

# Nutritious Biscuits for Celiac Patients: Effect of Different Cereals and Legumes Blends

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## ABSTRACT

This study was aimed to prepare and evaluate some biscuit products as gluten free products (GFPs) for Celiac disease (CD) patient. Four biscuits including control and three treatments according to raw material content have been prepared. The control (100% wheat flour), B<sub>1</sub> (45% yellow corn flour, 35% faba bean flour and 20% corn starch), B<sub>2</sub> (45% broken rice flour, 35% chickpea flour and 20% corn starch) and B<sub>3</sub> (45% sorghum flour, 35% lima bean flour and 20% corn starch). Chemical composition, mineral content, anti-nutritional factors in addition to sensory properties were evaluated. The results revealed that crude protein contents of different products were 8.29, 9.62, 7.52 and 8.18 g/100g for control, products B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub>, respectively. Potassium was the predominant minerals especially in product (B<sub>2</sub>), which contained the highest contents of potassium (353.47 mg/100g) and sodium (32.71 mg/100g). Phytic acid was ranged from 17.53 to 100.53mg/100g and tannins were ranged from 5.04 to 10.90 mg/100g. Germination, boiling, drying of raw materials and backing process caused increased of protein digestibility, eliminated of anti-nutritional factors and enhanced sensory and nutritional quality. According to sensory evaluation all GFB products were improved significantly compared to control product.

**Keywords:** Gluten free products, Pretreatment of cereals and legumes, Biscuits, Chemical composition, Sorghum, Broken rice, Yellow corn, Chickpea, Faba bean and lima bean.

## INTRODUCTION

Celiac disease is defined as an immune-mediated enteropathy induced by exposure to dietary gluten which is defined as the material that can be separated from wheat flour when starch, and other minor components, are removed by washing under running water (Cann and Day, 2013). Gliadin is the causative agent in wheat while other prolamins are the causative agents in rye (secalin), oat (avenin), barley (hordein) maize (zeins), rice (oryzins), and (kafirins) of millet and sorghum (Badiu *et al.*, 2014). CD, the body's immune system responds abnormally to gluten, resulting in inflammation of the intestinal villi caused by antibodies that fight against the presence of glutamine and the subsequent enzyme transglutaminase in the small intestine (Badiu *et*

*al.*, 2014). With the continuous inflammation of intestinal mucosa and villous atrophy, it causes of damage to the lining of the small intestine, and reduced absorption of iron, calcium, vitamins A, D, E, K, and folate (Rosell and Garzon, 2015). The mainstay of treatment for CD is a strict lifelong adherence to a GFD (Lee *et al.*, 2007). Gluten plays a principal role in backed development by giving cohesiveness and promoting the retention of the CO<sub>2</sub> produced during fermentation. Thus, gas expansion causes breads to gain volume and attain acceptable crumb texture (Deora *et al.*, 2014). Because it contains two major protein fractions: glutenin (a rough rubbery mass when fully hydrated) and gliadin (a viscous fluid mass) Many of the commercial GFPs are made from one or more ingredients which do not contain gluten such as some legumes (faba bean, chickpea and lima bean), and some cereals (rice, corn and sorghum) (Giménez *et al.*, 2013).

Fabian and Ju (2011) reported that rice flour is recommended for use in GFPs processing instead of wheat flour because it possesses no gluten. While Masure *et al.* (2016) showed that the most commonly to obtain an acceptable GFP are yellow corn flour after rice flour. Also Ciacci *et al.* (2007) noticed that sorghum is the fifth largest crop produced worldwide and has been shown to be safe for people with CD. Giuberti *et al.* (2015) noticed that faba bean flour enhance nutritional properties of gluten free products. Where Polesi *et al.* (2011) postulated that chickpea starch presents a similar amount of amylose as wheat, and were already used as an ingredient in GFP. Cai *et al.* (2003) mentioned that lima bean able to enhance the nutritional properties of GFP (Campos *et al.*, 2010).

This study aimed to prepare and evaluate biscuit products as GFPs for CD patient. Four biscuits included control and three treatments according to raw material contents have been prepared. These products were prepared based on the local available pretreated ingredients such as cereals (yellow corn flour – broken rice flour – sorghum flour) and legumes (chickpeas flour – faba bean flour – lima bean flour). Chemical composition, mineral content, anti-nutritional factors in

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addition to sensory properties were evaluated in the products to achieve the main goal of this study.

## MATERIALS AND METHODS

### Materials:

Yellow corn (*Zea mays*, L.) varieties Giza 162, sorghum (*Sorghum bicolor*, L.) varieties Giza 15, chickpea (*Cicer arietinum*, L) variety Giza 131, lima bean (*Phaseolus lunatus* L.) varieties Giza 6 and faba bean (*Vicia faba*, L.) varieties Giza 461 were obtained from Field Crops Research Institute, Agricultural Research Center Giza– Egypt. While broken rice was obtained from rice polishing company, Beheira governorate, Egypt.

### Methods:

#### Preparation of raw materials

Yellow corn, sorghum and broken rice grains were cleaned physically to eliminate dust particles, seeds of other crops and other contaminants like as weeds and metals, and then milled in the laboratory mill (Brabender Duisburg, Germany). Chickpeas, faba bean and lima bean were cleaned from the impurities, soaked for 12 hr. and germinated for 3 day at (25°C) according to the method of (Sidky *et al.*, 1991). After germination procedure taking place germinated chickpeas, faba bean and lima bean were cooked separately in boiling water for 15 min, and they dehulled by hand and drying in oven at (40°C) for 24 hr. then milled in a laboratory mill (Brabender Duisburg, Germany). All dried samples were passed through 60 mesh screen and stored in polyethylene bags in deep freezer at -20°C until used (El-Adawy, 2002)

#### Preparation of formulas:

Four formulas were prepared using the previous ingredients. These ingredients and their amounts are illustrated in Table (1).

#### Biscuit baking procedure

The biscuits dough was prepared as follow: 35% powdered sugar, 2% dry egg and 35% shortening were mixed for 2 min at speed four using a mixer (model SM-

5D, China Sinma Machine). The dry ingredients (flour as in Table 1, baking powder 3%, salt 1% and vanillin 1%) were weighed and mixed with 10% milk for 3 min at speed 3 to get biscuit dough. Then, it was sheeted to a thickness of about 5 mm; the sheeted dough was cut into square shape using a 35 mm diameter. The biscuits dough was baked at 180 °C for 12-15 min. Biscuits were kept at room temperature for 8-10 minutes after baking before sensory evaluation, Obeidat *et al.* (2012). The rest of biscuits were kept in polyethylene bags at refrigerator (4°C) until physical and chemical analyses.

#### Chemical analysis:

Moisture, crude protein, crude fat, ash and crude fiber were determined according to the official methods of AOAC (2005). Total carbohydrates were calculated by difference. *In vitro* digestibility of protein was determined by successive pepsin trypsin enzyme system according to method of Chavan *et al.* (2001). Minerals were determined using Perkin Elmer (Model 3300, USA) Atomic Absorption Spectrophotometer according to AOAC (2005). Trypsin inhibitor activity was assayed by the method of Gatta *et al.* (1988). Phytate was extracted and determined according to the procedure described by Camire and Clydesdale (1982) as modified by Mohamed *et al.* (1986). Tannins were determined using vanillin hydrochloric acid method as described by Burn (1971).

#### Organoleptic evaluation of biscuit products:

Sensory evaluations of biscuits were carried out by panel of 15 experienced guides from the staff of the Food Tech. Res. Institute, Agric., Res. Center, Giza, Egypt. Assigning scores for various quality attributes such as appearance, color, flavor, texture, taste and overall acceptability were done using 9-point hedonic scale(1= disliked extremely, to 9 = like extremely) Chinma *et al.* (2012).

#### Statistical analysis

Statistical analysis was carried out according to Fisher (1970). (LSD) Least significant difference test was used to compare the significant differences between means of treatments (Waller and Duncan, 1969).

**Table 1. Main ingredients of biscuit formulas**

Ingredients	Control formula	Formula.(B <sub>1</sub> )	Formula.(B <sub>2</sub> )	Formula.(B <sub>3</sub> )
	%	%	%	%
Wheat	100	---	---	---
Yellow corn	---	45	---	---
Sorghum	---	---	---	45
Broken rice	---	---	45	---
Faba bean	---	35	---	---
Chickpea	---	---	35	---
Lima bean	---	---	---	35
Maize starch	---	20	20	20

## RESULTS AND DISCUSSION

### Proximate chemical composition and protein digestibility of biscuit products

Biscuit products were analyzed for determining proximate composition and the data were presented in Table (2). The results were significantly different. The result showed that the moisture content was between 5.30 - 6.40 %. Control product contained the highest content of carbohydrate compared with all other products, it was in the second order of protein digestibility, but it had the lowest content of crude fat (12.18 %) and ash content (0.79 %). Product B<sub>1</sub> recorded considerable amounts of crude fat (14.64%), crude fiber (2.06 %) and crude protein (9.62%), but it had the lowest values of the total carbohydrates (66.99%), moisture (5.30%) and protein digestibility (79.04). Whereas, product B<sub>2</sub> contained the highest value of protein digestibility (86.35%) due to its containing of rice flour, but it had the lowest contents of crude protein (7.52%) and crude fiber (1.25%). Product B<sub>3</sub> contained the highest contents of moisture and ash, it characterized by the high content of fat (14.50%). Adebayo and Okoli (2017) found that the addition of lima flour to cereals has enhanced the nutritional composition of the biscuits. These results were agreed with Sharoba *et al.* (2014) who reported that in biscuit products crude protein content was ranged from 8.00 to 10.35%, crude fat ranged from 8.00 to 11.00%, ash content ranged from 1.50 to 4.20% and crude fiber ranged from 0.70 to 2.92%.

From the same Table 2 it could be noticed that crude protein content ranged from 7.52% to 9.62%, carbohydrates content ranged from 66.99% to 70.96%, crude fat content ranged from 12.18% to 14.64 %, crude fiber content ranged from 1.25% to 2.06% and ash content ranged from 0.79% to 1.73%. These results were similar to the biscuit composition presented by Sakac *et al.* (2015) and Mohammad (2017) who conducted a study on production of high nutritional

value biscuit from broken rice supplemented with some legumes flour and found that the chemical composition of its biscuit product was 6.49% for moisture, 8.85% for protein, 26.3% for fat, 0.5% for ash and 0.48% for fiber.

### Mineral contents of biscuit products

Biscuit products were analyzed to determine mineral contents and the data are presented in Table (3). The results were significantly different. Data revealed that all products had higher content of macro elements than control product. Kupper (2005) reported that there was a risk of nutritional deficiencies in the group of people with celiac disease that were following gluten-free diet. The risk was applied mainly to insufficient intake of calcium, iron, zinc and selenium. Potassium was the main element in the all biscuit products, and it was the predominant minerals especially in product B<sub>2</sub>, which contained the highest contents of potassium (353.47 mg/100g) and sodium (32.71 mg/100g). Control product characterized by the highest content of zinc (1.90 mg/100g), but it had the lowest content of all macro elements. While product B<sub>1</sub> contained high content of macro elements, but it had the lowest content of iron (1.84 mg/100g). Product B<sub>3</sub> recorded the highest content of calcium, magnesium and iron (81.73mg/100g), (103.30mg/100g) and (2.95mg/100g), respectively, but it had the lowest content of zinc (1.20 mg/100g). The noticed increment of important minerals in biscuit products was as result of mixing some cereals and some legumes. These results were in agreement with Priscila *et al.* (2015) they showed that the minerals content of biscuit which made from legume blended with rice flour had 3.31 mg/100gm iron and 1.78 mg/100gm zinc.

From the same Table it could be observed that sodium content ranged from 7.92 to 32.71 mg/100g, potassium content ranged from 210.22 to 353.47 mg/100g, calcium content ranged from 22.71 to 81.73 mg/100g, magnesium content ranged from 53.85 to 103.30 mg/100g, iron content ranged from 1.84 to 2.95 mg/100g and zinc ranged from 1.20 to 1.90 mg/100g.

**Table 2. Proximate chemical composition and protein digestibility of biscuit products (% as wet basis)\***

Parameters	control	B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>	LSD 0.05
Moisture	5.92±0.16 <sup>ab</sup>	5.30±0.23 <sup>b</sup>	6.29±0.21 <sup>a</sup>	6.40±0.23 <sup>a</sup>	0.70
Crude protein	8.29±0.02 <sup>b</sup>	9.62±0.03 <sup>a</sup>	7.52±0.07 <sup>c</sup>	8.18±0.02 <sup>b</sup>	0.14
Crude fat	12.18±0.44 <sup>b</sup>	14.64±0.35 <sup>a</sup>	12.99±0.08 <sup>b</sup>	14.50±0.62 <sup>a</sup>	1.38
Ash	0.79±0.03 <sup>d</sup>	1.39±0.04 <sup>b</sup>	1.09±0.06 <sup>c</sup>	1.73±0.05 <sup>a</sup>	0.16
Crude fiber	1.86±0.08 <sup>a</sup>	2.06±0.09 <sup>a</sup>	1.25±0.07 <sup>c</sup>	1.54±0.04 <sup>b</sup>	0.24
Carbohydrate (by difference)	70.96	66.99	70.86	67.65	
Protein digestibility	84.19±0.65 <sup>b</sup>	79.04±0.25 <sup>c</sup>	86.35±0.53 <sup>a</sup>	83.33±0.52 <sup>b</sup>	1.67

\*Means in a column not sharing the same superscript are significantly different at p<0.050.

**Table 3. Mineral contents of different biscuit products (mg/100g as wet basis)\***

Minerals	Control	B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>	LSD 0.05
Sodium	7.92±0.43 <sup>d</sup>	18.71±0.24 <sup>c</sup>	32.71±2.09 <sup>a</sup>	27.97±1.42 <sup>b</sup>	4.21
Potassium	210.22 ±8.30 <sup>c</sup>	320.28±14.08 <sup>ab</sup>	353.47±9.05 <sup>a</sup>	314.78±12.51 <sup>b</sup>	36.68
Calcium	22.71±0.98 <sup>d</sup>	71.43±2.02 <sup>b</sup>	59.77±1.56 <sup>c</sup>	81.73±1.51 <sup>a</sup>	4.96
Magnesium	53.85±2.65 <sup>c</sup>	77.53±3.41 <sup>b</sup>	66.70±4.06 <sup>b</sup>	103.30±3.37 <sup>a</sup>	11.13
Iron	2.39±0.20 <sup>b</sup>	1.84±0.04 <sup>c</sup>	2.42±0.14 <sup>b</sup>	2.95±0.04 <sup>a</sup>	0.42
Zinc	1.90±0.07 <sup>a</sup>	1.36±0.04 <sup>b</sup>	1.80±0.07 <sup>a</sup>	1.20±0.06 <sup>b</sup>	0.21

\*Means in a column not sharing the same superscript are significantly different at p<0.050.

These results were in agreement with Mohammad (2017), who found that the mineral contents of biscuit were iron (1.69 mg/100gm), zinc (1.86 mg/100gm), magnesium (62.50 mg/100gm) and calcium (119 mg/100gm).

#### Anti-nutritional factors of biscuit products

The results of anti-nutritional factors in biscuit products are presented in Table (4). The results showed significant differences between total phytic acid, tannins or trypsin inhibitor contents in resultant different biscuit products. It could be noticed that control product contained the highest amount of total phytic acid (100.53mg/100g) and tannin (10.90mg/100g) compounds, but it was free of trypsin inhibitor, while product B<sub>1</sub> and B<sub>2</sub> contained low amounts of phytic acid and total tannins compared with other products. Tannins are known to present in food legumes that decrease the protein quality of foods and interfere with dietary iron absorption (Tadelel, 2015). Product B<sub>2</sub> contained the highest amount of trypsin inhibitor (0.28 TIU/ mg), but it had the lowest contents of phytic acid (17.53 mg/100g) and tannins (5.04mg/100g). On the other hand, product B<sub>3</sub> was in the second order in phytic acid and tannins contents. These results were agreed with Hawa *et al.* (2018). Phytate content is high in legumes and decreases the bioavailability of essential minerals and bioavailability of proteins by forming insoluble phytate-mineral and phytate protein complexes (Admassu, 2009).

It was clear that germination followed by boiling and drying of raw materials and backing process decreased the anti-nutritional factors, where the reduction ratios were: trypsin inhibitor (81 to 83%), phytic acid (58-68

%) and tannins (72-76%). According to Adeparusi (2001), tannins affect protein digestibility and adversely influence the bioavailability, obtained from plant sources lead to poor iron and calcium absorption.

#### Organoleptic evaluation of biscuit products

The sensory evaluation of biscuit products were performed for appearance, color, taste, texture, flavor and overall acceptability (out of 9). Scores for sensory attributes of biscuit as fresh products are presented in Tables (5). There was significant difference between treatments in the all properties. Taste is the primary factor that determines the acceptability of any product which has the highest impact as far as market success of product is concerned. The results revealed that product B<sub>1</sub> had the highest value of taste followed by control product then product B<sub>2</sub>. Control product had the highest value of overall acceptability followed by products B<sub>1</sub> then B<sub>2</sub>.

Results in Table (5) for sensory evaluation of fresh products showed that control product and products B<sub>1</sub> and B<sub>2</sub> were excellent; while control had the highest overall acceptability (8.06), color, texture and flavor. While product B<sub>1</sub> was in the second order of overall acceptability (7.86), it had the highest value of appearance, taste and texture this may be related to its contents of yellow corn flour. Product B<sub>2</sub> was excellent in color (8.53) this may be related to its contents of rice flour and chickpeas flour. Color is a very important tool in judging properly baked products, which not only reflect the suitable raw materials used for the preparation but also provides information about the formulation and quality of the product (Ojinnaka *et al.*, 2013).

**Table 4. Anti-nutritional factors contents of biscuit products (as wet basis)\***

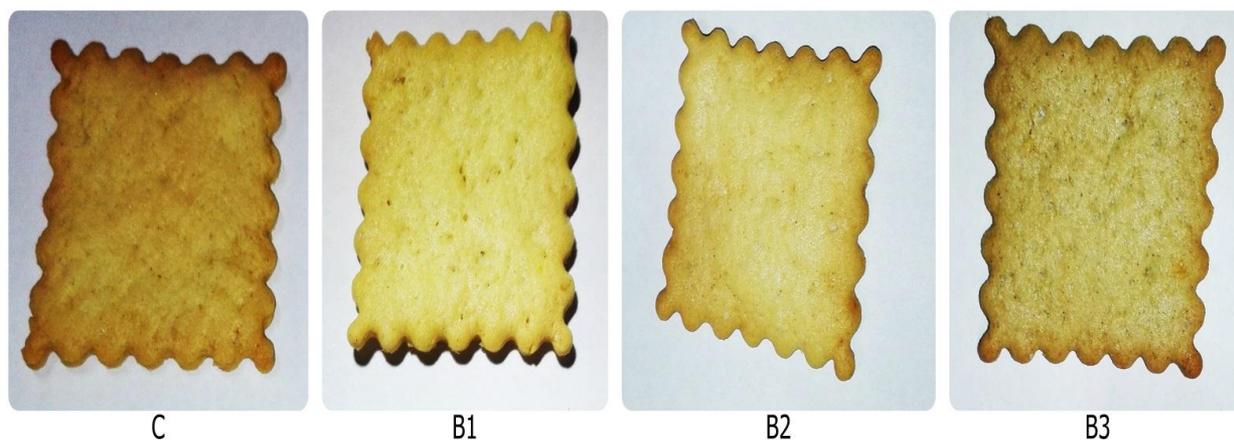
Product	Trypsin inhibitor TIU/mg	Phytic acid (mg/100g)	Tannins (mg/100g)
Control	0.00±0.00 <sup>c</sup>	100.53±5.60 <sup>a</sup>	10.90±0.44 <sup>a</sup>
B <sub>1</sub>	0.13±0.00 <sup>b</sup>	29.86±2.25 <sup>c</sup>	5.46±0.32 <sup>c</sup>
B <sub>2</sub>	0.28±0.01 <sup>a</sup>	17.53±1.00 <sup>c</sup>	5.04±0.11 <sup>c</sup>
B <sub>3</sub>	0.12±0.00 <sup>b</sup>	61.63±6.42 <sup>b</sup>	9.69±0.27 <sup>b</sup>
LSD 0.05	0.03	14.47	1.02

\*Means in a column not sharing the same superscript are significantly different at p<0.050.

**Table 5. Sensory evaluation of the biscuit products**

Properties	Control	B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>	LSD 0.05
Appearance	8.13±0.21 <sup>a</sup>	8.26±0.20 <sup>a</sup>	7.93±0.26 <sup>a</sup>	6.66±0.31 <sup>b</sup>	0.72
color	7.86±0.25 <sup>ab</sup>	7.66±0.28 <sup>b</sup>	8.53±0.13 <sup>a</sup>	6.13±0.37 <sup>c</sup>	0.78
Taste	8.00±0.27 <sup>ab</sup>	8.06±0.24 <sup>a</sup>	7.40±0.40 <sup>ab</sup>	7.00±0.40 <sup>b</sup>	0.96
texture	8.06±0.24 <sup>a</sup>	8.06±0.28 <sup>a</sup>	7.60±0.33 <sup>a</sup>	7.33±0.33 <sup>a</sup>	0.85
Flavor	8.06±0.30 <sup>a</sup>	7.86±0.23 <sup>ab</sup>	7.40±0.36 <sup>ab</sup>	7.00±0.29 <sup>b</sup>	0.85
Overall acceptability	8.06±0.22 <sup>a</sup>	7.86±0.21 <sup>a</sup>	7.56±0.35 <sup>ab</sup>	6.80±0.40 <sup>b</sup>	0.88

\*Means in a column not sharing the same superscript are significantly different at  $p < 0.050$ .

**Figure 1. Different biscuit products**

### CONCLUSION

Gluten free biscuit products prepared from mixing of some cereals and legumes are considered a valuable addition for celiac patient. Some technological treatments e.g. germination, boiling, drying and backing process are recommended to enhance the nutritional characteristics and reduce the anti-nutritional factors of the products.

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## الملخص العربي

### بسكويت مغذي لكرض السيلياك: تأثير خلطات مختلفة من الحبوب والبقول

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الحسى. ووفق التحليل الكيماوى كانت نسبة البروتين الخام فى المنتجات (٨,٢٩ ، ٩,٦٢ ، ٧,٥٢ ، ٨,١٨ جرام/١٠٠ جرام) فى الكنترول ومعاملات ١ و ٣ و ٢ على الترتيب. كما اتضح ان عنصر البوتاسيوم هو العنصر الاعلى وخاصة فى المنتج رقم (٢) والذى احتوى اعلى تركيز من عنصرى البوتاسيوم (٣٥٣,٤٧ مجم/١٠٠ جرام) و الصوديوم (٣٢,٧١ مجم/١٠٠ جم). كما وجد ان محتوى المنتجات من حمض الفيتك تتراوح من (١٧,٥٣ الى ١٠٠,٥٣ مجم/١٠٠ جم) و محتواها من التانينات يتراوح من (٥,٠٤ الى ١٠,٩٠ مجم/١٠٠ جم). كذلك وجد ان عملية الانبات و المعاملات الحرارية تعمل على زيادة الهضمية المعملية للبروتين وزيادة التقبل الحسى والقيمة التغذوية.

هدفت الدراسة الى اعداد وتقييم منتجات بسكويت كمنتجات خالية من الجلوتين لمرضى حساسية الجلوتين (السيلياك). حيث تم إعداد المنتج من اربع معاملات عينة كنترول وثلاث معاملات مختلفة تبعا لمكونات كل معاملة من المواد الخام حيث ان عينة الكنترول تم تحضيرها من ١٠٠% دقيق قمح والعينة B<sub>1</sub> تم تحضيرها من ٤٥% دقيق الذرة الصفراء + ٣٥% دقيق الفول البلدى + ٢٠% نشا الذرة و العينة B<sub>2</sub> تم تحضيرها من ٤٥% دقيق كسر الارز + ٣٥% دقيق الحمص + ٢٠% نشا الذرة اما العينة B<sub>3</sub> تم تحضيرها من ٤٥% دقيق السورجم + ٣٥% دقيق الفاصوليا + ٢٠% نشا الذرة.

تم تقييم التركيب الكيماوى ومحتواها من العناصر المعدنية وكذلك المواد المضادة للتغذية بالاضافة الى التقييم