Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(3): 1760-1764
Received: 18-03-2020
Accepted: 22-04-2020

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Formulation and standardization of gluten free flour mix

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DOI: https://doi.org/10.22271/phyto.2020.v9.i3ac.11568

Abstract
People with severe form of gluten intolerance for long period are at risk for several complications leading to malnutrition. The only dietary treatment for celiac disease is to follow a gluten-free diet. The gluten free diet is a lifetime requirement. Gluten free diet improves symptoms, heals intestinal damage, and prevents further damage. A study was undertaken with an objective to formulate and standardize Gluten free flour mix. Gluten free flour mix was formulated by conducting 6 treatments using millets and legume *viz*. Ragi, Amaranth, Buckwheat and Fava bean. Result revealed that Gluten free flour mix 25 g of ragi, 25 g of amaranth, 25 g of fava bean flour and 25 g of buckwheat flour was found to be the best acceptable formulation. The acceptable combination can be used in the preparation of various food products.

Keywords: Formulation, millets, legumes, gluten free flour mix

1. Introduction
Celiac Disease is precipitated by the ingestion of gluten, a component of wheat protein-gliadin, in genetically susceptible persons. A defect in the enzyme system that splits this protein fraction along with atrophy of jejunal mucosa may be the specific cause of celiac disease. It usually develops within the first three years of life. When gluten-free foods are given there is a dramatic recovery in the symptoms and the reversal of villi to normal growth. Dietary management of celiac disease is of crucial significance as it is related not only to the frequency and severity of morbidities but also mortality of the patient, usually a child. The only dietary treatment for celiac disease is to follow a gluten-free diet. Gluten refers to the combination of gliadin (prolamin) and glutenin (glutelin) fraction of wheat (Wrigley *et al.*, 2006) [12]. The gluten protein fraction displays unique structure building properties that are used in food processing. These structure building properties are also reflected in the terminology, as gluten is essentially the Latin translation of "glue" (El-Chammas K. and Danner E., 2011) [3]. Gluten in wheat flour forms a three-dimensional protein network upon proper hydration and mixing.
Millet is gluten-free, therefore an good option for people suffering from celiac diseases often irritated by the gluten content of wheat and other more common cereal grains. It is also useful for person who is suffering from atherosclerosis and diabetic heart disease (Gélinas *et al.*, 2008) [4]. Park *et al.* (2008) [8] reported that protein concentrate of Korean foxtail millet and proso millet significantly elevated plasma adiponectin and HDL cholesterol levels and caused major decreases in insulin levels relative to a casein diet in type 2 diabetic mice. In addition, proso millet protein concentrate has protective effects against D-galactosamin-induced liver injury in rats (Ito *et al.*, 2008) [6]. Devi *et al.* (2011) [2] review the nature of polyphenols and dietary fiber of finger millet and their role with respect to the health benefits associated with millet. Faba bean, commonly named broad bean, horse bean and field bean, belongs to the family of Fabaceae. It has an important place in the traditional diets of the Indian and has considerable importance as a low-cost food rich in proteins and carbohydrates. Broad beans are harvested at vegetative stage when the pods and seeds are fresh and green, and used as a vegetable. Interestingly, in contrast to cereals, Faba bean was found to contain high levels of lysine and arginine, which may complement the low levels of those in cereals (Imma *et al.*, 2016) [5].
Millets and legumes, which have a high nutritional value, were applied in the production of enriched gluten-free food products. Diet based on gluten-free products is often characterized by low contents of some nutritional components, as well as not- nutritional but physiologically important components like dietary fiber (Krupra *et al.* 2011) [7]. This is the reason; efforts are made to enrich gluten-free products especially in protein, macro, and microelements.

The present research study is therefore undertaken to formulation and standardization of Gluten-Free Flour Mix (GFFM).

2. Materials and Methods

The present investigation was undertaken to study the formulation and standardization of gluten free flour mix. The methodological details of experiment conducted during the course of investigation have been portrayed under the following sub headings:

2.1. Procurement of raw materials

Raw materials namely ragi, amaranth, fava bean and buckwheat were procured in bulk from Sahazadpur, Akbarpur market of Ambedkar Nagar District, Uttar Pradesh (India).

2.2. Processing of Millets: Ragi, Buckwheat and Amaranth were cleaned by sorting out foreign materials such as sticks, leaves, stones and washed and sundried. Dried grains were milled using minigrain mill and sieved into fine flour.

2.3. Processing of Fava bean: Fava bean was prepared by using different processing methods (soaking, germination, drying and baking). Fava beans were cleaned by sorting out contaminants such as sand, sticks and leaves and were washed and covered with several times their volume of water and soaked for 15 hours. After draining water keep the fava bean for the germination (36 hours). At the same time, it should be kept in mind that if it has sufficient moisture, the germination will be done. After germination dried in sunlight for 2 days. Then roast fava bean in hot air oven for 30-35 minutes at 75ºC. Roasted fava beans were later milled using minigrain mill and sieved into fine flour.

Fig 1: Preparation of Fava Bean flour

2.4. Formulation of gluten free flour mix

Gluten Free Flour Mix (GFFM) was prepared by ragi flour, amaranthflour, buckwheat flour and fava bean flour in different ratio (Table 1).

Table 1: Formulation of GFFM by using different ratios

Ingredients	Control	T1	T2	T3	T4	T5
Wheat flour(g)	100	-	-	-	-	-
Ragi(g)	-	26.6	25	22.3	21.6	20
Amaranth(g)	-	26.6	25	22.3	21.6	20
Buckwheat(g)	-	26.6	25	22.3	21.6	20
Fava bean(g)	-	20.2	25	30.1	35.2	40

2.5. Standardization of Gluten Free Flour Mix

GFFM were developed in the laboratory of Foods and Nutrition. Batches of 100g of GFFM were produced. For standardization of GFFM chapatti was prepared by using different ratio of Ragi, Amaranth, Buckwheat and Fava bean flour (Fig 2). Chapatti are unleavened flat breads made from whole wheat flour and are served as the staple food in the Indian sub-continent. Chapatti is one of the commonly consumed products in the lunch and dinner. Adding fava bean flour additionally improve the nutritional quality of the product. Different proportion of GFFM was used to prepare chapatti and replace wheat flour from chapatti and make it consumable for Celiac Disease patients.

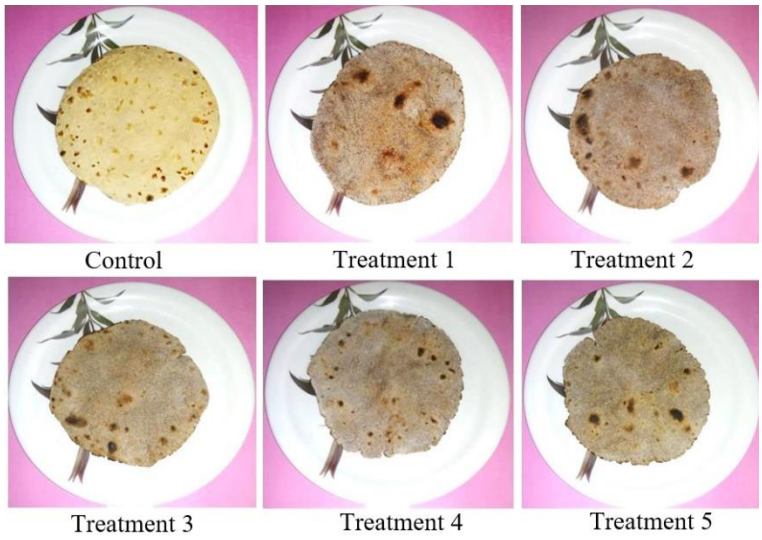

Fig 2: Chapatti prepared with different proportion of GFFM

2.6. Sensory Evaluation:

The prepared Gluten Free Flour Mix chappati were organoleptically evaluated by ten panels of judges. For the selection of semi-trained panel members threshold test is used. The Gluten Free Flour Mix was evaluated for various sensory quality characteristics such as color, flavor, taste, texture, appearance, and overall acceptability. Evaluation of the chappati was done on the basis of 9 point hedonic scale.

The test sample was given in triplicates with control. Control sample was prepared from the usual recipes. All samples were coded to avoid any type of biasness.

2.7. Nutritional Composition of GFFM
Nutritive value of GFFM was calculated by using Indian Food Composition Table (IFCT, 2017) published by National Institute of Nutrition (NIN).

2.8. Statistical Analysis
Statistically, all the collected data on organoleptic evaluation were analyzed. Data were presented as mean ± S.D. Analysis of Variance (ANOVA) was used to assess the sensory characteristics of Gluten Free Flour Mix chappatis. Statistical analysis was performed using O.P. STAT Software.

3. Result and discussion
3.1. Standardization of Gluten Free Flour Mix Chappati
Table 2 depicted the result of organoleptic evaluation of GFFM chapatti at different levels. Sensory scores reveal that the GFFM chapatti fell in the category of "liked very much" to "like moderately". Perusal of the sensory scores as evident from the Table 2 reveals that T2 obtained highest scores ratio i.e. 8.62 ±0.08 for color, 8.70 ± 0.09 for flavor, 8.72 ± 0.08 for texture, 8.65 ± 0.18 for appearance, 8.72 ± 0.04 for taste and 8.70 ± 0.07 for overall acceptability when compared to other counter parts. It is also clear that from overall acceptability point of view T2 scored the highest of 8.70 ± 0.07, Control 8.37±0.09, 8.02 ± 0.17 for T1, 7.67 ± 0.15 for T3, 7.57 ± 0.06 for T5 and 7.52 ± 0.13 for T4 respectively when compared to all other treatments. The sensory scores obtained in case of GFFM chapatti depicted that on an overall basis control was "liked very much" followed by T2, T1, T3, T5 and T4. Reveling that the ratio of GFFM increases in the sensory scores up to 25% ratio level, but then the sensory scores continuously decreased with T1, T3, T4 and T5, therefore T2 chapatti of GFFM was selected for further study (Fig.1). Similarly Radhika *et al.* (2019) [9] made a study on the development of gluten free cookies by incorporation of gluten-free ingredients in different proportions. Gluten free raw ingredients *i. e.* finger millet (FM), pearl millet (PM), soya bean (SB) and groundnut (GN) they concluded that these ingredients are a rich source of crude fibre, protein, fat and mineral content and the formulation containing all the four ingredients in equal proportion (25% each) was liked most and scored highest on 9-point hedonic rating scale.

Table 2: Mean acceptability scores of Gluten Free Flour Mix chapatti

Treatments	Color	Flavor	Texture	Appearance	Taste	Overall acceptability
Control	8.17±0.10	8.22±0.07	8.12±0.18	8.77±0.09	8.45±0.10	8.37±0.09
T1	7.82±0.10	7.80±0.07	7.87±0.28	8.60±0.09	7.97±0.21	8.02±0.17
T2	8.62±0.08	8.70±0.09	8.72±0.08	8.65±0.18	8.72±0.04	8.70±0.07
T3	7.77±0.26	7.75±0.13	7.52±0.22	7.47±0.11	7.92±0.20	7.67±0.15
T4	7.62±0.04	7.97±0.29	7.37±0.08	7.65±0.27	7.52±0.15	7.57±0.06
T5	7.42±0.08	7.27±0.25	7.35±0.18	7.77±0.25	7.52±0.12	7.52±0.13

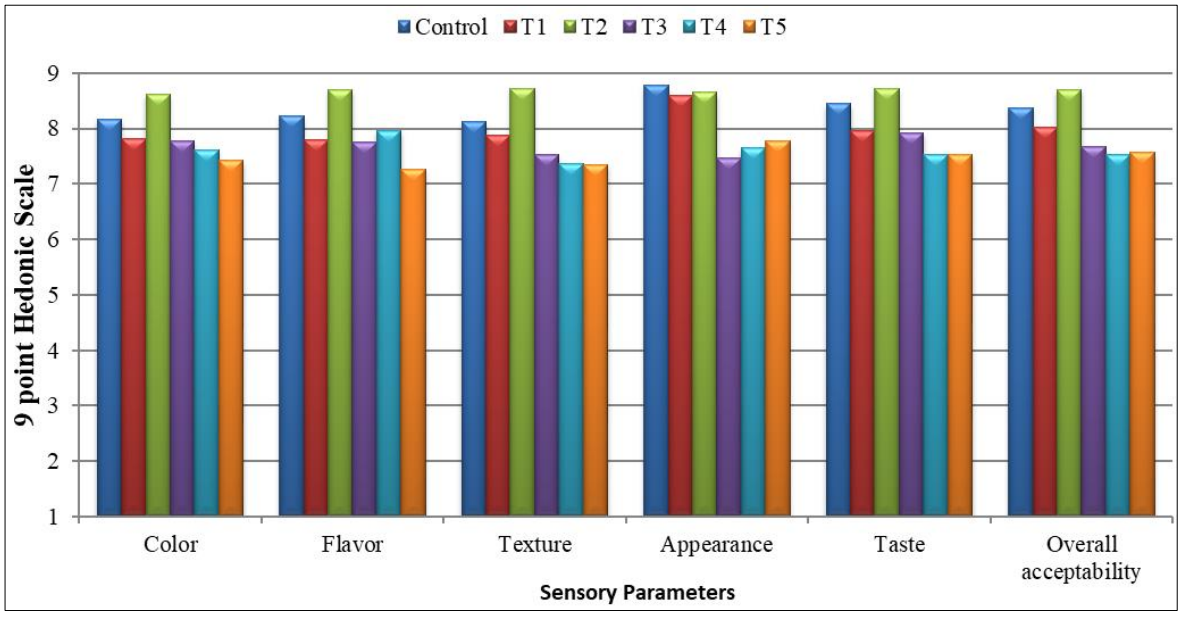

Fig 3: Mean acceptability scores of GFFM chapatti

3.2 Formulation of Gluten Free Flour Mix:
Six different trials were conducted for formulation of Gluten Free Flour Mix and organoleptic evaluation for the same was done. The most acceptable formulation was selected.

Table 3 represent the computed nutritive value for Gluten Free Flour Mix Trial 1 which is composed of 26.6 g. of ragi, 26.6 g. of amaranth, 26.6 g. of fava bean and 20.2 g. of buckwheat. It shows that total protein was 13.71g, fat 2.95g, fibre 12.11g, carbohydrate 62.03g, energy 907.42 kcal, calcium 182.58 mg and iron 9.16 mg/100 g of the Gluten Free Flour Mix. Table 4 represent the computed nutritive value for Gluten Free Flour Mix Trial 2 which is composed of 25 g of ragi, 25 g of amaranth, 25 g of fava bean and 25 g of buckwheat. It shows that total protein was 14.5g, fat 2.88g, fibre 12.94g, carbohydrate 62.46g, energy 874kcal, calcium 178mg and iron 9.02 mg/100 g of the Gluten Free Flour Mix. Table 5 represent the computed nutritive value for Gluten Free Flour Mix Trial 3 which is composed of 22.3 g of ragi, 22.3 g of amaranth, 22.3 g of fava bean and 30.1 g of buckwheat. It shows that total protein was 15.27g, fat 2.82g,

fibre 13.73g, carbohydrate 62.15g, energy 838.34 kcal, calcium 172.97 mg and iron 8.86mg/100 g of the Gluten Free Flour Mix.

Table 6 shows the mean sensory scores of trial 1, 2 and 3. The overall acceptability was higher in Trial 2 (8.70±0.07), compared to Trial 1 (8.02±0.17) and trial 3 (7.67±0.15). The color was higher in Trial 2 (8.62±0.08), compared to Trial 1 (7.82±0.10) and trial 3 (7.77±0.26). The flavor was higher in Trial 2 (8.70±0.09), compared to Trial 1 (7.80±0.07) and trial 3 (7.75±0.13). The texture was higher in Trial 2 (8.72±0.08), compared to Trial 1 (7.87±0.28) and trial 3 (7.52±0.22). The appearance was higher in Trial 2 (8.65±0.18), compared to Trial 1 (8.60±0.09) and trial 3 (7.47±0.11). The taste was higher in Trial 2 (8.72±0.04), compared to Trial 1 (7.97±0.21) and trial 3 (7.92±0.20).

Table 7 represent the computed nutritive value for Gluten Free Flour Mix Trial 4 which is composed of 21.6 g of ragi, 21.6 g of amaranth, 21.6 g of fava bean and 35.2 g of buckwheat. It shows that total protein was 16.01g, fat 2.67g, fibre 14.52g, carbohydrate 61.77g, energy 800.82 kcal, calcium 167.58 mg and iron 8.68 mg/100 g of the Gluten Free Flour Mix. Table 8 represent the computed nutritive value for Gluten Free Flour Mix Trial 5 which is composed of 20 g of ragi, 20 g of amaranth, 20 g of fava bean and 40 g of buckwheat. It shows that total protein was 16.8g, fat 2.6g, fibre 15.35g, carbohydrate 61.57g, energy 767.4 kcal, calcium 162.82 mg and iron 8.56 mg/100 g of the Gluten Free Flour Mix. Table 9 shows the mean sensory scores of Trial 4 and 5. The overall acceptability was higher in Trial 5 (7.57±0.06), compared to Trial 4 (7.52±0.13). The color was higher in Trial 4 (7.62±0.04), compared to Trial 5 (7.42±0.08). The flavor was higher in Trial 4 (7.97±0.29), compared to Trial 5 (7.27±0.25). The texture was higher in Trial 4 (7.37±0.08), compared to Trial 5 (7.35±0.18). The appearance was higher in Trial 5 (7.77±0.25), compared to Trial 4 (7.65±0.27). The taste was higher in Trial 4 (7.52±0.15), compared to Trial 5 (7.52±0.12).

Initial trials for formulation of Gluten free flour mix were based on equal proportion of Ragi, Amaranth, Buckwheat and Fava bean flour trail 2 (25:25:25:25) were highly acceptable. Hence the Fava bean flour quantity increases in trail 3, 4 and 5 (Table 5, 7, 8) for increase in protein and fibre with 30.1, 35.2 and 40 percent. With increase in percent of fava bean flour and decrease in millets flour it was slightly difficult to prepare dough and roll a good quality chappati. The overall acceptability score with trial 3, 4 and 5 was 7.67, 7.57 and 7.52 decreased respectively. Hence the trail no 2 was finally selected for further studies. Similar studies for development of gluten-free biscuits from the following flours mixture: maize flour (MF), rice flour (RF) and soybeans flour (SF) was developed by Simona *et al*., (2014) [10], reported that the blend formulated with flour levels 30:30:40 (MF:RF:SF) were most acceptable.

Beenu and Meenakshi (2017) [1], developed and standardized gluten free cookies from the following flours mixture: bajra flour (BF), buckwheat flour (BWF) and ragi flour (RF). Four experimental variants (gluten-free cookies) were obtained by varying the proportion of flours and found that the blend A3 with flour levels BWF 60g: RF 40g: BF100g led to the highest acceptability. Udaybeer *et al.* (2017) [11], investigated the alternate flours sorghum/pearl millet for the preparation of gluten free cookies as compared to conventional wheat flour cookies and found that the cookies with pearl millet and soy flour combination had higher fat, protein, ash and calorific values as compared to control cookies and also the maximum sensory overall acceptability scores were found for cookies prepared from combination of pearl millet and soy flour followed by pearl millet and control cookies. When millet flour was fortified with soy flour it gives high level of protein of 12.60% of cookie. Fat content increased from 19.12% for control cookies to 17.57% for 100% incorporation mixed based cookies.

Table 3: Nutritional composition of Gluten Free Flour Mix trial 1

	Ragi	Amaranth	Buckwheat	Fava Bean	Total
Quantity(g)	26.6	26.6	26.6	20.2	100
Protein(g)	1.90	3.88	2.73	5.2	13.71
Fat (g)	0.5	1.52	0.63	0.3	2.95
Fibre(g)	2.97	1.86	2.28	5.0	12.11
Carbohydrate(g)	17.17	15.95	17.31	11.6	62.03
Energy(kcal)	356.97	396.34	85.91	68.2	907.42
Calcium (mg)	96.82	48.14	17.02	20.6	182.58
Iron(mg)	1.22	2.48	4.12	1.34	9.16

Table 4: Nutritional composition of Gluten Free Flour Mix trial 2

	Ragi	Amaranth	Buckwheat	Fava Bean	Total
Quantity(g)	25	25	25	25	100
Protein(g)	1.79	3.64	2.57	6.5	14.5
Fat (g)	0.48	1.43	0.6	0.37	2.88
Fibre(g)	2.79	1.75	2.15	6.25	12.94
Carbohydrate(g)	16.70	14.99	16.27	14.5	62.46
Energy(kcal)	335.5	372.5	80.75	85.25	874
Calcium (mg)	91	45.25	16	25.75	178
Iron(mg)	1.15	2.33	3.87	1.67	9.02

Table 5: Nutritional composition of Gluten Free Flour Mix trial 3

	Ragi	Amaranth	Buckwheat	Fava Bean	Total
Quantity(g)	23.3	23.3	23.3	30.1	100
Protein(g)	1.67	3.40	2.40	7.8	15.27
Fat (g)	0.44	1.33	0.55	0.45	2.82
Fibre(g)	2.60	1.63	2.0	7.5	13.73
Carbohydrate(g)	15.58	13.99	15.18	17.4	62.15
Energy(kcal)	313.08	347.61	75.35	102.3	838.34
Calcium (mg)	84.92	42.22	14.93	30.9	172.97
Iron(mg)	1.07	2.17	3.61	2.01	8.86

Table 6: Mean sensory scores of trial 1, 2 and 3

Trial	Color	Flavor	Texture	Appearance	Taste	Overall acceptability
1	7.82±0.10	7.80±0.07	7.87±0.28	8.60±0.09	7.97±0.21	8.02±0.17
2	8.62±0.08	8.70±0.09	8.72±0.08	8.65±0.18	8.72±0.04	8.70±0.07
3	7.77±0.26	7.75±0.13	7.52±0.22	7.47±0.11	7.92±0.20	7.67±0.15

Table 7: Nutritional composition of Gluten Free Flour Mix trial 4

	Ragi	Amaranth	Buckwheat	Fava Bean	Total
Quantity(g)	21.6	21.6	21.6	35.2	100
Protein(g)	1.54	3.15	2.22	9.1	16.01
Fat (g)	0.41	1.23	0.51	0.52	2.67
Fibre(g)	2.41	1.51	1.85	8.75	14.52
Carbohydrate(g)	14.43	12.95	14.06	20.3	61.77
Energy(kcal)	289.87	321.84	69.76	119.35	800.82
Calcium (mg)	78.62	39.09	13.82	36.05	167.58
Iron(mg)	0.99	2.01	3.34	2.34	8.68

Table 8: Nutritional composition of Gluten Free Flour Mix trial 5

	Ragi	Amaranth	Buckwheat	Fava Bean	Total
Quantity(g)	20	20	20	40	100
Protein(g)	1.43	2.91	2.06	10.4	16.8
Fat (g)	0.38	1.14	0.48	0.6	2.6
Fibre(g)	2.23	1.40	1.72	10	15.35
Carbohydrate(g)	13.36	11.99	13.02	23.2	61.57
Energy(kcal)	268.4	298	64.6	136.4	767.4
Calcium (mg)	72.8	36.02	12.8	41.2	162.82
Iron(mg)	0.92	1.86	3.1	2.68	8.56

Table 9: Mean sensory scores of trial 4 and 5

Trial	Color	Flavor	Texture	Appearance	Taste	Overall acceptability
4	7.62±0.04	7.97±0.29	7.37±0.08	7.65±0.27	7.52±0.15	7.52±0.06
5	7.42±0.08	7.27±0.25	7.35±0.18	7.77±0.25	7.52±0.12	7.57±0.13

4. Conclusion

Gluten Free Flour Mix with 25 g of ragi, 25 g of amaranth, 25 g of fava bean and 25 g of buckwheat was found to be the best formulation. This combination can be used in the preparation of various food products for celiac disease patients.

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