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ORIGINAL ARTICLE

Physicochemical and sensory cake characteristics, Gluten-free enriched with iron and folic acid and different concentrations of guar gum

[1]Rojina Khodarahmi Roozbahani, [2]Javad Keramat, [3]AbdolReza Mohamadi Nafechi
[1]MSc in Food Science and Industry ,Islamic Azad University,Damghan Branch
[2]PhD in Chemical and Food Technology Engineering ,the University of Reading, England
[3]Biopolymer engineering doctorate from the University of USM, Malaysia
Email: RozhinRouz@gmail.com

ABSTRACT
The aim of this research, is Producing gluten free cake enriched with iron and folic acid for fixing the nutritional needs of patients with celiac disease. For this purpose, was used rice Flour and corn flour to make cakes without gluten. To optimize the formulation, was used Guar gum at three levels of 0, 5.0 and 1% (by the weight of rice flour). Physico-chemical properties of ash in acid soluble, Moisture, acidity, fat, energy, color, tissue firmness, specific volume, amount of iron, and also microbiological and sensory characteristics were evaluated for 21 days. The amount of iron remained stable during storage, with increasing levels of guar gum in the formulation Special volume significantly increased ($p<0.05$). And simples' tissue firmness also decreased significantly ($p<0.05$). As a result of sensory evaluation, treatment with 0.5% of guar gum was recognized as the best treatment.
Key words: celiac, gluten free cake, enriched with iron and folic acid, rice flour, corn flour, guar gum.

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INTRODUCTION

Celiac disease, (Known as non-tropical diarrhea, Gluten sensitive enteropathy, diarrhea celiac, gluten and celiac disease adults) is a situation in which the person reacts to gluten [1]. Removing gluten from the diet leads to improved clinical symptoms and mucosal injury and the only way for treated the celiac disease is Avoiding gluten a lifelong. It causes stretching the dough and crispy texture of final product. In fact, the most important factor of gluten is about the size and texture uniformity, efficiency and water absorption and gas retention ability, taste and quality of wheat flour products [2, 3, and 4]. Grains Protein are gluten-free such as white rice and corn [2]. In situations gluten-free flours such as white rice or corn flour used in product manufacturing .Can be used a variety of hydrocolloids such as guar gum, fix problems caused by the absence of gluten. Gularte *et al* [5] looked the effect of soluble fiber (inulin and guar gum) and insoluble (barley fiber) individually and combined to use up to 20% of rice flour on dough properties and gluten-free layer cake. The results showed that except of inulin fiber composition, paste viscosity increase. Gluten-free cake enriched with barley fiber composition And inulin have more volume. Except of combination of guar gum and barley, the presence of fibers resulting in cake to become brighter color. And generally the fiber composition of barley with inulin, Get better results in gluten-free cake made from rice flour [5]. Also Turabi and *et al* [6] compared the Characteristics of gluten free cakes made from rice flour containing xanthan gums, Guar, carob, κ xanthan-guar -Karagynan and mixed with gluten free cake without gum as compared to the control sample. It was observed that the addition of different types of gum effect on present of pores of rice cakes. Most of the pores is in the cake containing xanthan and guar were mixed Zantan- [6]. In a study in 1989, Accept cake with three different levels of guar gum (0, 5, 10 and 15% relative to the flour) using hedonic sensory evaluation examined although the cakes prepared with more than 10% guar gum, significantly have lower admission Compared to the control sample

(containing 0% guar) However, all treatments had a mean score above 5 that means it is acceptable [7]. Naghipour and et al (2011), in a study investigate the impact of Guar and xanthan gums each one in three levels: 5.0, 0.1 and 5/1% on the quantity and quality of gluten free cakes. The results showed that the addition of guar gum and xanthan gum in all three levels significantly ($p<0.05$) Can reduce firmness, 24 and 72 hours after baking than the control sample gum; However, that between 0.1 and 5.1 percent guar gum xanthan gum significantly different ($P<0.05$)there is decrease in firmness, in both time periods (8). Celiac disease increases the need for folic acid (9). Kiskini et al (2007) in research, examined the taste Characteristics of gluten-free breads fortified with iron. They use, ferric pyrophosphate of, ferric pyrophosphate and emulsifier, NaFeEDTA, electric iron, ferrous gluconate, ferrous lactate and ferrous sulfate to enrichment. And then taste Characteristics of such as mouth feel, texture, color, aroma and taste of the products were evaluated. Ferric pyrophosphate preparations which were produced with an emulsifier, sensory acceptance gained the most points (01).

Materials and methods:

Raw materials used to produce cakes include sugar (11), eggs (12), oil (13), flour (14), vanilla (15) and guar gum (16), premix Powder for flour fortification was prepared from medicinal chemistry Shafei. All chemicals used in chemical and biological experiments were also laboratory.

Methods:

The physic-chemical tests on flour.

- Organoleptic characteristics: Sensory properties of corn flour and rice flour such as smell and taste, Color, pesticides and foreign matter in accordance with the national standard 10497 and 11136, respectively, were studied.
- Humidity: Flour, rice and corn moisture measurement in the usual way oven in accordance with the national standard of Avon Investment No. 2705 (17) was performed.
- Protein: Measured according to National Standard number 2863 (18) was performed. The principles of this method is the oxidation of organic material in the sample by thick sulfuric acid and in the presence of a catalyst, as a result of nitrogen in the sample will be converted to ammonium sulfate And then by ammonium sulfate Released ammonium and distillation it And release ammonia absorbs acid a specific title After titration to calculate the amount of free ammonia And the amount of ammonia nitrogen account in the sample And by multiplying the amount of nitrogen In the body weights of the test sample determines the amount of protein is followed.
- Ash: Measurement of ash according to the national standard number 2706 (Determination of ash grain and its products) was performed. The acid insoluble ash measurement is accordance with ISO 11136 (rice flour, specifications and test methods) was performed thus:

The amount of 2 to 5 g of sample accurately to fixed weight was previously weighed In Chinese plant.Then was placed in a furnace at 600 ° C temperature until the white Color become gray Plants were removed from the furnace to cool at room temperature. Plants were removed from the furnace to cool at room temperature. 25 ml of 5 N hydrochloric acid was added to it and then covered with a watch glass and was boiled for 10 minutes in the bainmarie. Sometimes stirred and then cleared by filter paper. Filter paper washed with distilled water so as to be free from hydrochloric acid (at blue litmus paper was investigated). The filter paper in the same plant again burned and in the furnace become ash. Plant after desiccating cooled and then weighed. On the other hand, 25 ml of hydrochloric acid by a similar paper filtered And in a Chinese plant that has already reached a constant weight, was placed. Then it burned and in the furnace-like sample ashe and cold desiccator and then weighed. Finally, through the following formula insoluble ash in acid per 100 grams of sample were obtained:

$$\text{Ash insoluble in per 100 g acid sample} = \frac{(w_1 - w) - \left(w_1^{'} - w^{'}\right)}{w_2} \times 100$$

In which:

W1: weight of the plant containing the ashes Insoluble in sample acid in grams.

W: weight of the empty plant in grams

W'_1: weight of the plant containing the ashes Insoluble in acid Filter paper in grams.

W': The weight of empty plant to determine insoluble of ash in acid used filter paper in grams.

W2: weight in grams

- pH: PH measures in accordance with the national standard 11136 as follows: 10 grams of flour with 100 ml of freshly boiled distilled water were thoroughly mixed and 20 minutes to be deposited, then, without smoothing, the upper solution by pH meter instrument, previously was determined, according to the pH of the solution was adjusted with a buffer solution.

- 1 Particle size: The size of the constituent particles of flour in accordance with the national standard 11136 took place: Strainers in size 106 to 475 microns in order from bottom to top on the device vibrate sieve, was put with the rotational motion. 100 gram samples of flour weighted and on the 475 micron sieve is poured and then the power is turned on To work for 5 minutes, After this time, the device was turned off. And the remaining flour on the each Strainers and the bottom sifting (underside) was weighing and flour Particle size was calculated based on present.
- Flour fortification with iron and measuring the amount of iron in flour: Business powder mixed with flour was added by micro feeder. The addition of premixes according to the instructions of the Ministry of Health of 200 grams to a ton of flour (19). Flour enrichment program in Iran spot test because of its simplicity, is considered in the factory. Spot test is the semi-quantitative that shows the presence of iron-enriched in flour. And by comparing the color intensity and the number of them can be quite the amount of iron in the flour to be identified. The method of determining the exact amount of iron in fortified flour, is spectrophotometer method. The amount of iron in the flour of this study was determine by both methods.

Preparing the cake

For each treatment, the amount of one kilogram cake was created. To prepare, First of all egg (16.34%) with vanilla (0.02%) good staring and then sugar (22.6%) were added. And stirred so that the liquid volume to be doubled .The oil (3.76%) is added and stirred another minute. Half of boiled water (3.76%) was added and the mixture is stirred little else. Then half of the flour (corn flour, rice flour 30.26% and 5.44%) is added. The rest of the flour and gradually add boiling water and after a good staring mix, the mixture is poured into the mold. How set molding the dough can be achieved as well as expansion and to gain the necessary volume. To prevent adhesion of cake batter into the mold, the mold was placed on with special paper. Then the molds in the oven for 45 minutes with the temperature of 175 ° C was placed. After curing, samples were cooled so that the remaining moisture from sweating on the cake and thus prevent dough cake texture that cause the lowers quality and providing the conditions for microbial spoilage. This combination is intended as a witness and to evaluate the effect of the enrichment process and the effect of guar gum on physicochemical and sensory properties of the formulation Guar gum at two levels of 0.5and 1 percent rice flour was added to the formulation. In addition to the effect of flour fortification on physicochemical properties, Microbial and sensory cake, a series of treatments with the enriched flour were produced.

Table 1: Treatments and its compounds.

The combination of treatment	Naming treatment	enrichment	The amount of guar gum
No gum, not enrichment gluten free cake	B	-	0.0%
Gluten-free cake enriched without gum	F	+	
Gluten-free cake not enriched containing 5/0% gum	B1	-	0.5%
Gluten-free cake enriched containing 5/0% gum	F1	+	
Gluten-free cake not enriched containing 0.1% gum	B2	-	0.1%
Gluten-free cake enriched containing 0.1% gum	F2	+	

Physicochemical tests on samples of cake:

4.1 Acidity of extracted fat: measured according to Iran National standard number 37, for this purpose, 5.2 to 3 grams extracted oil in a 250-ml Erlenmeyer flasks weighted, And 30 ml of warm neutralized alcohol And 2 ml of phenolphthalein added And soda solution 0.01 to appearance the color pink And fixed for 30 seconds to become headline.

Free fatty acids are based on oleic acid in per 100 grams of sample was calculated according to the following formula:

$$Q = \frac{28/2 \times N \times V}{W}$$

In which: V: is the volume consumed benefit (ml)

N: normality consumed soda solution.

W: weight of sample (gr).

Q: free fatty acids based on oleic acid.

Acid soluble ash: This test with the relevant standard completely described in the assessment flour acid soluble ash.

Energy: Atwater system used to measure the energy of the cake samples. According to this system, the amount of protein, carbohydrate (excluding fiber) and fat content in food to be determined. Then For

each gram of protein and carbohydrate 4 kcal for each gram And 9 kcal for each gram of fat is calculated (21).

Ferric: Measure the amount of iron in the sample based on the national standard No. 5688 (grain and its products Iron spectrum measurement survey) was conducted. Under this method, Dry or wet ash sample, Solving it and converting trivalent iron which is probably in there With hydroxyl amine hydrochloride divalent iron or alpha - alpha-dipyridyl in an acid environment. To a complex blend of red orange in the pH range between 2 and 9 is stable. After that, absorbance is determined by spectrum detector and the iron content from the the reading curve is measured.

Color: Colorimetric samples were measured using a Hunter Lab Colorimeter. For the colorimetric value of the sample put inside the chamber. And the biggest square of the software that can colorimeter to be able to cover the greatest surface of sample as the surface Colorimeter was selected. Color expression using color parameters L *, a *, b * was done. Colorimeter using a black and white paper was standardized. The test was performed in triplicate for each treatment.

Special Size: To measure the amount of cakes of alternative volume with the canola seed according to standard AACC, 2000 No. 72-10 was used.

For this purpose, a piece with dimensions of 20 × 20 × 20 mm of the geometric center of the cake preparation and the specific volume was determined.

Texture: To test the firmness of texture was used of Texture Analyzer. For this purpose, After cutting the cake samples with dimensions of 20 × 20 × 20 mm of the geometric center of the cake, In containers impervious to air and were capping. In texture profile analysis, Samples up to 50% of the initial height (depth 10 mm) were pressed by machine. Used probe diameter 36 mm and the penetration rate was 5.0 mm per second.

- Microbiological tests on samples of cake: Bacterial tests in accordance with the national standard 2935 first amendment (22) was performed. The culture medium used for the total count of yeasts and molds was Rose Bengal culture medium .And rabbit citrate plasma .From culture medium lauryl sulfate double And AC broth for coli bacteria. Simon environments citrate agar, blood agar, urea agar-agar and brilliant green lactose broth, Tetrathionate broth, Salmonella, Shigella Agar and the TSA was used.
- Sensory tests on samples of cake:
  In evaluating the sensory characteristics of appearance, texture, flavor, color and overall acceptability of the samples were tested. Hedonic test criteria with 5 score were selected for evaluation. For this purpose, 7 Evaluation of patients with celiac disease, celiac association of judges were selected. Necessary training on how conduct the test And the interval between tests were given to them. Then they were asked before the start of the original study, a glass of water that was provide them to eat till all their Mouth conditions become similar.Reviews was conducted in this way that all evaluators examined the control sample,Then attempt to fill out the form evaluated.5 minutes after filling the form, again like before a glass of water were consumed. And the same process was repeated. Evaluators were unaware from the first sample thatis the Control samples. The Respective use of the samples was the same for all referees. An example of an evaluation questionnaire is provided in the Annex A.For statistical analysis study, to each of the comments received give a score as follows:5 = very good, 4 = good, 3 = average, low = 2, bad = 1.
- Statistical analysis: for data Statistical analysis Software (SPSS statistics .19) was used. A category factorial as a completely randomized design was selected for this study. Due to the time involved in assessments, data Statistical test of analysis variance (ANOVA with repeated measure) was analyzed. If the difference were significance the comparison between each treatment means using (LSD).Statistical surveys at 95 percent ($p < 0.05$) was performed. All tests were repeated three times. Charts with software (Excel .2007) were drawn.
- In the sensory evaluation test(Friedman)was selected. This test when the data are not normal, a good alternative for one-way analysis of variance (ANOVA with repeated measure one-way)When evaluating operating At the same time with Other factors considered. To check for significant differences between individual group of test (Wilcoxon test)were used.

RESULTS

Test results sensory characteristics of flour: Each of the flour used like international number 11136 and 10497 has its own flavor, Natural color and no pests and foreign substances there isn't in them.

Results of physico-chemical characteristics of flour: The average three times physicochemical tests moisture, pH, Protein on a dry matter(percent by weight), Ash on dry matter (percent by weight), Ashes in soluble in acid based on the dry matter(percent by weight) and the particle size of the rice

flour and corn meal used in this study are listed in Table2. All results of the national standard features of corn starch 10497and No.11136 was consistent national standard properties of rice flour.

Table2: The physico-chemical properties of flour used in this study

Approximately acceptable standard	Some results	Flours	Features
10 Maximum	69/9±05/0	Rice	Moisture(percent by weight)
Maximum 15	11/13±03/0	Corn	
7-6	21/6±00/0	Rice	pH
7-6	19/6±05/0	Corn	
5/7	11/8±23/0	Rice	Protein on dry matter basis (weight percent)
Minimum 5/8	96/8±16/0	Corn	
Maximum 9/0	42/7±05/0	Rice	Ash on dry substance (percent by weight)
9/0 betweento5/1	93/9±00/0	Corn	
Maximum 06/0	05/0±00/0	Rice	Ashes in soluble in acid based on the dry matter(percent by weight)
Not mentioned in the standard	-	Corn	
Particles larger than 475 mm, maximum 1 Particles larger than 180 mm, maximum 40 Particles larger than 125 mm, maximum 25 Particles smaller than 125 micrometers, maximum 30	Particles larger than475 micrometers, Particles larger than180 micrometers, 29/1 Particles larger than125 micrometers, 96/23 Particles smaller than125 micrometers, 3/13	Rice	particle size
100%passing5.0mmflourof sieve 100%passing1mmflour of sieve For flour semi-rough	Particles larger than475micrometers, 1 Particles larger than180 micrometers, 67/30 Particles larger than125 micrometers, 18/31 Particles smaller than125 micrometers, 2/11	Corn	

Specified numbers mean±standard deviation is repeated three times.

Test results measuring the amount of iron in flour: The results of measuring the amount of iron in the samples by the tests pot, And spectrophotometric method are listed in Table 3. Flour, rice and corn each of them has iron in them, Thus enriching the flour Add the Ministry of Health guidelines, As expected, the final iron is 30ppm , The iron rice flour and corn flour, respectively 33ppm and 5/30 ppm increased.

Table 3: quantities of iron-enriched flour

The amount of iron	Flours	method
ppm45-30about	Rice	Spot test
ppm 45-30about	Corn	
00/33±00/0ppm	Rice	Spectrophotometry
50/30±00/0ppm	Corn	

The results of physicochemical cake:

Acidity: Over time, significantly acidity of samples increases (P <0.05). The reason of this increase, breaks down fats into fatty acids by lipase enzyme [2] Among other treatments Acidity difference was not significant.

Energy: The amount of cake energy products produced in this study, was measured using at water. The average energy cake samples 02/0 ± 7/301 kilo calorie,was calculated.

Ashes: By increasing the amount of hydrocolloid in the formulation, The amount of ash significantly increased (P<05/0). The amount of ash-enriched in samples also significantly is higher than the other samples (P <05).Due to iron overload in these samples compared to those that not rich, it is obvious.

Iron: The amount of iron in samples enriched with iron significantly is higher than non-enriched (P <0.05). This increase is quite obviously. With the passage of time during the storage period of 21 days, samples did not change the amount of iron.

Color: The reason for this is the improvement of the cake by hydrocolloids, This means that hydrocolloid to attract and retain more water during the storage Makes the cake texture is more smooth surface and therefore is able to reflect more light.

Increase the amount of gum in the formulation resulted in a significant increase in volume (P<0.05).The reason for this is, The ability to attract and retain more moisture hydrocolloid in the process of create a paste and baking. Over time, the sample size was reduced significantly, The reason of the volume loss, is loss of moisture and firmness.

Firmness of texture: Increasing the amount of hydrocolloids in texture, firmness dramatically decreases. Due to hold more moisture, is resulting in a softer texture by hydrocolloid. Over time, the amount of hardness is faced a significant increase. Part of this increase is due by the moisture loss, and partly due to the staling phenomenon. Enrichment with iron showed there is nosignificant effect on the firmness of texture:(P <0.05)

Humidity: By increasing the amount of guar gum in the formulation, ,The amount of moisture due to high capacity of guar gum to absorb and hold water during the curing process and maintenance, significantly increased (P<0.05). The moisture in the treatment enriched with iron is lower than the other one that isn'tenriched with iron. But this difference was not statistically significant. Over time, the amount of moisture content of samples on the seventh day to the first day significantly decreased. But with increasing shelf life more than 7 days, there was no significant change in moisture content (P <0.05). Low humidity in the first week due to water loss based on evaporation from the formulation is due to package; But with more time, The combination of water and air of package has reached equilibrium and moisture content will barely change.

Bacterial cake Test Results :The results of this study are given in Table 4. Amount of *Escherichia coli*, *Staphylococcus aureus* and *coagulase*-positive in each gram and in each 25 grams samples were negative for salmonella. Amount of Enterobacteria in all cases is less than10 CFU/g And was in the standard range. Amount of mildew and yeast, which in all cases is less than100CFU/g and was in the standard range.

Table 4: Results of microbial treatments in different intervals.

Experimental treatments						Maintenance time (the day)	Type of microorganism
F2	B2	F1	B1	F	B		
10 >	10 >	10 >	10 >	10 >	10 >	1	(CFu/g) Enterobacteriaceae
10 >	10 >	10 >	10 >	10 >	10 >	7	
10 >	10 >	10 >	10 >	10 >	10 >	14	
10 >	10 >	10 >	10 >	10 >	10 >	21	
Negative	Negative	Negative	Negative	Negative	Negative	1	*E.coli* (gram)
Negative	Negative	Negative	Negative	Negative	Negative	7	
Negative	Negative	Negative	Negative	Negative	Negative	14	
Negative	Negative	Negative	Negative	Negative	Negative	21	
Negative	Negative	Negative	Negative	Negative	Negative	1	*Staphylococcus coagulase*+ (gram)
Negative	Negative	Negative	Negative	Negative	Negative	7	
Negative	Negative	Negative	Negative	Negative	Negative	14	
Negative	Negative	Negative	Negative	Negative	Negative	21	
Negative	Negative	Negative	Negative	Negative	Negative	1	Salmonella (in 25gram)
Negative	Negative	Negative	Negative	Negative	Negative	7	
Negative	Negative	Negative	Negative	Negative	Negative	14	
Negative	Negative	Negative	Negative	Negative	Negative	21	
100 >	100 >	100 >	100 >	100 >	100 >	1	Mildew (CFU/g)
100 >	100 >	100 >	100 >	100 >	100 >	7	
100 >	100 >	100 >	100 >	100 >	100 >	14	
100 >	100 >	100 >	100 >	100 >	100 >	21	
100 >	100 >	100 >	100 >	100 >	100 >	1	(CFU/g) Yeast
100 >	100 >	100 >	100 >	100 >	100 >	7	
100 >	100 >	100 >	100 >	100 >	100 >	14	
100 >	100 >	100 >	100 >	100 >	100 >	21	

Sensory cake Test Results:

In this study, iron-fortified cake samples and in samples 33ppm was not rich cake. So the amount of iron in the samples enriched with iron are significantly more than non-enriched samples ($p<0.05$) And due to the increase of iron in this sample compared to non-enriched samples, along with an increase in the amount of ash hydrocolloids significantly increased ($P <0.05$).Another factor is color. Color is one of the important aspects of the quality of processed and unprocessed and it reflects changes in the chemical changes in the thermal processes such as browning, caramelization, roasting and drying [25]. The results of the study by Naghipour *et al* [8] also matched. Polis and Salvadori [26] described improvement the texture of the cake by hydrocolloids.

That is a hydrocolloid with attracting and keeping more water during the storage makes the texture of the cake will have a more smooth surface and therefore has the ability to reflect more light. According to the research by Karadzhov *et al* [27] in Russia, adding 15-35 mg of iron per kg flour darker color would be flour.

In this Kuyper and Koch [28] also flour fortification with calcium darker brown color of the flour became white.

Loss of moisture and the resulting increase in concentration caused by the browning colors, which are insoluble in water [29]. In general, the color yellow cake can be carotenoids found in egg yolk and attributed to corn flour [30]. Enriched with iron showed no impact on the amount of jaundice.. Dexter [31] showed that micronutrient status Pasta flour mixed with thiamin, riboflavin, niacin and iron. The results showed that enrich impact the color of spaghetti. An increase in resin volume will increase and the volume decrease with time and cause of this decline is the loss of moisture and firmness. In the study Naghipour *et al* [8] found that the use of xanthan gum and guar especially in combination, volume increased and improved sensory properties. In addition, firmness also fell.

Sedaghti *et al* [32] also uses whey protein to produce bread without gluten for celiac patients, respectively. The results indicated that the specific volume of bread increased with increasing dose of whey protein [32]. With the increase, the hydrocolloids in texture reduced firmness dramatically. Hydrocolloids due to hold more moisture and thus softening the tissue. Over time, the hardness is faced with a significant increase. Part of this increase is related to the loss of moisture, and partly related to the staling phenomenon. Ahlborn and colleagues have suggested that the use of hydrocolloids according to a woven fabric is similar to the gluten network. As well as the absorption of water in cereal products improves the quality of these products.

The process of enrichment with iron and folic acid significantly different in terms of acceptance of flavor didn't occur in terms of evaluating. The points significantly decreased flavor over time. According to lose moisture during storage, we can say that it has been lost probably fragrance and taste of the water-soluble compounds. On the other hand part of the volatile essential oils are separated from the products. Changes fragrance and flavor of staling are also effective in the field and to extend the shelf, the mean score in tissue samples containing hydrocolloids significantly increased compared to the non-hydrocolloid and hydrocolloid high ability to retain moisture and stay soft tissues as a result of its products ut much moisture is in the desired product will leading to a softening .IN addition, it will also provide growth and spoilage microbes and finally in the twenty-first treatments of gum got 0.5% points more than t 1% of gum treatments. Vatankhah *et al* [33], with increasing gum tragacanth and orchids in gluten-free bread made from flour was slow than the process moisture loss. Finally add a lot of value not only improves the quality of gum tissue samples were obtained, but also strongly reduced quality. Other experts have also concluded . Evaluation of gluten-free cake samples containing 0.5 gum was recognized as the best samples during storage did not cause a change in the enrichment process evaluation. In research by Arestani Brass *et al* [34] fortify wheat flour with iron, folic acid, zinc and calcium had no significant effect on the sensory properties and shelf life of bread.

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