Current Science International
Volume: 11 | Issue: 04| Oct. – Dec.| 2022

EISSN:2706-7920 ISSN: 2077-4435
DOI: 10.36632/csi/2022.11.4.39
Journal homepage: www.curresweb.com
Pages: 504-515

Preparation and Evaluation of Gluten-Free Low Carbohydrates Kaiser Bread

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Received: 10 Nov. 2022 **Accepted:** 11 Dec. 2022 **Published:** 30 Dec. 2022

ABSTRACT

From the point of an increasing demand for producing and improvement of gluten-free products. The objective of this study was to investigate this demand to produce of gluten-free low carbohydrates kaiser bread by asing gluten-free low materials such as sesame, pumpkin, sunflower and golden flax seed powder in the existence of karish cheese, and psyllium husks, affected the quality of GLKB, its chemical composition, physical measurements, color parameters, sensory evaluation, as well as its texture profile analysis, shelf life, and their representative of recommended dietary allowance for children (4-8y), males and females (31-50y). Rice flour (RF) was used to make the control kaiser bread, and the other ingredients in T_1 were sesame powder and pumpkin powder (30, 20%), in T_2 were sesame powder and sunflower powder (30, 20%) and in T_3 were sunflower powder and flaxseed powder (30, 20%). 100% fresh cheese + 5% psyllium husks were added to each of the three samples. The results showed that all of the three formulations improved the kaiser bread quality by increasing loaf volume and crumb distribution, enhancing general appearance, taste, odor, sponge crust color, crumb color and distribution of crumb. In addition, there was a double increase in protein, fibre, minerals, and calories, with starch diminishing and carbohydrates.

Keywords: Gluten-free, low-carbohydrate, karish cheese, Sesame, Sunflower, Pumpkin, Golden flaxseeds, kaiser bread.

1. Introduction

Most of the total dietary energy intake by different global population groups comes from carbohydrates. Globally, scientists are focusing on developing functional food products that are healthy and low in calories. Consumers are more concerned for their health and lifestyle. The demand for the production of low-calorie, high-fiber food products is increasing, especially bakery products (Gupta *et al.*, 2017). Flour-based bread is an important part of the daily diet of most people around the world because it is a source of energy, protein, and minerals (Rosell, 2011).

Rice (*Oryza sativa*) is the primary ingredient in the most widely used gluten-free bread products. For certain special diets, such as gluten-free food items, rice flour, free of gluten, low in sodium, protein, fat, and crude fibers, and high in easily digestible carbs, is preferred. Rice, however, have several quality issues such as low volume, bad texture, poor color, and poor crumb structure because to its limited gas retention capability, therefor, gums are a common food additive showed be used in gluten-free food formulations to solve these issues (Matos *et al.,* 2014). Sesame seeds (*Sesamum indicum* L.), of the Pedaliaceae family, are widely grown and have a mellow flavor and high nutritional value. sesame is used as food, feed, and cosmetics, and the health-food applications of sesame are increasing. It is rich in protein, lipids, and lignan-like active ingredients. Sesame seeds have many health benefits, such as antioxidant, anti-inflammatory, and anti-tumor effects, cholesterol reduction, blood lipid regulation, liver and kidney protection, and cardiovascular system protection (Wei *et al.*, 2022). Pumpkin seeds

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(Cucurbita maxima), belong to the Cucurbitaceae family, and have been frequently used as a functional food. It is utilized as dried or roasted food because it is a good source of proteins, fibers, polysaccharides, minerals (like iron, zinc, magnesium, and calcium), polyunsaturated fatty acids, phytosterols, tocopherols, and phenolic acids (Patel, 2013). Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops grown in the world. Sunflower seeds contain unsaturated fatty acids, proteins, fibers, vitamins (E, B, folate and niacin), minerals (selenium, copper, zinc, and iron) and phytochemicals (Anjum *et al.*, 2012 and Islam *et al.*, 2016). Flaxseeds (*Linum usitatissimum* L.), commonly known as flaxseeds or linseeds, are mainly grown for oil, fiber, food, and feed purposes. It has gained worldwide recognition as a health food because of its plenteous supply of varied nutrients such as oils, fatty acids, proteins, fiber, lignans, mucilage, and micronutrients. These constituents attribute a multitude of beneficial properties to flaxseed, which makes its use possible in several applications, such as functional foods, health supplements, nutraceuticals, biomaterials, and cosmetic products (Sanmartin *et al.*, 2020 and Mueed *et al.*, 2022). Popular traditional Egyptian dairy item karish cheese (kind of soft cheese) is delectable, soft, and nutritious. It is suitable for all age groups, has nutritional and therapeutic benefits, and it is frequently produced in rural Egypt and consumed in their diet due to its high protein content, low fat content, and reasonable price (Abou-Donia, 2008). It is also recognized as one of the foods with the highest levels of calcium and phosphorus which are essential for the development of teeth and bones, and usually recommended for people who are obese, have high cholesterol, or have heart disease (Fayed *et al.*, 2014). Psyllium husks are a source of natural dietary fiber with marked water absorbability and gelling properties, which makes it an attractive functional ingredient for applications in the food industry, such as gluten free bread and breakfast cereals. The main functional component of psyllium husk is a complex branched heteroxylan (Ren *et al.*, 2020).

Low-carbohydrate high fat diets are a highly queries current issue in nutrition (Noakes and Windt 2017). It can be an effective weight-loss tool for overweight and obese individuals and type 2 diabetes. It can improve glycemic control, reduce glycated haemoglobin and fasting glucose, and improve blood glucose levels in overweight and obese people (Bazzano *et al.*, 2014 and Tay *et al.*, 2015).

The lack of gluten has a high influence on dough properties, the bread making process, and the final quality and shelf life of gluten-free bread (GFB) (El Khoury *et al*., 2018 and Capriles *et al.*, 2020). As a result, obtaining high-quality GFB remains a major challenge for food scientists and producers, with increasing demand due to the growing number of individuals following a GF diet (Capriles *et al.*, 2020). There has been a rise in the number of people adhering to the gluten-free (GF) diet partly due to an increased prevalence and awareness of gluten-related disorders, especially celiac disease, which has become a notorious public health problem worldwide but mainly due to the widespread belief that a GF diet is healthier and more suitable for weight management (Melini and Melini, 2018; Kami-nski *et al.*, 2020). Thus, this increasing demand and consumption of GF products is becoming a trend in the global food sector (Capriles *et al.*, 2020).

Currently, consumers have been looking for varied and functional bread, among them gluten-free, low-carbohydrate, or high-protein bread. There is a need for new researches on low-carbohydrate or high-protein bread (Dhinda *et al.*, 2012 and Bourekoua *et al.*, 2018). The human population that is intolerant to gluten is increasing, and most gluten-free flour types have low-protein content, which affects the nutritional value of bread (Hager *et al.*, 2012).

In order to address the problem of low nutritional value and high carbohydrate content in the meals of patients with gluten intolerance, this study proposes a number of mixtures that are acceptable in terms of physical characteristics, sensory acceptance, shelf life and feeling full after eating to suit children of 4 to 8 years old, men and women of 31 to 50 years.

2. Materials and methods

2.1. Materials

Rice (*Oryza sativa*) flour, sesame (*Sesamum indicum)* seeds, pumpkin (*Cucurbita ficifolia)* seeds, psyllium seeds (*Plantago ovata Forsk*), fresh whole eggs and salt were obtained from local markets, Cairo, Egypt. Sunflower (*Helianthus annuus* L., Sakha 53 variety) seeds and golden flaxseed (*Linum usitatissimum L*., Sakha 5 variety) were obtained from the Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. Karish cheese was obtained from milk processing unite in Faculty of Agriculture, Cairo University, Giza, Egypt. All chemicals used in this study were of analytical grades. El-Gomhouria Company for Trading Drugs, Chemicals and Medical Supplies.

2.2. Methods

Raw, and low-carbohydrate components were chosen for the GLKB bread formula and the control bread dough used made from 100% rice flour (Table1)

Table 1: Formula of the gluten-free low-carbohydrate kaiser bread (GLKB).

Ingredients	Formula (g)			
	Control Con.	T_1	T_2	T_3
Rice flour	100	-	-	-
Karish cheese (fresh)	-	100	100	100
Sesame seeds powder	-	30	30	-
Pumpkin seeds powder	-	20	-	-
Sunflower seeds powder	-	-	20	30
Flaxseed powder	-	-	-	20
Psyllium husks	-	5	5	5
Fresh egg (whole)	-	35	35	35
Baking powder	2	2	2	2
Corn oil	6	6	6	6
Salt	2	-	-	-
Arabic gum	1	-	-	-

Control = 100 % rice flour. T_1= 100% fresh karish cheese+30 % sesame powder +20 % pumpkin seeds powder + 5% psyllium husks. T_2= 100% fresh karish cheese+ 30 % sesame powder + 20 % sunflower seeds powder + 5 % psyllium husks. T_3=100% fresh karish cheese+ 30 % sunflower powder + 20 % flaxseed powder + 5 % psyllium husks

2.2.1. Preparation of raw materials

Fresh karish cheese and eggs are used at room temperature. The seeds (sesame, pumpkin, sunflower, and golden flaxseed) are also purified from impurities, then ground well in a laboratory mill (Athelzion, HZ: 50, H: I, V: 220, Italy) and packed in polyethylene bags until used.

2.2.2. Preparation of gluten-free low carbohydrates kaiser bread (GLKB)

Fresh karish cheese was mixed first until it became creamy by hand blender (THB - 600 W). Next, eggs and ground seeds were added in the selected amounts for each sample and thoroughly mixed. Psyllium husks were then added, and the mixing process was continued until all of the ingredients were well homogenised. The mixture was then transferred to a bowl, covered, and allowed to sit for 10 to 15 minutes. Following that, the baking powder was added and well combined, after which the dough is shaped and put on a silicone mat on a baking sheet. The bread was baked for 20 minutes at a temperature of 200 ºC in an oven, and then left in the oven to cool at room temperature until it is ready to eat. following the modified procedure by Clark and Aramouni (2018).

2.2.3. Proximate analysis of main raw materials and bread

Protein, fat, ash, and crude fibers content were determined in the main raw materials and gluten-free low-carbohydrates kaiser bread, however carbohydrates were calculated by difference, [carbohydrates = 100 - (protein+ crude fibers+ ash+ fats)] (AOAC, 2019). Total calories were calculated by the formula of Livesey (1995) (total calories= fat x 9 + (protein + total carbohydrates x 4). Mineral (*e.g.,* potassium, calcium, magnesium, iron, zinc, and manganese contents of the main raw materials and gluten-free low-carbohydrates kaiser bread samples was determined according to (AOAC, 2019) using the Agilent Technologies Microwave Plasma Atomic Emission Spectrometers (Model-4210-MPAES, USA). RDA (%) is calculated as follows: Value of the nutrient in the kaiser sample × 100 / RDA for the same nutrient (RDA, 1989).

2.3. Physical properties of gluten-free low-carbohydrate kaiser bread (GLKB)

2.3.1. Specific volume of GLKB

The weight of kaiser bread loaves was determined after cool at room temperature, and bread loaf volume was measured by the alfalfa seeds displacement method as described by AACC (2016). Specific volume (cm^3/g) of bread was also, calculated by dividing volume (cm^3) by weight (g).

2.3.2. Color measurement of GLKB

Gluten-free low-carbohydrate kaiser bread (GLKB) crust and crumb colors were measured by a hand-held Tristimulus reflectance colorimeter Minolta Chromameter (model CR-400, Konica Minolta, Japan). Color readings were expressed by Minolta for L^*, a^* and b^* and reported values were the means of triplicate determinations. L^* values measured the lightness to darkness (100 for lightness and zero for darkness), a^* values measured redness when were positive and greenness when were negative. b^* values measure yellowness when were positive and blueness when it negative.

2.3.3. Texture profile analysis (TPA) of GLKB

A texture analyzer (Brookfield CT3 Texture Analyzer-Operating Instructions Manual, Stable Micro Systems, M08- 372-C0113, USA) was used as described by according (AACC, 2016). The texture profile was used to evaluate the kaiser bread in terms of the samples hardness (N), cohesiveness, gumminess (N), chewiness (mj), and springiness (mm). The samples were twice compressed to 40% of their original height using the parameters of Test-TPA, probe-36 mm (2.5 cm height, and 4 cm diameter) cylindrical, test speed of 2 mm/s, pre-test speed of 2 mm/s, and post-test speed of 2 mm, and the trials were carried out in ambient circumstances at 0, 24, 72 and 96 hours after baking and cooling the laboratories of the Agricultural Research Centre, Giza, Egypt.

2.3.4. Evaluation of shelf life of GLKB

Gluten-free low-carbohydrate kaiser bread (GLKB) were packaged after baking and cooling in polyethylene bags and stored at 27 ± 3 ºC. Bread samples were daily investigated for visible mold growth as outlined by Del Nobile *et al.,* (2003).

2.3.5. Sensory evaluation of GLKB

Gluten-free low-carbohydrate kaiser bread (GLKB) was evaluated for sensory characteristics by well-trained ten panelists from the staff of the Bread and Pasta Research Department, Food Technology Research Institute, ARC. The scoring scheme was established according to the method described by AACC (2016) as follows: texture (15), general appearance (15), taste (15), odor (15), sponge (10), crust color (10), crumb color (10), distribution of crumb (10)**,**

2.4. Statistical analysis

The data from this study were statistically analyzed using the Costat statistical software (version 6.451) for means and standard deviations (Steel *et al*., 1997), via one-way analysis of variance (ANOVA).

3. Results and Discussion

3.1. Proximate chemical composition of the main ingredients

The approximate composition of used raw materials and mineral contents (on a dry weight basis) in rice flour, sunflower powder, pumpkin powder, sesame powder, flaxseed powder, psyllium husks and karish cheese are represented in Table (2). The data indicated that pumpkin powder, sunflower powder, sesame powder, flaxseed powder and karish cheese, had the highest contents of protein (30.18, 27.75, 24.70, 24.26, 12.06 and 3.00%, respectively) compared with rice flour (7.6%). It could be pointed out that rice flour had the highest content of carbohydrates (91.11%) and the lowest content of ash and fat (0.41 and 0.60%, respectively). Sesame had the highest content of fat (46.14%), followed by pumpkin powder (40.01%) then, flaxseed powder, sunflower powder, karish cheese and finally psyllium husks 33.01, 30.13, 2.92 and 2.80%, respectively, compared with rice flour. From the same Table, it could be pointed out that flaxseed powder had the highest content of K, Mg, and Zn (803.00, 420.00, and 4.70 mg/100g, respectively).Also from the results presented in Table (1), sesame powder respectively, which had the highest content of Ca and Zn (418.00 and 2.50 mg/100g, respectively), followed by sunflower powder (159.00 and 1.30 mg/100g, respectively), then pumpkin powder (58.20 and 1.8 mg/100g, respectively), while it was seen that psyllium husks contain a high content of Fe and Zn (6.50 and 4.48mg/100g, respectively), compared with rice flour. These results agreed with Allam *et al.,* (2017); Akusu *et al.,* (2019) and Ziemichód *et al*., (2019).

Table 2: Proximate chemical composition of the main ingredients (% on dry weight basis).

Major components	Rice flour	Sunflower powder	Pumpkin powder	Sesame powder	Flaxseed powder	Karish cheese	Psyllium husk
Protein	7.60^{e} ±0.08	27.75^{b}±0.35	30.18^{a} ±0.42	24.26^{c}±0.61	24.70^{c}±0.78	12.06^{d}±0.09	3.00^{f}±0.09
Fats	0.60^{f}±0.04	30.13^{d}±1.57	40.01^{b} ±0.99	46.14^{a} ±2.06	33.01^{c} ±0.60	2.92^{e} ±0.14	2.80^{e}±0.55
Ash	0.41^{e} ±0.10	5.29^{a}±0.02	4.68^{ab} ±0.11	4.45^{bc} ±0.37	3.83^{c} ±0.03	1.02^{e} ±0.07	2.50^{d}±0.99
Crude fiber	0.28^{d}±0.02	3.00^{c}±0.21	3.11^{c}±0.08	5.51^{b}±0.52	5.85^{b}±0.35	0.00^{d}±0.00	75.24^{a}±0.15
Carbohydrates	91.11^{a}±0.24	33.83^{c} ±2.14	22.02^{d}±1.60	19.64^{d}±3.32	32.61 ^{c}±0.29	86.00^{b}±0.29	16.46^{e}±0.55
				Minerals (mg/100g)			
Potassium (K)	89.00^{f}±0.50	750.00^{b}±0.10	290.00^{d}±0.40	330.00^{c}±0.02	803.00^{a}±0.55	180.00^{e}±0.21	0.59^{g}±0.01
Calcium (Ca)	13.00^{f}±0.05	159.00^{d}±0.42	58.20^{e}±0.70	418.00^{b}±0.33	230.00^{c}±0.22	480.00^{a}±0.30	0.17 ^{g}±0.02
Magnesium(Mg)	48.00^{e}±0.20	240.00^{b}±0.10	70.33^{d}±0.02	210.00^{c}±0.04	420.00^{a}±0.07	30.00^{f}±0.50	0.12 ^{g}±0.01
Iron (Fe)	1.40^{d}±0.14	3.80^{b}±0.18	2.50^{c}±0.02	6.20^{a}±0.11	3.50^{b}±0.04	0.20^{e}±0.03	6.50^{a}±0.45
Zinc (Zn)	0.60^{e}±0.50	1.30^{d}±0.05	1.80^{c}±0.12	2.50^{b}±0.15	4.70^{a}±0.02	0.20^{f}±0.03	4.48 ^{a}±0.23
Manganese(Mn)	0.70^{e}±0.11	5.40^{a}±0.20	3.90^{b}±0.80	1.66^{d}±0.23	2.80^{c}±0.01	0.60^{e}±0.02	2.60^{c}±0.20

Values are means of three replicates ±SD, value in the same row followed by the same letter is not significantly different at 0.05.

3.2. Proximate chemical composition of gluten- free low carbohydrate kaiser bread

The approximate composition of gluten- free low carbohydrate kaiser bread (GLKB) was determined noticed and according to the obtained results presented in Table (3), it could be that the protein, fat, ash, crude fibers and energy content of GLKB prepared by sesame + pumpkin (T_1), sesame + sunflower (T_2) and sunflower + flaxseed (T_3) is higher than of control

Table 3: Proximate chemical composition of the gluten-free low-carbohydrates kaiser bread (GLKB) (% on dry weight basis).

Bread samples Constituents (%)	Control	T_1	T_2	T_3
Protein	7.69 ^{b} ±0.14	30.05 ^{a} ±0.10	29.56 ^{b} ±0.09	30.00 ^{a} ±0.20
Fats	5.61^{c}±0.05	26.63 ^{a} ±0.55	24.65 ^{b} ±0.28	20.42 ^{c} ±0.12
Ash	2.16^{d}±0.02	3.72 ^{a} ±0.03	3.845 ^{a} ±0.50	3.81 ^{a} ±0.44
Crude fiber	0.38 ^{d}±0.03	2.41 ^{a} ±0.05	2.39 ^{a} ±0.15	2.21 ^{a} ±0.45
Carbohydrates	84.16 ^{a}±0.21	37.19 ^{c} ±0.07	39.56 ^{c} ±0.14	43.54 ^{b} ±0.72
Starch	53.10^{a} ±0.68	12.00^{b} ±0.05	12.50^{b} ±0.18	12.68^{b} ±0.18
Total sugars	21.86^{a} ±0.09	10.17 ^{c} ±0.05	9.69 ^{d} ±0.09	12.94^{b} ±0.11
Energy (kcal/100g)	165.41^{d}±0.22	508.63 ^{a} ±0.77	498.33 ^{b} ±0.06	477.94 ^{c} ±0.23
		Minerals (mg/100g)		
Potassium (K)	85.20^{d}±0.60	340.97 ^{c}±0.28	436.7 ^{b}±0.25	573.3 ^{a}±0.44
Calcium (Ca)	11.50^{d}±0.75	445.33 ^{b}±0.03	480.10 ^{a}±0.03	416.6 ^{c}±0.03
Magnesium (Mg)	45.03^{d}±0.22	108.64 ^{c}±0.19	153.10 ^{b}±0. 66	198.1^{a}±0.22
Iron (Fe)	1.01^{c}±0.15	3.16 ^{a}±0.08	3.10 ^{a}±0.02	2.311 ^{b}±0.23
Zinc (Zn)	0.40^{c}±0.18	1.71^{b}±0.13	1.91^{b}±0.21	2.255 ^{a}±0.04
Manganese (Mn)	0.50^{b}±0.13	15.63 ^{a}±0.45	15.60 ^{a}±0. 50	16.20 ^{a}±0.14

Control = 100 % Rice flour.
T1= 100% fresh karish cheese+30 % sesame powder +20 % pumpkin seeds powder + 5% psyllium husks
T2= 100% fresh karish cheese+ 30 % sesame powder + 20 % sunflower seeds powder + 5 % psyllium husks
T3=100% fresh karish cheese+ 30 % sunflower powder + 20 % flaxseed powder + 5 % psyllium husks
Values are mean of three replicates ±SD, number in the same row followed by the same letter are not significantly different at 0.05 level.

The increase was due to the sesame, pumpkin, sunflower and flaxseeds which are good sources of these nutrients, and low in carbohydrates, starch and sugars. Consequently, GLKB is nutritious and beneficial for patients with celiac disease who depends on gluten-free bread that is high in carbohydrates and low in protein, fat and fiber. These result was in agreement with that found by Mukherjee and Abraham (2022).

In the same Table, the minerals content of GLKB was found to be higher than that of control. The increase was mainly because of the used seeds which contain high amounts of minerals. Thus, the bread made from these seeds with the use of psyllium husks and karish cheese improves the nutritional status of patients with digestive disorders (El-Sayed *et al*., 2016). Feinman *et al.,* (2006) observed that some of the low carbohydrate high fat diets focus on seeds affords a diet that is nutrient-dense and providing most of the essential nutrients.

3.3. Physical measurements of GLKB

The absence of a cohesive protein matrix, extensibility and elasticity of the cereal based gluten free batters is declined and lessened loaf volumes (Hager *et al.*, 2012). Results presented in Table (4) showed the effect of different formula on the physical measurements (weight, volume and specific volume) of GLKB. It could be observed that, the control sample which prepared from 100% rice flour had a weight of 27.00 g and volume of 30.00 cm^3 with specific volume of 1.11 cm^3/g, while the preparation of GLKB by 30% sesame powder + 20% pumpkin seeds powder (T_1), 30% sesame powder + 20% sunflower seeds powder (T_2) and 30% sunflower seeds powder + 20% flaxseed powder (T_3) caused an equal gradual increase in the volume and specific volume, and a decrease in weight of prepared bread parallel with the all different formula. The increase in GLKB volume may be due to the decrease in starch content which characterized by sesame, pumpkin, flaxseed and the use of psyllium husks. These results are agreement with Al-Subhi (2014).

Table 4: Physical measurements of gluten-free low-carbohydrates kaiser bread (GLKB**)**

Samples	Weight (g)	Volume (cm^3)	Specific volume(cm^3/g)
Control	27.00 ^{a}±0.20	30.00 ^{d} ±0.11	1.11 ^{b} ±0.33
T_1	20.50 ^{c} ±0.50	65.00 ^{c} ±0.40	3.17 ^{a} ±0.66
T_2	21.00 ^{bc}±0.99	67.00 ^{b} ±0.22	3.19 ^{a} ±0.21
T_3	22.00 ^{b} ±0.81	70.00 ^{a} ±0.80	3.18 ^{a} ±0.02

Control= 100 % Rice flour,
T1= 100% fresh karish cheese+30 % sesame seeds powder +20 % pumpkin seeds powder+5% psyllium seed husk**,**
T2= 100% fresh karish cheese+ 30 % sesame seeds powder + 20 % sunflower seeds powder + 5 % psyllium seed husk.
T3=100% fresh karish cheese+ 30 % sunflower seeds powder + 20 % flaxseeds powder + 5 % psyllium seed husk.
Values are mean of three replicates ±SD, number in the same column followed by the same letter are not significantly different at 0.05 level.

3.4. Color parameters of the GLKB

Table (5) displays the color measurements of the gluten-free low-carbohydrate kaiser bread (GLKB). Data indicated that the addition of different powder to the bread formula significantly ($p<0.05$) decreased the lightness (L^*) values of the crust and crumb of the bread samples compared with the control (T_1). T_1 bread had the lowest lightness values, and this may be due to the color of the powder. In comparison to control, the redness (a^*) values for the crust of different bread samples were higher. The different formula showed the maximum yellowness (b^*) values of the bread crumb, whereas the bread crust showed lower yellowness (b^*) values compared with control. Besides, T_2 recorded the highest crust b^* value (14.65) and T_1 recorded the highest crumb b^* value (24.75) compared with other bread samples. Gluten free control bread containing rice flour has a much lighter color than bread prepared from flaxseed powder (Ziemichód *et al.*, 2020).

Table 5: Color parameters of gluten-free low-carbohydrates kaiser bread (GLKB) (bread crust and crumb).

Bread samples	Crust			Crumb		
	*L**	*a**	*b**	*L**	*a**	*b**
Control	68.13^a±0.55	9.52^b±0.79	27.00^a±0.09	84.68^a±0.44	-1.55^c±0.11	13.31^c±0.71
T_1	56.50^c±0.12	10.04^b±0.53	13.02^c±0.34	82.36^b±1.70	-1.53^c±0.21	24.75^a±0.28
T_2	56.61^c±0.41	9.71^b±0.19	14.65^b±0.91	82.35^b±0.31	0.15^a±0.05	22.94^b±0.82
T_3	58.55^b±0.93	11.13^a±0.27	14.63^b±0.88	82.91^{ab}±0.88	-1.12^b±0.07	24.33^a±0.66

*L**= lightness, *a**= redness, *b**= yellowness, **Con.** = 100 % Rice flour.
T1= 100% fresh karish cheese+30 % sesame powder +20 % pumpkin seeds powder + 5% psyllium husks
T2= 100% fresh karish cheese+ 30 % sesame powder + 20 % sunflower seeds powder + 5 % psyllium husks
T3=100% fresh karish cheese+ 30 % sunflower powder + 20 % flax seed powder + 5 % psyllium husks.
Values are mean of three replicates ±SD, number in the same column followed by the same letter are not significantly different at 0.05 level.

3.5. Sensory evaluation of GLKB

Sensory evaluation is a crucial factor for an acceptable evaluation of applying sesame, pumpkin, sunflower, flaxseed and psyllium husks in the different formulation. The sensory evaluation results are illustrated (Table 6) demonstrated that texture, general appearance, taste, odor, sponge, crust color, crumb color, distribution of crumb and overall acceptability of all samples were not significantly different from each other compared to the control. The control sample had the lowest in all characteristic. The samples (T_1, T_2 and T_3) had the highest score in the all examinations. This shows that using sesame, pumpkin, sunflower and flaxseed in the formulation improved gluten-free bread. Samples with substitution had a darker crust color than the control sample.Gluten-free bread is made based on rice flour and has a light-yellow color which is not favorable. By applying the sesame, pumpkin, sunflower, and flaxseed in the formulation of bread, the color could be improved. This result coincides with those found by Al-Subhi, (2014). Regarding sponge scores, the control sample had the lowest sponge, while the T_1, T_2, and T_3 bread samples had the highest sponge and had the best taste. All samples were more acceptable to the consumer.

Table 6: Sensory evaluation of GLKB.

Samples	Texture (15)	General appearance (15)	Taste (15)	Odor (15)
Control	10.00^c±0.08	10.00^b±0.03	10.00^d±0.55	10.00^c±0.80
T_1	14.00^a±0.24	14.00^a±0.24	15.00^a±0.25	15.00^a±0.04
T_2	14.00^a±0.40	14.00^a±0.24	14.00^b±0.20	14.00^b±0.06
T_3	13.00^b±0.50	13.00^a±0.24	12.00^c±0.17	14.00^b±0.10
Samples	**Sponge (10)**	**Crust color (10)**	**Crumb color (10)**	**Distribution of crumb(10)**
Control	5.00^b±0.02	5.00^b±0.04	5.00^c±0.10	5.00^c±0.21
T_1	10.00^a±0.11	10.00^a±0.11	10.00^a±0.33	10.00^a±0.26
T_2	10.00^a±0.30	10.00^a±0.55	10.00^a±0.22	10.00^a±0.80
T_3	10.00^a±0.12	9.00^a±0.50	9.00^b±0.20	9.00^b±0.01

Control = 100 % Rice flour .
T1= 100% fresh karish cheese+30 % sesame powder +20 % pumpkin seeds powder + 5% psyllium husks
T2= 100% fresh karish cheese+ 30 % sesame powder + 20 % sunflower seeds powder + 5 % psyllium husks
T3=100% fresh karish cheese+ 30 % sunflower powder + 20 % flaxseed powder + 5 % psyllium husks
Values are mean of ten replicates ±SD, number in the same column followed by the same letter are not significantly different at 0.05 level.

3.6. Texture profile analysis of GLKB

The findings of the texture analysis for fresh bread, for zero time, 24, 48, 72, and 96 hours held at room temperature (27±3°C) are shown in Fig. (1). Psyllium reduces the hardness and chewiness of the bread and increases cohesion and resilience. The same figure, also, the decrease in bread hardness may be due to the presence of arabinoxylans in psyllium. Water was formerly believed to be the cause of this effect because it functions as a plasticizer in the gluten-starch composite matrix, reducing the stiffness of the final produced product. On the other hand, psyllium appears to work to improve water retention and, therefore, less drying out of the breads. The decrease in hardness may also be caused by a decrease in starch level. Additionally, all the seeds used to prepare bread have higher fat contents than the control sample, which increases the softness of product and decreases its hardness (Belorio *et al.,* 2020).

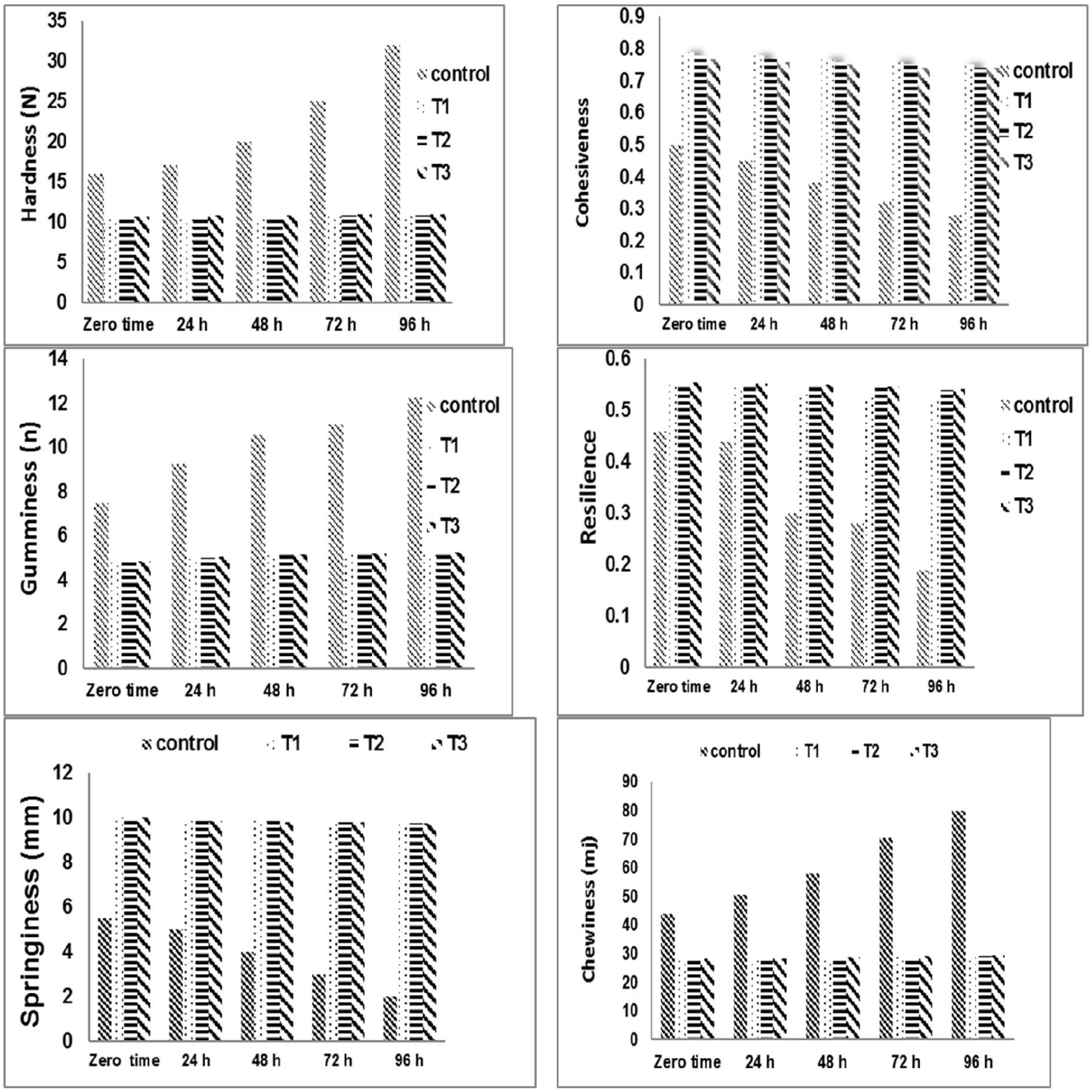

Fig. 1: Texture profile analysis of gluten-free low-carbohydrate kaiser bread (GLKB).

3.7. Shelf life of gluten-free low-carbohydrates kaiser bread during storage at different period at room temperature (27±3ºC)

The extension of shelf life by delaying bread staling is one of the biggest challenges for baking industry currently (Plessas *et al.*, 2008). The shelf life of bread is relatively short, mainly due to a number of physicochemical alterations that occur after baking and during storage. These alterations are known as bread staling, which is responsible for the disposal of large quantities of bread (8–10%), resulting in economic losses (Katina *et al.*, 2006). The quality of bread is rapidly lost not only due to staling but also due to microbial spoilage. Mould grows under ambient conditions, on well packaged wheat bread within 4 to 6 days (Sluimer, 2005). Shelf life of bread is generally limited due to the staling phenomenon and fungi spoilage, in particular moulds. Industrially produced bread normally operates with a shelf life of several days at room temperature (27±3°C). According to the results presented in Table (7), there were no signs of bread fungal spoilage after 3 days of inspection. The first signs of bread rope spoilage were noticed in the control (1) after 3 days, followed by T_3 and T_1 after 4 days, and T_2 showed the first signs of fungi spoilage after 5 days. This may be because each of the seed powder (sesame, sunflower, flaxseed, and pumpkin) contains antioxidants that are present in a particular order. According to Senanayake *et al.* (2019), these findings are consistent. The addition of psyllium seed husks caused water to bond, lengthening the shelf life. These findings are in the same line with Ren *et al.* (2020). Nsimba *et al.* (2008) observed that the indications of bread spoilage involved black, green and yellow coloration due to the presence of diverse fungi species, and the reason for the long bread shelf life was recognized to the presence of antioxidant compounds in seeds. El Khoury *et al.* (2018) mentioned that the absence of gluten in bread affect dough properties, bread quality and shelf life of gluten-free bread.

Table 7: Shelf life of gluten-free low-carbohydrates kaiser bread *

Samples	Storage period (days)					
	0	1	2	3	4	5
Control	-	-	-	+	++	+++
T_1	-	-	-	-	-	++
T_2	-	-	-	-	-	++
T_3	-	-	-	-	+	++

*Inhibition levels of growth fungi: (-)= no inhibition around the filter disc; (+)= inhibition with weak, almost undetectable zone; (++)= inhibition with detectable zone (diameters of 1- 2 cm); (+++)= strong inhibition with clear but irregular zone (diameters of 2- 4 cm).
Con. = 100 % Rice flour .
T1= 100% fresh karish cheese+30 % sesame powder +20 % pumpkin seeds powder + 5% psyllium husks
T2= 100% fresh karish cheese+ 30 % sesame powder + 20 % sunflower seeds powder + 5 % psyllium husks
T3=100% fresh karish cheese+ 30 % sunflower powder + 20 % flaxseed powder + 5 % psyllium husks

3.8. Nutritional characteristics of kaiser bread

Table 8 presented the calculated nutritional characteristics of kaiser bread based on Table (3) results. Results clarified that control sample covered 40.47; 9.50; 3.74; 1.00; 33.85 ; 10 and finally 6% of the RDA for children (4-8 years) in protein, energy, K, Ca, Mg, Fe and Zn, respectively. Besides, they also covered 13.03; 6.24; 2.53; 1.00; 10.78; 12.50 and 2.73% of the RDA for males (31-50 years) of these nutrients, respectively. Moreover, they also covered 15.38; 6.62; 3.31; 1.00; 13.75; 5.56 and 3.75% of the RDA for females (31-50years) in these nutrients, respectively. Concerning nutritional characteristics of T_1, it covered 158.16; 29.20; 14.82; 44.53; 83.57; 31.60 and 34.20% of the RDA for children in protein, energy, K, Ca, Mg, Fe and Zn, respectively. Moreover, they also covered 50.93; 19.19; 10.03; 44.53; 25.87; 39.50 and 15.55% of the RDA for males of these nutrients, respectively. Additionally, they also covered 60.10; 20.35; 13.11; 44.53; 33.95; 17.56 and 21.38% of the RDA for females of these nutrients, respectively. Concerning T_2, results demonstrated that each 100 g of T_2 bread provide 155.58; 28.20; 18.99; 48.01; 117.77; 31 and 38.20% of the RDA for children in protein, energy, K, Ca, Mg, Fe and Zn, respectively. Moreover, for males they provide 50.10, 18.80, 12.84, 48.01, 36.45; 38.75 and 17.36% of the RDA in these nutrients, respectively. Likewise, they covered 59.12; 19.93; 16.80; 48.01; 47.84; 17.22 and 23.88% of the RDA for females of these nutrients, respectively. Concerning RDA for T_3, data revealed that they covered 157.89; 27.44; 24.93; 41.66; 152.3; 23.11 and 45.10% of the RDA for children in protein, energy, K, Ca, Mg, Fe and Zn respectively. Additionally, for males they also covered 50.85; 18.04; 16.86; 41.66; 47.17; 28.88 and 20.50% of the RDA of these nutrients, respectively. For females they covered 60.00; 19.12; 22.05; 41.66; 61.91; 12.84 and 28.19% of the RDA of these nutrients, respectively. Dietary protein intake should represent about 15% of total calories in a gluten-free diet (Gorinstein *et al.*, 2002).

Table 8: Nutritional characteristics of some nutrient provided from 100g of GLKB for children (4-8 y), males and females (31-50 y).

			RDA from 100g GLKB samples (%)			
Age group	**Nutrients**	**RDA***	**Control**	**T_1**	**T_2**	**T_3**
Children (4-8 years)	**Protein (g)**	19	40.47	158.16	155.58	157.89
	Energy (K cal)	1742	9.50	29.20	28.20	27.44
	K (mg)	2300	3.74	14.82	18.99	24.93
	Ca (mg)	1000	1.00	44.53	48.01	41.66
	Mg (mg)	130	33.85	83.57	117.77	152.30
	Fe (mg)	10	10.00	31.60	31.00	23.11
	Zn (mg)	5	6.00	34.20	38.20	45.10
Males (31-50 years)	**Protein (g)**	59	13.03	50.93	50.10	50.85
	Energy (K cal)	2650	6.24	19.19	18.80	18.04
	K (mg)	3400	2.53	10.03	12.84	16.86
	Ca (mg)	1000	1.00	44.53	48.01	41.66
	Mg (mg)	420	10.78	25.87	36.45	47.17
	Fe (mg)	8	12.50	39.50	38.75	28.88
	Zn (mg)	11	2.73	15.55	17.36	20.50
Females (31-50 years)	**Protein (g)**	50	15.38	60.10	59.12	60.00
	Energy (K cal)	2500	6.62	20.35	19.93	19.12
	K (mg)	2600	3.31	13.11	16.80	22.05
	Ca (mg)	1000	1.00	44.53	48.01	41.66
	Mg (mg)	320	13.75	33.95	47.84	61.91
	Fe (mg)	18	5.56	17.56	17.22	12.84
	Zn (mg)	8	3.75	21.38	23.88	28.19

Con. = 100 % Rice flour.
T1= 100% fresh karish cheese+30 % sesame powder +20 % pumpkin seeds powder + 5% psyllium husks
T2= 100% fresh karish cheese+ 30 % sesame powder + 20 % sunflower seeds powder + 5 % psyllium husks
T3=100% fresh karish cheese+ 30 % sunflower powder + 20 % flaxseed powder + 5 % psyllium husks
RDA (%) = Value of nutrient in the sample of kaiser bread × 100 / RDA for the same nutrient based on Table 3 results.
*Source RDA (1989).

4. Conclusion

It is conceivable to draw the conclusion that adding sesame, sunflower, pumpkin, and golden flaxseed powder, karish cheese and psyllium husks to make gluten-free bread is both feasible and beneficial, producing high-quality bread with a sufficient nutrient. Thus, this new-formulated bread can be included in the diet of every age group, which will enhance sensory characteristics and increase nutrient intake while maintaining good health and promoting immunity against infections. The use of a gluten-free low carbohydrate, high nutritional kaiser bread is a desirable opportunity for overweight and diabetic patients.

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