

## Research Paper

# Development of gluten free composite flour for children with attention deficit hyperactivity disorder

A. Thahira Banu and Sindhuja P.

Department of Home Science, Gandhigram Rural, University, Tamilnadu, India.

\*Corresponding author E-mail: [thaaze@gmail.com](mailto:thaaze@gmail.com)

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Objective of this study was to develop gluten free composite flour suitable for children with ADHD children. The multigrain namely: brown rice, ragi, sorghum, maize, lentils, chickpeas and Italian millet were used for development of the composite flour. Three variations with different proportion of maida and multigrain flour was taken [ $V_1$  (75:25) and  $V_2$  (50:50)] and  $V_3$  (100% gluten free flour + 1.7g of xanthan gum) for understanding the suitability. The modified flour was evaluated for their physicochemical properties, proximate nutrients, antioxidant activity, shelf life and structure of starch granules. The results of the study revealed that the moisture content was low in  $V_1$  variation (6.6 %). Protein, fat, minerals, vitamins, water absorption and foam stability

was increased in  $V_3$ . Amino acids such as cysteine, tyrosine, phenyl alanine and glycine were present in  $V_3$ . Scanning Electron Microscopy (SEM) analysis of the modified gluten free composite flour showed formation of an embedding material and fibril formation because of xanthan gum. The EDAX measurement showed the presence of elements like calcium and magnesium. Antioxidant activity of modified gluten free composite flour was 89.88 % in aqueous extract. The Shelf-life of the flour was one month.

**Key words:** Gluten-Free Composite Flour, Millets, ADHD, Xanthan gum

## INTRODUCTION

Gluten is a protein found in cereals. In some people, eating or drinking anything containing gluten can cause different types of undesirable reactions. Gluten - free diet can help the human body ward off viruses and germs, just because many of the foods you eat will be full of antioxidants and essential vitamins and minerals (Feillet and Dexter, 1996). Attention deficit hyperactivity disorder (ADHD) is a disorder affecting many children. This disorder appears more in boys than girls. ADHD is a behavioural disorder manifested in childhood with symptoms that may persist in adulthood. As the name implies, individuals with ADHD struggle to pay attention to activities and have poor impulse control and hyperactivity (Research\*Eu, 2015).

In the US study, it was found that children with ADHD had significantly lower brain iron levels than children without ADHD. The Italian study investigated the possible consequences of brain iron deficiency. More specifically, it focused on the association of brain iron levels with brain functioning and sleep quality/quantity, using magnetic resonance imaging, electroencephalograms, somatosensory evoked potentials and polysomnography. Significant correlation was found between brain iron levels and the number of periodic movements in sleep. This suggests that low brain iron levels can disrupt sleep, which, in turn, contributes to cognitive deficits and further aggravates ADHD symptoms. Of note, no differences were found in

serum ferritin levels in children with and without ADHD in both studies. ADHD affects around 5 % of children globally and study results suggest that about 15 % of such children have brain iron deficiency. Alleviation of ADHD symptoms through early iron supplementation has the potential to benefit this subset of children and vastly improve their quality of life (Research\*EU, 2015).

In typical functioning of gastrointestinal tract the enzymatic activity breaks protein into peptides and transforms peptides into amino acids. The intestinal lining then absorbs the amino acids into the blood stream. This carries the amino acids to the rest of the body providing nutrition. The opioid excess theory, allergies in ADHD can result from disruptions to this process. According to the theory some individuals suffer from inadequate production of gluten related digestive enzymes and increased gut permeability adequate levels of digestive enzymes, peptides derived from gluten fail to become amino acids in large numbers (Austin *et al.*, 2009). Increased observance to a gluten-free diet remains effective treatment for celiac disease throughout the patients' lifetime (Murray, 1999). An observance to a gluten-free diet remains effective treatment for celiac disease throughout the patients' lifetime (Murray, 1999). Gluten free diet has benefits such as the recovery of the villi of the small intestine and reduced risk of malignant complications (Seraphin and Mobarhan, 2002). Since the diet of celiac patients must be completely free of any gluten, so all the products from wheat, rye, barley and oat must be replaced with corn, rice, millet equivalents and various types of starch (corn, rice and potato) or appropriate mixtures (Lazaridou *et al.*, 2007 and Hegazy *et al.*, 2009).

The study was an attempt to develop a gluten free modified flour that can be used for preparation of various food items that can be relished by children with ADHD and gluten sensitivity.

## MATERIALS AND METHODS

### Sample collection and flour preparation

All grains, which were used in this study passed through winnowing and hand sorting in order to remove stones, dust materials, glumes, stalks, and broken, undersized and immature grains.

Grains namely brown rice (20 gm), Italian millet (20 gm), sorghum (20 gm) corn (15 gm), lentils (10 gm), ragi (5 gm), chickpeas, and xanthan gum were used for the improvement of gluten free composite flour and the raw ingredients used were brought from the local market in Dindigul district Tamilnadu India.

The selected raw ingredient were cleaned and washed, soaked in water for 3 h and sun-dried. Roasted and the grains were milled through a grinder (Model, the manufacturer, Country origin the (Butterfly mixer grinder

India). The flour was taken in different mixtures. The three mixtures formulated were coded as V<sub>1</sub>, V<sub>2</sub> and V<sub>3</sub>. mixtures V<sub>1</sub> had maida and gluten free composite flour in the ratio of 75:25 and V<sub>2</sub> with Maida and gluten free composite flour in the ratio of 50:50, whereas the third mixtures V<sub>3</sub> had 100 % gluten free composite flour mixed with 1.7 g of xanthan gum/100g of composite flour. The amount of xanthan gum required was standardized and found that the optimum amount was 1.7 gm of xanthan gum per 100 gm of the flour.

### Nutrient composition of the modified flour

Proximate compositions like moisture content, crude protein, crude fat, crude fiber and total ash were analyzed using standard method (AOAC, 2000). The carbohydrate contents were determined by differences. The carbohydrate contents were determined with the method described by Dubois *et al.* (1956). Minerals namely: calcium, zinc was determined using standard method (AOAC, 1990) and iron (Raghuramulu *et al.*, 2003). Vitamins namely:  $\beta$ -carotene (AOAC, 2000) and vitamin C (Sadsivam and Manikam, 1987) were determined. Qualitative amino acids test were carried out to find the type of amino acids present in the developed gluten free composite flour (Sadsivam and Manikam, 1987).

### Estimation of antioxidant activity of modified gluten free composite flour

The determination of antioxidant activity of developed gluten free composite flour was done through the 1,1 Diphenyl- $\beta$  picrylhydrazyl (DPPH) radical scavenging method described by Braca *et al.* (2001).

### Physicochemical properties of modified gluten free composite flour

#### Water absorption capacity

The determination of water absorption capacity was carried out according to the method as described by Sosulski *et al.* (1976). After mixing 20 ml distilled water with 1 g gluten free flour, the contents were allowed to rest at 30°C for 30 min and then centrifuge at 3000 rpm for 15 min and finally the water absorption capacity of the flour was expressed as grams of water absorption by 1 g of gluten free composite flour.

#### Swelling power

The determination of swelling power was carried out

according to the method described by Leach *et al.* (1959). After mixing 15 ml distilled water with 0.5 g gluten free flour, the contents were allowed to heat at 90°C for 30 min with vigorous shaking every 5 min and then centrifuge at 3000 rpm for 15 min and finally the swelling power of the flour was expressed as grams of water absorption by 0.5 g of gluten free composite flour.

### **Foam capacity and foam stability**

The method of Narayana and Narasinga Rao, (1982) was used to determine the foam capacity and stability of developed gluten free composite flour. 50 ml water was taken in a cylinder, into which 2 g of flour samples were mixed keeping the temperature at 30±2°C. The suspension was properly shaken to foam and the volume of the foam after 30 sec was recorded in ml as foam capacity while the foam volume (ml) recorded after 1 h of whipping was recorded as foam stability.

### **Bulk density**

It was determined according to the method of Okaka and Potter, (1977). 50 g of developed gluten free composite flour was taken into a 100 ml volumetric cylinder. The cylinder was tapped several times on a laboratory bench to attain a constant volume. The bulk density (g/cm<sup>3</sup>) was then calculated as weight of developed gluten free composite flour divided by flour volume (cm<sup>3</sup>).

### **Morphology of granules of the modified gluten free composite flour**

The morphology of developed gluten free composite flour granules was evaluated by scanning electron microscope (SEM) (QUANTA FEG 250 ESEM Bruker Nano, Germany). Samples were mounted on circular aluminum stubs with double-sided sticky tape. The starch granules were distributed on the surface of the tap. The samples were then coated with 12 nm gold, examined and photographed at an accelerating voltage of 5 kv with a magnification of \* 1000 and \* 5000.

### **Shelf life analysis of the modified gluten free composite flours**

Assessment of microbial count in the gluten free composite flours was done to find out the total bacterial count on the first day and 60<sup>th</sup> day of storing the flour in normal room temperature in high density package and air tight container. The serial dilution pour plate method was done

by Goff *et al.* (2003).

### **Cost of the modified gluten free flour**

The cost of the best accepted variation of gluten free composite flour was calculated taking into account the cost of ingredients used, processing and packaging statistical measure of mean was used. A gluten free diet (GFD) is a diet that excludes gluten, protein composite present in rye, barley, wheat, and all their kinds and hybrids like spelt, and triticale. The addition of oats in gluten free diet is debatable. Venin found in oats can be also harmful for celiac people. Its toxicity is based on the cultivar eaten. Also, oats is usually cross contaminated with gluten-containing cereals.

### **Proximate composition of modified gluten free composite flour**

The results of the Proximate nutrient, vitamin and mineral content of the modified gluten free composite flour of the developed gluten free flour are presented in the (Table 1). The carbohydrate content of V<sub>1</sub> (69.4 g) was higher than V<sub>2</sub> (65.1 g) and V<sub>3</sub> (56.3 g). The protein content of the V<sub>3</sub> (11.302 %) was higher than V<sub>1</sub> (11.05 %) and V<sub>2</sub> (11.15%). Among the blends of flour V<sub>3</sub> had higher amount of iron (3.5 mg), calcium (45.75 mg) and zinc (2.25 mg) than the other two blends. The β-carotene and vitamin C contents found to be 77.3 µg and 0.15 mg, respectively in V<sub>3</sub> variation, which is also higher than the other two blends. Vitamin content was also comparatively higher in V<sub>3</sub> variation than the other two. This may be due to the presence of different grains that has improved the nutrient content of V<sub>3</sub> variation.

### **Amino acid composition of the modified gluten free composite flour**

The amino acid screening was done to understand the presence of essential amino acids. The results of the qualitative analysis of amino acid present in the composite flour are (Table 2).

Amino acids such as tyrosine and phenyl alanine were present in the V<sub>3</sub>. It is found that cysteine, cystine, tyrosine, phenyl alanine and glycine are present in the developed gluten free composite flour. V<sub>3</sub> gluten free composite flour had the essential amino acid phenylalanine and tyrosine, which is a very beneficial amino acid for ADHD children. It is also found that sulphur containing amino acid cysteine and cystine are present.

Thus, the developed composite flour contains essential amino acids. The presence of amino acids may be due to the addition of pulses and millets.

Table 1. Proximate nutrient, vitamin and mineral content of the modified gluten free composite flour

Nutrients / 100g	V <sub>1</sub>	V <sub>2</sub>	V <sub>3</sub>
Carbohydrate (g)	69.4±0.2	65.1±0.2	56.31±0.02
Protein (g)	11.05±0.01	11.15±0.02	11.30±0.001
Fat (g)	1.03±0.002	1.43±0.02	1.96±0.002
Crude fiber (g)	1.05±0.002	1.81±0.02	3.32±0.002
Iron (mg)	2.90±0.002	3.11±0.02	3.53±0.02
Calcium (mg)	28.6±0.2	34.3±0.2	45.75±0.02
Zinc (mg)	12323	1.57±0.001	2.25±0.002
β carotene (μg)	31.52±0.02	51.1±0.2	77.3±0.2
Vitamin C (mg)	<1	<1	<1

Table 2. Functional properties of the modified gluten free composite flour.

Properties	V <sub>1</sub>	V <sub>2</sub>	V <sub>3</sub>
Moisture (%)	7.69±0.01	6.6±0.02	6.9±0.2
Bulk density	0.16±0.015	0.29±0.002	0.22±0.02
Water absorption index (g/g)	1.81±0.02	1.83±0.015	2.35±0.02
Swelling power (g/cm <sup>3</sup> )	5.61±0.002	7.93±0.001	5.46±0.001
Foam capacity (%)	0.63±0.002	0.24±0.015	0.63±0.001
Foam stability (%)	100±0.001	99±0.001	100.36±0.01

### Antioxidant activity of modified gluten free composite flour

Table 1 showed the DPPH-radical scavenging activity of composite flour. The results indicate a positive DPPH radical scavenging activity for composite flour in aqueous extract with 89.88 percent and in ethanolic extract, it was found to have only 68.22 percent. The highest activity was detected in the aqueous extraction (89.88 percent).

### Physicochemical properties of the modified gluten free composite flour

The physicochemical properties of the developed composite flour were determined and the results are presented in (Table 2). The bulk density of a good material is important in relation to the packaging. As shown in the (Table 2), the bulk density was lowest (0.16 g/cm<sup>3</sup>) in V<sub>2</sub> and highest (0.285 g/cm<sup>3</sup>) in V<sub>1</sub>. Increase in moisture content may increase the bulk density of the flour. It is found that as the proportion of grains to maida decreases, bulk density decreases.

The bulk density of the blends flour varied from 0.285 to 0.16 g/cm<sup>3</sup>. The moisture content was found to have significant effect on bulk density of the flour (Patil, 1990). The highest water absorption capacity (2.35 g/g) was found in V<sub>3</sub>. Water absorption index increases with increase in protein and carbohydrate. Further it is also increases with addition of gums to flour, stickiness also increase water absorption index as shown in Table 2.

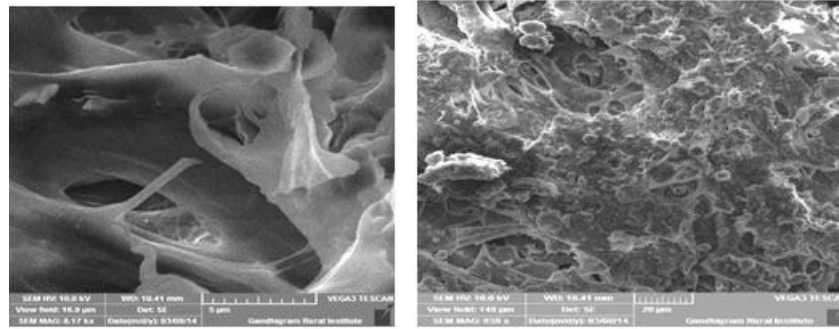
Swelling power of the flour varied from 5.466 – 7.928. As proportion of the composite flour increased the swelling

power decreased. The reasons for flour to have high swelling power may be due to starch gelatinization and degradation during preparation (Kim and Tanhehco, 2005).

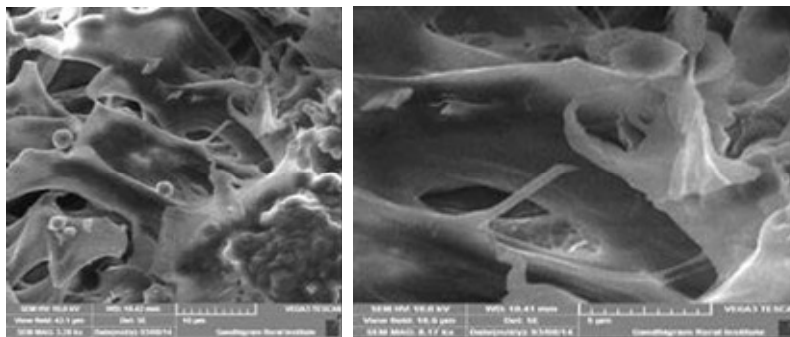
The results of foam capacity and stability of the different blends flour showed that V<sub>3</sub> was found to be higher when compared with other variations. The addition of Xanthan gum in variation (V<sub>3</sub>) would have also attributed to the high water absorption index and foam stability (Gracia *et al.*, 2005). It is reported that adding the xanthan gum improves the flour quality such as enhanced viscosity and improved dough handling (Gracia *et al.*, 2005).

### Morphological properties and particle size distribution of the modified gluten free composite flour

The morphological properties and particle size distribution of the developed gluten free composite flour are presented in the (Figures 1 and 2). From Figure 1a, it is clear that the starch and protein bodies are seen as discrete body. On adding the polysaccharide (xanthan gum) it has formed as an embedding material for the composite flour particles especially for the heat stable starch and protein globules. The image in Figure 1b shows a closer view of the round shape starch molecules and protein structures. Figure 2a and 2b shows the binding of the xanthan gum. This image shows a clear view of the fibril formation which is due the xanthan gum. Further the recently, it has also been demonstrated that addition of emulsifiers together with hydrocolloids into gluten-free formulations is critical, since the complex formed by hydrocolloid, emulsifier, and dough components have an important role in the enhancement of dough handling ability and product quality (Demirkesen *et*



Figures 1a and a. Structure of the flour added with Xanthan gum



Figures 2a and b. The fibril formation in the flour with Xanthan gum

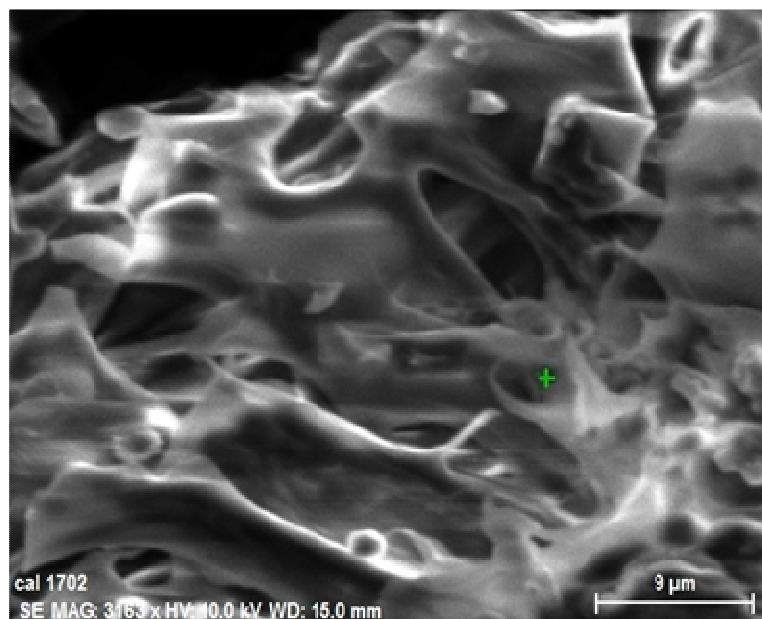


Figure 3. EDAX measurement of V3.

*al.*, 2010, Dewettinck and Depypere 2011). Therefore, in the presence of xanthan–guar gum high quality baked products are possible. EDAX measurement shows the element like

calcium and magnesium, which is essential for ADHD children (Newman *et al.*, 1996). The results of total bacteria count in the developed gluten free composite flour is show

**Table 3.**Total bacterial count of the modified gluten free composite flour.

Variations of the composite flour	TBC (High Density Package)CFU/gm		TBC( Air Tight Package)CFU/gm	
	1 <sup>st</sup> day	60 <sup>th</sup> day	1 <sup>st</sup> day	60 <sup>th</sup> day
V <sub>2</sub> (75:25)	Nil	To high	Nil	264x10 <sup>5</sup>
V <sub>1</sub> (50:50)	Nil	To high	Nil	122x10 <sup>5</sup>
V <sub>3</sub> (100% composite flour + 1.7g of Xanthan gum)	Nil	276x10 <sup>1</sup>	Nil	To high

in (Table 3). The shelf life for the prepared flour is about one month abundant bacterial growth was absorbed in flour which has

been prepared before two months. Thus to get best results, frequent purchase of the flour is necessary to avoid any health disorders.

### Cost analysis of the modified gluten free composite flour

The overall acceptability and best rated gluten free composite flour was V<sub>3</sub> blend. The total cost for producing 500 g of composite flour was found to be Rs. 44.26/or 0.737USD. The cost is slightly more than the commercially available flour. Since the product is a value added, commercialization will not be a problem due to changing trend in eating pattern of the consumer. The common supplements available in the market for ADHD and gluten sensitive individuals are very costly hence the developed flour is found to be very cost effective.

### Conclusion

Results from the study show that modified gluten free composite flour has a lot of potential benefits. The prepared composite flour can be cooked into different dishes like extruded products, bread, biscuit and steamed dishes thus the developed gluten free composite flour is very useful for individual suffering with ADHD and celiac disease.

### Authors` declaration

We declare that this study is an original research by our research team and we agree to publish it in the Journal.

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