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ORIGINAL ARTICLES

Microbiological Analysis of Fortified Cakes with Peanut and Chickpea to Celiac Patients

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ABSTRACT

The present work was conducted to evaluate gluten-free butter cake obtained by partially fortified corn and rice flours with ground peanut or chickpea as potential healthy ingredients with different concentrations at 10%, 15% and 20% to improve nutritive value, sensory and microbiological characteristics estimated during storage period for four weeks. The chemical compositions were determined in raw materials. Twelve different formulas of butter cake were evaluated chemically, organoleptically and microbiologically compared with two control samples made from pysllium as a replacement for gluten at two various ratios (3% and 6%) and using rice and corn without any supplementation. The peanut and chickpea flour containing gluten-free butter cake were evaluated of their total protein, fat, carbohydrates, dietary fiber, ash as well as their minerals content composition. Results could be summarized that the fortification of the butter cake with 20% peanut or chickpea was the best treatments and improved its macro and micronutrients than that of the corresponding control sample with pysllium without any supplementation. Essential amino acid contents pointed out that peanut or chickpea upgraded the nutritive values. Sensory characteristics can be affected by the efficiency of ingredient agents in dough. Moreover, microbiological as total bacterial and fungal count were estimated in butter cake made from peanut and chickpea during storage period (four weeks) at room temperature and the results showed that the butter cake is safe from any microorganisms. Peanut and chickpea were acceptable to most panelists when compared to control butter cake. From the obtained results we concluded that fortified at 15% and 20% levels led to an acceptable quality and high nutritional value due to the complementary nature of the amino acids derived from these raw materials, compared with those made without any replacement. The attributes of these butter cake conform to the expert's nutritional recommendations for the gluten-free diet. These results confirm that the peanut and chickpea can represent a healthy alternative to frequently used ingredients in gluten-free products.

Key words: Celiac disease - Psyllium – Gluten free Cake - Peanut - Chickpea - Nutritive values – Organoleptically evaluation.

Introduction

New recent epidemiological data show that celiac disease (CD) is a common disease in the world. It's now understood to affect as many as 1:266 people worldwide. However, its symptoms are common to many other conditions and can range in severity. CD is often overlooked or misdiagnosed, often as irritable bowel syndrome, chronic fatigue syndrome or fibromyalgia. Consequently, individuals may remain undiagnosed and untreated for many years (Saturni *et al*. 2010).

Furthermore, food fortification can be an economical, flexible, and socially acceptable to improve the nutrient intake of groups at risk in order to ensure nutritional adequacy of the diet being an option by which people have access to milled or processed food (Granato and Ellendersen 2009). The gluten-free diet for celiac disease at first sight seems very restrictive, monotonous, expensive and difficult, although no one would argue that it is more challenging. To remove gluten technologically, great quantities of fat are added for preparations (Kennedy and Feighery 2000). Butterworth *et al*. (2004) reported that the celiac patients are not able to develop appropriate recipes in accordance with their diets. Indeed, dietary habits have a protective effect on gluten intolerance leading to a later CD onset with atypical and milder symptoms, which are more difficult to diagnose (Cataldo and Montalto 2007).

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Consumption of gluten free whole grains (rice and corn) not only provide an opportunity to help prevent or delay the onset of lifestyle diseases, but they also help individuals on a gluten free diet obtain adequate amounts of vitamins and minerals that are often lacking in the diet, and are necessary for good health and wellbeing (Hegazy *et al*., 2009).

Chickpea is a very important staple food for developing countries because they provide a cheaper form of protein than expensive animal sources. In addition, they are easy to grow, even in harsh arid environments (Biomed 2006). The chickpea belongs to legumes which are rich in protein, the ratio ranged from 23.7 – 28.0%, moisture 7.0 – 9.2%, fat 4.8 – 6.6%, ash 1.4 –3.0% and carbohydrates 68.0 – 70.3%. Amino acid composition of chickpea protein (gm/ 16 gm N) showed that it had valine 4.43; isoleucine 4.10; leucine 7.93; threonine 3.63; methionine 1.34; phenylalanine 4.11; tyrosine 4.9; lysine 6.99; histidine 4.65 and arginine 7.30 (g/16 g N) (Halaby *et al.*, 2000).

Sabanis *et al.* (2006) noticed that, gluten free dough with chickpea flour presented protein contents that ranged between 13.0 and 18.36 %. While, Duranti (2006) published that the protein level in chickpea added to gluten free dough might be considered as nutraceutical properties attributed to legume proteins, confirmed by Saturni *et al.* (2010). In particular lysine, the limiting amino acid in cereals can be found in high amounts. The high contents of arginine and histidine both essential for infants and children for the nutrition of CD children.

Peanuts are a versatile source of nutrition. Some Egyptian raw peanut varieties had (5.36 - 9.9) moisture; (24.7 – 29.0) protein; (38.5 – 51.2) fat; (17.5 – 24.5) carbohydrates; (2.0 – 2.9) ash and (1.7 – 2.5) fiber according to Gad (1985), compared with the same nutrients analyzed by Halaby *et al.* (2000) who published 4.9, 26.4, 44.9, 21.7, 2.4 and 2.9% (gm/ 100gm DW). Amino acids content of raw peanut were 4.8, 1.3, 11.4, 5.6, 18.2, 3.8, 4.3, 3.8, 5.0, 4.2, 3.5, 1.1, 2.6, 1.1, 6.4, 3.4, 2.4 and 11.2 (gm/ 100gm) for serine, cystine, aspartic, glycine, glutamic, alanine, proline, tyrosine, phenylalanine, valine, lysine, methionine, threonine, tryptophan, leucine, isoleucine, histidine and arginine, respectively. According to Granato and Ellendersen (2009), the enrichment of cookies processed with Peanut and almond ingredients leads to the improvement of their nutritional qualities.

Psyllium was chosen because it is one of the safest, being similar to gluten in food, gentlest laxatives available, acting on constipation and diarrhea alike and providing additional benefits to protect from hemorrhoids, bowel disorders, cholesterol and blood sugar levels. These properties allow its application in the food industry; pysllium can be a substitute for fat or act as an emulsifier or thickening agent (Zandonadi *et al.*, 2009).

Spoiled food may be defined as a food that has been damaged or injured so as to make it undesirable for human consumption. Bakery products are an important part of a balanced diet and a wide variety of such products can be found on supermarket shelves. However, bakery products, like many processed foods, are subject to physical, chemical and microbiological spoilage. While physical and chemical spoilage limits the shelf life of low and intermediate moisture bakery products, microbiological spoilage by bacteria, yeast and molds is the concern in high moisture products. Many industrially produced baked goods emerge from the baking process with a surface that is essentially sterile but post bake handling can quickly lead to fungal, microbial surface contamination as a result of exposure to airborne contaminants as well as equipment contact. This microbial spoilage of bakery products and its control preservatives focused by Saranraj and Geetha (2012).

The objectives of the present study are to evaluate the effect of replacing gluten with psyllium at 3% and 6% on chemical and nutritional value of gluten-free dough (as a control samples), to produce butter cake gluten free with maximum nutritive value to maintain normal growth and health through suggesting new formulas with various concentrations using peanut or chickpea flours at 10%, 15% and 20%, to determine the existed correlation between physical properties and consumers acceptance of sensory characteristics of the modified preparations. It should be mentioned that backing are prepared to be used for all ages of celiac patients as nutritive value. Moreover, microbiological analysis was estimated in butter cake during storage period.

Materials And Methods

Materials:

Maize (Zea mays L.), rice (Oryza Sativa L.) peanut (Arachis hypogaea L.) and chickpea (Cicer arienum L.) were obtained from Field Crops Research Institute of the Agricultural Research Centre, Cairo, Egypt.

Psyllium and skim milk were purchased from local market. French butter, vanilla, sugar and baking power were purchased from local market.

Methods:

Chemical constituents of raw materials and cake:

Protein, total fat, ash and crude fiber were determined in raw materials (chickpea, peanut, yellow maize, white rice and psyllium) according to the method outlined in AOAC (2000). Minerals content sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and zinc (Zn) were determined in the diluted solution of ash raw materials using the atomic absorption spectrophotometer (3300 Perkin-Elme) as described in by AOAC (2000) method. While, phosphorus (P) was determined using the Unican SP 1800 Spectrophotometer at wave length 650 nm.

Moreover amino acids content of peanut and chickpea were determined according to the procedure described by Olson *et al.* (1978).

Preparation of butter cake:

The ingredients of raw materials (yellow maize, white rice, psyllium, chickpea and peanut) were used in the preparation of butter cake gluten-free according to Mizukoshi *et al.* (1979). Psyllium at level 3 or 6% was added separately to equal weight (50gm.) from yellow maize and white rice and mixed using 50gm french butter, 100gm. sugar, 3.0gm. baking powder, 75.0 g. egg, 3.0gm. vanilla and 7.0gm. skim milk were added to give 3 and 6% psyllium control. Chickpea or peanut was added separately to control 3 and 6% at levels 10, 15 and 20% to give twelve blends, from 1 to 6 formulas for peanut and chickpea formulas from 7 to12, respectively.

The butter cake formulas were baked at 190° C for 25 minutes in an electric oven. Butter cake was allowed to cool on racks for about one hour before evaluation.

The organoleptically evaluation for different blends of butter cake was estimated by ten experienced panelists according to A.A.C.C. (1985). Also, the chemical compositions and the minerals content were determined in all formulas. Biological activity as total count of bacteria and fungi were determined in butter cake formulas during storage period for four weeks at room temperature.

Determination of microbiological analysis:

Biological activity as total count of bacteria and fungi were estimated by the plate methods of Martin (1950) and Allen (1959) in butter cake which made from chickpea and peanut during storage at room temperature for four weeks. Plates of biological activity were incubated in incubator at 30 °C for three days.

Statistical analysis:

The data obtained were analyzed by using SPSS statistical software (version 13 SPSS Inc., Chicago. USA). The results were expressed as mean ± SD, and tested for significance using one-way analysis of variance "ANOVA" according to Armitage and Berry, (1987).

Results And Discussion

Chemical composition of raw materials of yellow maize, white rice, psyllium, peanut and chickpea have been determined and the results are listed in Table (1). The parameters in Table (1) point out that yellow maize and white rice have the highest content of carbohydrates (85.01 and 90.74%), and the lowest content of ash (1.16 and 0.38%) and crude fiber (1.2 and 0.48%). On the contrary, psyllium had the highest content of ash, crude fiber, K, Ca and Zn contents. These results are in agreement to the results that have been obtained by Stevens and Rashid (2008).

Results in the same table indicate that the peanut and chickpea present the highest protein content (25.80% and 20.30%), they are considered as good protein sources. Values recorded here are in the same range (24.7% - 29% protein in peanut) as reported by Halaby *et al.* (2000). On the other hand, protein in chickpea ranged from 22.0% to 24.0% investigated by Sabanis *et al.* (2006) and Iqbal *et al.* (2006). In contrast to most other pulses and cereals, peanut have a relatively high fat content at 49.24%. This makes them an important energy source for vegans and those without regular access to meat and dairy products confirmed by Biomed (2006). The crude fat content in chickpea flour was 6.04% higher than those reported in other chickpea cultivars (5.69%), Zhao *et al.* (2005) and 5.2%, Iqbal *et al.* (2006).

The peanut and chickpea flours showed a crude fiber content of 8.54% and 10.43% respectively. These ratios were lower than those reported for other both cultivars which ranged from 19.1% to 14.33% (Rosin *et al.*, 2002). An adequate intake (20-35g/d) of fiber has been recommended in CD subjects by Saturni *et al.* (2010).

Total carbohydrates content in the peanut and chickpea were 14.09% and 60.75% slightly higher than that reported by Hoover and Zhou (2003) for other cultivars. At the same time, peanut and chickpea are characterized by high contents of minerals dominated by Na, K, Ca, Mg, Fe and Zn which depend on the species and on the characteristics of the soil where the plants are grown, confirmed by Biomed (2006).

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Table 1: Chemical analysis of raw materials (on dry weight basis)

Chemical composition (g/100 g)	Yellow maize	White rice	Psyllium	Peanut	Chickpea
Protein	8.49	7.72	1.33	25.80	20.30
Total fat	4.14	0.68	3.66	49.24	6.04
Ash	1.16	0.38	24.7	2.33	2.48
Crude fiber	1.20	0.48	60.33	8.54	10.43
T.C	85.01	90.74	9.98	14.09	60.75
Minerals (mg/100g)					
Na	15.72	12.30	54.62	18.25	24.31
K	192.35	185.64	811.37	905.0	875.0
Ca	20.85	32.39	34.54	92.0	105.0
Mg	3.59	1.20	51.82	168.0	115.0
Fe	4.60	4.51	20.91	4.58	6.24
Zn	2.72	2.30	2.73	3.27	3.43

Amino acids percent in peanut and chickpea:

Amino acids content in peanut and chickpea protein are presented in Table (2). The results show that the arginine, cysteine, isolucine, leucine, histidine, phenylalanine, tyrosine, theronine and valine have the highest amounts 10.6, 2.6, 8,2, 11.4, 3.6, 8.4, 8.2, 5.0 and 9.5 g/ 100 g protein of the total amino acids in peanut protein respectively. Whereas, for chickpea protein, the results show that the methionine, lysine, aspartic, proline, serine, glutamic, glycine and alanine have the highest amounts 2.5, 6.7, 7.2, 4.7, 4.8, 8.2, 4.0 and 4.2 g/100g protein, respectively.

From these results, peanut and chickpea proteins have considerable biological value which can supplement the essential amino acids requirements of animals. These results are in agreement with **Biomed (2006).**

Table 2: percent of amino acids content in peanut and chickpea g/100 g. protein

Amino acids	Peanut	Chickpea
Arginine	10.6	8.5
Cysteine	2.6	1.9
Methionine	1.8	2.5
Isoleucine	8.2	7.6
Leucine	11.4	9.9
Lysine	6.0	6.7
Histidine	3.6	2.5
Phenylalanine	8.4	6.7
Tyrosine	8.2	7.6
Threonine	5.0	4.0
Valine	9.5	7.1
Aspartic	2.3	7.2
Proline	2.4	4.7
Serine	2.2	4.8
Glutamic	7.5	8.2
Glycine	3.2	4.0
Alanin	4.0	4.2

Chemical composition of butter cake formulas gluten free:

Results in Tables (3 and 4) indicated that the control samples of gluten free butter cake with 3% and 6% psyllium had the lowest percentage of nutrients compared to that of gluten free butter cake made by using various ingredients. It could be recorded that total protein, fat, ash and crude fiber contents were 9.17, 3.40, 2.90 and 4.70% with 3% psyllium and the contents were 10.24, 4.20, 3.30, and 5.81% respectively, with 6% psyllium (as control samples).

Granato and Ellendersen (2009) have published that the psyllium and other flours including rice and maize have potential in very important recipes for patients with celiac disease.

In the present study, the chemical composition of gluten free butter cake formulas fortified separately with 10%, 15% and 20% peanut or chickpea has been considered. It could be noticed that total nutrients (including total protein, fat, ash and crude fiber) were gradually increased to reach 14.37, 20.23, 3.91 and 6.57% at 3% psyllium with 20% peanut. While, with 6% psyllium and the same ratio of peanut, the values increased to reach 15.43, 21.39, 4.77 and 8.25%, respectively.

Recently, Granato and Ellendersen (2009) published that the consumption of 100g of peanut cookie would provide 16% of the total daily recommended value of protein (DRV) for adults. FAO/WHO (1990) recommended the ingestion of 25-30 g/day of proteins for children aged 5 to 19 years old, which means that the consumption of 100g of any cookie produced would provide about 44% of the daily recommended value for those consumers.

In this concept, gluten free butter cake supplemented with 20% peanut was higher in various nutrients, it could be noticed from the results that, addition of peanut flour improved the nutrients including protein, fat, ash more than chickpea, while fiber was higher in chickpea compared with peanut. In fact, total dietary fiber in dough with chickpea was higher than that recorded in other studies (6.36%), Saura-Calixto *et al.* (2000) and (2.91%) Rosin *et al.* (2002). While, similar levels to those were reported in chickpea-added pasta (9.41 %), Goñi and Gamazo, (2003).

Dietary fiber in these preparations is important due to its functional effects in the gut. For instance, viscous fiber-containing foods may elicit low postprandial glycemic responses due to delayed glucose absorption (Tovar *et al.*, 1992). Furthermore, control butter cake gluten free exhibited higher total carbohydrate level (79.83% and 76.45%) than the gluten free butter cake supplemented with different ingredients. Hence, addition of peanut or chickpea flour resulted in reduced total carbohydrate values compared to the control gluten free butter cake (Tables 3 & 4).

On the same direction, butter cake gluten free supplemented with 15% and 20% peanut or chickpea were also higher in mineral contents including K, Ca, Mg, Fe and Zn, than the corresponding control samples. The results in this study are related to those obtained by Granato and Ellendersen (2009) and Saturni *et al.* (2010). The data suggest a possible commercial exploration of the produced free gluten butter cake. So the number of these types of products could be increased in the markets, offering new nourishing options of consumption for gluten-intolerants.

Table 3: Chemical composition of gluten-free butter cake supplemented with different levels of peanut and psyllium (on dry weight basis)

Chemical analysis Parameter	Different levels of peanut and psyllium at 3% and 6 %							
	Control		10 %		15 %		20 %	
	3 %	6 %	3 %	6 %	3 %	6 %	3 %	6 %
Protein	9.17	10.24	12.48	12.65	13.42	13.59	14.37	15.43
Total fat	3.40	4.20	15.35	16.86	17.32	19.12	20.23	21.39
Ash	2.9	3.30	3.42	3.67	3.76	4.12	3.91	4.77
Crude fiber	4.70	5.81	5.00	6.61	5.97	7.56	6.57	8.25
Total carbohydrates	79.83	76.45	63.75	60.21	59.53	55.61	54.92	50.16
Minerals mg/100g								
Na	32.29	47.23	26.32	27.47	27.38	28.53	27.95	28.94
K	313.7	339.9	441.3	465.7	476.6	550.9	511.8	596.2
Ca	129.8	270.2	224.9	234.7	229.2	238.9	233.4	243.2
Mg	20.59	34.93	63.24	65.98	67.9	68.74	71.75	74.49
Fe	4.64	5.31	2.13	2.93	2.51	2.71	2.62	3.11
Zn	2.63	3.16	1.86	1.91	1.95	1.97	2.01	2.10

Table 4: Chemical composition of gluten-free butter cake supplemented at different levels of chickpea and psyllium (on dry weight basis)

Chemical analysis Parameter	Different levels of chickpea and psyllium at 3% and 6 %							
	Control		10 %		15 %		20 %	
	3 %	6 %	3 %	6 %	3 %	6 %	3 %	6 %
Protein	9.17	10.24	11. 83	11. 99	12. 45	12. 61	13. 07	13. 23
Total fat	3.40	4.20	10.04	10.53	11.61	11.94	12.05	12.75
Ash	2.9	3.30	3.44	3.60	3.79	3.94	3.99	4.39
Crude fiber	4.70	5.81	6.70	8.34	7.21	9.35	8.71	9.95
Total carbohydrates	79.83	76.45	67.99	65.54	64.94	62.16	62.18	59.68
Minerals mg/100g								
Na	32.29	47.23	28.36	28.93	29.07	29.92	29.87	30.27
K	313.7	339.9	521.3	536.2	549.3	564.2	577.3	592.2
Ca	129.8	270.2	226.2	235.9	231.1	240.9	235.9	245.8
Mg	20.59	34.93	55.95	58.69	56.05	61.79	60.15	63.89
Fe	4.64	5.31	3.03	3.56	3.23	4.28	3.62	4.96
Zn	2.63	3.16	1.96	1.98	2.05	2.09	2.14	2.19

Organoleptic evaluation of the gluten free butter cake (GFBC) fortified with peanut or chickpea grounds:

Data obtained in Tables (5 and 6) for sensory properties showed that the control samples of gluten free butter cake at 3% and 6% psyllium had the lowest degree of sensory acceptance. Furthermore, psyllium can be replaced by gluten in preparations confirmed by Hegazy *et al.* (2009), in fact, butter cake gluten free samples with supplementation at various ratios of peanut and chickpea grounds exhibited good sensory properties and there were significant differences between those samples and the control in all organoleptically.

Sensory characteristics can be affected by various concentrations of the ingredients agents in dough. Peanut and chickpea at 15% and 20% with psyllium at 6% were acceptable to most panelist regarding to taste, odor, texture, color and general appearance. On the other hand, there was a significant decrease in sensory characteristics with control butter cakes at 3% and 6% psyllium without using any ingredients. Also there was a decrease in sensory evaluations with gluten free fortified butter cake at a 10% of the ingredients that's for

texture, volume, color and general appearance when compared to other samples supplemented with 15% and 20% ingredients.

Table 5: Effect of peanut and psyllium on the sensory evaluation of butter cake.

Types of additions	Taste 20	Odor 20	Texture 15	Crust color 15	Crumb color 15	General Appearance 15	Overall acceptability 100
Control 3%	16.10^{d} ±0.76	16.00^{d} ±0.79	12.35^{c} ±0.93	11.89^{cd} ±0.64	11.52^{cd} ±0.93	12.40^{c} ±0.63	80.26
Peanut 10%	17.40^{bc} ±1.06	17.15^{bc} ±0.76	13.21^{bc} ±0.25	12.10^{c} ±0.86	12.21^{c} ±0.72	12.74^{c} ±0.12	84.81
15%	18.00^{b} ±1.06	17.30^{bc} ±0.65	13.45^{b} ±0.77	12.48^{c} ±0.95	12.45^{c} ±0.12	13.60^{b} ±0.63	87.28
20%	18.45^{ab} ±0.56	17.50^{bc} ±0.13	13.63^{b} ±0.66	12.90^{bc} ±0.11	13.60^{b} ±0.55	14.05^{ab} ±0.99	90.13
Control 6%	17.15^{bc} ±0.21	17.62^{bc} ±0.35	13.85^{b} ±0.35	12.31^{c} ±0.34	12.18^{c} ±0.87	13.34^{b} ±0.43	86.45
Peanut 10%	17.57^{bc} ±0.23	18.00^{b} ±0.12	14.35^{ab} ±0.28	13.65^{b} ±0.48	13.68^{b} ±0.09	14.31^{ab} ±0.67	91.56
15%	18.12^{b} ±0.09	19.10^{a} ±0.63	14.22^{ab} ±0.97	14.24^{a} ±0.74	14.30^{ab} ±0.72	13.91^{b} ±0.04	93.89
20%	19.30^{a} ±1.06	19.20^{a} ±0.93	14.35^{ab} ±0.97	14.78^{a} ±0.74	14.89^{a} ±0.61	14.89^{a} ±0.99	97.43

Table 6: Effect of chickpea and psyllium on the sensory evaluation of butter cake

Types of additions	Taste 20	Odor 20	Texture 15	Crust color 15	Crumb color 15	General Appearance 15	Overall acceptability 100
Control 3%	16.10^{d} ±0.76	16.00^{d} ±0.79	12.35^{c} ±0.93	11.89^{cd} ±0.64	11.52^{cd} ±0.93	12.40^{c} ±0.63	80.26
Chickpea 10%	16.05^{d} ±0.76	16.62^{d} ±0.99	12.76^{c} ±0.48	12.54^{c} ±0.79	12.71^{c} ±0.642	12.27^{c} ±0.658	82.95
15%	17.00^{bc} ±1.08	17.83^{b} ±0.89	12.95^{bc} ±0.97	12.69^{c} ±0.78	13.17^{bc} ±0.23	13.27^{bc} ±0.74	86.91
20%	17.60^{bc} ±0.06	18.05^{b} ±0.98	13.38^{b} ±0.95	12.96^{bc} ±0.99	13.82^{b} ±0.22	13.75^{b} ±0.77	89.56
Control 6%	17.15^{bc} ±0.21	17.62^{bc} ±0.35	13.85^{b} ±0.35	12.31^{c} ±0.34	12.18^{c} ±0.87	13.34^{b} ±0.43	86.45
Chickpea 10%	17.49^{bc} ±0.187	17.94^{b} ±0.95	13.96^{ab} ±0.19	13.14^{b} ±0.87	13.74^{b} ±0.96	13.20^{bc} ±0.96	89.47
15%	17.94^{b} ±0.44	18.00^{b} ±0.54	14.39^{a} ±0.32	13.74^{ab} ±0.33	14.12^{a} ±0.83	14.27^{a} ±0.69	92.46
20%	18.84^{ab} ±0.63	19.88^{a} ±0.69	14.89^{a} ±0.321	13.82^{ab} ±0.11	14.32^{a} ±0.37	14.20^{a} ±0.69	95.95

Biological activity in butter cake:

Microbiological spoilage is often the major factors limiting the shelf life of bakery products. Spoilage from microbial growth causes economic loss for both manufacturers and consumer. These losses could be due to many individual cases such as, packaging, sanitary practice in manufacturing, storage conditions and product turnover (Saranraj & Geetha 2012).

Biological activity as total bacteria and fungi count were estimated in butter cake with psyllium at 3% and 6% made from peanut and chickpea at level 10, 15 and 20% respectively, during storage period for four weeks and the results are reported in Tables 7 & 8. The results from the tables showed that the total count of bacteria and fungi were increased in peanut than chickpea butter cake during the storage period. Total count of bacteria for butter cake from peanut was ranged from 32.2 to 45.4× 10^{-6} CFU at 3% psyllium, and 33.6 to 47.6× 10^{-6} CFU at 6% psyllium after four weeks and the total count of fungi was parallel to the bacteria count. Peanut cake products are good sources of proteins and oil, the results obtained from microbial analysis of peanut cakes product show that there were a significant increase in oil and rancidity during storage period. Moreover, no variation was obtained in butter cake chickpea during storage period.

Mold growth is by far the major factor limiting shelf life of high and intermediate bakery products. In general, mold growth on bakery products is a serious problem that results in economic losses. Furthermore, losses of products due to mold spoilage are between 1 and 5 percent depending on the type of product, season, and the method of processing (Malkki and Rauha 2000).

Adjou *et al.* (2012) investigated the detection of spoilage fungi and mycotoxins contamination in peanut cake product, popularly called ''kulikuli''. Forty five major markets were sampled and peanut cake products were analyzed. The results showed that the total coliform count ranged between 1.6 × 10^{1} and 14.0 × 10^{2} CFU g^{-1}, while the fungal count was ranged from 1.0 to 8.1 × 10^{2} CFU g^{-1} and total bacteria count was between from

5.4×10^4 to 1.4×10^6 CFU g^{-1}. The high total bacterial and coliform count may have been as a result of the low level of hygiene maintained during the processing and sale of peanut cake product. This includes the handlers, quality of ingredients used for dressing and the utensils. The open-air exposure of products, while they were displayed for sale, can also serve as source of contamination.

Table 7: Total bacteria and fungi count in butter cake made from peanut during storage period

Types of additions	Total bacteria count × 10^{-6} CFU			Total fungi count × 10^{-2} CFU		
	0Time	2weeks	4weeks	0 Time	2weeks	4weeks
Control 3%	18.7	21.5	32.2	29.5	36.9	37.4
Peanut 10%	19.1	22.2	39.9	33.8	44.6	47.1
15%	19.5	24.3	42.1	34.1	47.2	50.1
20%	19.8	25.4	45.4	35.3	47.5	51.9
Control 6%	19.5	23.3	33.6	30.9	37.4	39.2
Peanut 10%	20.2	25.1	40.2	35.2	45.5	49.9
15%	20.5	27.4	42.8	36.5	49.3	51.3
20%	20.7	27.2	47.6	37.9	50.1	52.4

Table 8: Total bacteria and fungi count in butter cake made from chickpea during storage period.

Types of additions	Total bacteria count× 10^{-6} CFU			Total fungi count× 10^{-2} CFU		
	Time	2weeks	2weeks	Time	2weeks	2weeks
Control 3%	18.7	21.5	32.2	29.5	36.9	37.4
Chickpea10%	18.7	19.6	25.9	29.8	30.3	37.4
15%	18.9	19.9	26.5	30.1	31.1	35.7
20%	18.9	20.4	27.8	30.3	32.7	36.2
Control 6%	19.5	23.3	33.6	30.9	37.4	39.2
Chickpea10%	19.7	20.8	27.6	31.2	31.5	38.3
15%	19.8	21.1	28.1	31.7	33.1	40.1
20%	19.8	21.9	29.8	32.5	33.5	41.3

Commercially, prepared gluten-free products are expensive and difficult to find. Products such as peanut or chickpea flour used in this study allow patients to consume healthful and tasty products made at home with low cost. Therefore, it could be recommended that preparation of butter cake free gluten from peanut and chickpea at level 15 and 20% gave an acceptable quality with high nutritional value products and more safe from microbiological contamination.

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