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UTILIZATION OF GLUTEN-FREE COMPOSITE FLOUR NUTRITIONAL, PHYTO-CHEMICAL AND FUNCTIONAL PROPERTIES EVALUATION

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ABSTRACT: Celiac disease an antibody-mediated enteropathy which is a lifelong condition where there is intolerance towards gluten. The only treatment till now is the gluten-free diet. The aim of this study was to investigate proximate, phytochemical and functional property analysis of gluten-free composite flour. The proximate analysis of composite flour for the most acceptable sample showed moisture (10.97±0.0), ash (3.35±0.2), fat (4.03±0.1), fiber (4.88±0.2) and protein (18.01±0.5), carbohydrate (61.72±0.4), respectively. Micronutrients like calcium (248.6±1.9), iron (5.03±0.9) and phosphorus (359.6±0.2), respectively, were also present in composite flour. Gluten content had been found 0.02 g/100g. This is the negligible amount that is allowable for celiac patients. The composite flour also contains several phytochemicals such as flavonoids, saponin, tannin, glycosides, alkaloids, and steroids. Some functional properties were assessed, namely water absorption capacity (WAC), oil absorption capacity (OAC), emulsion capacity (EC), foaming capacity (FC) and gelation capacity (GC). So this composite flour is useful for celiac patients and being nutritionally sufficient, conditions like anemia, diabetes and osteoporosis can also be prevented. Composite flour can be utilized as a functional food and used in the development of food products which can be helpful in the development and promotion of health.

INTRODUCTION: According to studies, a combination of flour prepared from mixtures of flours from cereals, roots, tubers, legumes, or other raw materials which can be used for the formulation of traditional or new products such as pasta and bread is referred to as composite flour. Composite flour is used as a functional food because of the health benefits it has and a wide variety of extruded and baked products have been made using it. The acceptability of the products among the population is also checked by sensory evaluation¹. Various components are also used in making the composite flour in the present study.

Each component is carefully chosen, namely proso millet, adzuki bean, and basil seeds. The nutritional and functional attribute of the product developed is decided by the components selected. Proso millet provides many macro and micronutrients such as protein, energy, healthy fats, vitamins and minerals. But they are low in functional properties such as low pasting viscosity and low water holding capacity. Being opposite to this, adzuki bean has higher oil and water absorption and high foaming and emulsifying properties.

Basil seeds, on the other hand, are rich in antioxidants and phytochemicals as compared to proso millet and adzuki bean. It is also a rich source of essential fatty acid (linoleic acid) which has various health-enhancing properties². Therefore, prepared composite flour is gluten-free along with good nutritional qualities and higher fiber content than the normal flours that can also deal with other health problems because gluten-free

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extruded products are generally formulated with flours of rice or maize that have low fiber and protein content. When patients with celiac disease consume these flours, it can lead to constipation in patients as well³. Taste and structure are the other major problems related to gluten-free products. However, incorporation of pros millet, adzuki bean and basil seeds to make composite flour can solve these problems⁴ because all of them found to be good in overall acceptability such as colour, aroma, crispiness, texture and taste to the extruded products⁵.

Thus, as a result, composite flour-based extruded products will have good amount of macro and micronutrients and also the products made from them will have good texture and consistency as it will also have good functional property. Therefore, the main objective of the study is to evaluate proximate composition, phytochemical screening and functional properties analysis. It can be further direct research towards its applications for gluten-free food product development in the market.

MATERIALS AND METHOD:

Collection of Plant Material: The seeds were collected from the local market of Jaipur, India. The seeds were dried at 100 °C in an oven for half an hour. The dried sample of seeds was milled with a mechanical blender and stored in air-tight containers in a refrigerator for further analysis.

Determination of Proximate Composition:

Composite flour was taken in a clean, dry, and weighed crucible. It was oven-dried later on at 110 °C. It was weighed repeatedly until a constant weight was acquired. The crucible was cool down in desiccators every time before weighing. Proximate analysis included the estimation of moisture ash, fat, protein, crude fiber and carbohydrate of seeds. Total ash was estimated by weighing the furnace in incinerated residue at 550 °C for 12 h. Protein was analyzed by using the micro-Kjeldahl distillation method. Carbohydrate content was determined by a different methods.

Determination of Minerals: Chemical estimations were carried out for determining calcium (Ca), iron (Fe) and phosphorus (P). The estimation of Ca, Fe and P was done by atomic absorption spectrophotometer (AAS) (model VGP 210, Buck Scientific, USA).

The data recorded for respective elements was done in triplicate measurements for its authentication and used for standard deviation calculation.

Phytochemical Screening: Composite flour was screened for phytochemicals (tannins, steroids, alkaloids, glycosides, saponin, and flavonoids) according to the procedure as described by^{6,7}.

Functional Property Analysis: Functional properties were analyzed of composite flour, namely water absorption capacity (WAC), oil absorption capacity (OAC), foaming capacity (FC) and emulsion capacity (EC)⁸, and gelation capacity (GC)⁹.

Statistical Analysis: All the results were shown in Mean and Standard Deviation.

RESULTS AND DISCUSSION: Table 1 showed the proximate analysis of composite flour. The Mean \pm SD (g/100g) of moisture, ash, protein, fat, fiber, carbohydrate, calcium, iron, and phosphorus content is shown in the given table. The macronutrients analysis showed that the moisture content (g/100g) was low in all the samples of composite flour that is sample a (10.97 \pm 0.0), sample B (11.09 \pm 0.1), and sample C (11.17 \pm 0.2) in comparison to standard (13.9 \pm 0.7). This is helpful for prolonging the shelf life of the flour.

Also, the products made out of that flour will ensure better shelf life. Julianti *et al.*, (2016) conducted a study where a similar amount of moisture was found in other gluten-free composite flour that is 10.5 g/100g. The ash content of the composite flour in all the ratios was found to be more than standard (0.5 \pm 0.6). The amount of ash in any food sample informs about the nutritionally significant minerals. The most acceptable ratio of composite flour that is sample A obtained was (3.35 \pm 0.2) which was higher when compared to the study carried out by 11 that is 0.3 g/100g. The fat content of composite flour of variant A was (4.03 \pm 0.1) which was again higher than the standard (1.3 \pm 0.2). Similarly, the study was conducted by 12 in which the fat content was low with a value (1.4 \pm 0.0) g/100g in raw rice in comparison to our composite flour. The fiber content was found in good amount in gluten-free composite flour in all the three samples, sample A (4.88 \pm 0.2), sample B (5.26 \pm 0.2) and sample C

(5.54±0.4) while comparing to the study of 13 the fiber content of other gluten-free composite flour (50% rice flour and 50% African yam bean flour) was 4.6 g/100g which was comparable with the current study. Fiber plays a very important role for patients suffering from celiac disease as it helps in the prevention of constipation. The protein content of composite flour of sample A was (18.01±0.5) which was higher than the observation retrieved in other composite flour by¹⁴ which is 15.3±0.0 respectively. The carbohydrate content (61.72±0.4) in the composite flour was rich. Related study was done by¹⁵ in which carbohydrate content with a

value (60.28 g/100g) was found which was comparable with the current study. Calcium content in all the samples of composite flour sample A was (248.6±1.9), sample B (226.96±0.3), and sample C (204.70±0.8) found in considerable quantity. On the opposite side, a reduced amount was seen in a study performed by 16 that is (52.8±0.3) mg/100g. The amount of phosphorus in sample A was (359.6±0.2)g/100gm, which is considered a good amount. On the contrary, the study was conducted by¹⁷ on composite flour made up of sweet potato in which phosphorus amount (20.68 ± 0.01) was found lowest.

TABLE 1: PROXIMATE COMPOSITION OF DIFFERENT VARIANTS OF COMPOSITE FLOUR

Nutrients (per 100g)	Standard (Wheat Flour)	Sample A	Sample B	Sample C
Moisture (g)	13.9±0.7	10.97±0.0	11.09±0.1	11.17±0.2
Ash (g)	0.5±0.6	3.35±0.2	3.25±0.2	3.07±0.1
Fat (g)	1.3±0.2	4.03±0.1	4.39±0.1	4.71±0.0
Fiber (g)	2.7±0.8	4.88±0.2	5.26±0.2	5.54±0.4
Protein (g)	9.4±0.5	18.01±0.5	16.97±0.1	15.94±0.3
Carbohydrate (g)	77.7±0.1	61.72±0.4	61.93±0.4	62.06±0.8
Calcium (mg)	47.0±0.7	248.6±1.9	226.96±0.3	204.70±0.8
Iron (mg)	3.6±0.3	5.03±0.9	5.11±0.2	5.18±0.3
Phosphorus (mg)	108.0±0.5	359.6±0.2	337.1±0.1	316.7±0.8
Gluten (g)	34.1±0.5	0.02±0.0	0.02±0.0	0.02±0.0

Phytochemicals are also known as phytonutrients. They are called as phytochemicals because they are non-nutritive plant chemicals that is responsible for defensive or disease protective properties. The major prevention was against various chronic diseases like including cardiovascular diseases (CVDs), cancer, and diabetes¹⁸. For the phytochemical screening, qualitative analysis of the aqueous extracts of composite flour was done. It revealed positive results for the presence of flavonoids, tannin, saponin, glycosides, alkaloids and steroids, which are presented in **Table 2**. Various animal studies show regular consumption of whole grains, vegetables and fruits lower oxidative damage-related risk of chronic disorders. The previous study also suggests that glycosides and flavonoids report an extensive range of biological activities such as anti-inflammatory,

anti-allergic, and antioxidant¹⁹. Flavonoids can prevent the development of atherosclerosis as it can put off the oxidation of low-density lipoprotein (LDL). Saponins are also analgesic and anti-nociceptive, which have a positive effect on cold-blooded animals. Alkaloids whereas have a good amount of anti-viral and anti-bacterial effects²⁰. Several studies confirmed that the tannins also display anti-microbial and anti-inflammatory properties. Tannins-rich foods have curative and valuable health effects on a human being. One of its properties is the ability to form a leather resistance on the exposed tissues; that is why the tannin can also be used as a drug to heal the burning wound and/or on cuts to discontinue bleeding²¹. Steroids were known for their antifungal property specifically linked with membrane lipids²².

TABLE 2: PHYTOCHEMICAL SCREENING OF COMPOSITE FLOUR

Phytochemical	Standard	Sample A	Sample B	Sample C
Flavonoids	+	+	+	+
Tannin	+	+	+	+
Saponin	+	+	+	+
Glycosides	+	+	+	+
Alkaloids	-	+	+	+
Steroids	-	+	+	+

Functional Properties Analysis: The higher water absorption capacity of all the samples of composite flour was found in comparison to the standard (0.89±1.0). Among the composite flour, the highest was found in sample A (1.7±0.1). In food production, water absorption capacity is essential for dough management²³. Oil absorption capacity is vital for food formulation as it has significance in improving the mouthfeel, and it keeps the flavor of food products²⁴. The oil absorption capacity of variant A was (2.6±0.2), which was similar to the findings of (Kisambira *et al.*, 2015) in yam bean flour. Emulsion capacity in composite flour was (35.2±0.0), which was low in comparison with the findings of other composite flour, which had a range between (47.2±24) to (48.6±3.74) as reported

by²⁶. Foaming capacity was found (10.6±0.2) in composite flour. Similar findings were found in a study conducted by Mepba H *et al.*²⁷ that is (12.0±0.6). Foaming capacity is advantageous characteristic for flours that are used for the manufacturing of a variety of extruded, baked and other kinds of products such as pasta, chakli, cookies, paratha, dosa and cakes²⁸. Gelatin capacity was found to be (56.0±0.5) in sample A of composite flour. In a study conducted by Chandra S *et al.*²⁹, comparable amount was found which had range between (56.2±0.5) to (60.5±0.0) in other composite flours at different ratio. So, gluten-free composite-based products will have improved texture and appearance.

TABLE 3: FUNCTIONAL PROPERTIES ANALYSIS OF COMPOSITE FLOUR

Functional Properties	Standard	Sample A	Sample B	Sample C
Water absorption capacity (g/g)	0.89±1.0	1.7±0.1	1.5±0.1	1.3±0.2
Oil absorption capacity (g/g)	0.90±0.0	2.6±0.2	2.4±0.0	2.2±0.0
Emulsion capacity (%)	43.80±0.2	35.2±0.0	23.1±0.0	27.4±0.3
Foaming capacity (%)	12.90±0.3	10.7±0.2	9.6±0.1	7.8±0.1
Gelation capacity (°C)	59.20±0.5	56.0±0.4	50.0±0.5	49.0±0.4

CONCLUSION: It can be concluded from a study that the composite flour is very useful for celiac patients or people not consuming gluten at all. This gluten free composite flour has good nutritional composition. The combination used of millet, bean and seeds is advantageous in various diseases like diabetes, anemia, eye and bones related disorders, cancer and CVD's. This can be considered as complete nutrition because of its high quality of macro and micro nutrients. It also has good amount of antioxidants and phytochemicals.

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