



Preparation and evaluation of Quinoa-Kishk as a novel functional fermented dairy product

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Abstract Quinoa is gaining more attention throughout the world because of its high nutritional, antioxidant and antimicrobial impacts. This study aimed to develop a novel functional Kishk prepared from wheat burghul replacement with quinoa seeds at 0, 25, 50, 75, and 100% levels. Changes in chemical, microbial, and sensory properties were followed during storage at room temperature for 3 months. The obtained results revealed that Kishk samples fortified with quinoa seeds had higher protein (17.18–18.37%), fat (3.00–5.99%), ash (6.64–8.01%) and fiber (1.32–2.05%) compared to control sample (16.52, 2.82, 6.00 and 1.18%), respectively for fresh samples. Furthermore, incorporation of quinoa into Kishk formulations improved amino acid profile, mineral contents, antioxidant activity and total phenols. However, addition of quinoa affected the color attributes and significant decreases in L* and b* values were noticed compared to control sample. During the storage period, overall bacterial and lactic acid bacteria counts for all samples were reduced. Coliform, mould and yeast counts of all fresh samples were less than 10 CFU/g and not detected throughout the storage. Sensory evaluation results revealed that Kishk fortified with 50% quinoa seeds exhibited good sensory properties. Therefore, fortification with quinoa

could improve nutritional and functional properties of fermented dairy products.

Keywords Kishk · Quinoa seeds · Wheat burghul · Laban Rayeb · Fermented dairy product · Functional properties

Abbreviations

AOAC	Association of Official Analytical Chemists
DPPH	2,2-Diphenyl-1-picrylhydrazyl
TAB	Total aerobic bacteria
LAB	Lactic acid bacteria
FAO	Food and Agriculture Organization
WHO	World Health Organization
UNU	United Nations University
CFU	Colony forming unit

Introduction

Functional ingredients include dietary fiber, proteins, carotenoids, polyunsaturated fatty acids, phenolic compounds, minerals, vitamins, prebiotics, and probiotics are potentially beneficial compounds found naturally in or applied to foods (Betoret et al. 2011). Many traditional food products, containing fruits, vegetables, soya, whole grains and fermented dairy products have been found to contain components with potential health benefits.

One of the main popular foods in Upper Egypt is the Egyptian Kishk. In Egypt and the eastern Mediterranean, Kishk is widely consumed and is regarded as an important diet in many populations. It is natural, nutritious and has an appealing taste for the consumers. Laban Zeer (fermented milk) combined with boiled, dried and crushed whole wheat grains (Burghul) are the essential ingredients of Kishk. It is rich in nutrients and considered as a source of

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various vitamins, growth factors and other beneficial constituents (EL-Gendy 1983). Different types of cereals such as rice, wheat, corn, or sorghum were combined with natural local sour milk like Laban Khad, Laban Zeer, or Laban Rayeb to produce the Kishk. Laban Rayeb is one of the fermented dairy milk which widely consumed in Egypt, obtained from natural lactic acid fermentation of milk. A good quality Laban Rayeb is a firm and uniform consistency with a clean acid taste and a smooth, glossy surface and free from cracks or gas bubbles.

Quinoa is widely cultivated for its seed, a pseudo-cereal grain which is one of the worldwide common foods. It is a gluten free grain and considered one of the best sources of vegetal protein (with high bioavailability for all essential amino acids), as its protein levels are close to those found in milk and higher than those present in cereals such as wheat, rice and maize (Gordillo-Bastidas et al. 2016). It is a good source of minerals with significant quantities of potassium, calcium, magnesium, copper, iron, and manganese and, the ratio of calcium and phosphorus is better than those of conventional cereals. Furthermore, the seeds of quinoa are rich in lipids which contain high unsaturated fatty acids such as linoleic and linolenic acids and antioxidants, with five-fold higher levels than other cereals (Koziol 1992). Due to its high nutritional quality, quinoa has recently been used as a novel functional food ingredient. It is used in the preparation of salads, soups, porridges, stews, fried patties, drinks, breakfast cereals, granola bars, beer, snacks, bread, and biscuits.

Keeping in mind the necessity for increasing the nutritional quality, additional plant food sources are needed. Wheat based diets are poor regarding essential amino acid content especially lysine as the first limiting amino acid. Thus, whole grains have the potential to make a good contribution in this respect as recognized sources of essential amino acids, dietary fiber, minerals, vitamins, and antioxidants. Several studies have been carried out to enhance the nutritional quality of Kishk by replacing burghul using various kinds of cereal such as oats, freekeh and chickpeas (Nassar et al. 2016). Therefore, this study aimed to use quinoa seeds, as a novel functional food ingredient, in partially or complete replacement of wheat burghul for producing Kishk with high nutritional characteristics. The influence of quinoa seeds on Kishk properties was assessed by determining the physicochemical, microbial, minerals contents, color, and sensory properties.

Materials and methods

Materials

Cow milk used in Laban Rayeb preparation was obtained from dairy farm at El-Kharga City, New Valley Governorate, Egypt. Quinoa seeds genotype *Regalona* were obtained from the New Valley Research Station (Desert Research Center), Egypt. Wheat burghul and salt were purchased from local market in Asuut City, Asuut Governorate, Egypt. All chemicals were of analytical grade and purchased from Sigma-Aldrich Co. This study (main experiment) was carried out in laboratories of Dairy Science Department, Faculty of Agriculture, New Valley University and some chemical compositions were carried out in Food Technology Department, Faculty of Agriculture, Suez Canal University, Egypt.

Methods

Manufacture of Laban Rayeb

The raw cow milk was filtered and then left up to 48 h at 25 ± 2 °C for coagulation. After gelation, the resultant product is called “Rayeb” according to the method described by EL-Gendy (1983). For preparation of Laban Rayeb samples which will use in Kishk making, the milk was filtered and heated to 73 °C for 15 s in water bath and cold down to 37 °C. The starter culture (lactic acid bacteria was isolated from the prepared Rayeb) was added at 1% under sanitary conditions. The inoculated milk was left at room temperature for 24 h until curdling completed. The produced Laban Rayeb contains total solid 9.21%, protein 2.65%, fat 2.74%, ash 0.65%, acidity 1.25%, pH value 4.85 and carbohydrate 3.17%.

The resultant Laban Rayeb was filtered through cheesecloth (double layer) to be concentrated (23.45% total solids) and used in making of the Kishk.

Preparation of grains

Wheat burghul and quinoa seeds were cleaned from straw, dirt and weed seeds. After that, they washed using tap water several times (this step was carried out to remove the saponins from quinoa). The grains were soaked in water in a large pan for 24 h at room temperature. Then the cereals were heated until boiling for 30 min. The cooked cereals were washed with water to remove starch and peels.

Manufacture of Kishk

In five formulas Kishk samples were prepared as following: B, Control Kishk made from 100% wheat burghul; BQB, wheat burghul was replaced by 25% quinoa seeds; BQ, wheat burghul was replaced by 50% quinoa seeds; QBQ, wheat burghul was replaced by 75% quinoa seeds and Q, wheat burghul was replaced by 100% quinoa seeds. A mixture of wheat burghul and quinoa seeds were mixed with concentrated Laban Rayeb at level of 1:2 (w/w) respectively, then kept 1 day at room temperature for fermentation. The next day, the amount of Laban Rayeb was increased until the ratio reaches 1:4 (w/w) and the mixture was kneaded again. The salt was added to the mixture with a ratio of 1%. Afterwards, the mixture was formed into small balls, placed on plates, and then dried in a hot air oven at $50\text{ }^{\circ}\text{C} \pm 2$ for 48 h. The resultant Kishk samples were packed in plastic bags and stored at room temperature for 90 days (Tamime et al. 1997). When it was fresh and after 1, 2, and 3 months, resultant Kishk samples were analyzed for physicochemical, microbial, and sensory properties. Furthermore, minerals, amino acids, antioxidant activity, and color attributes were analyzed when fresh.

Chemical analysis

The total solids, protein, ash, fiber, acidity, and pH values of wheat burghul, quinoa seeds and Kishk samples were analyzed according to Association of Official Analytical Chemists (2007). The carbohydrate content was calculated by difference according to the following equation: Carbohydrate % = $100 - (\% \text{ moisture} + \% \text{ protein} + \% \text{ ash} + \% \text{ fat} + \% \text{ fiber})$.

The calorific value of Kishk samples was calculated from the proximate analysis results using the following equation:

$$\text{Kcal}/100\text{ g} = (\text{protein } \% \times 4) + (\text{fat } \% \times 9) + (\text{carbohydrate } \% \times 4)$$

Minerals and amino acids analyses

The mineral contents of Kishk samples including calcium (Ca), phosphorus (P), potassium (K), Magnesium (Mg), iron (Fe), zinc (Zn), and copper (Cu) elements were determined using Perkin-Elmer Atomic Absorption Spectrophotometer as reported by AOAC (2007).

The amino acid contents were analyzed as reported by Association of Official Analytical Chemists (2007) using High Performance Amino Acid Analyzer (Biochrom 20, Auto sampler version, Amersham Pharmacia Biotech., Sweden). Sample (100 mg) was hydrolyzed with 5 mL of 6 N HCl at $110\text{ }^{\circ}\text{C}$ for 24 h. The hydrolyzed sample was re-dissolved in Na citrate buffer (pH 2.2) and filtered using

a $0.2\text{ }\mu\text{m}$ membrane filter then injected into the amino acid analyzer. The contents of the various recovered amino acids were presented as grams per 100 g of protein.

Preparation of methanolic extracts

The samples were homogenized to obtain fine powder. The resultant powder (2.5, 5.0, 7.5, or 10.0 g) and methanol (40 mL) were placed in flasks, kept in a magnetic agitator overnight, and then filtered through filter paper (Whatman No. 1). The methanolic extracts represented 50, 125, 187.5, and 250 mg of sample per mL, respectively.

Total antioxidant capacity was measured using DPPH radical scavenging activity method, according to Ravichandran et al. (2013). Sample extract (0.1 mL) was mixed with 3.9 mL of DPPH solution (6×10^{-5} M), and left to react for 30 min. The absorbance of the mixture was measured at 515 nm using a spectrophotometer (model 6505 UV/Vis, JENWAY, UK). The DPPH solution without extract was analyzed as a control. The antioxidant activity was calculated as follows:

$$\text{DPPH radical - scavenging activity } (\%) = \left[\frac{Ac - As}{Ac} \right] \times 100$$

where Ac is the absorbance of control and As is the absorbance of sample at 515 nm.

The IC₅₀ value (mg/mL) was defined as the concentration of the extract which was required to quench 50% of the initial amount of DPPH· under the given experimental conditions.

The total phenolic compounds content was determined according to Barros et al. (2011). An aliquot of the extract solution was mixed with Folin-Ciocalteu reagent (5 mL, previously diluted with water 1:10 v/v) and sodium carbonate (75 g/L, 4 mL). The tubes were vortexed for 15 s and allowed to stand for 30 min. Absorbance was measured at 765 nm using a spectrophotometer (model 6505 UV/Vis, JENWAY, UK). Gallic acid was used as standard and the results were expressed as mg of gallic acid equivalents (GAE) per g.

Color attributes determination

Color attributes of the samples were estimated by measuring the L* (100 = white; 0 = black), a* (+, red; −, green) and b* (+, yellow; −, blue) values using a Minolta color reader (Minolta Camera, Co., Ltd., Osaka, Japan). Values are the mean of three determinations.

Enumeration of microorganisms

Counts of microorganisms were determined and examined by the method of Marshall (1992). For the enumeration of

total aerobic bacteria (TAB), lactic acid bacteria (LAB), yeast and mould (YM) and coliform bacteria, samples of Kishk powder (10 g) were dispersed in 90 mL sterile physiological saline solution (0.85%). The count of TAB was enumerated by Plate Count Agar (Oxoid) after incubation at 37 °C for 48 h. LAB was counted on MRS agar containing 0.1 g/L of cycloheximide after incubation at 30 °C for 72 h in anaerobic conditions. Dichloran Rose Bengal Chloramphenicol Agar (Oxoid) was used for YM enumeration and plates were incubated at 25 °C for 5 days. Coliform bacteria were enumerated on Violet Red Bile Agar (Oxoid) after incubation at 37 °C for 24 h.

Sensory evaluation

Kishk samples were sensory evaluated by the staff members of the Dairy Science Department, Faculty of Agriculture, New Valley University. The samples were prepared as soups by mixing dried Kishk (20 g) with 170 mL of water, then heated with stirring to boiling for few mins, and cooled to 40 °C. The samples were introduced in identical glass containers for organoleptic panellists and served at 40 °C. The scoring system for sensory attributes was as follows: the flavor (1–45 points), body and texture (1–30 points), appearance and color (1–15 points) and acidity (1–10 points), according to the method of Abou-Donia et al. (1991). In another trial, 5% spice mixture containing tomato paste, paprika, onion powder, peppers red hot at ratio 1:2:2:1 respectively, were added to soups to improve the soups taste.

Statistical analysis

The obtained data were expressed as mean \pm SD of triplicates and subjected to analysis of variance (ANOVA) and Duncan's multiple range test was used to detect significant differences between samples. Significant differences were defined at $p < 0.05$. All analyses were performed using SPSS program (version 17.0 SPSS Inc).

Results and discussion

Chemical composition of raw materials

The chemical compositions of wheat burghul and quinoa seeds are presented in Table 1. The quinoa seeds have higher protein (14.10%), fat (6.17%), ash (3.80%) and fiber contents (3.67%) than those in wheat burghul; protein (10.95%), fat (2.10%), ash (1.95%) and fiber (1.35%). On the other hand, wheat burghul has higher carbohydrate content than quinoa seeds. In general, the chemical composition of quinoa seeds was in a good agreement with the

data obtained by other authors (Miranda et al. 2013), and that of wheat burghul was in agreement with those obtained by of Yousif et al. (2018). Furthermore, Navruz-Varli and Sanlier (2016) found that protein content of quinoa seeds was higher than those found in rice, corn, rye, barley, sorghum, and wheat. Quinoa is better known than other grains for its protein quality; quinoa protein is made up of amino acids, of which eight are considered important for both children and adults alike (Repo-Carrasco-Valencia et al. 2003). Quinoa protein can provide more than 150% and 200% of schoolchildren and adults requirements, respectively (Yamani and Lannes 2012). The fiber content in quinoa seeds is higher than that reported previously in wheat or rice and are comparable with those determined for legumes (Sobota et al. 2020). Dietary fiber is considered necessary for optimal digestive health, and imparts various functional benefits. Moreover, quinoa seeds are rich in fat, which represents a source for calories, and helps in the absorption of fat-soluble vitamins such as vitamin E. Dini et al. (1992) reported that lipid content of quinoa seeds is 14.5% with an unsaturated level of about 70%, having linoleic and oleic acids in percentages of 38.9% and 27.7% respectively.

The pH and acidity values of quinoa seeds (6.51 and 0.32%) are similar to those of wheat burghul (6.67 and 0.22%), respectively. These values are important when food additive is added as a replacement for another grain crops which will not affect the characteristics of the final product. The pH value for quinoa fell within the range reported by Pellegrini et al. (2018) for six commercial genotypes of quinoa (6.42–6.63).

Table 1 illustrates the DPPH· radical scavenging activities (expressed as IC₅₀) and total phenolic compounds content in quinoa and wheat burghul samples. The results indicated that quinoa has lower IC₅₀ almost two times (146.00 mg/mL) than that of wheat burghul (271.67 mg/mL) this can be attributed to the higher phenolic compounds in quinoa (119.55 mg GAE/100 g) compared to wheat burghul (35.03 mg GAE/100 g). Therefore, quinoa seeds can be utilized as a natural potent antioxidant.

3.2 Minerals and amino acid content of wheat burghul and quinoa seeds

The minerals content of wheat burghul and quinoa seeds are shown in Table 1. Quinoa is a good source of minerals. It had the greatest macro elements; K, Mg and Ca contents and microelements; Fe, Zn, and Cu than wheat burghul. Furthermore, K was found to be the most abundant minerals and Cu was the lowest one. K is an important chemical element that helps to prevent muscle weakness, respiratory insufficiency, and hypotension in humans. Quinoa seeds are rich in minerals than other grains and

Table 1 Chemical composition, antioxidant activity, total phenols, minerals and amino acid content of wheat burghul and quinoa seeds

Component	Wheat burghul	Quinoa seeds
<i>Chemical composition</i>		
Moisture (%)	10.67 ± 0.23b	9.65 ± 0.51a
Dry matter (%)	89.33 ± 0.23a	90.35 ± 0.51b
Protein (%)	10.95 ± 0.35a	14.10 ± 0.40b
Fat (%)	2.10 ± 0.28a	6.17 ± 0.32b
Ash (%)	1.95 ± 0.35a	3.80 ± 0.10b
Fiber (%)	1.35 ± 0.64a	3.67 ± 0.32b
Carbohydrate (%)	72.97 ± 1.15b	62.61 ± 0.79a
Acidity (%)	0.22 ± 0.06a	0.32 ± 0.14a
pH	6.67 ± 0.02a	6.51 ± 0.16a
<i>Antioxidant activity (DPPH)</i>		
Scavenging activity—IC50 (mg/mL)	271.67 ± 1.87a	146.00 ± 1.87b
<i>Total phenolic compounds (mg GAE/100 g)</i>		
Total phenols	35.03 ± 1.47b	119.55 ± 0.45a
<i>Minerals (mg/100 g)</i>		
Calcium	100.87 ± 3.67a	156.30 ± 9.04b
Phosphorus	430.57 ± 14.32b	397.70 ± 19.38a
Potassium	513.43 ± 38.57a	876.48 ± 184.19b
Magnesium	64.80 ± 16.00a	141.15 ± 30.02b
Iron	4.60 ± 0.26a	10.83 ± 6.75b
Zinc	1.53 ± 1.07a	4.35 ± 0.54b
Copper	0.42 ± 2.79a	1.17 ± 0.24b
<i>Amino acid (g/100 g Protein)</i>		
Threonine	2.85 ± 0.07a	3.33 ± 0.35b
Valine	4.30 ± 0.14a	4.27 ± 0.32a
Isoleucine	3.20 ± 0.00a	3.60 ± 0.26b
Leucine	6.80 ± 0.00b	6.43 ± 0.47a
Tyrosine	1.37 ± 0.13a	3.20 ± 0.71b
Phenylalanine	3.80 ± 0.08a	4.71 ± 0.25b
Histidine	2.15 ± 0.21a	3.03 ± 0.15b
Lysine	2.65 ± 0.07a	5.87 ± 0.42b
Methionine	1.75 ± 0.07a	3.27 ± 0.25b
Tryptophan	1.10 ± 0.14b	0.73 ± 0.15a

Means (three different determinations) ± standard deviation (SD)

contain high levels of Mg, Ca, Zn, Fe, and Cu when compared to the recommended daily allowances (Repo-Carrasco-Valencia et al. 2003).

Table 1 illustrates the essential amino acids content in wheat burghul, and quinoa seeds. The data revealed that quinoa seeds are the highest in the contents of amino acids such as lysine, threonine, isoleucine, tyrosine, phenylalanine, histidine and methionine. On the other hand, wheat burghul was higher in valine, leucine and tryptophan contents than quinoa seeds. Miranda et al. (2012) found that quinoa proteins are rich in the essential amino acids, especially lysine, methionine, and threonine which are the limiting amino acids in most traditional cereal grains. Moreover, the essential amino acid content of quinoa

protein is equal to or greater than that of FAO/WHO/UNU (1985) reference patterns and similar to the amino acid composition of milk casein. Additionally, Vega-Gálvez et al. (2010) found that the essential amino acid content of quinoa protein was higher than other whole cereal grains, such as wheat, barley, rice, and/or corn.

Chemical composition of Kishk samples

The chemical composition of the resultant quinoa and wheat burghul Kishk samples are shown in Table 2. Significant differences were noticed in the chemical compositions among Kishk samples. Use of quinoa seeds (Q, 100%) as a substitute for wheat burghul gave a product

Table 2 Effect of enrichment with quinoa seeds on the chemical composition of Kishk samples during storage

Kishk samples	Storage period (month)	Moisture	Protein	Fat	Ash	Fiber	Carbohydrate	Gross energy (kcal)
B	Zero	9.69 ± 0.23cA	16.52 ± 0.42dA	2.82 ± 0.19cA	6.00 ± 0.25dD	1.18 ± 0.03cC	63.79 ± 0.08aA	346.62 ± 0.76bA
	One	8.75 ± 0.45cB	16.11 ± 0.24dAB	2.66 ± 0.11cAB	6.75 ± 0.07cC	1.21 ± 0.02cC	64.51 ± 1.22aA	346.45 ± 4.20aB
	Two	6.81 ± 0.32dC	15.69 ± 0.32dBC	2.54 ± 0.11 dB	7.52 ± 0.11 dB	1.29 ± 0.01cB	66.15 ± 0.15aA	350.25 ± 1.38bB
BQB	Three	6.13 ± 0.13dD	15.13 ± 0.15cC	2.43 ± 0.12cB	8.00 ± 0.24dA	1.33 ± 0.02bA	66.97 ± 1.31aA	350.31 ± 4.25aB
	Zero	9.50 ± 0.21cA	17.18 ± 0.21cA	3.00 ± 0.14cA	6.64 ± 0.13cD	1.32 ± 0.26bC	62.36 ± 0.26bA	345.14 ± 0.70bA
	One	9.19 ± 0.18cA	16.86 ± 0.13cB	2.91 ± 0.19cA	7.20 ± 0.13dC	1.36 ± 0.12bB	62.48 ± 0.70aB	343.58 ± 4.01aB
BQ	Two	7.61 ± 0.16cB	16.52 ± 0.15cBC	2.88 ± 0.13cA	7.97 ± 0.13cB	2.15 ± 0.25bAB	62.86 ± 0.47aAB	343.45 ± 0.43aB
	Three	7.30 ± 0.15cB	16.29 ± 0.16bC	2.77 ± 0.14cA	8.31 ± 0.13cdA	2.45 ± 0.15aA	62.87 ± 0.42bAB	341.60 ± 1.05aB
	Zero	10.62 ± 0.32bA	17.60 ± 0.31bcA	4.33 ± 0.39bA	7.15 ± 0.10bC	1.88 ± 0.13aC	58.41 ± 0.21cA	343.05 ± 2.40aA
QBQ	One	9.98 ± 0.14bB	17.32 ± 0.26bA	4.16 ± 0.19bA	7.50 ± 0.18cB	2.19 ± 0.05aB	58.85 ± 2.56bAB	342.14 ± 9.33aB
	Two	8.02 ± 0.19cC	16.74 ± 0.36cB	3.96 ± 0.24bA	8.03 ± 0.16cB	2.42 ± 0.10aA	60.82 ± 0.64cB	345.91 ± 2.66bB
	Three	7.52 ± 0.11cD	16.38 ± 0.23bB	3.96 ± 0.24bA	8.50 ± 0.20cA	2.58 ± 0.16aA	61.06 ± 0.79cAB	345.44 ± 4.98aB
Q	Zero	11.05 ± 0.18abA	17.99 ± 0.15abA	4.34 ± 0.28bA	7.47 ± 0.25bC	1.98 ± 0.14aB	57.18 ± 0.15cC	339.71 ± 2.57cAB
	One	10.37 ± 0.22abB	17.52 ± 0.26ab	4.29 ± 0.23bA	8.00 ± 0.14bB	2.13 ± 0.05abB	57.69 ± 0.24bB	339.43 ± 1.88aB
	Two	8.71 ± 0.14bC	17.35 ± 0.22aB	4.07 ± 0.18bA	8.30 ± 9.17bB	2.24 ± 0.06abAB	59.33 ± 0.20bA	343.35 ± 1.56aA
Q	Three	8.33 ± 0.21bD	17.04 ± 0.19aB	3.84 ± 0.24bA	8.96 ± 0.16bA	2.47 ± 0.29aA	59.36 ± 0.19cA	340.18 ± 1.30aAB
	Zero	11.29 ± 0.25aA	18.37 ± 0.44 aA	5.99 ± 0.13aA	8.01 ± 0.12aD	2.05 ± 0.12aB	54.29 ± 0.66dA	344.55 ± 1.31aA
	One	10.66 ± 0.41aB	17.89 ± 0.17aA	5.71 ± 0.15aA	8.46 ± 0.07aC	2.20 ± 0.18aB	55.08 ± 1.07bA	343.25 ± 6.12aB
Q	Two	9.30 ± 0.38aC	17.24 ± 0.45abB	5.33 ± 0.21aB	8.76 ± 0.13aB	2.45 ± 0.08aA	56.93 ± 0.62dA	344.59 ± 0.92aBC
	Three	9.04 ± 0.10aC	17.03 ± 0.24aB	5.04 ± 0.13aB	9.27 ± 0.15aA	2.54 ± 0.12aA	57.08 ± 0.71dA	341.83 ± 2.58aC

Results are the mean of three different determinations ± standard deviation. Means that are followed by the same letter in the row and the same capital letter in the column did not differ significantly ($p < 0.05$). B, Control Kishk made from 100% wheat burghul; BQB, replaced at 25% Quinoa seeds; BQ, replaced at 50% Quinoa seeds; QBQ, replaced at 75% Quinoa seeds; Q, replaced at 100% Quinoa seeds

with high moisture, fat, protein, ash, and fiber values whilst, low carbohydrate content compared to the control (B, 100% wheat burghul) and the rates increased with increasing the substitution level of quinoa seeds (BQB, BQ, QBQ and Q). The data declared that incorporation of 100% of quinoa seeds (Q) increased the protein, fat, ash, and fiber contents in fresh Kishk by around 10.07, 52.92, 25.09, 42.44%, respectively but decreased carbohydrate by around 17.50%. Results of Kishk blends with quinoa seeds were close to the data previously reported by Curti et al. (2017) who found that yogurt fortified with quinoa resulted in an increment in the nutrients and produced yogurt with higher protein, ash, fiber, and lipids contents than that of the control yogurt. The results also indicated that moisture content increased with increasing quinoa seeds levels. This might be due to the starch in quinoa added some functional properties like water holding capacity and high viscosity which may increase the moisture holding in Kishk samples. The higher protein, fat, ash, and fiber content of quinoa-Kishk samples can be attributed to the higher contents of these constituents in quinoa seeds than in wheat burghul. The total carbohydrate content of Kishk samples blended with quinoa seeds (25–100%) was lower than that in control due to the content of protein, lipids, ash, and fiber in Quinoa-Kishk being higher than wheat Kishk. On the other hand, moisture, protein, and fat were decreased whereas ash, fiber, and carbohydrate, were increased during storage.

The gross energy values of Kishk samples are shown in Table 2. Small differences were noticed for the gross energy values between the Kishk samples when processed fresh and during storage. As the chemical compositions vary within and between ingredients, the measured component may often differ. Fat, protein and carbohydrates in foods provide energy for body functions and physical activities. It could be seen that the differences between Kishk samples with respect to energy values, may be due to the greater level of fat and protein in quinoa seeds and opposite the greater level of carbohydrates in wheat burghul. It is recognized that carbohydrates, protein, and fat are considered as fuel for all organisms which contribute to about 55–75, 10–15, and 15–30% respectively, of energy required by the organisms (WHO 1990).

Figure 1 shows the acidity and pH values for the different Kishk samples. The rates of acidity were increased and pH values were decreased throughout the storage. Moreover, acidity values were higher in Kishk samples fortified with quinoa seeds (BQB, BQ, QBQ and Q), than control sample (B) when processed fresh and up to the end of storage. It can be concluded that the hydrolysis of quinoa proteins by LAB during fermentation could enhance their growth and metabolic activity toward the release of organic acids during storage periods (Moore et al. 2008). Significant decrease in pH and an increase in acidity was

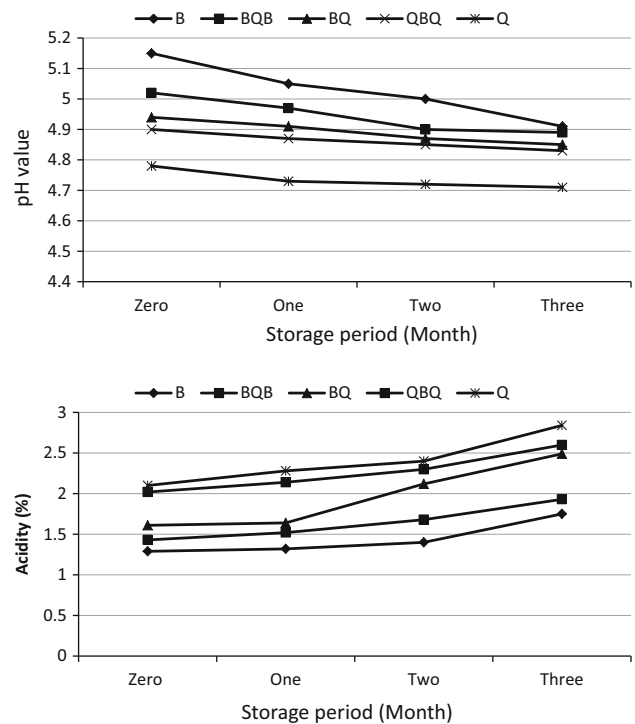


Fig. 1 Effect of enrichment with quinoa seeds on pH values and acidity (%) of kishk during storage. B, Control Kishk made from 100% wheat burghul; BQB, replaced at 25% Quinoa seeds; BQ, replaced at 50% Quinoa seeds; QBQ, replaced at 75% Quinoa seeds; Q, replaced at 100% Quinoa seeds

found for Kishk samples prepared from quinoa seeds up to level 100%. Curti et al. (2017) found that yogurt samples fortified with quinoa flour revealed a much greater decrease in the pH value and increases in the total acidity values comparatively with the non-supplemented control sample during the fermentation process, possibly because of the high amino acid and mineral contents of quinoa flour that are required for yogurt starter culture to develop.

Minerals content of fresh Kishk samples

Mineral contents of fresh Kishk made with various ratios of quinoa seeds were presented in Table 3. Significant differences in minerals content between control (B) and all blends with quinoa seeds (BQB, BQ, QBQ and Q) were noticed. The results indicated that K, P, Mg and Ca were the most predominant elements and Fe, Zn and Cu were the lowest among all Kishk samples. Mineral contents in Kishk samples were increased as levels of quinoa seeds increased in Kishk formulation. Kishk sample contains 100% quinoa seeds (Q) were higher in K, Mg and Ca than control (B, 100% wheat burghul). The increase in these minerals in quinoa Kishk samples might be due to the high mineral content of quinoa seeds than those in wheat burghul. It was previously reported increases in the mineral contents after

Table 3 Effect of enrichment with quinoa seeds on minerals content, amino acid, antioxidant activity, total phenols and color attributes of fresh Kishk samples

Component	Kishk samples				
	B	BQB	BQ	QBQ	Q
<i>Minerals (mg/100 g)</i>					
Calcium	173.33 ± 0.79e	188.13 ± 0.92c	210.32 ± 1.80b	222.11 ± 2.30a	182.32 ± 0.91d
Phosphorus	527.26 ± 0.85a	495.46 ± 0.55b	476.93 ± 1.79c	462.35 ± 1.96d	452.60 ± 0.80e
Potassium	681.46 ± 0.77e	715.21 ± 4.03d	812.99 ± 2.43c	875.26 ± 1.79b	964.53 ± 1.76a
Magnesium	86.30 ± 0.93e	121.28 ± 1.83d	171 ± 0.71c	232.47 ± 1.69b	261.17 ± 1.60a
Iron	4.44 ± 0.23e	6.61 ± 0.10d	9.08 ± 0.09c	13.50 ± 0.50b	15.33 ± 1.17a
Zinc	1.37 ± 0.16e	1.91 ± 0.04d	2.62 ± 0.09c	3.36 ± 0.07b	4.12 ± 0.15a
Copper	0.41 ± 0.02c	0.62 ± 0.04b	0.64 ± 0.12b	0.87 ± 0.09a	0.99 ± 0.015a
<i>Amino acid (g/100 g protein)</i>					
Threonine	3.15 ± 0.09b	3.17 ± 0.04b	3.28 ± 0.15ab	3.23 ± 0.12ab	3.410.03a
Valine	4.71 ± 0.03a	4.66 ± 0.21a	4.62 ± 0.11a	4.56 ± 0.10a	4.53 ± 0.20a
Isoleucine	3.42 ± 0.02a	3.48 ± 0.23a	3.57 ± 0.16a	3.63 ± 0.24a	3.72 ± 0.06a
Leucine	7.40 ± 0.05a	7.15 ± 0.12b	7.12 ± 0.10b	7.00 ± 0.12b	7.04 ± 0.07b
Tyrosine	1.55 ± 0.04e	2.14 ± 0.07d	2.46 ± 0.12c	2.94 ± 0.06b	3.37 ± 0.07a
Phenylalanine	4.11 ± 0.13c	4.25 ± 0.10c	4.56 ± 0.11b	4.71 ± 0.07b	4.91 ± 0.10a
Histidine	2.31 ± 0.09d	2.44 ± 0.13 cd	2.62 ± 0.19bc	2.74 ± 0.09b	3.1 ± 20.09a
Lysine	3.05 ± 0.06d	3.32 ± 0.11d	4.53 ± 0.31c	5.46 ± 0.11b	6.17 ± 0.06a
Methionine	1.52 ± 0.10e	2.26 ± 0.09d	2.66 ± 0.08c	3.44 ± 0.09b	4.64 ± 0.13a
Tryptophan	ND	ND	ND	ND	ND
<i>Antioxidant activity (DPPH)</i>					
Scavenging activity-IC50 (mg/mL)	290.27 ± 2.01a	245.64 ± 2.51b	193.23 ± 2.04c	170.68 ± 1.45d	149.87 ± 0.59e
<i>Total phenolic compounds (mg GAE/100 g)</i>					
Total phenols	33.45 ± 1.13d	49.04 ± 2.03e	64.56 ± 1.51c	81.13 ± 1.02b	111.64 ± 1.58a
<i>Color attributes</i>					
L*	68.08 ± 1.61a	61.20 ± 2.77b	56.53 ± 0.84c	52.40 ± 1.86d	46.96 ± 1.72d
a*	3.67 ± 0.20d	3.79 ± 0.11 cd	4.04 ± 0.16c	4.34 ± 0.11b	4.76 ± 0.12a
b*	23.60 ± 0.93a	20.48 ± 1.12b	17.45 ± 1.29c	14.50 ± 0.64d	13.79 ± 0.98d

Results are the mean of three different determinations ± standard deviation. Means that are followed by the same letter in the row did not differ significantly ($p < 0.05$). ND, not detected. B, Control Kishk made from 100% wheat burghul; BQB, replaced at 25% Quinoa seeds; BQ, replaced at 50% Quinoa seeds; QBQ, replaced at 75% Quinoa seeds; Q, replaced at 100% Quinoa seeds. All measurements were taken for fresh samples (at zero time)

the addition of quinoa flour into some foods such as tarhana and bread (Demir 2014; Bilgiçli and İbanoğlu 2015). Ballester-Sánchez et al. (2019) reported similar behavior with regard to the minerals content of bakery products fortified with quinoa. Besides, the bioavailable forms of Ca, Mg, and K are present in quinoa, so their content is considered sufficient for a healthy diet.

Amino acid content of fresh Kishk samples

The main characteristic of quinoa seeds is the unique quality of its amino acid composition. Table 3 shows the amino acid composition of Kishk samples made from wheat burghul replacement with different ratios of quinoa

seeds (25–100%). It is clear that incorporation of quinoa in Kishk formulations had improved the amino acid profile of the resultant Kishk samples. An increase in the essential amino acids; threonine, tyrosine, isoleucine, phenylalanine, histidine, lysine, and methionine was noticed by the addition of quinoa seeds to Kishk formulations but the contents of valine and leucine were lower when compared with the control (B). The values of leucine followed by valine, phenylalanine and lysine were higher than other amino acids. Tryptophan was not detected in all samples, because of acid hydrolysis during the digestion of samples for analysis or by destroying during the processing of Kishk samples as a result of fermentation and drying. Quinoa protein is characterized as a better amino acid balance due

to the presence of methionine, lysine, and cysteine in relatively higher amounts than that are deficient in cereal proteins such as wheat and corn. Hence, quinoa is one of plant foods that able to provide human body with all essential amino acids with values close to those adopted by FAO and its amino acid composition is similar to that of milk protein (Kozioł 1992).

Antioxidant activity and total phenolic compounds

The DPPH· radical scavenging activities (expressed as IC50) and total phenolic compounds contents of samples made from wheat burghul and quinoa seeds are shown in Table 3. It can be seen that the substitution of wheat burghul with quinoa seeds resulted in increased antioxidant activity of Kishk, i.e. lower IC50 values and higher total phenolic compounds. The obtained IC50 values depended on the level of substitution (BQB, BQ, QBQ and Q), and decreased dose-dependently and significant differences ($p < 0.05$) between IC50 values at all levels of substitution with quinoa were noticed. Increased antioxidant activity of quinoa-enriched Kishk could be attributed to the significantly higher total phenolic content and in all samples as a result of quinoa inclusion in Kishk samples (Table 3). These results are in parallel with those obtained by Jan et al. (2018) who found an increase in total antioxidants in cookies supplemented with quinoa flour. These results suggested that quinoa seeds possess advanced amounts of antioxidant activity and phenolic compounds.

Color analysis of fresh Kishk samples

Color of foods is an important feature and determines the acceptance or rejection of final product by the consumer. The color of processed Kishk samples is measured using the L^* a^* b^* values and presented in Table 3. It could be noticed that using of quinoa seeds in Kishk formulations affected the color values and significant differences were noticed between the color attributes of the different Kishk samples. Control Kishk (B) had the highest L^* value (68.08) whilst Kishk (Q, 100% quinoa seeds) were the lowest one (46.96) and lightness L^* values decreased by increasing the ratio of quinoa seeds in Kishk formulas. These results may be attributed to the light color of wheat burghul and dark color of quinoa seeds; in addition, some minerals like Fe and Cu catalyzed some non-enzymatic browning reactions which lower the color values of Kishk incorporated with quinoa seeds (Bilgiçli and İbanoğlu 2015). On the other hand, darker color of quinoa is probably due to its higher ash content and phenolic pigments (Tang et al. 2015). The a^* values (redness) indicated that Kishk prepared using quinoa seeds at different levels was different in its color from those of control. The a^* values of

Kishk samples were significantly increased ($p < 0.05$) when higher levels of quinoa seeds were added. Similarly, Wang et al. (2015) found that the darkness and redness of bread increased when the levels of quinoa flour were raised. The b^* values (yellowness) in control Kishk was a significant increase when compared to other treatments. Color of bulghul is mainly due to natural pigments (carotenoids) that are present at different levels in wheat which, give it yellowish color lead to increase customer acceptability. The b^* value showed a significant reduction when the proportion of quinoa seeds was increased. The obtained results are similar to those reported by Abd-Rabou et al. (2020) who found that addition of quinoa seeds to wheat grains caused increase in the values of redness a^* and decrease in the values of lightness L^* and yellowness b^* in all fortified camel milk kishk samples. Similarly, Abou-Zaid et al. (2012) reported that increasing the percentage of added quinoa meal to wheat flours, caused decrease in the values of lightness L^* and yellowness b^* in all fortified samples.

Microbiological analysis

Despite the extended period of fermentation and drying of the Kishk samples, no spoilage or contamination by pathogenic microbes were observed, because the nature of Kishk product in terms of acidity and low water activity did not support the growth of these microorganisms (Tamime et al. 1997). The results of the microbial analyses of Kishk samples are given in Table 4. It could be noticed that total bacterial and lactic acid bacteria counts of experimental Kishk samples were higher than that of control Kishk (B) in fresh and through the storage period. Dallagnol et al. (2013) reported that hydrolysis of quinoa proteins was faster, reaching 40–100%, while hydrolysis of wheat protein has only 0–20% after 8 h of incubation, resulted in greater quantities of peptides and free amino acids were found in quinoa compared to wheat. Moreover, the nature of starch of the quinoa seeds may also influenced the microbial growth. Although, quinoa seed has a considered amount from Saponin most of it concentrated in the husk that is nutritional component approximately 4.7–30.8 g/kg had antifungal and antibacterial activities (Gómez-Caravaca et al. 2014). However, washing, soaking, and other processing steps for quinoa seeds preparation represent a way of decreasing the Saponin component (Quispe-Fuentes et al. 2013). As expected quinoa seeds used in Kishk did not affect the total bacterial and lactic acid bacteria counts but the enumeration of microorganisms of Kishk samples increased with quinoa seeds incorporation. However, total bacterial and lactic acid bacteria counts of all samples were decreased during the storage period. This decrease could be evidently attributed to the increase in acidity which

Table 4 Effect of enrichment with quinoa seeds on microbiological quality of kishk during storage

Microbiological analysis	Storage period (month)	Kishk samples				
		B	BQB	BQ	QBQ	Q
Total bacterial count (Log CFU/g)	Zero	8.04 ± 0.10dA	8.34 ± 0.11bA	8.58 ± 0.04cA	8.76 ± 0.01bA	8.95 ± 0.09aA
	One	8.00 ± 0.06dA	8.12 ± 0.11cdB	8.25 ± 0.07cB	8.62 ± 0.04bB	8.77 ± 0.05aA
	Two	7.23 ± 0.11cB	7.43 ± 0.03bC	7.66 ± 0.05aC	7.72 ± 0.03aC	7.81 ± 0.16aB
	Three	6.15 ± 0.05cC	6.17 ± 0.06cD	6.23 ± 0.05cD	6.38 ± 0.03bD	7.57 ± 0.08aC
Lactic acid bacteria (Log CFU/g)	Zero	7.13 ± 0.01eA	7.22 ± 0.02dA	7.31 ± 0.02cA	7.43 ± 0.03bA	7.59 ± 0.04aA
	One	6.88 ± 0.02cB	6.85 ± 0.05cB	7.14 ± 0.03bB	7.17 ± 0.01bB	7.38 ± 0.03aB
	Two	6.05 ± 0.04eC	6.34 ± 0.03dC	6.47 ± 0.06cC	6.64 ± 0.09bC	6.76 ± 0.02aC
	Three	5.22 ± 0.09dD	5.44 ± 0.03cD	5.68 ± 0.03bD	5.61 ± 0.04bD	5.83 ± 0.04aD
Coliform bacteria, mold and yeast counts (CFU/g)	Zero	< 10	< 10	< 10	< 10	< 10
	One	ND	ND	ND	ND	ND
	Two	ND	ND	ND	ND	ND
	Three	ND	ND	ND	ND	ND

Results are the mean of three different determinations ± standard deviation. Means that are followed by the same letter in the row and the same capital letter in the column did not differ significantly ($P < 0.05$). ND, not detected. B, Control Kishk made from 100% wheat burghul; BQB, replaced at 25% Quinoa seeds; BQ, replaced at 50% Quinoa seeds; QBQ, replaced at 75% Quinoa seeds; Q, replaced at 100% Quinoa seeds

controlled the rate of bacterial growth or acted as a bactericidal agent. In addition, this may be attributed to the decrease of moisture content in samples that would inhibited the growth of microorganisms. Regarding the coliform counts, mould and yeast counts of all fresh samples were less than 10 CFU/g and not detected throughout the storage period. The Kishk samples have some special characteristics including low pH (3.3–5.0) and moisture content (6–10%) resulted in a harsh environment for pathogenic microbes. In such conditions, food spoilage may not occur and the shelf life will extend.

Sensory evaluation

Consumer rejection of any of the sensory characteristics could negatively influence the overall perception of food. Organoleptic characteristics of Kishk samples are shown in Table 5. Panelists had evaluated the flavor, body and texture, appearance, and color as well as acidity during the storage period. We noticed that wheat bulghul substitution by quinoa seeds affected the sensory properties of Kishk samples. There were slight differences ($p < 0.05$) between the soup samples of Kishk in flavor and texture scores. However, significant differences existed with regard to appearance and color as well as acidity, this is may be due to the unconventional color and sour taste that are different from those of wheat Kishk. Statistically, 25% supplemented with quinoa seeds containing sample (BQB) had the same sensory scores compared to control Kishk. Also, addition of quinoa seeds improved all sensorial properties.

Tang et al. (2015) reported that quinoa protein has WHC higher than that of oat, soybean, and wheat proteins, thus in various food applications it is expected that quinoa will improve the texture characteristics. Moreover, Demir and Kılınç (2017) found that addition of quinoa flour improved all sensorial properties of cookies samples. Also, the authors stated that more favorable cookies can be made using quinoa flour up to levels 20%. These results showed that more satisfying Kishk can be manufactured using quinoa seeds up to levels 50% (BQ), and being preferred over the control sample. It is clear that, the addition of quinoa seeds more than 50% resulted in lower total acceptability. Curti et al. (2017) found that addition of higher concentrations of quinoa flour into yogurts formulations resulted in reduction in acceptability of aroma and flavor. Although the color of the final product was darkness and thus not so appealing but, sensory color values of Kishk soup samples containing quinoa seeds were not significantly different and most of the soups samples were accepted in position of sensory color values and have the potential to be well received by consumers.

In the second trial (Fig. S1), during sensory evaluation of Kishk samples which prepared as soups by using spices mixture (tomato paste, paprika, onion powder and peppers red hot) at level 5% to improving the taste. This treatment improved the score acceptably of soup properties in terms and enhanced the sensory properties. Some arbitrators reported that addition of the spices has enhanced the Kishk taste and the replacement of wheat bulghul with quinoa seeds could be increased to 100%.

Table 5 Effect of enrichment with quinoa seeds on sensory evaluation of Kishk during storage

Kishk samples	Storage period (month)	Flavor (45)	Body and texture (30)	Appearance and color (15)	Acidity (10)	Total acceptability (100)
B	Zero	38.17 ± 1.04bA	21.83 ± 1.76cB	12.17 ± 0.76aA	9.00 ± 0.50aA	81.17 ± 3.21dAB
	One	41.33 ± 1.53aA	25.33 ± 2.08abA	14.00 ± 1.00aA	9.50 ± 0.50aA	90.17 ± 5.01bA
	Two	42.00 ± 1.00aAB	26.17 ± 0.76aA	14.00 ± 1.00aA	9.50 ± 0.50aA	91.67 ± 0.76aAB
	Three	38.00 ± 1.00bAB	23.17 ± 0.76bcB	13.00 ± 1.00aA	8.67 ± 0.29aA	82.83 ± 0.58cAB
BQB	Zero	37.00 ± 1.00bA	25.17 ± 0.76bA	12.17 ± 0.76bAB	8.00 ± 0.50aAB	82.33 ± 1.76bAB
	One	41.33 ± 0.76aAB	26.00 ± 1.00abA	14.00 ± 1.00abAB	9.00 ± 1.00aB	90.33 ± 3.40aAB
	Two	42.00 ± 1.00aBC	27.00 ± 0.50aA	14.00 ± 1.00aAB	8.83 ± 0.29aAB	91.83 ± 0.76aBC
	Three	38.33 ± 0.29bAB	25.00 ± 1.00bA	12.00 ± 1.00bB	8.00 ± 1.00aAB	83.33 ± 2.25bB
BQ	Zero	37.00 ± 0.50cA	26.00 ± 0.50bA	13.00 ± 0.87abA	7.00 ± 0.50cBC	83.00 ± 1.00cA
	One	42.00 ± 0.50aA	27.00 ± 1.00abA	14.00 ± 0.50aA	8.00 ± 0.50bB	91.00 ± 1.73bA
	Two	43.00 ± 1.00aA	28.00 ± 1.00aA	14.00 ± 0.50aA	9.00 ± 0.50aAB	94.00 ± 1.32aA
	Three	39.00 ± 1.00bA	26.33 ± 0.76bA	12.00 ± 0.50bB	8.00 ± 0.50bAB	85.33 ± 0.29cA
QBQ	Zero	35.00 ± 1.00 dB	26.00 ± 1.00aA	11.00 ± 1.00aBC	6.00 ± 1.00bC	78.00 ± 3.61cB
	One	39.00 ± 1.00bBC	27.00 ± 1.00aA	12.00 ± 1.00aBC	7.00 ± 0.58abB	85.00 ± 3.00abAB
	Two	41.00 ± 0.58aBC	27.00 ± 1.00aA	12.00 ± 1.00aB	8.00 ± 1.00aB	88.00 ± 2.50aCD
	Three	37.00 ± 1.0cB	25.17 ± 1.00aA	12.00 ± 0.50aB	7.00 ± 0.50abBC	81.17 ± 0.29bcBC
Q	Zero	33.00 ± 1.00cC	24.50 ± 0.50cA	11.00 ± 0.50bC	6.00 ± 0.50bC	74.50 ± 2.00dC
	One	38.17 ± 1.26abC	26.33 ± 0.76abA	11.33 ± 0.76abC	7.00 ± 0.50abB	82.83 ± 0.58bB
	Two	39.67 ± 0.76aC	27.50 ± 1.00aA	12.00 ± 1.00aB	8.00 ± 1.00aB	87.67 ± 1.76aD
	Three	37.00 ± 1.00bB	25.50 ± 1.04bcA	11.00 ± 0.50abB	6.00 ± 1.00bC	79.50 ± 1.04cC

Results are the mean of three different determinations ± standard deviation. Means that are followed by the same letter in the row and the same capital letter in the column did not differ significantly (*p* < 0.05). B, Control Kishk made from 100% wheat burghul; BQB, replaced at 25% Quinoa seeds; BQ, replaced at 50% Quinoa seeds; QBQ, replaced at 75% Quinoa seeds; Q, replaced at 100% Quinoa seeds

Conclusion

The present study showed that inclusion of quinoa seeds into Kishk formulations increased the protein, fat, ash, fiber, mineral contents and antioxidants. In addition, the sensory evaluation results revealed that acceptability of the Kishk samples were enhanced by the addition of quinoa seeds. Generally, substitution levels up to 50% quinoa seeds received the most acceptable sensory scores for Kishk samples. Modification of the technological procedures afforded the inclusion of high levels of quinoa in Kishk processing and gave the possibility to develop a novel highly nutritive product. Thus, supplementation with quinoa is not only important in developing countries but also in developed countries where there is a need to introduce new and more nutritious food products and further studies could be performed to formulate other products with quinoa as raw material.

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Declarations

Conflict of interest Authors have no conflicts of interest to declare for this article.

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