Total flavanoid content using UV-Vis colorimetric method

Total flavanoid content in the ethanol extract was determined by the UV-Visible spectrophotometry [20]. The extract was dissolved in DMSO. Out of this 250 µl extract was added to 4.5 ml distilled water and 0.3 ml 5% NaNO₂ and kept for 5 mins. Added 0.3 ml 10% AlCl₃ and incubate for 6 mins. Add 2 ml 1M NaOH to it and make up the final volume to 10 ml with distilled water. Vortex all the test tubes and take OD at 510 nm. A 5 points calibration curve was made with Rutin as standard.

2.6.2 Total Phenol estimation by FC method

The method was given by Slinkard & Singleton. 100 µl of sample was added to 250 µl FC reagent with a further addition of 1.15 ml distilled water. Vortex the test tubes and added 1.5 ml 20% sodium carbonate, incubation for 2 hrs add 2 ml distilled water followed by measuring the OD at 765 nm. Standard curve generated using Gallic acid as standard [21].

2.6.3 Total Tannins estimation

Estimation of tannin content of the flower was done by the method of Van Buren (1981). To 1 gm sample 100 ml water was added and allowed to shake for 1 hr in an incubator shaker.

After that the solution was filtered and 5 ml filtrate obtained was taken in a test tube. To this 1 ml of 0.1M FeCl₃ made by dissolving it in 0.1N HCl was added with further addition of 1 ml 0.008M potassium ferrocyanide, OD was measured at 615 nm within 10 mins. A standard curve was generated using tannic acid as standard [22].

2.6.4 Crude alkaloid content

Alkaloid content was determined by gravimetric method given by Harborne (1973). 5 gm sample was added to 10% ammonium hydroxide stirred and allowed to stand for 4 hrs and filtered. Filtrate obtained was evaporated to 1/4th of its original volume using a hot plate. To this conc. NH₄OH was added drop wise, so that the precipitation of alkaloids takes place. The precipitates were filtered using a pre weighed filter paper and washed with 10% ammonium hydroxide solution. The precipitates were dried with the filter paper in an oven for 30 mins at 60 °C and reweighed [23]. The amount of alkaloids present was calculated in % alkaloid in the sample by the formulae:

$$\% \text{ Alkaloid} = \frac{W_2 - W_1 \times 100}{W}$$

Where: W₁- weight of the wattman filter paper

W₂- weight of the wattman filter paper with alkaloids

W- Weight of sample

2.7 Antibacterial activity

In order to determine the antibacterial activity of ethanolic extract, the Agar well diffusion method was performed. Antibacterial activity of raw formulation was tested against *Bacillus subtilis* (gram +ve), *Staphylococcus epidermidis* (gram+ve) and *Escherichia coli* (gram -ve), *Salmonella enterica* (gram -ve) and *Bacillus cereus* (gram +ve). The test bacteria were grown in sterile Nutrient broth tubes respectively. The broth cultures of bacteria were then aseptically transferred to the agar plates by pour plate method. Wells of 6 mm diameter were created in the inoculated plates using sterile cork borer. Different concentrations of the extracts were prepared by dissolving in DMSO (100 mg and 50 mg in 1 ml DMSO) and were filled in labelled wells. The plates were incubated at 37 °C for 24 hours and the zone of inhibition was measured [24].

Methanol and DMSO were separately plated and used as a control for the experiment.

3. Result and Discussions

3.1 Nutritional Analysis

Nutritional profiling of the best formulation (F1) revealed that the product is a healthy source of nutrition in all respects. Moisture and Dry matter (ash) content is very important to determine because they directly affect the stability and storage of food.

Table 1: Nutritional profiling of Product formulated

Nutritional attributes	Formulation 1
Moisture content	18.325%
Ash content	6.335%
Protein content	9.18%
Dietary fiber content	18.94%
Fat content	2.04%
Carbohydrate content	64.12%
Energy value	374.44 Kcal/100 g

The mineral composition of raw formulation was analyzed by ICP-OES. The results revealed that the formulation contains high amount of Calcium, magnesium and phosphorus. Calcium is very good for bones, helps in prevention of osteoporosis and fractures [25]; phosphorus also helps in healthy bone formation, helps in repairing cells and tissues, it improves digestion, keeps the hormonal balance etc. Magnesium on the other hand enables nerves to function and helps creating energy out of food. Some recent studies prove it to be helpful in reducing the risk of heart diseases, hypertension and diabetes [26, 27, 28].

Results are shown in table 2.

Table 2: Mineral content estimation of raw formulation

Analyte	Concentration (ppm)	Analyte	Concentration (ppm)
B	0.7	Sr	2.50
Ba	0.1	Zn	12.50
Ca	180	Cu	5
P	765	Fe	23.75
Sr	2.50	Mg	230
Mn	7.50	Pb	00
Mo	00	Cd	00
Ni	2.13		

3.2 Phytochemical analysis

Polyphenolic substances are the major category of phytochemicals found in higher plants are the cause behind the antioxidant attributes. The intake of these compounds is very important for health promotion. These bioactive compounds act as radical scavengers and inhibit lipid autoxidation, therefore, are essential antioxidants that protect against the propagation of the oxidative chain. The important classes analyzed here were phenol, flavanol, tannins and alkaloids performing similar functions.

The results are given below in table 3:

Table 3: Phytochemical analysis of raw formulation

Analyte (Ethanol extract)	Content
Total Phenols	16.71 µg GAE/ gm sample
Total Flavanoids	85.26 µg RE/ gm sample
Crude Alkaloid content	0.96%
Total Tannins	15.24 mg TAE/gm sample

3.3 Antioxidant Activity

3.3.1 DPPH radical scavenging assay

DPPH is nitrogen centered; stable free radical having maximum absorption at 517 nm in alcoholic solution. It becomes a stable diamagnetic molecule on accepting an electron or hydrogen atom [29]. In the presence of an extract capable of donating a hydrogen atom, the free radical nature of DPPH is lost and the purple color changes to yellow (diphenylpicrylhydrazine). The bleaching of DPPH radical is one of the most widely used strategies to evaluate the antioxidant activity of the extracts. The results are given in table 4.

3.3.2 FRAP assay

The method described measures the ferric reducing antioxidant power. In acidic medium the ferric-Trotopyridyltriazine (Fe III-TPTZ) complex is reduced to ferrous (Fe II) form and an intense blue color with an absorption maximum at 593 nm develops. However the formulation's ethanol extract showed a very good antioxidant activity against DPPH free radical. The calibration curve revealed highly positive linear relation between FRAP values and BHT standard [19]. The results are given in table 4.

Table 4: Antioxidant activity of raw formulation's ethanol extract

Test	Antioxidant activity
DPPH scavenging assay	81.35% inhibition
FRAP assay	33.61 µg BE/mg sample

3.4 Determination of Antibacterial Activity

The antibacterial activity of the extract was tested against 3 gram +ve strains *Bacillus subtilis*, *Staphylococcus epidermidis* and *Bacillus cereus* and 2 gram -ve strains namely *Escherichia coli* and *Salmonella enterica*. The methanol extract of 1st formulation (F1) showed maximum antibacterial activity against *Bacillus cereus* and *Staphylococcus epidermidis*, *Staphylococcus aureus* and methanol extracts of 2nd formulation (F2) sample showed maximum activity against *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella enterica* and *Staphylococcus aureus*. The table below depicts the results of antimicrobial activity against different concentrations of samples in tabular form.

Table 5: Antibacterial activity of raw formulation's extract

Bacterial strain	Zone of inhibition (in cm)	
	Extract conc. (100 mg)	Extract conc. (50 mg)
<i>Bacillus subtilis</i>	-	-
<i>Staphylococcus epidermidis</i>	1.7	-
<i>Bacillus cereus</i>	2.6	0.9
<i>Escherichia coli</i>	-	1.0
<i>Salmonella enteric</i>	-	-

3.5 Sensory Evaluation of Product

Sensory Evaluation is defined as "A scientific discipline used to evoke, measure, analyze, and interpret those responses to products that are perceived by the senses of sight, smell, touch, taste, and hearing" [30]. The sensory quality of a food product is

the individual most significant factor influencing its success in the market.

Sensory evaluation is done by a panel of 7 students from the Food Technology Dept. who tested the product for sensory and give their opinion about its appearance, taste, flavor, aroma, texture and aftertaste on a hedonic scale numbered between 0-9 based on increasing likability's of attributes.

Table 6:

Panelists	Attributes tested				
	Appearance	Taste	Flavor	Aroma	Aftertaste
1	7.5	8	7	7	7
2	8.5	8	6.5	8	8
3	7	7	6.5	8.5	8
4	8.5	7.5	7	8.5	8.5
5	7.5	7	6	7	7
6	8	8	6.5	7	8
7	8	7	6.5	7	7
Average scores	7.9	7.5	6.5	7.5	7.6

3.6 Reconstitution: Two gram of product formed was reconstituted in 20 ml of boiling water to check the solubility of powder formed. The powder was easily reconstituted in water without any lumps formation. Ready to mix infant powder was also checked to reconstitution in milk and it was found good in that also. The overall taste and reconstitution of Ready to mix infant powder was excellent with milk.

4. Conclusion

The raw formulation taken to prepare infant food was examined for their phytochemical and antioxidant efficacies. Further the product formulated was subjected to nutritional analysis. The product contained 40% gorgon nut flour, 40% samak rice and 20% banana powder was found nutritious, easy to digest and low in protein which is beneficial for infants. Milk Powder and sugar were added to increase the taste of the product. The formulation was found to have a good mineral content having calcium, iron and zinc were 180 ppm, 23.75 ppm and 12.50 ppm per gram of sample respectively. The raw formulation was extracted in methanol and subjected to various phytochemical, antioxidant and showed a good amount of each. The product prepared contained good nutraceutical properties besides nutrition, which is very health benefiting in the early stage of growth of infants.

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