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Nutritional and Sensory Evaluation of the Formulated Gluten Free Flour and Its Effect on the Gastrointestinal Symptoms of Celiac Patients

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ABSTRACT

The present study was aimed to developing a gluten free substitute from indigenous sources for celiac disease patients with premium nutritional and sensory characteristics, particularly for the preparation of *chapatti*. Various recipes were formulated using corn, rice, sorghum, millet grams and pulses. These recipes were evaluated for their nutritional and sensory characteristics. The selected recipe comprised of rice flour, corn flour and mung bean flour with small additions of xanthan gum. The flour was prepared in bulk and fed to 50 celiac patients for a period of four months. Patients were assessed for the presence of gastrointestinal (GI) symptoms before and after the feeding trial. Induction of gluten free flour (GFF) resulted in significant (p<0.05) reduction in abdominal pain, heart burn, sucking sensation, nausea, vomiting, abdominal distension, increased stool frequency, loose stools, hard stools, evacuation urgency and steatorrhea. It was further observed that all GI symptoms were more prevalent in patients aging 1-8 and 19-50 years. These symptoms were least in patients aging 14-18 years. Significant (p<0.05) reduction in GI symptoms occurred in the age group of 1 to 13 and of 31 to 50 years.

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SAN conceived and designed the study and supervised the work. SI executed the field work, clinical trials, analyzed the data and wrote the article. OU helped in statistical analysis. SK and ZH helped in article writing.

Key words
Celiac, gluten free flour, chapatti, gastrointestinal symptoms

INTRODUCTION

Celiac disease is an auto immune disorder with chronic inflammation and subsequent atrophy of proximal small intestine with increased secretion of water and solute. Various studies have demonstrated a variety of gastrointestinal symptoms in celiac patients including stomach pain, distention, bloating, chronic diarrhea and steatorrhea (Holtmeier and Caspary, 2006). Female celiac patients have been rated significantly higher on the gastrointestinal symptom rating scale (Svedlund *et al.*, 1988) as compared to control patients who have been on a gluten free diet for several years (Midhagen and Hallert, 2003). A high incidence of abdominal pain in some patients has been attributed to maldigestion as well as high prevalence of *Helicobacter pylori* (Murray *et al.*, 2004). Anderson (2014) reported that people with celiac disease could complain of constipation, weight gain and heartburn rather than diarrhea, weight loss and stomach pain.

Celiac disease can be treated by avoidance of gluten found in wheat, rye and barley. Substitute for gluten containing flours are the flours naturally devoid of gluten. According to Codex Alimentarius gluten free labeled food products should contain less then 20ppm of gluten (Codex, 2008). Rice, corn, lentil, bean and pulse based gluten free flours are being used to enhance the quality of gluten free products (Boye *et al.*, 2010). Hydrocolloids are used in small amounts (0.3-0.4%) for sensory characteristics improvement in these products. The maximum acceptable limit set by WHO for xanthan gum as a food additive is 1% by weight (Preichardt *et al.*, 2011).

The beneficial effect of a gluten free diet on gastrointestinal symptoms has been debatable. Some studies have suggested full and some partial mucosal recovery (Holtmeier and Caspary, 2006). It is, however, agreed that severity of the symptoms was reduced after treatment with a gluten free diet. Few studies have found that most symptoms were resolved with a gluten free diet in an average time of four weeks. Although non compliance has been stated as the main reason for persistent or recurrent symptoms, these have also been attributed to partially healed celiac disease, an associated medical problem or a medical complication. Improvement in symptoms did not reflect complete histological recovery as this could take a long time. Histological improvement has been found to be quicker and more complete in children than in adults (Murray *et al.*, 2004).

Many recipes of gluten free flour are available; however, no effort has been made to formulate an appropriate blend for *chapatti* (unleavened flat bread).

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Furthermore, the nutritional profile of gluten free flour has been a neglected concern. For the Pakistani population of celiac patients this feature is crucial because of the underlying malnutrition caused by disease which is further aggravated by poverty. The present study was carried out to develop gluten free flour of high quality sensory characteristics as well as good nutritional profile. The effect of the formulated gluten free flour (GFF) on gastrointestinal symptoms of celiac patients is reported.

MATERIALS AND METHODS

Formulation of gluten free flour (GFF)

GFF was formulated using gluten free grains, grams and pulses. Three varieties of grams (*Cicer arientinum*) including CM, Punjab and bittle, sorghum PSC 10/0 (*Sorghum bicolor*), millet HP-50 (*Pennisetum glaucum*), maize 919 (*Zea mays*), super basmati rice (*Oryza sativa*), pulses including mung bean (golden gram-*Vigna radiate*), whole mash bean (black gram -*Vigna mungo*) and dal chana (Split Bengal Gram- *Cicer arientinum*) were procured from Punjab Seed Corporation, Pakistan. The proximate analysis of the above mentioned gluten free grains was carried out using AOAC (2005) methods (Table I).

Preliminary sensory evaluation of the prepared formulations was accomplished by the experts at Supreme flour Mills. Grams were added to enhance the protein quality but were excluded from the recipes after initial evaluation as they were not contributing the desired texture to the *chapatti*. Sorghum and millets attributed a bitter aftertaste and a darker color. Rice flour was best in terms of taste although it made the texture slightly gritty. Addition of xanthan gum (0.5% by weight) was found to be appropriate as it conferred reasonable stickiness which made the preparation of *chapatti* easy.

Keeping in view the foregoing facts, grams were replaced by pulses including de hulled mung bean, dal chana and whole mash beans to add good quality protein as well as elasticity to the *chapatti* dough. A total of nine recipes were developed and analyzed for sensory properties, nutritional composition and cost.

Sensory evaluation

For final sensory evaluation 'preference test' was carried out using British Standard, BS5929 (BS Method, 1992). Each recipe was scored for its taste, texture, aroma, appearance and overall acceptability. For each sample, 120 g of flour was kneaded with 72 ml of water. The dough was then divided in half and allowed to rest for half an hour at 30ºC. Each dough ball (60g) was rolled into the shape of a *chapatti* on a flat surface and gently cooked on a *tawa* (hot plate) at a temperature of 220- 250ºC for 2 min on both sides until golden brown. Each *chapatti* was cut into four equal pieces so that each panelist could have at least quarter *chapatti* for testing. The *chapatti* was served hot immediately after baking to reduce flavor loss and discoloration. Sensory scoring was compared for final recipe selection (Table II).

Nutritional evaluation of the recipes

Nutritional evaluation (Table III) of the recipes was carried out by AOAC (2005) methods using proximate analysis scheme.

Cost evaluation of the recipes

The cost of the recipes was calculated by the sum total of the cost (Pak Rupees) of grains, xanthan gum, processing (electricity units used in grinding and mixing) and packaging (Table II).

Bulk production of the flour

Recipe 7 containing 30g rice flour, 20g mung bean and 10g corn flour was selected and prepared in batches of 300 kg whenever required. The grinding, mixing and packing was carried out at a local factory. Any chance of contamination with wheat was ruled out. The whole grinding unit was thoroughly washed and dried before the production of flour. The corn was thoroughly cleaned, de hulled and degermed in a separate corn processing unit. This resulted in improved taste and shelf life of the corn flour and consequently of the gluten free flour. Remaining grains were cleaned and de stoned manually. At all stages of the recipe preparation, personal hygiene of the working staff was ensured. The grains were weighed on a digital weighing scale to ensure exact proportion of the recipe. A meal mixer (Butcher Boy Company) with a capacity of 300kg and operative rpm of 25 was used to blend and mix various components of the recipe. During mixing 1.5 kg xanthan gum (0.5% by weight) was added. It was ensured that all the grains and gum were thoroughly mixed. Each flour bag was filled with 5kg flour and sealed. This flour was retested for its proximate principles and gluten content in ppm using sandwich ù-gliadin ELISA (Skerritt *et al.*, 1991) using a Gluten Detection kit obtained from Imutest Diagnostic Innovation Limited, UK. The flour thus produced contained gluten less than 20ppm.

Biological evaluation

The formulated GFF was tested for its efficacy in reducing gastro intestinal symptoms in celiac patients.

Selection of the study sample

A total of 50 diagnosed celiac patients (25 male and

Table I.- Proximate analysis of indigenous gluten free grains and legumes.

Varieties	Moisture	Ash	Fat	Protein	Crude fiber	NFE
Grams (CM)	8.75±0.04a	2.06±0.12a	4.90±0.17ab	23.72±0.95a	3.36±0.51a	57.72±1.56a
Grams (Punjab)	8.98±0.15a	2.28±0.07b	4.51±0.19a	23.07±0.86a	6.79±1.00b	53.38±0.25b
Grams (Bittle)	8.77±0.09a	2.18±0.11ab	4.73±0.14ab	21.67±0.46b	5.73±0.60c	56.57±0.03a
Sorghum	11.45±0.05b	1.21±0.19c	3.84±0.17c	11.73±0.67c	2.57±0.21a	68.90±0.49cd
Millet	9.62±0.21c	1.45±0.11d	5.67±0.33d	14.13±0.89d	2.90±0.50a	67.06±1.92d
Rice	11.50±0.09b	0.26±0.01e	0.38±0.11e	9.41±0.35e	0.37±0.04d	78.16±0.29e
Maize	10.19±0.26d	1.25±0.03c	4.60±0.44a	11.35±0.25c	2.55±0.07a	69.79±0.54c
Mung beans (dehulled)	4.99±0.14e	2.76±0.06f	5.20±0.35bd	27.90±1.41f	0.12±0.01d	57.53±1.27a
Dal Chana	4.76±0.13e	2.58±0.06fg	3.00±0.21f	26.43±2.12f	1.10±0.03d	62.13±2.01f
Mash beans (whole)	11.10±0.14f	2.53±0.42g	0.99±0.01g	17.85±0.21g	3.5±0.15a	64.03±.13f

Mean values followed by different letter in a column are significantly different at $p<0.05$

Table II.- Sensory scoring of the formulated recipes.

Recipe	Sensory evaluation scoring*	Cost/kg (Rs)
1	16.71±1.89abde	62.1
2	16.43±3.69bfg	61.4
3	19.14±2.54af	65.6
4	13.71±1.98g	63.1
5	16.86±2.12abcde	76.4
6	18.57±2.88abcf	72.3
7	19.29±1.89ace	68.9
8	15.71±1.89dg	64.4
9	17.14±3.39def	70.6

Mean values followed by different letter in a column are significantly different at $p<0.05$
*Maximum score=25
1US$=100 Rs approximately

25 female) selected from the Gastroenterology and Pediatric Department of Sheikh Zayed Medical Complex, Lahore and Mayo Hospital, Lahore, were included in the study. Both antibody (IgG and IgA) tests and endoscopy results were used as the diagnostic criteria. All required formalities of the Institution Review Board were met. Patients selected belonged to the middle or lower socio-economic status (Nagra *et al.*, 1984).

Gastrointestinal symptom rating scale (GSRS)

Gastrointestinal rating scale (Svedlund, Sjodin and Dotevall, 1988) with the addition of steatorrhea was used to assess the presence and intensity of commonly found gastrointestinal symptoms before and after the GFF trial.

Statistical analysis

Descriptive statistics were worked out for all the parameters studied. One way analysis of variance and multiple comparisons of means were made by LSD test using SPSS version 17. Correlation was also worked out where necessary.

RESULTS AND DISCUSSION

Recipe 7 was rated the best in the sensory and nutritional evaluation (Tables II, III). The number of patients with varying degree of gastrointestinal symptoms was reduced with use of GFF. Significant ($p<0.05$) reduction occurred in abdominal pain, heart burn, sucking sensation, nausea and vomiting, distension, increased stools, loose stools, hard stools, urgency and steatorrhea (Table IV). Patients aged between 31-50 years scored the highest and 14-18 years scored the lowest on the gastrointestinal symptom rating scale before the use of GFF. Gastrointestinal symptom score was significantly ($p<0.05$) reduced in the patients of 1-13 and of 31-50 years after GFF induction (Table V). Mean score for males, prior to the use of GFF, was higher on the gastrointestinal symptom rating scale. After treatment reduction in the mean score of males was more obvious as compared to the females (Table V). All gastrointestinal symptoms except decreased and hard stools, and evacuation urgency, were found to be significantly ($p<0.05$) and positively correlated to wheat intake (Table VI).

The primary goal of the study was to formulate flour recipes by making use of gluten free grains and pulses in varying proportions. Recipes thus developed were evaluated for their sensory characteristics, cost effectiveness (Table II) and nutritional composition (Table III).

The amount of rice flour appeared directly proportional to the better sensory characteristics, especially taste. Nutritionally, rice flour contained low protein and fiber as compared to other cereals. However, its lysine, thiamine, riboflavin, niacin, and vitamin E content have been reported highest amongst the other cereals. Biscuits made from rice flour had better structure-mechanical properties and became harder with

Table III.- Nutritional composition of recipes (g / per 60 g of recipes).

Recipe	Moisture	Ash	Fat	Protein	Crude Fiber	NFE	Calories
1	5.18±0.06ac	0.65±0.01a	1.64±0.08a	7.19±0.25a	0.63±0.01a	44.58±0.03a	221.74±0.20a
2	5.15±0.07ac	0.63±0.01b	1.42±0.06b	7.04±0.32a	0.72±0.01b	45.05±0.37bc	221.07±0.79ae
3	5.31±0.03ae	0.55±0.01c	1.19±0.06c	6.98±0.25a	0.42±0.01c	45.43±0.03c	220.30±0.33ae
4	5.21±0.00ae	0.77±0.01d	1.93±0.09d	8.65±0.17b	0.65±0.04a	42.56±0.27d	222.17±0.93abc
5	4.37±0.05b	0.72±0.00e	1.22±0.05bc	8.34±0.35b	0.16±0.01d	44.90±0.18ab	223.87±1.67bd
6	4.66±0.01bc	0.80±0.00f	1.66±0.08a	8.86±0.36bc	0.38±0.00c	43.36±0.14e	223.78±1.24cd
7	4.60±0.06bd	0.76±0.01d	1.67±0.13a	9.47±0.40c	0.39±0.01c	41.94±0.11f	220.60±0.78ae
8	5.75±0.08e	0.68±0.01g	1.65±0.12a	8.64±0.34b	0.52±0.02e	42.54±0.01d	219.53±0.32e
9	5.64±0.71ae	0.73±0.00e	1.25±0.09bc	8.47±0.23b	0.73±0.04b	42.58±0.03d	215.39±0.01f

Mean values followed by different letter in a column are significantly different at $p<0.05$

Table IV.- Prevalence of GI symptoms in the celiac patients before and after treatment with gluten free flour.

GI Symptom	Absent		Mild		Moderate		Severe		Chi square
	1†	2†	1†	2†	1†	2†	1†	2†	
Abdominal pain	20	32	10	16	12	2	8	0	$\chi2=19.30$, p=0.00
Heart burn	33	40	4	8	10	2	3	0	$\chi2=10.34$, p=0.02
Regurgitation	37	44	4	4	6	2	3	0	$\chi2=5.60$, p=0.13
Sucking sensation	34	46	1	2	11	2	4	0	$\chi2=12.36$, p=0.01
Nausea and vomiting	33	44	6	6	7	0	4	0	$\chi2=12.57$, p=0.01
Borborygmus	26	37	9	6	11	6	4	1	$\chi2=5.79$, p=0.12
Distension	22	29	8	16	10	5	10	0	$\chi2=15.29$, p=0.00
Eructation	42	44	3	4	3	2	2	0	$\chi2=2.39$, p=0.50
Increased flatus	27	33	5	8	14	8	4	1	$\chi2=4.73$,0.19
Decreased stools	48	49	1	1	1	0	0	0	$\chi2=1.01$, p=0.60
Increased stools	24	40	17	10	9	0	0	0	$\chi2=14.82$, p=0.00
Loose stools	24	37	12	12	9	1	5	0	$\chi2=14.17$, p=0.00
Hard stools	44	50	5	0	1	0	0	0	$\chi2=6.38$, p=0.04
Urgency	22	38	3	4	14	8	11	0	$\chi2=17.05$, p=0.00
Incomplete evacuation	32	42	4	3	11	5	3	0	$\chi2=6.74$, p=0.08
Steatorrhea	27	40	6	6	13	2	4	2	$\chi2=11.26$, p=0.01

†1=pre treatment †2Post treatment

the addition of corn flour (Juliano, 1993). After the final sensory evaluation it was concluded that recipes containing 50 % rice flour were rated superior. Various studies have suggested the addition of sorghum and millet due to their high protein content (Saleh *et al.*, 2013). However, due to poor palatability their incorporation in various recipes was kept minimal. Addition of 10% leguminous flour (chick pea and pea flour) in bakery products had also been recommended for the improvement in sensory parameters of baked products (Boye *et al.*, 2010). Addition of gram flour resulted in poor sensory characteristics of the chapattis. Despite the fact that it enhanced the quantity and quality of protein, its use could not be encouraged in patients with diarrhea due to recent diagnosis or non compliance. Therefore, grams were replaced by pulses. The potential of lentil and bean flour had been explored recently in many studies and it was found that they improve the nutritional profile of gluten-free baked goods. They are rich in fiber, protein (17–30%) and have a high profile of amino acids such as lysine, leucine, aspartic acid, glutamic acid and arginine. They are good sources of iron and potassium (Kohajdova *et al.*, 2013). Pulses have good water holding and emulsifying properties. An addition of 10% of lentil and bean flours in wheat flour enhanced the acceptability of the finished products, whereas, further increase in their amount, deteriorated sensory characteristics (Boye *et al.*, 2010). In the present study, the best sensory results were achieved by the addition of 33% bean flour (mung bean flour) in combination with rice and corn flour. It was noted that addition of corn flour of more than 17% (10g in a standard 60 g chapatti) resulted in off taste.

Table V.- Effect of gluten free flour on GI symptom rating scale score of celiac patients of different age groups and gender.

	GI Symptoms		
	Pre-treatment	**Post-treatment**	**% reduction**
Age (years)			
1-3	13.33±7.66	2.50±2.17*	80.29
4-8	11.75±7.05	3.08±2.64*	64.46
9-13	8.35±9.16	3.24±5.03*	63.64
14-18	5.50±7.14	1.75±2.36	69.05
19-30	16.00±13.62	5.50±7.34	43.47
31-50	19.20±10.50	12.20±8.07*	36.87
Gender			
Male	12.16±9.57	2.80±4.03*	16.44
Female	10.92±9.65	5.52±6.36*	4.76

Means in a row followed by asterisk (*) are significantly different at $p<0.05$
Mild, 1-16; Moderate, 17-32; Severe, 33-48.

Most of the commercially available imported GFFs include white starch and have little nutritional value (Hager *et al.*, 2012). In the present study most of the cereals used were dehulled to reduce the fiber content for easy digestibility, increased shelf life, improved sensory characteristics and better nutritional profile, especially in terms of increased proteins and B vitamins.

To keep the *chapatti* intact during preparation, 0.5% by weight of xanthan gum was added. Increased amounts resulted in the baked product texture becoming heavy, gummy and slimy (Preichardt *et al.*, 2011). It was observed in the present study that a further reduction (<0.05%) in the amount of gum made the preparation of *chapatti* difficult.

After thorough evaluation of the nutritional profile, sensory characteristics and cost effectiveness of the recipes, Recipe 7 was rated as the most appropriate. It scored highest on sensory characteristics with a mean score of 19.3 out of 25 points. Nutritionally, this recipe contained protein 15%, ash 2.8%, fat 1.3% and fiber 0.65%. It was expected to be suitable for malnourished patients with sensitive intestines and recurrent diarrheal episodes and offered an optimal compromise on cost effectiveness. This recipe was finally evaluated for its efficacy in the improvement of gastrointestinal symptoms.

Patients were assessed for the presence and severity of gastrointestinal symptoms before and after treatment with GFF (Table IV). It was observed that abdominal pain, distension, increased stools, loose stools and urgency of some intensity were present in more than half of the patients before treatment. Presence and intensity of gastrointestinal symptoms reduced after the introduction of GFF. It is a well established fact that a variety of gastrointestinal symptoms are associated with celiac patients and it has been reported that in approximately 50% of the celiac adults, diarrhea was the major symptom which led to disease diagnosis (Murray *et al.*, 2004). Major symptoms reported in various studies included stomach pain, gas and bloating, diarrhea, weight loss, anemia, edema, bone or joint pain (Holtmeier and Caspary, 2006). In the present study, out of a total of 50 patients, abdominal pain was persisting in 30 patients before treatment. After treatment, 18 patients still complained of abdominal pain but the intensity reduced predominantly to the mild category. Murray *et al*. (2004) reported a high incidence of abdominal pain in some patients. They attributed it to maldigestion as well as high prevalence of *H. pylori*. The pain was settled in most patients after starting GFF. Tursi *et al.* (2003) postulated that small intestinal bacterial overgrowth could result in continuing gastrointestinal symptoms even after omission of gluten from their diet. Although *H. pylori* was not ruled out in the patients of the present study, the continuing abdominal pain in at least some of the patients may be suspected due to the presence of *H. pylori*. Most of the patients were slum dwellers and residing in over populated unhygienic suburbs of Lahore, where its incidence has been reported to be high (Khan *et al.*, 2012).

Anderson (2014) reported that people with celiac disease often complained of constipation and heartburn rather than diarrhea. In the present study, heartburn and regurgitation was experienced by 34% and 26% of the patients, respectively, before treatment. After the GFF trial the number of patients with these complaints reduced. The presence of these symptoms in celiac patients has been reported by Nachman *et al.* (2011) and Boettcher and Crowe (2013). They had also suggested that a gluten free diet could result in the improvement of

Table VI.- Correlation between wheat intake (g) and gastrointestinal symptoms before treatment.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. Wheat	-																	
2. Abdominal pain	.47**	-																
3. Heart burn	.43**	.46**	-															
4. Regurgitation	.30*	.43**	.69**	-														
5. Sucking sensation	.36*	.73**	.40**	.52**	-													
6. Nausea / vomiting	.50**	.57**	.42**	.59**	.65**	-												
7. Borborygums	.48**	.35*	.55**	.54**	.24	.34*	-											
8. Distention	.50**	.51**	.18	.24	.42**	.32*	.43**	-										
9. Eructation	.43**	.27	.35*	.59**	.26	.49**	.42**	.46**	-									
10. Increased flatus	.31*	.28*	.16	.24	.06	.16	.43**	.55**	.46**	-								
11. Decreased stools	-.10	-.09	.26	-.11	-.13	-.12	-.10	-.13	-.08	-.16	-							
12. Increased stools	.65**	.58**	.21	.30*	.51**	.55**	.44**	.68**	.40**	.41**	-.18	-						
13. Loose stools	.62**	.52**	.14	.18	.54**	.48**	.41**	.64**	.35*	.38**	-.17	.87**	-					
14. Hard stools	.25	.13	.27	.03	.10	.23	-.15	-.05	-.14	-.30*	.42**	-.06	-.11	-				
15. Urgency	.23	.19	-.04	.16	.20	.18	.35*	.62**	.49**	.48**	-.10	.43**	.53**	-.20	-			
16. Incomplete evacuation	.42**	.22	.22	.34*	.35*	.49**	.33*	.49**	.46**	.25	-.14	.46**	.42**	.10	.33*	-		
17. Steatorrhea	.42**	.39**	.23	.43**	.40**	.38**	.43**	.61**	.47**	.33*	-.16	.61**	.58**	-.10	.50**	.38**	-	.
18. No of stools per day	.59**	.62**	.34*	.44**	.57**	.60**	.46**	.67**	.51**	.46**	-.10	.90**	.83**	-.03	.50**	.57**	.66**	-

** p< 0.01, * p< 0.05

reflux symptoms which is indicative of intestinal recovery.

Sucking pain in the stomach was experienced by 16 patients before treatment and by 4 patients after treatment. Nausea and vomiting was reduced to half after introduction of GFF. Previous studies have reported that young celiac patients were more commonly presented with vomiting (Murray *et al.*, 2004). The present study, however, revealed that among patients with ongoing treatment, nausea and vomiting was more common in patients of 31-50 years.

Improvement was evident for the symptoms of borborygmus and abdominal distension. However, even after treatment some degree of distension was persistent, although the severity reduced tremendously. Eructation and increased flatus was also reduced in intensity in most patients. Almost half of the study sample experienced increased frequency of stools and loose stools before treatment. After treatment marked reduction in their frequency and intensity was also observed. Persistence of diarrhea in some celiac patients on a gluten free diet has been observed and attributed to poor compliance and other conditions including microscopic colitis, exocrine pancreatic disorder, dietary lactose or fructose malabsorption, fecal incontinence due to compromised function of the anal sphincter and irritable bowel syndrome (Fine *et al.*, 1997; Rubio-Tapia *et al.*, 2013).

More than 50% of the patients experienced urgency in stool elimination and 36% complained of incomplete evacuation. Signs of steatorrhea were also reported by 46% of patients before treatment. Frequency of these symptoms and their severity was lessened by the use of GFF.

It was thus concluded that the frequency of patients with varying intensities of gastrointestinal symptoms was reduced significantly (p<0.05) in the case of abdominal pain, heartburn, sucking sensation, nausea and vomiting, distension, increased stools, loose stools, hard stools, urgency and steatorrhea. When different age groups were compared for the presence of gastrointestinal symptoms (Table V), it was found that all the gastrointestinal symptoms were more prevalent in the patients aging 1-8 and 19-50 years. They were least in the patients of age 14 to 18 years. Significant (p<0.05) reduction in gastrointestinal symptoms occurred in the age group of 1-13 and of 31-50 years with GFF use. Maximum flour consumption has also been reported to be by the study participants of 9-13 years (Imran *et al.*, 2015).

Celiac women have been rated significantly higher on the gastrointestinal symptom rating scale as compared to the controls even when on a gluten free diet for several years (Midhagen and Hallert, 2003). When compared

according to gender total pre treatment gastrointestinal score of males was slightly higher than females. After treatment, males show greater reduction in symptoms compared to females (Table V).

In the landmark prevalence study on celiac disease, investigators found that 60% of children and 41% of adults diagnosed had no obvious symptoms (Anonymous, 2005). In the present study also, many patients did not experience gastrointestinal symptoms. This was attributed to good compliance as all of them had some sort gastrointestinal symptom which led to their diagnosis. It could also be concluded that patients without gastrointestinal symptoms still remained largely undiagnosed in Pakistan. From Pearson correlation results, it was evident that most of the gastrointestinal symptoms were significantly positively correlated (p<0.05) to wheat intake (Table VI). Improvement in gastrointestinal symptoms has been considered an indicator of histological recovery, however constant monitoring for adherence and repeated serological testing has been recommended by various investigators as this improvement did not suggest absolute histological recovery (Boettcher and Crowe, 2013; Anderson, 2014).

CONCLUSION

Combination of rice, corn and mung bean flour turned out to be the best in terms of nutritional and sensory characteristics. Feeding of the selected recipe resulted in a significant (p<0.05) reduction in gastrointestinal symptom score in both genders especially of age 1-13 and 31 -50 years.

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Conflict of interest statement

Authors have declared no conflict of interest.

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