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Shelf-stability of Gluten Free Cakes Produced from Quinoa Flour and Chia Gel

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Abstract: *Bakery products, particularly cakes, represent one of the most consumed foods around the world. The development of gluten-free cakes with the same flavour and texture properties similar to the conventional wheat flour may be an interesting objective. In the market there is a challenge in increasing shelf life for gluten-free cakes, so the aim of this study was to assess shelf-stability of new formulations cupcake. Cake samples were prepared by replacing quinoa flour with wheat flour and chia mucilage gel with shortening at varying levels (0%, 25%, 50%, and 100%). The cake samples stored at 4, 20 and 37ºC for 8 daysand analysed every two days. Microbial contents were determined using serial dilutions method on Nutrient agar medium for bacteria. Mold and yeast were detected on Potato-Dextrose (PDA) Agar. The results showed that the microbial, mold and yeast contents didn't appear at 4, 20 or 37ºC up to 6 days for all the examined cakes. So, the cake that prepared by replacing quinoa flour with wheat flour and chia mucilage gel with shortening at varying levels (0%, 25%, 50%, and 100%) are safe and ready to eat when the storage temperature is (25±2ºC).*

Kay words: *Shelf-stability, Gluten, Cakes, Quinoa, Flour, Chia Gel*

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I. Introduction

From a commercial perspective, the development of gluten-free cakes with the same flavor and texture properties similar to the conventional wheat flour maybe an interesting objective. Bakery products, particularly cakes, represent one of the most consumed foods around the world. Cupcakes, sponge cakes, muffins, or traditional small cakes (madeleine) are connected in the consumer's mind as a delicious product with particular organoleptic characteristics (Matsakidou *et al*., 2010). The worldwide market of cakes currently grows about 1.5% a year. There are challenges in this market include cost reduction, increased shelf life, and quality control (Wilderjans *et al*., 2013).

Substitution of wheat flour requires finding other flours from rice, beans, and the "ancient grains" like quinoa, millet, amaranth, teff and sorghum, which do not have gluten. Some of these flours do not generate the same texture, and these alternative flours can generate different flavor profiles (Wilson, 2011).

Currently at marketplace, many of available gluten-free cakes are of low quality, exhibiting poor mouth-feel and flavor (Peressini *et al*., 2011). The allergen-and gluten-free bakery industry typically must select from a broad range of ingredients to achieve the same level of functionality as in conventional formulas. During gluten-free baking, these ingredients require to replace the attributes that gluten lends to breads or baked products. When formulating products with gluten-free flour, moisture content is sensitive. If baking things that is expected to rise and the dough is dry, it will be too dense. If the dough is too moist, the rise will be good but it will fall down during breaking (Martínez-Monzó *et al*., 2013).

In the baking industry, lipids provide characteristics such as tenderness, moistness, lubricity, mouth feel, flavor, structure, and shelf life. The lipids enhance the air incorporation during mixing to give a softer structure and avoid a dry mouth feel. The trapped air bubbles accumulate water vapor, and the gas provided by the dough (Casas Moreno *et al*., 2015).

Some previous studies have focused on improving sensory and nutritional profiles of cakes (Agrahar-Murugkar *et al*., 2016, Casas Moreno *et al*., 2015) with attention to proper diet. Additional studies have focused on developing low-fat cake recipes using emulsifiers, fat mimetics, and inter esterified shortening made from sunflower oil (Felisberto *et al*., 2015, Marconi *et al*., 2014, Martínez-Cervera *et al*., 2011, Psimouli and Oreopoulou, 2013).One previous study, Marconi *et al*., (2014) used 60% and70% of high oleic sunflower oil and water to replace saturated fatty acids in shortbread and concluded that high oleic sunflower oil is a good saturated fatty acids replacer because it had the best sensory profile.

Quinoa known as a complete food because its high nutritional value (Jancurová *et al*., 2009). It can use either as whole or parts of the plants (Mujica and Jacobsen, 2006).This seed has been attracting attention because of the nutritional value and quality of its proteins (Ranhotra *et al*., 1993). It is rich in the essential amino acid lysine that makes it a more complete protein than many vegetables. It does not contain gluten, so it can be eaten by people who have celiac disease as well as by those who are allergic to wheat (James, 2009). Quinoa is especially rich in an antioxidant called quercetin and the researchers conclude that quinoa and other traditional crops from the Peruvian Andes have potential in developing effective dietary strategies for managing type 2 diabetes and associated hypertension (Ranilla *et al*., 2009).Also, the studies show the effective in helping with appetite control (Berti *et al*., 2005).Quinoa has a good effect on lowering free fatty acid levels and triglyceride concentrations (Berti *et al*., 2004).

The use of chia may be in the form of whole seeds, mucilage, flour and oil seed (da Silva Marineli *et al*., 2014**).**Chia seed is an excellent source of omega-3/omega-6 fatty acids, soluble dietary fiber and contains appreciable amount of proteins and phytochemicals. It thus has nutritional attributes which support the prevention of several non-communicable diseases such as hypertension, obesity, cardio-vascular disease (CVD's), cancer and diabetes (Suri *et al*., 2016).The aim of this study was to assessshelf-stability of newformulations cupcake made by replacing quinoa flour with wheat flour and chia mucilage gel with shortening at varying levels (0%, 25%, 50%, and 100%) and microbial counts at different temperatures were determined.

II. Materials and Methods

1-Materials used

All cake ingredients (quinoa flour, chia seed, wheat flour, sucrose, shortening, whole fresh egg, milk powder, baking powder and vanilla) were collected from local markets in Jeddah, Saudi Arabia.

2- Chia mucilage gel preparation:

The chia mucilage gel (CMG) was prepared according to Felisberto *et al*., (2015). It was prepared from 20 g grind chia seed then and mixing with a Mixer (Philips HR1456) with 100 ml water and leaving to rest for 30 min. at room temperature.

3- Cakes preparation:

Cup cakes (control samples) were prepared as described by Bennion *et al*., (1997) with the following recipe of wheat flour, sucrose, shortening, whole fresh egg, milk powder, baking powder and vanilla. Quinoa and chia mucilage gel cup cakes were prepared with the same recipe but shortening was replaced by CMG at different levels of substitution: 25, 50 and 100 %. Also, wheat flour was replaced by quinoa flour at different levels of substitution: 25, 50 and 100 %.Table 1 gave the percentage formulas for quinoa and chia mucilage gel cup cakes.

Table1: The percentage Quinoa and Chia in different formulas of the 4 prepared quinoa and chia mucilage gel cup cakes.

Cake type	Type 1 (C)	Type 2 (Q1)	Type 3 (Q2)	Type 4 (Q3)
Ingredients (g)	Quinoa and Chia (0.0%)	Quinoa and Chia (25%)	Quinoa and Chia (50%)	Quinoa and Chia (100%)
Quinoa Flour (g)	-	25	50	100
Chia Gel(g)	-	25	50	100
Wheat Flour (g)	100	75	50	-
Shortening (g)	100	75	50	-
Sucrose (g)	85	85	85	85
Whole Fresh Egg (g)	110	110	110	110
Milk Powder (g)	3.0	3.0	3.0	3.0
Baking Powder (g)	3.8	3.8	3.8	3.8
Vanilla (g)	2.0	2.0	2.0	2.0
Orange Peel (g)	5.0	5.0	5.0	5.0

Cake samples were prepared by creaming of shortening and sugar using a kitchen machine (Philips HR1456)at medium speed (for gluten cake samples, shortening was replaced by CMG and mixing with sugar), egg-vanilla mixture was gradually added and beaten for 5 min. Free gluten cake samples were prepared by replacing wheat flour by quinoa flour. The different flour samples (wheat flour and quinoa flour) and baking powder mixture were gradually added to above mixture and mixed at high speed for 5 min. The batter was scaled at 110 gram in baking pans then, placed in a preheated oven and baked at 180°C for 35 min. After baking

different prepared cake samples were allowed to cool for 30 min., then packed in polyethylene bags at room temperature until analysis.

4-Shelf-stability:

The cakes were packaged in polyethyleneplastic bags and stored at 4±2ºC, 20±2ºC and 37±2ºC for bacterial analysis and at 25±2ºC for fungal analysis. Total microbial count and fungi count were determined every two daysfor 8 days according to FDA's Bacteriological Analytical Manual(FDA-BAM, 2001).Total microbial contents were determined using serial dilutions method. Bacterial counts were carried out on plates containing Nutrient agar medium. Fungi were detected on Potato-Dextrose Agar (PDA) medium.

Procedure for analysis of cake:

One gram of each stored sample was used and mixed with 5 ml sterile dist, water until homogenate was appeared. Using separate sterile pipettes, serial dilutions of $10^{-9}$of food homogenate was prepared using sterile dist. water. About 10 ml of each dilution were added to 90 ml of diluents. All dilutions were shaked and 1 ml of each dilution was spread on a Petri dish by cotton swab. Incubate Petri dishes promptly for 8 days at 4, 20 and 37°C for bacteria and 25°C for fungi.

Counting of bacteria and fungi:

Colonies are most readily counted using a plate counter. The plate counter has a light source and a magnifying glass making colonies easier to see. In the former case, the colonies, run together, and, in the latter, there are too few to allow statistically accurate counts. Once you count the colonies, multiply by the appropriate dilution factor to determine the number of CFU/g in the original sample (Bennet, 1984).

One gram of the tested cake sample was homogenized in 10 ml dist. water. Serial decimal dilutions were made using 0.85% NaCl solution. Once the desired concentration was reached, 0.1 ml of the solution was spread on the surface of the prepared Potato dextrose agar medium and incubated at 25°C for 8 days and all plates were examined each 2 days for fungal growth. Colony counts were determined per gram of the prepared and preserved cakes at 25°C (Upasen and Wattanachai, 2018).

5- Sensory evaluation:

Sensory quality characteristics of the four formulations were evaluated by a panel of 20 untrained volunteer consumers from King Abdulaziz University, Jeddah, in laboratories of Faculty of Home Economic at 9 April 2018. The evaluation was done by using a 9-point Hedonic scale (1=extremely dislike, 9=extremely like). They were asked to score the internal characteristics of cake samples i.e. taste, texture, crumb color, odor and overall acceptability(Stone *et al*., 2012).

6- Statistical analysis:

All data obtained were analysed using SPSS (version 23). Collected data were presented as mean± standard deviation (SD). Student t-test was used to determine any significant differences between samples, $p \leq 0.05$ considered significant.

III. Results and Discussions

In our days, reducing Fat is a major concern for healthy food and market demands lower fat products was increased. Fat replacement by other ingredient is a great importance whereas bakery products contained higher levels of fat. Cakes are eaten universally and contained from 17% fat in the product. Fat had numerous importance to cakes. It gave cake higher volume, softness and inhibited gas-bubbles (Bobbio and Bobbio, 2003). Different ingredient was tested for substitute fat in food like gums, fibers or Chia which was newly studied as a probable fat replacer. Seed of Chia (*Salvia hispanica* L.) grown mainly in Mexico but now transported all over the world and many uses due to high nutritional value (Felisberto *et al*., 2015).

Shelf-stability is a complex phenomenon and it means all changes that occur in cake after baking. Higher values of shelf-stability mean lower freshness of the cake and vice versa. The growth of bacteria in cake was apparently affected by active packaging and packaging material (Janjarasskul *et al*., 2016). The prepared cake was preserved at four different temperatures, 4, 20, 25 and 37°C and bacteria and fungi were determined. All tested plates must be in the range of 25–250 CFU/g/plate (Juodeikiene *et al*., 2018). In this experiment, the examined plates were contained no bacterial colonies (0.0), very small numbers (≤10) or detectable numbers.

It is well known that microbes were classified according to their optimum temperature at which they can grow. The growth rates were the highest at the optimum growth temperature for the organism which can vary according to other environmental factors. For many years, diverse organisms called psychrotrophs**,** also known as psychrotolerant, prefer cooler environments such as refrigeration temperature about 4°C, thus they were the cause of spoilage of refrigerated food. From the tabulated data, it could be observed that no detected

bacterial contents and no growth at 4°C (refrigeration temperature) was appeared up to 6 days but little contamination appeared for control cakes (C) as in Table 2. At 20°C (room temperature), no bacterial growth was noticed until the fourth day but in sixth day the bacterial contents increased to about 10^3cfu/gat the eighth day for control cake. For prepared gluten free cakes (Q1 (25%), Q2 (50%); and Q3 (100%), at the eighth day, lower bacterial contents or no microbial contents were noticed compared to control cake (Table 3). From the tabulated data, it could be observed that the bacterial contents at 37°C appears <10 CFU/g after 4 and 6 days of incubation for all different cake samples. After 8 days, the counts were more than >10 CFU/g and reached to $0.79x10^3$cfu/g for control. Lower counts were obtained for Q1,Q2 and Q3 whereas the counts were in the range of 0.59- $0.66x10^2$cfu/g. Mesophilic bacteria are best active at median temperatures (15°–40°C) with optimum temperatures at 30–37°C. Also, they take a part in the web of micro-organic activity that forms the human and animal pathogens. At the beginning of the process, another group of bacteria, psychrophylic bacteria, start the process because they are active in lower temperatures from below zero up to 20°C and generate heat in the process which encouraged mesophilic bacteria to grow. Several species of fungi also take part in each stage but almost all fungi grow well at 25°C. The data in Table 5 showed that the fungi contents at 25℃during the tested period appears<10 CFU/g for different cakes sample. According to food safety guidance of the Department of Medical Sciences, Ministry of Public Health, fungi counts must not exceed 10^2 CFU/g (Upasen and Wattanachai, 2018).

In study of Ammar and El-Razik, (2013) there was a gradual decrease in freshness for wheat cake samples during storage period at room temperature. Cake samples prepared from mixture of cassava, pumpkin and potato flours have a high freshness value. According to Iglesias-Puig and Haros, (2013) the inclusion of chia seeds produce breads with a significant increase in specific volume. The volume increase could be due to the interaction of the mucilage network, which interacts with the gluten network. Hydrocolloids give a more porous structure to the gluten network, permitting greater stability and greater expansion of the dough during fermentation. Peroxide values is a key product of lipid oxidation and has been used as a marker of oxidative damage in both biological samples. During the accelerated shelf-life test, the changes in peroxide values of the low saturated fatty acid shortbread stored at 55 °C for 20 days were increased during storage(Marconi *et al*., 2014).Similarly in a study aimed to determine the microbiological quality of chocolate cake samples, microbiological examination of cake samples included total viable mesophilic bacterial count at 30 °C and total thermophilic bacterial count and total fungi count at 25°C. Their obtained results showed that, the counts of bacteria were 1×10^2 - 2×10^5 colony forming unit/ gram (cfu/g). On contrast to our results, five isolates of fungi were isolated from cake samples and they were identified as *Rhizopus stolonifer* and *Aspergillus niger*. Concerning, our study, lower counts of bacteria and fungi were obtained which may due to replacement of wheat flour and Shortening with different percentages of Quinoa Flour and Chia Gel.

Quinoa belongs to the Chenopodiaceae family, has a higher nutritive value than traditional cereals (Jancurová *et al*., 2009, Vega-Gálvez *et al*., 2010). It has high protein content (10-18%) with balanced amino acid composition, supplying high contents of lysine and methionine (Nowak *et al*., 2016). The fat content of raw quinoa seeds was 9.7% (dry-weight basis) and it has similar fatty acid composition with soybean oils with high amounts of essential fatty acids linoleic and linolenic acids (Jancurová *et al*., 2009). These essential fatty acids required for good health, cannot be synthesized in human body and must be obtained from the diet (Costantini *et al*., 2014). It contains a significant amount of fibers, more calcium, magnesium, potassium, iron, copper, riboflavin (B2), α-Tocopherol (vitamin E), β-Carotene and ascorbic acid (vitamin C) than wheat and barley (Jancurová *et al*., 2009). Furthermore, quinoa does not contain gluten and can be used safely in the production of foods (Pagano, 2006). Moreover, *Salvia hispanica* L., an oilseed plant, is commonly known as chia and is native from southern Mexico and northern Guetamala (Norlaily *et al*., 2012). Chia seed contains 25 to 40% oil with high essential fatty acids (omega) fiber, vitamins, minerals and antioxidants (Norlaily *et al*., 2012). Due to its unique nutritional composition, especially its high unsaturated fatty acids, its consumption decreases the risk of various types of diseases such as cardiovascular diseases, cancer, diabetes, inflammatory and autoimmune diseases (Munoz *et al*., 2013). Thus, we aimed this study to formulate gluten-free cakes prepared from quinoa and chia flour to increase the nutritional value of gluten-free cakes and these cakes can be preserved at cold (4°C), room (20°C) or warm (37°C) temperatures and consumed safely up to 8 days.

Sensory evaluation of the prepared cakes was determined using questionnaire. According to a 9-point Hedonic scale, 20 randomly persons evaluated four portions of cakes samples. In table 7, statistical analysis showed that cake sample prepared from 100% wheat flour and 100% shortening as control sample was more acceptable, followed by cake sample prepared from 50% quinoa flour and 50% chia gel (Q2). Then cake sample prepared from25% quinoa flour and 25% chia gel(Q1) has less acceptable.There were no significant differences between Q2 and Q1 in overall acceptability compared to control. Gluten free cake sample (Q3) was a lowest acceptability.

Table (2):Microbial content of the four prepared cakes stored at 4℃ for 8 days

Preservation Temperature	Type of cake	CFU/g			
		Day 2	Day 4	Day 6	Day 8
4℃	C (Control)	0.0 ± 0.0*	0.0 ± 0.0	0.0 ± 0.0	$0.1x10^3$ ± 0.02
	Q1 (25% Quinoa & 25% Chia)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0**
	Q2 (50% Quinoa & 50% Chia)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0**
	Q3 (100% Quinoa & 100% Chia)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0**

0.0*: no bacterial growth, ,**: significant results at p< 0.05 compared to control at the same time, no of replicates (n) = 10.

Table 3.Bacteriological content of the four prepared cakes stored at 20℃ for 8 days

Temperature	Sample	CFU/g			
		Day2	Day4	Day6	Day8
20℃	C (Control)	0.0 ± 0.0*	0.0 ± 0.0	<10**	$0.54x10^3$ ± 0.11
	Q1 (25% Quinoa & 25% Chia)	0.0 ± 0.0	0.0 ± 0.0	<10	$0.54x10^3$ ±0.14
	Q2 (50% Quinoa & 50% Chia)	0.0 ± 0.0	0.0 ± 0.0	<10	<10***
	Q3 (100% Quinoa & 100% Chia)	0.0 ± 0.0	0.0 ± 0.0	<10	<10***

0.0*: no bacterial growth, <10**: less than 10 colonies/ g, ***: significant results at p< 0.05 compared to control at the same time, no of replicates (n) = 10.

Table (4): Microbial content of the four prepared cakes stored at 37℃ for 8 days

		CFU/g			
Temperature	Sample	Day2	Day4	Day6	Day8
37℃	C (Control)	0 ± 0*	0 ± 0	<10**	$0.79x10^3$ ± 0.16
	Q1 (25% Quinoa & 25% Chia)	0 ± 0	0 ± 0	<10	$0.59x10^2$ ± 0.10***
	Q2 (50% Quinoa & 50% Chia)	0 ± 0	0 ± 0	<10	$0.64x10^2$ ± 0.15***
	Q3 (100% Quinoa & 100% Chia)	0 ± 0	0 ± 0	<10	$0.66x10^2$ ± 0.24***

0.0*: no bacterial growth, <10**: less than 10 colonies/ g, ***: significant results at p< 0.05 compared to control at the same time, no of replicates (n) = 10.

Table (5):Fugal content of the four prepared cakes stored at 25℃ for 8 days

Temperature	Sample	Spore/ g			
		Day2	Day4	Day6	Day8
25 ℃	C (Control)	0 ± 0*	0 ± 0	<10	$0.32x10^2$ ± 0.14
	Q1 (25% Quinoa & 25% Chia)	0 ± 0	0 ± 0	<10	$0.39x10^2$ ± 0.22
	Q2(50% Quinoa & 50% Chia)	0 ± 0	0 ± 0	<10	<10
	Q3 (100% Quinoa & 100% Chia)	0 ± 0	0 ± 0	<10	<10

0.0*: No bacterial growth, <10**: less than 10 colonies/ g, Number of replicates (n) = 10.

Table 6: Sensory characteristics of different prepared cake samples.

Sample	Control C	25% Quinoa & 25% Chia Q1	50% Quinoa & 50% Chia Q2	100% Quinoa & 100% Chia Q3
Color	8.70 ± 0.57	7.95 ± 1.19	7.95 ± 1.09	6.90 ± 1.71
Taste	8.00 ± 1.52	6.25 ± 1.88*	6.70 ± 1.92*	5.85 ± 2.11*
Odor	8.15 ± 1.46	7.45 ± 1.50	7.45 ± 1.84	6.45 ± 2.21*
Texture	7.45 ± 2.06	7.45 ± 1.87	7.85 ± 1.46	7.50 ± 1.27
Overall Acceptability	8.25 ± 1.25	7.30 ± 1.59	7.60 ± 1.27	6.80 ± 1.73*

*: significant value at $p \leq 0.05$

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