



Proximate analysis of processed and unprocessed samples for development of quality composite flour

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Abstract

The present investigation was carried out in the Department of Food Science and Nutrition at Mangalore University, Konaje, Mangalore (Karnataka) during 2017-18. To develop quality composite flour, ingredients like; whole wheat (*Triticum aestivum*), little millet (*Panicum sumatrense*), moth bean (*Vigna aconitifolia*) and mango ginger (*Curcuma amada*) were used. The samples were divided into two categories *i.e.* batch 1 consisting of washed and sundried whole wheat, little millet, moth bean and mango ginger flours and batch 2 consisting of washed, soaked, sprouted and sundried whole wheat, little millet and moth bean flours. Whereas, mango ginger powder was prepared directly after proper cleaning and drying. Based on proximate analysis, the processed sample (batch 2) was selected, where crude fibre, total protein and iron content were higher than unprocessed sample (batch 1). The processed sample was further divided into two batches based on composition of ingredients. The ratios of different ingredients *i.e.* flour of whole wheat, little millet, moth bean and mango ginger for batch-1 were 50:20:20:10 (Batch 1) and for batch-2 30:30:30:10 (Batch 2), respectively. The comparative studies of both batches further revealed that the composite flour developed in the ratio of 30:30:30:10 of whole wheat, little millet, moth bean and mango ginger, respectively gave better nutritional properties particularly high values of protein, iron, crude fibre, ash content, besides oil absorption and emulsifying activities, which are essential for development of cookies. The replacement of wheat flour and increasing content of little millet and moth bean has improved the quality of composite flour. The use of mango ginger has further added advantage of medicinal properties. From the present investigation, it is suggested that quality composite flour can be prepared by using flour of sprouted whole wheat, little millet, moth bean and mango ginger (un-sprouted) in the ratio of 30:30:30:10, respectively. The cookies prepared by such composite flour will address the problem of anaemia to some extent.

Keywords: Composite flour, cookies, proximate analysis, *Curcuma amada*, *Panicum sumatrense*, *Triticum aestivum* and *Vigna aconitifolia*

Introduction

Access and affordability of quality diet to every citizen is a worldwide concern with highest prevalence in developing countries. Nutritional anaemia is a major concern found especially among women of child bearing age, young children, during pregnancy and lactation. It is established that two-third of pregnant and one half of non-pregnant women in developing countries are affected with nutritional anaemia (Swaminathan, 1974). Overall, 72.7 % of children up to the age of three years in urban areas and 81.2 % in rural areas are anaemic (Swaminathan, 1983). By far the most common cause of nutritional anaemia is iron deficiency, and less frequently folate or vitamin-B₁₂ (Park, 2017). Iron deficiency can arise due to inadequate intake or poor bioavailability of dietary iron or due to excess losses of iron from the body (Ramchandran, 1978). Women lose considerable amount of iron during menstruation. Some of the other factors leading to anaemia are malaria and hookworm infestations. In addition, mothers who have children at close intervals become anaemic due to additional demands of rapid pregnancies and loss of blood in each delivery. The need for strategic development in the use of inexpensive local resources in the production of staple foods has been promoted by organizations such as Food and

Agricultural Organization (FAO) and the United Nations (Awogbenja and Ndife, 2012). This led to the initiation of composite flour programme, the objective of which was to seek ways of substituting flours, starches and protein concentrates from indigenous crops, for as much wheat as possible in baked products (FAO/WHO, 1994).

In fact, composite flours may be considered firstly as blends of wheat and other flours for the production of (1) leavened breads, (2) unleavened baked products, (3) pastas, (4) porridges and (5) snack food or secondly, wholly non-wheat blends of flours or meals, for the same purpose (Chandra *et al.*, 2015). The quality of composite flour be supplemented with other locally available cheaper but nutritionally rich ingredients. The legumes usually improve the quality of cereal protein by supplementing them with limiting amino acids such as lysine and sometimes tryptophan and threonine. Among food grains, millets are the cheapest and nutritionally wholesome food for people of all age groups. Besides nutritional properties, the biological activities of mango ginger include antioxidant activity, antibacterial activity, antifungal activity, anti-inflammatory and anti-allergic activity (Policegoudra *et al.*, 2011).

For preparation of cookies, the raw material mainly

used is wheat flour, which is most important staple food for more than one third of the world population and contributes more calories and proteins to the world diet than any other cereal crops. It is also an important source of minerals, b-complex, vitamins and dietary fiber (Kumar *et al.*, 2011) but generally low in lysine and certain other amino acids. Enrichment of common food products with essential micronutrient especially iron may solve the problem of malnutrition specially anemia. Thus, the formulation of composite flour using whole wheat (cereal), moth bean (pulse), mango ginger (tuber) and little millet (coarse grain) were considered in this investigation as they have a high iron content. The proximate analysis of both processed and unprocessed forms of samples were done to develop composite flour. Further different ratios of composite flour are required to be assessed for standardizing quality composite flour which can be used in preparation of cookies.

Materials and Methods

The investigation was carried out in Department of Food Science and Nutrition at Mangalore University, Mangalore (Karnataka) during 2017-18. The materials and methods used in this investigation are given here under;

(a). For unprocessed and processed samples

In this investigation, all ingredients like whole wheat (*Triticum aestivum*), little millet (*Panicum sumatrense*), moth bean (*Vigna aconitifolia*) and mango ginger (*Curcuma amada*) were procured from a authentic source. The samples were divided into two categories *i.e.* Batch 1 consisting of washed and sundried whole wheat, little millet, moth bean and mango ginger flours and Batch 2 consisting of washed, soaked, sprouted and sundried whole wheat, little millet and moth bean flours. Whereas, mango ginger powder was prepared directly after proper cleaning and drying. The proximate nutritional properties like; total moisture content, ash content, crude fiber, total carbohydrate, total protein, total carbohydrates and iron were studied by following standard methods of (AOAC, 2000).

To determine moisture content of every ingredients, 3g of the sample was weighed in a previously dried petriplate and placed in the oven for 75 minutes. The time was reckoned from the moment the oven attained 120°C after the dishes have been placed. After 75 minutes, the samples were taken out of the oven, cooled and weighed. The sample was placed in oven at half hour intervals until constant weight was achieved. The moisture content was calculated using standard formula and expressed in percentage.

$$\text{Moisture content (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

For total ash content, 1g of sample was weighed accurately into a crucible (which was previously heated to about 600°C and cooled) the crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 3-5 hours at 600°C. It was then cooled and weighed. To ensure completion of ashing, the crucible was

again heated in a muffle furnace for half an hour, cooled and weighed. This was repeated till two consecutive weights were obtained as almost equal and the ash was white or grayish white in color. The total ash content was calculated using standard formula and expressed in percentage;

$$\text{Percentage ash content (wet basis)} = \frac{\text{Weight of crucible with ash} - \text{Weight of crucible}}{\text{Weight of crucible with sample} - \text{Weight of crucible}} \times 100$$

$$\text{Percentage ash content (dry basis)} = \frac{\text{Percentage ash content (wet basis)}}{100 - \text{Percentage of moisture}} \times 100$$

To determine total crude fibre, 2 g of sample was boiled with 200 ml of sulphuric acid for 30 minutes. It was filtrated through muslin cloth and washed with boiling water until washing was no longer acidic. It was further boiled with 200 ml of sodium hydroxide for 30 minutes, filtered again through muslin cloth and washed with water ensuring it is no longer alkaline. The residue was removed and transferred to ashing dish (pre weighed dish W_1). The residue was dried at 120±2 °C for 2 hours, cooled and weighed (W_2). It was ignited at 600±15 °C for 30 minutes, cooled and weighed (W_3). The percentage of crude fibre in ground sample was calculated using standard formula;

$$\text{Crude fibre (\%)} = \frac{\text{Loss in weight on ignition } (W_2 - W_1) - (W_3 - W_1)}{\text{Weight of sample (g)}} \times 100$$

The total lipid content was determined where 0.5 g of oven dried culture was homogenized with 5 ml of chloroform methanol mixture (2:1) and filtered. The extraction was repeated thrice with the residue till the filter paper was made free from the lipids. To the filtrate, 4 ml of distilled water was added and mixed well. The water soluble impurities were diffused away from the solvent and reached the top position in the separating funnel. Solvent containing lipid (bottom layer) was collected. This filtrate was transferred to the pre-weighed beaker and was dried using an evaporator and thereafter final weight was determined. The difference in initial and final weight gave the total lipid content and expressed in percentage of dry weight.

The total protein content was quantified by TCA acetone extraction. Adopting this method, 2g of fine ground sample was treated with 10 ml of cold TCA-2Mercaptoethanol acetone solution and incubated at a temperature of -20 °C for one hour. It was then centrifuged at 5000 rpm for 15 minutes and the supernatant was discarded. The pellet was dried and the precipitate was treated with 10% TCA. Then, it was centrifuged to settle the precipitate and the supernatant was discarded. The pellet was re-suspended in 2N sodium hydroxide solution. Thereafter, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of working standard was pipette out into a series of test tubes. The amount of protein present in the sample was calculated using BSA graph and expressed in g 100g⁻¹. Similarly, total carbohydrate content was calculated by Phenol-sulphuric acid method, where 100 g of sample was weighed in a test tube. It

was hydrolyzed by keeping in boiling water bath for three hours with 5 ml of 2.5N HCl and cooled to room temperature. It was neutralized with solid sodium carbonate until effervescence ceases. The volume was made up to 100 ml and centrifuged. The supernatant was collected and 0.2 ml aliquots were used for analysis. One ml of phenol solution was added to each sample and mixed well. After 10 minutes, the content in the test tube were placed in water bath at 25-30 °C for 20 minutes. Absorbance was measured at 490 nm. The blank was set using 1.0 ml of distilled water. The amount of total carbohydrate present in the sample was calculated using glucose standard graph.

For determination of iron content, 5g of powdered sample was taken in a porcelain crucible and heated over a flame till all the material was completely charred. It was followed by heating in a muffle furnace for about 3-5 hours at 600°C. Then the sample was cooled, moistened with 1 ml distilled water and ash was collected. 5 ml of HCl was added, evaporated to dryness on boiling water bath and this step was repeated again. Then added 4 ml HCl and 1ml distilled water and warmed. It was then filtered into 50 ml volumetric flask cooled and made up the volume. The aliquots were used for estimation of iron in ash solution. 1ml - 5 ml of standard solutions were pipetted out into a series of test tubes. The volume was made up to 5 ml by adding distilled water. Blank is prepared by adding 5 ml of distilled water in a test tube. Test is prepared by taking 1 ml of test solution in test tube and made up to 5 ml using distilled water. Then 30% sulphuric acid was added to all the test tubes making the volume up to 6 ml. Then 1 ml of Potassium persulphate solution and 1.5 ml of Potassium thiocyanate was added to all the test tubes and then incubated for 20 minutes at room temperature. The intensity of the standard is observed at 540 nm against blank. The amount of iron (ferric form) in the sample was calculated using standard curve and expressed as mg/100g of the sample.

(b.) For composite flour development

After proximate analysis of both batches samples, the composite flour was developed from processed samples only. Two formulations were made by mixing the ingredients in different ratios. The batch 1 was in the ration of 50:20:20:10 for wheat, moth bean, little millet and mango ginger respectively while the batch 2 was in the ratio of 30:30:30:10 for wheat, moth bean, little millet and mango ginger respectively. The functional properties like bulk density, water and oil absorption capacity, foaming properties, emulsifying properties, swelling properties, percentage solubility, least gelation property of formulated composite flours in different ratios were also studied. While determining, bulk density 100g of the flour by volume was measured in measuring cylinder (250ml) after tapping the cylinder on laboratory slab until no visible decrease in volume was noticed, and based on the weight and volume the bulk density was calculated. The water and oil absorption, foaming capacity and stability, emulsifying activity and stability, swelling power and percent solubility as well as gelation property were determined using standard methods.

Results and Discussion

Proximate analysis of samples

The proximate analysis for different nutritional parameters was carried out for all the samples using standard protocols (Table 1). Among different unprocessed samples, moisture content of mango ginger was maximum (85.00%) and dehydrated moth bean was minimum (5.33%) while in processed samples, it was maximum in wheat (9.70%) and minimum in little millet (6.45%). In fact, moisture determination is one of the most common tests in foods since the water content in foods has an important relationship between shelf life and the chemical, physical and microbiological changes during the storage (Sampaio *et al.*,

Table 1. Nutritional composition of unprocessed and processed samples.

Samples	Moisture (%)		Ash (%)		Crude fibre (%)		Total lipid (%)		Total protein (%)		Total carbohydrate (%)		Iron (mg/100g)	
	US*	PS**	US	PS	US	PS	US	PS	US	PS	US	PS	US	PS
Wheat	7.15	9.70	1.43	1.36	1.71	1.93	1.65	0.95	7.71	8.65	82.30	72.29	3.56	3.68
Moth bean	5.32	8.11	3.66	3.37	4.49	5.06	1.12	0.96	20.60	24.24	56.86	37.05	9.08	10.26
Little millet	6.43	6.45	1.72	1.70	6.41	6.87	2.54	1.23	8.93	8.98	60.53	60.03	7.00	7.40
Mango ginger	85.00	7.22	1.30	0.90	1.00	1.20	1.06	1.01	1.30	1.10	10.00	9.81	3.00	3.00
Mean	29.98	7.87	2.03	1.83	3.40	3.76	1.59	1.04	9.64	10.74	52.42	44.80	6.32	6.07
SD±	39.36	1.40	1.10	1.08	2.51	2.66	0.69	0.13	8.04	9.71	30.43	27.52	3.90	3.39

* US=Unprocessed Samples; ** PS=Processed Samples

2009). In general, moisture content was more in processed samples than unprocessed samples except mango ginger. The ash content was maximum (3.66% and 3.37%) in moth bean and minimum mango ginger (1.03 % and 0.90%), both in unprocessed and processed samples, respectively. The ash content mainly refers to the amount of mineral present in food. The crude fibre content increased in processed samples than unprocessed. The maximum crude fibre content was found in processed little millet (6.87%) followed by moth bean (5.06%) and minimum in mango ginger (1.20%). High fiber content is beneficial for healthy gut, proper absorption of nutrients and also in lowering glucose and cholesterol levels (Tosh and Yada, 2010). The total lipid content was higher in unprocessed samples than processed samples with maximum value of total lipid was 2.54% in little millet and minimum in processed wheat i.e. 0.95%. Fat is essential for health but it is important to know the fatty acid profile of foods since a high intake of saturated fat contributes to the development of coronary heart disease and trans fatty acids have also been associated with adverse effects, such as raising low density lipoprotein cholesterol (LDL) and lowering high density lipoprotein cholesterol (HDL) (Tavella, *et al.*, 2000).

In general protein content increased in processed samples than unprocessed sample except mango ginger. The protein content of sprouted moth bean was maximum (24.24%), where as it is minimum in mango ginger (1.10%). High protein content has a nutritional importance in most developing countries, where many people especially children can rarely take adequate foods with high protein content because of the costs (Edem, *et al.*, 2001), whereas high protein content may be harmful for people suffering from kidney diseases. Among different samples, the carbohydrate content of sun dried wheat sample was maximum (82.3%) and dried

mango ginger was minimum (9.81%). This is beneficial for the development of composite flour as the wheat was partially replaced by ingredients such as mango ginger, moth bean and little millet which have lower carbohydrate content than wheat flour, thus decreasing the calories obtained from carbohydrate. The iron content in processed sample was also increased in all ingredients except mango ginger. The highest iron content of processed samples was in moth bean (10.26 mg) followed by little millet (7.40 mg), wheat (3.68 mg) and minimum in mango ginger (3.0 mg). This is only because the sprouted ingredients synthesized more iron in presence of light than unprocessed samples. The mean and standard deviation values showed that there was high variation in different nutritional composition of samples used in present study. This was primarily due genetically dissimilar ingredients were taken for the development of composite flour without compromising with major nutritional composition and added advantage of medicinal attributes by using mango ginger. Overall, processed samples were better than unprocessed samples in view of increased fibre, protein and iron content. Thus, for development of composite flour only processed samples were considered.

Evaluation of composite flour

The nutritional, functional, foaming and emulsifying properties of composite flour was also assessed for both batches separately. The data presented in table 2 shows that average moisture content of batch 2 was higher (3.51%) than batch 1 (2.55%). This is only due to lowering the ratio of wheat flour and increasing the ratio of moth bean and little millet. Low moisture content is beneficial as it ensures long shelf life of the food product (Hussein, *et al.*, 2006). The average ash content of batch 2 was also higher (3.69%) than batch 1 i.e.,

Table 2. Nutritional, functional, foaming and emulsifying properties of composite flour.

Parameters	Nutritional value		
	Batch-1 (50:20:20:10)	Batch-2 (30:30:30:10)	Percent change Batch-1 over Batch-2
Moisture content (%)	2.55	3.51	+27.35
Ash content (%)	2.92	3.69	+20.87
Crude fibre (%)	2.07	2.78	+25.54
Crude Lipid (%)	6.59	8.31	+20.70
Total carbohydrates (%)	65.22	62.21	-4.62
Total protein (%)	13.94	14.38	+3.06
Total Iron (mg/ 100g)	11.37	18.01	+36.87
Bulk density (g/ml)	6.67	6.67	0.00
Gelation value	36.00	32.00	-11.11
Least percent solubility (%)	0.40	0.40	0.00
Water absorption capacity (ml/ g)	1.80	1.90	+5.26
Oil absorption capacity(ml/ g)	0.60	1.00	+40.00
Foam capacity (%)	10.00	10.00	0.00
Foam stability (%)	40.00	40.00	0.00
Swelling power (g/g)	0.82	0.40	-51.22
Emulsifying activity (%)	1.13	1.16	+2.59
Emulsifying stability (%)	50.00	51.00	+1.96

2.92%. High ash content can be attributed to increase in mineral content (Hussein *et al.*, 2006) as the amount of moth bean and little millet flour increased and the amount of wheat flour decreased in batch 2. The average crude fiber content was higher in batch 2 (2.78%) than batch 1 (2.07) which is beneficial for healthy gut, proper absorption of nutrients and also in lowering glucose and cholesterol levels. The average crude lipid content was also high in batch 2 (8.31%) than batch 1 (6.59%). The average carbohydrate content of batch 2 was less (62.21%) than batch 1 (65.22%) mainly due to decreased ratio of wheat flour. The average protein content of batch 2 was higher (14.38%) than batch 1 (13.94%). This is only due to higher ratio of moth bean which is low in carbohydrate and high in protein content. Higher total protein content (14.38%) was recorded in batch 2 than batch 1 (13.94%) because of the higher protein containing ingredient. The average iron content of batch 2 was higher (18.01mg) than batch 1 (11.37mg) which fulfils the main purpose of development of iron rich composite flour.

In the present study, various functional properties of flours were also analyzed. The bulk density remained unchanged which was 6.67g/ml for both batches. The bulk density depends on particle size and initial moisture content of flours. The high bulk density suggests their suitability in food preparations. In contrast, low bulk density would be an advantage in the formulation of complementary foods. The gelation value for batch 2 was lower (32%) than batch 1 (36%). The gelling agents in foods are usually polysaccharides and proteins and gelation depends on factors such as temperature, the presence of ions, pH and concentration of gelling agents (Chandra *et al.*, 2015). The percentage solubility of both the batches was same *i.e.*, 0.4% while water absorption capacity of batch 2 was better (1.9ml/g) than batch 1 (1.8ml/g). High water absorption capacity can be attributed to the presence of high amount of carbohydrate (starch) and fiber in the flour. Water absorption capacity was slightly better in batch 2 which is a critical function of protein in various food products like soups, dough and baked products.

The foaming property was also analyzed with the help of foaming capacity and foam stability for both batches of composite flours. The result showed that foaming capacity as well as the foam stability of both the batches of composite flour was same *i.e.* 10% and 40, respectively. Foam consists of a gaseous phase, a liquid phase and a surfactant. There are different types of foams, among them solid foams are essential for the production of cakes and other baked goods. Swelling property and percentage solubility was also used for analyzing both the flours and it was observed that swelling power of batch 1 was higher (0.82g/g) than batch 2 (0.4g/g).

The oil absorption capacity of batch 2 was higher (1ml/g) than batch 1 (0.6 ml/g). Binding of fat with food components influences the texture and flavor of food. The ability of protein to absorb and retain fat is essential in food formulations such as dough preparation, production of breads, cakes, pastries and doughnuts. Protein being the surface active agents can form and stabilize the emulsion by creating

electrostatic repulsion on oil droplet surface. The analysis of emulsion activity (EA) and emulsion stability (ES) of both batches composite flours gave higher emulsion activity of batch 2 (1.16%) than batch 1 (1.13%). The EA of composite flours were found to be significantly increased with decreasing in the proportions of wheat flour. Whereas, emulsion stability can be greatly increased when highly cohesive films are formed by the absorption of rigid globular protein molecules that are more resistant to mechanical deformation. Thus, batch 2 was chosen as quality composite flour which gave better nutritional properties particularly high values of protein, iron, crude fibre, ash content, besides oil absorption and emulsifying activities.

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