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Quality Criteria of Beef Sausage Supplemented by Quinoa Seed Flour during Frozen Storage

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Abstract: This study was performed to evaluate the effect of Quinoa flour (QF) at different ratios (5, 10 and 15%) instead of meat on keeping quality characteristics of prepared beef sausage during storage at - 18°C for 3 months. Chemical compositions, physical, physicochemical and, cooking quality, sensory characteristics and microbiological criteria of different prepared beef sausage samples were determined. Results for proximate composition of quinoa seeds flour indicated that moisture is 9.20%, protein content 13.30%, ether extract content 5.72%, ash content 2.60%, crude fiber 3.80%, carbohydrate content 65.32 % and Energy 381.40 k cl/100g. Beef sausage supplemented with QF had a higher and a balanced contents of total essential amino acids compared with the control sample or FAO/WHO requirements. Quinoa flour increased cooking yield and decreased frying and cooking loss. The increase in TVN and TBA of substituted samples were lesser than the control sample. The TVN and TBA increased with increasing storage time. Texture properties of sausage are affected by substitute levels of quinoa seed flour of beef sausage. Also, beef sausage samples containing quinoa flour (QF) exhibited a good sensory characteristics and better acceptability, especially those contained 5 and 10 %, even after frozen storage for 3 months. It was concluded that using quinoa flour into beef sausage partial replacement of meat improving nutritional, functional, sensory evaluation and lowering the cost of product.

Key words: Quinoa Seed Flour · Beef Sausage · Quality Attributes · Frozen Storage

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is usually referred to as a pseudo-cereal since it is not a member of the Gramineae family, but it produces seeds that can be milled into flour and used as a cereal crop. It is an annual dicotyledonous plant usually standing 0.5-2.0 m high with large panicles of 1.8-2.2 mm long seeds produced at the end of the stem. The seed is usually pale yellow, but it may vary from almost white through pink, orange or red to brown and black. The embryo can hold 60% of the seed weight and it forms a ring around the endosperm that loosens when the seed is cooked [1].

Quinoa is famous for its well-balanced nutritional composition, ability to thrive in harsh climatic conditions, salinity and drought tolerance (boon for cultivators in dry areas) but is underutilized [2].

Quinoa is a seed crop, which has some healthy properties such as easy to digest and a good sources of protein, dietary fiber, minerals and essential amino acids e.g. lysine, methionine and histidine [3]. Additionally, the quinoa seed contains antioxidant compounds such as carotenoids and flavonoids [4,5].

Quinoa is considered as a complete food because of its high nutritional value including good quality edible oil, high protein content with balanced amino acid profile, minerals like iron and calcium [2,6]. In the last decade, the use of pseudocereals was increased not only in special diets for people allergic to cereals, but also in healthy diets [7].

Currently, there is also a growing interest in the food industry to obtain high added value products from quinoa grains, by isolating some of their chemical components such as starch, fibre, protein and oil, among others [8, 9].

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In the meat industry, quinoa products (seeds, flour and their coproducts) have been used in different products not only as a fat replacer, for its nutritional and healthy properties [10, 11], but also for its technological properties, mainly as extender or binder [12, 13].

The main objective of this research was to investigate the effect of partial replacement of meat by quinoa flour on the quality parameters such as chemical composition, cooking parameters, pH, color, texture, sensory characteristics and microbial aspects in beef sausage during storage at - 18°C for 3 months.

MATERIALS AND METHODS

Materials: Quinoa seeds (*Chenopodium quinoa* Willd) were obtained from Agriculture Research Center, Giza, Egypt.

Frozen Beef Meat and Beef Fat: Frozen beef meat (imported from Brazil), stored at - 18°C was obtained from a supermarket at Damanhour city, Egypt, fresh beef fat (Tallow) and the natural mutton casings (cleaned) were obtained from butcher shop, at Damanhour city, Egypt and immediately transported in ice box to the laboratory.

Other Ingredients: Spices (black pepper, cubeb, dried onion, dried garlic, sodium chloride, cumin, nutmeg, cloves, ginger, cinnamon and coriander), salt, wheat flour and starch were obtained from a supermarket at Damanhour city, Egypt. Sodium nitrate, glucose, ascorbic acid and sodium pyrophosphate were obtained from El-Nasr Co., Cairo, Egypt.

Methods

Preparation of Whole Meal Quinoa Seeds (Quinoa Flour): Quinoa seeds were cleaned and freed of broken seeds, dust and other foreign materials. Whole seeds were washed with cold water 4 - 5 times or until there was no foam to remove saponins, then oven-dried at 45± 1°C for 24 h or until being dry. The whole quinoa seeds were ground into flour using stainless steel electric grinder using a laboratorial disc mill and sifted through a 60 mesh, packed in polyethylene bags and stored at 4 ± 1°C until used [14].

Preparation of Beef Sausage: Beef sausage samples were prepared according to the method described by Zaika *et al*. [15], using the ingredients listed in Table (1). Meat and fat tissues were cut into small pieces and frozen at -18°C for 24 h. Meat and fat were ground through a meat mincer and then mixed in a bowel chopper with adding of salt. Iced water was added in three stages to save the temperature of mixture, other additives and spices were added to the beef bulk and continuously mixed for another 2 min. The mixture was served as a control (without quinoa flour). Other three batch of beef sausage were prepared as same previous method with adding whole quinoa meal as 5, 10 and 15% replacement as partial part from meat. The sausage was stored at -18°C for 3 months. The mixture was taken out of the bowl chopper and was filled into natural casings of 20 + 2 mm diameter. Beef sausage was put in plastic bag and stored at -18°C for 3 months. Samples were analysis (each a month) and subjected to chemical, physical, microbiological analysis and sensory evaluation.

Table 1: Formula of beef sausage contained different level of quinoa seed flour (%)

		Substitution level with quinoa		
Components (%)	Control	5 %	10 %	15%
Beef meat	70	65	60	55
Fat tissues	12.0	12.0	12.0	12.0
Sodium chloride	2.3	2.3	2.3	2.3
Water (as ice)	9.3	9.3	9.3	9.3
Dried onion	1.2	1.2	1.2	1.2
Dried garlic	1.0	1.0	1.0	1.0
Starch	3.0	3.0	3.0	3.0
Spices mixture	1.2	1.2	1.2	1.2
Quinoa flour	0.0	5	10	15
Total	100	100	100	100

Composition of Spices mixture

Spices mixture	Amount (g)
Black pepper	30.0
Red pepper	8.0
Cumin	15.0
Nutmeg	8.0
All spices	15.0
Cloves	8.0
Ginger	8.0
Coriander	8.0

Gross Chemical Composition: Moisture, crude protein (total N × 6.25), crude fat and ash contents were determined according to AOAC [16]. Total carbohydrates content were calculated by difference [17] as follows:

% Carbohydrate = 100-(% moisture + % protein + % fat +% ash).

Total energy was estimated as follows as described by AOAC [18]:

Caloric value (kcal/100 g) = (% carbohydrate x 4) + (% protein x 4) + (% fat x 9).

Amino Acid Composition: Amino acid composition was determined by acid hydrolysis according to Block *et al.* [19] using Automatic Amino Acid Analyzer (Beckman amino acid analyzer, Model 119 CL).

Physicochemical Properties of Beef Sausage Samples: Acid value (AV) was estimated as described by AOCS [20], peroxide value was determined according to Pearson [21]. Thiobarbituric acid value (T.B.A.) was colorimeterically determined as described by Pearson [21]. The pH values of beef sausage were measured using pH meter (Jenway 3510 pH meter -Germany) at 20°C according to the method described by Fernansez-Lopez *et al*. [22]. Water holding capacity (WHC) was determined by filter press method [23]. Total volatile nitrogen (T.V.N.) was measured according to the method of the Egan *et al.* [24] and the results were calculated as mg TVN/100g.

Cooking Procedure: Beef sausage samples were cooked in a pan (148°C) with little of oil. The sausage were cooked for 6 minutes, then turned and cooked for 4 minutes on the other side. The sausage was weighed before and after cooking to determine percentage cooking yield according to Choi *et al*. [25] as follows:

$$\text{Cooking yield (\%)} = (\text{Weight of cooked sausage/Weight of uncooked sausage}) \times 100$$

Cooking loss was calculated using the following equation:

$$\text{Cooking Loss \%} = \frac{\text{uncooked sausage sample weight} - \text{cooked sausage sample}}{\text{uncooked sausage sample weight}} \times 100$$

Change in beef sausage diameter and length (Shrinkage) was measured on cooked samples as mentioned by George and Berry [26] using the following equations:

$$\text{Shrinkage \%} = \frac{\text{Uncooked diameter or length (cm)} - \text{Cooked diameter or length (cm)}}{\text{Uncooked siameter or length 9 (cm)}} \times 100$$

Texture Profile Analysis: Texture profile Analysis (TPA) was determined according to the method of Bourne [27] as described follow : Samples were formed to 50 mm diameter cylinder with 40 mm height and texture was determined by a universal testing machine (Cometech, B type, Taiwan) provided with software. An aluminum 25 mm diameter cylinder probe was used in a TPA double compression test to penetrate to 50% depth at 1 mm /s speed test. Firmness (N), Gumminess (N), Chewiness (N), Cohesiveness, Springiness, Adhesiveness negative force (N) and resilience were calculated from the TPA graphic. Both springiness and resilience give information about the after stress recovery capacity. But, while as the former refers to retarded recovery, the latter concerns instantaneous recovery (immediately after the first compression, while the probe goes up.

Sensory Evaluation: Cooked sausage samples (in little of oil) were evaluated by 10 staff members in the Food Science and Technology Department, Faculty of Home Economic, Al-Azhar University. A 10- point hedonic scale (1 being dislike very much to 10 being like very much) for texture, odor, color, taste and overall acceptability were used to evaluate the sensory attributes of beef sausage sample according to Crehan *et al*. [28].

Microbiological Aspects: Total bacterial count (TBC) was determined using plate count agar (Difco) Difco [29], psychrophilic bacteria count were detected following Difco [29] method using nutrient agar medium, coliform bacteria count and *Salmonella* & *Shigella* bacterial counts were determined according to Bryan [30] using Macconky agar media and mold and yeast count were determined on potato dextrose agar according Oxoid [31].

Statistical Analysis: The obtained results were subjected to statistical analysis according using SPSS [32]. Significant differences among individual means analyzed by Duncan multiple range tests [33].

RESULTS AND DISCUSSION

The Proximate Chemical Composition of Quinoa Flour and Imported Frozen Meat: Results of analysis of the proximate composition of quinoa flour and imported frozen beef meat are presented in Table 2. Concerning the proximate chemical composition of QF, the obtained results showed 9.20% moisture, 13.30% crude protein, 5.72% crude fat, 2.60 ash, 3.80% crude fiber and 65.38% carbohydrates. In this respect, Moawad *et al*. [34] reported that whole meal quinoa flour had 11.36% moisture, 15.10% crude protein, 6.33 lipids, 3.80% crude fiber and 3.72% ash. On the other hand, imported frozen beef meat recorded of 74.67% moisture, 19.24% crude protein, 3.27% ether extract and 2.02% carbohydrates. Mousa [35] showed that imported frozen beef meat contained moisture, protein, fat, ash and carbohydrates values of 74.84, 21.46, 2.28, 0.98 and 0.44%, respectively.

Table 2: Proximate analysis of quinoa flour and frozen beef meat

Constituents (%)	Washed quinoa with water	Frozen beef meat
Moisture	9.2±0.5	74.67±0.1
Crude protein	13.30±0.3	19.24±0.1
Ether extract	5.72±0.07	3.27±0.2
Ash	2.6±0.4	0.75±0.08
Crude fiber	3.8±0.6	0.06±0.03
Carbohydrates*	65.38±0.03	2.02±0.12
Energy (Kcal/100g)	381.4±0.4	130±0.05

M±SD means and standard deviation

* Carbohydrates was calculated by deference

Table 3: Amino acids composition of beef sausage supplemented with different ratios quinoa flour

			Quinoa flour ratios				
Amino acids	Quinoa flour	Frozen beef meat	0 (Control)	5%	10%	15%	FAO/WHO
Essential amino acids							
Histidine	4.8	3.30	3.10	3.20	3.28	3.18	1.50
Isoleucine	2.2	6.18	3.20	3.40	3.66	3.60	3.00
Leucine	5.4	7.48	1.39	1.46	1.55	1.52	5.90
Lysine	5.3	7.82	7.50	7.80	7.86	8.10	4.50
Methionine	1.3	3.63	2.60	2.70	2.88	2.90	1.60
Phenylalanine	2.4	4.38	6.40	6.31	6.50	6.40	3.80
Threonine	3.9	4.21	0.74	0.80	0.85	0.80	2.30
Valine	3.2	3.72	4.20	3.80	3.70	3.76	3.90
Tyrosine	1.8	4.23	3.70	3.77	3.90	3.80	-
Cysteine	ND	0.83	1.80	1.93	2.01	1.98	-
Tryptophan	0.49	1.67	1.82	1.96	1.88	1.84	-
Total essential amino acids	30.3	47.43	36.45	37.10	37.87	37.88	26.5
Non-essential amino acids							
Aspartic acid	10.2	11.32	10.60	10.99	12.10	11.30	-
Serine	4.1	3.26	6.80	5.40	5.30	5.36	-
Glutamic acid	15.7	14.28	13.40	14.20	15.10	14.80	-
Proline	12.0	4.80	6.90	7.12	7.68	8.60	-
Glycine	5.6	5.93	6.10	6.70	6.90	6.82	-
Alanine	8.3	4.81	6.80	6.88	7.20	7.10	-
Arginine	8.7	5.74	4.80	4.60	4.70	5.82	-
Total non- essential amino acids	64.4	50.14	55.40	55.89	58.98	57.80	-
Total amino acids	94.7	97.57	91.85	93.02	96.85	97.68	
Ammonia	5.4						

Amino Acids Composition of Beef Sausage Supplemented with Different Ratios Quinoa Flour: From Table (3), it could be noticed that the increasing QF ratio in beef sausage samples result in increasing the content of lysine, methionine and threonine acids and decreasing the content of valine of beef sausage samples, especially in sausage sample containing 15 % QF. Also, sausage formula containing 15% QF had the highest of total essential amino acids which recorded 37.88%. Compared with the reference protein pattern of FAO/WHO [36], it could be observed that all sausage formulas which supplemented with QF were rich in essential amino acids such as phenylalanine, methionine, lysine, isoleucine and histidine. Therefore, fortified meat products such as sausage with quinoa flour can represent a healthy alternative for people and can plays a complementary role to the low protein sources in lysine and essential amino acids. Shokry [37] reported that the protein content of quinoa flour represents 14-20%, it has a good balance of the amino acids that make up the protein where particularly rich in essential amino acids such as lysine and methionine and so supplying a high-quality protein Quinoa is one of the few plants that provide all the amino acids necessary for human life and contrary to grain proteins poor in especially lysine, quinoa proteins are accepted as high-quality proteins [11].

Table 4: Chemical composition (%) of beef sausage prepared with different level of QF during frozen storage

Components (%)	Frozen storage period (month)	Whole quinoa flour ratios			
		0% (Control)	5%	10%	15%
Moisture	0	57.76±0.03^{Aa}	57.32±0.01^{Ba}	57.11±0.04^{Ca}	56.82±0.02^{Da}
	1	57.53±0.05^{Ab}	57.05±0.04^{Bb}	56.78±0.01^{Cb}	56.21±0.05^{Db}
	2	57.16±0.01^{Ac}	56.83±0.02^{Bc}	56.24±0.03^{Cc}	56.01±0.03^{Dc}
	3	56.83±0.02^{Ad}	56.23±0.03^{Bd}	56.04±0.02^{Cd}	55.87±0.01^{Dd}
Crude protein	0	15.54±0.04^{Aa}	15.21±0.01^{Ba}	14.90±0.03^{Ca}	14.64±0.02^{Da}
	1	15.33±0.03^{Ab}	15.02±0.02^{Bb}	14.78±0.05^{Cb}	14.55±0.03^{Db}
	2	15.16±0.01^{Ac}	14.92±0.04^{Bc}	14.68±0.02^{Cc}	14.43±0.01^{Dc}
	3	15.07±0.02^{Ad}	14.84±0.03^{Bd}	14.59±0.01^{Cd}	14.30±0.04^{Dd}
Ether extract	0	17.66±0.05^{Da}	18.50±0.03^{Ca}	18.63±0.04^{Ba}	19.77±0.02^{Aa}
	1	17.42±0.04^{Db}	18.44±0.05^{Ca}	18.51±0.02^{Bb}	19.54±0.01^{Ab}
	2	17.23±0.02^{Dc}	18.29±0.04^{Cb}	18.42±0.01^{Bc}	19.12±0.03^{Ac}
	3	17.02±0.03^{Dd}	17.88±0.02^{Cc}	17.94±0.03^{Bd}	18.65±0.04^{Ad}
Ash	0	2.93±0.01^{Dd}	3.12±0.02^{Cd}	3.55±0.04^{Bc}	3.69±0.03^{Ad}
	1	3.43±0.04^{Cc}	3.35±0.03^{Dc}	3.61±0.02^{Bc}	3.77±0.05^{Ac}
	2	3.76±0.02^{Bb}	3.47±0.01^{Cb}	3.74±0.03^{Bb}	3.84±0.04^{Ab}
	3	3.89±0.03^{ABa}	3.56±0.05^{Ca}	3.85±0.04^{Ba}	3.95±0.01^{Aa}
Carbohydrates	0	4.00±0.04^{Dd}	5.72±0.01^{Cd}	6.39±0.02^{Bc}	7.19±0.03^{Ad}
	1	4.17±0.05^{Dc}	6.07±0.02^{Cc}	6.39±0.03^{Bc}	8.05±0.04^{Ac}
	2	4.80±0.01^{Db}	6.36±0.02^{Cb}	7.05±0.05^{Bb}	8.49±0.02^{Ab}
	3	5.56±0.03^{Da}	7.43±0.04^{Ca}	7.64±0.01^{Ba}	8.86±0.05^{Aa}
Energy (Kcal/100g)	0	256.09±0.02^{Aa}	251.39±0.01^{Ca}	251.66±0.03^{Ba}	246.26±0.04^{Dc}
	1	253.86±0.01^{Ab}	250.95±0.03^{Bb}	250.64±0.02^{Cc}	247.18±0.05^{Da}
	2	251.92±0.05^{Ac}	250.90±0.02^{Cb}	251.53±0.01^{Bb}	246.75±0.01^{Db}
	3	250.37±0.02^{Bd}	250.54±0.04^{Ac}	249.84±0.05^{Cd}	245.82±0.02^{Dd}

Mean ± Standard deviation

In a row, means have the same capital superscript letter are not significantly different by Danken's Test at 5% level

In a column, means have the same small superscript letter are not significantly different by Danken's Test at 5% level

Chemical Composition of Beef Sausage Prepared with Different Level of QF During Frozen Storage: The chemical composition of beef sausage substituted with different ratios of quinoa flour is shown in Table (4). It could be observed that moisture content of beef sausage was decreased by increasing either quinoa flour substitute ratio from 0 to 15 % or frozen storage time from 0 to 3 months. Moisture content decreased from 57.76% in control sample (0% quinoa flour) to 56.82% for sample containing 15% QF at 0 month frozen storage. Crude protein percentage of sausage samples has the same trend as moisture content of sausage samples. Crude protein decreased from 15.61 to 14.51% by increasing QF substitute ratio from 0 to 15 % at 0 frozen storage time. This decrease in protein content may be due to protein hydrolysis by natural meat enzymes and bacterial enzymes that are produced as well as the loss of water soluble protein with separated drip [38]. On contrast, ether extract increased from 17.66 to 19.77 % by increasing QF substitute ratio from 0 to 15 %. This could be referred that QF contains higher ether extract % than meat (5.72 and 3.27%, respectively). Ether extract percentage decreased from 17.66 to 17.02 by increasing frozen storage time from 0 to 3 months for control beef sausage sample. Ash and carbohydrates contents increased by increasing ratios of QF in all sausage formulas.

Cooking Loss and Cooking Yield: Table (5) shows the effect of adding quinoa flour on cooking loss, cooking yield and shrinkage of sausage. It could be observed that all sausage samples which supplemented with QF had higher cooking yield and lower cooking loss comparing with the control sample. Cooking loss of the control beef sausage had the highest value of cooking loss (23.16%) and the lowest value of cooking yield (85.85%) among other studied samples. There was increment in the cooking yield from 85.85% for the control formula to 88.14 and 91.62% for QF 10 and QF 15%, respectively at zero time. Similar results were obtained by Baioumy *et al*. [11] who reported that cooking loss percent of beef burger decreased and cooking yield percent increased by increasing the concentration of added quinoa seeds. These results confirmed that addition of quinoa improves the quality attributes of beef sausage. However Yogesh *et al*, [39] found that cooking loss decreased significantly in treated meat batter with flaxseed powder when compared with the control sample, due to flax seed ability of keeping the moisture in the matrix.

Table 5: Some physical characteristics of beef sausage prepared with different ratio of quinoa powder during freezing storage (- 18°C for 3 months)

Cooking quality	Storage period (months)	T1	T2	T3	T4
Cooking loss (%)	0	23.16±0.01^{Ad}	21.93±0.03^{Bd}	19.87±0.02^{Cd}	17.39±0.04^{Dd}
	1	25.69±0.04^{Ac}	23.05±0.02^{Bc}	21.13±0.05^{Cc}	19.12±0.01^{Dc}
	2	27.75±0.03^{Ab}	24.74±0.01^{Bb}	22.68±0.04^{Cb}	20.81±0.02^{Db}
	3	29.62±0.02^{Aa}	26.30±0.05^{Ba}	24.64±0.01^{Ca}	21.77±0.03^{Da}
Cooking yield (%)	0	85.85±0.03^{Da}	87.27±0.02^{Ca}	88.14±0.04^{Ba}	91.62±0.01^{Aa}
	1	82.31±0.02^{Db}	85.96±0.01^{Cb}	86.87±0.03^{Bb}	89.89±0.05^{Ab}
	2	80.87±0.04^{Dc}	83.26±0.03^{Cc}	85.32±0.01^{Bc}	88.19±0.02^{Ac}
	3	78.39±0.05^{Dd}	81.71±0.04^{Cd}	83.36±0.02^{Bd}	85.24±0.04^{Ad}
Shrinkage (length %)	0	10.99±0.02^{Ad}	9.85±0.01^{Bd}	9.74±0.03^{Cd}	8.65±0.05^{Db}
	1	11.31±0.04^{Ac}	10.51±0.03^{Bc}	10.54±0.02^{Bc}	9.57±0.01^{Cc}
	2	12.33±0.01^{Ab}	11.35±0.04^{Bb}	11.32±0.05^{Bb}	10.11±0.02^{Cb}
	3	13.90±0.03^{Aa}	12.12±0.02^{Ca}	12.24±0.01^{Ba}	1.39±0.04^{Da}

Mean ± Standard deviation

In a row, means have the same capital superscript letter are not significantly different by Danken's Test at 5% level

In a column, means have the same small superscript letter are not significantly different by Danken's Test at 5% level

Table 6: Quality attributes of beef sausage supplemented with different ratios of QF during storage period at - 18°C for 3 months

		Quinoa flour ratios (%)			
Parameters	Storage periods (months)	0	5	10	15
Acid value (%)	0	0.86±0.01^{Ad}	0.64±0.04^{Bd}	0.50±0.03^{Cd}	0.47±0.02^{Cd}
	1	1.32±0.03^{Ac}	0.73±0.02^{Bc}	0.61±0.01^{Cc}	0.54±0.05^{Dc}
	2	1.67±0.04^{Ab}	0.97±0.03^{Bb}	0.79±0.02^{Cb}	0.81±0.01^{Cb}
	3	2.31±0.02^{Aa}	1.87±0.01^{Ba}	1.05±0.04^{Ca}	0.96±0.03^{Da}
Peroxide value (meq.O_2/Kg)	0	10.04±0.05^{Ad}	9.53±0.02^{Bd}	9.33±0.03^{Cd}	9.12±0.01^{Dd}
	1	12.07±0.01^{Ac}	10.33±0.04^{Bc}	10.16±0.02^{Cc}	9.87±0.03^{Dc}
	2	13.79±0.02^{Ab}	12.09±0.01^{Bb}	11.88±0.04^{Cb}	10.90±0.05^{Db}
	3	15.60±0.03^{Aa}	14.12±0.02^{Ba}	13.45±0.01^{Ca}	12.78±0.04^{Da}
TBA (mg/Kg)	0	0.34±0.01^{Ad}	0.33±0.03^{Ad}	0.31±0.02^{Ac}	0.28±0.05^{Ac}
	1	0.45±0.02^{Ac}	0.40±0.01^{ABc}	0.38±0.04^{Bb}	0.31±0.03^{Cbc}
	2	0.56±0.03^{Ab}	0.49±0.05^{Bb}	0.41±0.01^{Cab}	0.37±0.04^{Cab}
	3	0.68±0.05^{Aa}	0.58±0.0.4^{Ba}	0.46±0.03^{Ca}	0.41±0.02^{Ca}
pH values	0	5.40±0.02^{Dd}	5.67±0.01^{Cc}	5.80±0.04^{Bc}	5.92±0.05^{Ad}
	1	5.56±0.03^{Dc}	5.76±0.02^{Cb}	5.84±0.05^{Bc}	5.98±0.01^{Ac}
	2	5.72±0.01^{Db}	5.82±0.04^{Cb}	5.95±0.03^{Bb}	6.04±0.02^{Ab}
	3	6.03±0.04^{Ca}	6.11±0.05^{Ba}	6.15±0.02^{ABa}	6.20±0.03^{Aa}
WHC (%)	0	78.22±0.05^{Aa}	76.41±0.04^{Ba}	75.33±0.02^{Ca}	74.32±0.01^{Da}
	1	74.54±0.04^{Ab}	73.17±0.01^{Bb}	72.19±0.03^{Cb}	68.38±0.02^{Db}
	2	72.12±0.03^{Ac}	70.04±0.05^{Bc}	68.33±0.01^{Cc}	63.07±0.04^{Dc}
	3	66.37±0.01^{Ad}	64.78±0.02^{Bd}	62.06±0.04^{Cd}	60.45±0.03^{Dd}
TVN (mg/100g)	0	12.22±0.02^{Ad}	12.06±0.01^{Bd}	11.69±0.03^{Cd}	11.23±0.04^{Dd}
	1	13.17±0.05^{Ac}	13.05±0.02^{Bc}	12.43±0.01^{Cc}	12.21±0.03^{Dc}
	2	14.45±0.01^{Ab}	14.22±0.04^{Bb}	13.55±0.02^{Cb}	13.16±0.01^{Db}
	3	16.78±0.03^{Aa}	15.75±0.01^{Ba}	15.38±0.04^{Ca}	14.56±0.05^{Da}

Mean ± Standard deviation

In a row, means have the same capital superscript letter are not significantly different by Danken's Test at 5% level

In a column, means have the same small superscript letter are not significantly different by Danken's Test at 5% level

Quality Criteria of Beef Sausage Supplemented with QF: As shown in Table (6) acid value (AV) of beef sausage significantly ($p \leq 0.05$) decreased as substituted of QF increased in formula, whereas the AV of the control sample (0.86%) decreased to 0.64, 0.50 and 0.47% in beef sausage contained 5, 10 and 15% QF, respectively. The decrement in AV may be due to the presence of antioxidant compounds (sapnonin, polyphenols, flavonoids, vitamins E, C) which had antioxidant activity. From the obtained data, it could be observed that AV increased by increasing the period of frozen storage. Since, AV of beef sausage increased to 2.31, 1.87, 1.05 and 0.96% in control and samples contain 5, 10 and 15% QF, respectively.

It is shown from the same table that PV of beef sausage gradually decreased by substitution the QF ratio in its formula whereas PV of the control sausage sample was 10.04 meq. O_2/kg decreased to 9.53, 9.33 and 9.12 meq. O_2/kg in beef sausage contained 5, 10 and 15% QF, respectively. PV of all formula increased by increasing frozen storage period whereas PV in sausage samples contained 15% QF gradually increased during storage period but with lower rate compared with the control. This may be due to the presence bioactive compounds such as polyphenols and flavonoids which had antioxidant activity.

Also, the same table shows that TBA value decreased by increasing ratios of QF, whereas TBA value in control sample was 0.34 mg/kg decreased to 0.33, 0.31 and 0.28 mg/kg in sausage contained 5, 10 and 15% QF, respectively at zero time. TBA increased for all sausage with increasing storage time.

As shown in Table (6), pH value of the control beef sausage sample was 5.67 increased to 5.67, 5.80 and 5.92 in sausage contained 5, 10 and 15% QF, respectively. These results are in agreement with Dzudie *et al*. [40] who reported an increasing in pH in beef sausages with the addition of bean flour as extensor.

The control beef sausage sample showed highest WHC as compared with sausage contained QF at zero time which recorded 78.22%. This trend of results was in agreement with Oroszvari *et al*. [41] who reported that WHC values continuously reduce in all beef burgers with extending the frozen storage periods as the result of breakdown hydrogen bonding between the water molecules and gross chemical components of beef burgers.

Concerning TVN content of beef sausage containing QF, it was clearly decreased with increasing level of QF. It could be noticed that TVN value increased with increasing the storage period from 0 to 3 months. Degradation of beef burgers protein during storage resulting in formation of some basic compounds such as volatile nitrogen compounds, amines and hydrogen sulfide, leading to increase pH value [42].

Texture Profile Analysis of Beef Sausage Prepared with QF During Storage at - 18°C for 3 Months: Texture profile analysis (TPA) parameters better reflect the contribution of proteins to textural properties than the contribution of starch to texture [43]. Hardness, adhesiveness, resilience, cohesiveness, springiness, gumminess and chewiness were investigate the effect of quinoa flour substitution at different levels (5, 10 and 15%) and storage period at - 18°C for 3 months on the texture profile analysis of beef sausage. From the obtained data (Table, 7), it could be exhibited that hardness increased with the increased substitution level of quinoa flour. These results are agreement with Zapata and Rodriguez de la Pava [44] who reported that the hardness and shear force of the sausages increased with the addition of quinoa flour. This may be due to a lower moisture content was observed, which may explain the higher hardness values. Thus, QF affected the mechanical properties of beef burger and provides the final product its characteristic of juiciness and better texture [37]. From the obtained results, hardness decreased with increasing frozen storage time for all samples. This may be due to decreased in moisture content during frozen storage. These results are disagree with Aleson-Carbonell *et al*. [45] who reported that some substitutes may provide better texture to products made with ground beef because these ingredients absorb water, dissolve with the meat protein matrix and result in increased softness of the product [46, 47]. Adhesiveness is the negative force area of the first bite, or the work necessary to pull the molars away from the food [21]. From the obtained results, the control sausage sample had greater adhesiveness than those containing quinoa flours, especially after storage 2 months at - 18°C. Cohesiveness is defined as the degree to which the sample can be deformed before it breaks [12]. In this respect, AL-Juahimiet *et al*. [48] reported that the chewiness of uncooked meatballs increased with increment of moringa seed flour. When the quinoa flour was increased, the cohesiveness values were increased [12].

Quinoa level had a clear effect on springiness of sausage. Springiness can be defined as the rate at which the deformed beef meatball springs back after the compression [49]. From the obtained data, it could be observed that springiness was decreased by increasing the level of QF. Springiness, or elasticity, is the rate at which the compressed sample returned to its original height after the deforming force was removed [50].

The amount of quinoa flour displayed effect on the gumminess values of sausage samples. When the amount of quinoa flour in sausage formulation increased, the gumminess values decreased. The highest gumminess value found in the control sausage samples which recorded 4.14.

Chewiness is the energy required to chew a solid food until it is ready for swallowing. It is determined as the product of hardness, cohesiveness and springiness [21]. Sausage prepared with 15% quinoa flour had the

Table 7: Texture profile analysis of beef sausage prepared with QF during storage at - 18°C for 3 months

Level of QF	Storage periods (months)	Texture parameters						
		Hardness (N)	Adhesiveness (mJ)	Resilience	Cohesiveness	Springiness (mm)	Gumminess (N)	Chewiness (mJ)
0%	0	3.38	0.00	0.32	0.76	3.43	4.14	14.20
	1	3.39	0.00	034	0.74	3.30	3.25	10.70
	2	3.96	0.70	0.21	0.66	2.89	2.76	8.00
	3	4.39	0.20	0.25	0.70	2.81	2.92	8.20
5%	0	3.52	0.20	0.31	0.80	3.13	2.80	8.80
	1	3.76	0.00	0.23	0.61	2.72	2.49	6.80
	2	4.16	0.60	0.25	0.58	2.76	2.72	7.50
	3	4.42	0.50	0.28	0.75	3.11	3.13	9.70
10%	0	3.60	0.00	0.30	0.78	3.05	2.87	8.70
	1	3.78	0.10	0.26	0.63	2.72	2.55	6.90
	2	4.02	0.30	0.32	0.72	3.12	3.13	9.80
	3	4.85	0.90	0.24	0.65	2.88	2.99	8.60
15%	0	3.79	0.10	0.22	0.65	2.40	2.45	5.90
	1	3.93	0.10	0.27	0.68	2.95	3.49	5.30
	2	4.17	0.10	0.25	0.69	2.56	2.03	5.20
	3	5.22	0.10	0.27	0.71	2.96	2.98	8.80

Table 8: Sensory characteristics of beef sausage formulas prepared with different ratio of quinoa flour during freezing storage (at - 18°C for 3 months)

Parameter	Storage periods (month)	Quinoa flour ratios (%)			
		0	5	10	15
Texture	0	8.70 ± 0.01^{Ba}	8.90 ± 0.03^{Aa}	8.30 ± 0.02^{Ca}	8.20 ± 0.04^{Da}
	1	8.60 ± 0.03^{Ab}	8.30 ± 0.02^{Bb}	8.15 ± 0.04^{Cb}	7.70 ± 0.05^{Db}
	2	8.56 ± 0.02^{Ab}	8.10 ± 0.01^{Bc}	8.04 ± 0.05^{Cc}	7.60 ± 0.03^{Dc}
	3	8.30 ± 0.04^{Ac}	8.00 ± 0.05^{Bc}	7.60 ± 0.03^{Cd}	7.20 ± 0.02^{Dd}
Odor	0	$8.90\pm o.02^{Aa}$	8.80 ± 0.04^{Ba}	8.30 ± 0.05^{Cc}	8.10 ± 0.01^{Da}
	1	8.80 ± 0.01^{Ab}	8.80 ± 0.03^{Aa}	8.50 ± 0.02^{Ca}	8.00 ± 0.04^{Db}
	2	8.60 ± 0.03^{Ac}	8.70 ± 0.02^{Ab}	8.40 ± 0.01^{Bb}	8.00 ± 0.03^{Cb}
	3	8.0 ± 0.04^{Cd}	8.61 ± 0.05^{Ac}	8.40 ± 0.03^{Bb}	7.69 ± 0.02^{Dc}
Color	0	8.80 ± 0.01^{Ab}	8.10 ± 0.02^{Ba}	7.90 ± 0.04^{Cb}	7.60 ± 0.03^{Dc}
	1	8.60 ± 0.04^{Ac}	7.90 ± 0.03^{Bc}	7.60 ± 0.05^{Cc}	7.40 ± 0.02^{Dd}
	2	8.90 ± 0.01^{Aa}	8.15 ± 0.01^{Ba}	7.90 ± 0.02^{Db}	8.05 ± 0.04^{Cb}
	3	8.20 ± 0.02^{Cd}	8.00 ± 0.04^{Db}	8.60 ± 0.03^{Aa}	8.30 ± 0.01^{Ba}
Taste	0	9.00 ± 0.02^{Aa}	8.10 ± 0.04^{Ba}	7.60 ± 0.03^{Cb}	7.30 ± 0.03^{Da}
	1	8.70 ± 0.05^{Ab}	7.80 ± 0.03^{Bb}	7.50 ± 0.02^{Cc}	7.23 ± 0.04^{Db}
	2	8.40 ± 0.01^{Ac}	7.75 ± 0.02^{Bb}	7.80 ± 0.05^{Ba}	7.10 ± 0.03^{Cc}
	3	8.30 ± 0.04^{Ad}	7.70 ± 0.05^{Bc}	7.30 ± 0.01^{Cd}	7.00 ± 0.03^{Dc}
Overall acceptability	0	8.83 ± 0.01^{Ab}	8.23 ± 0.03^{Bb}	7.95 ± 0.02^{Cc}	7.60 ± 0.01^{Da}
	1	8.70 ± 0.05^{Ac}	8.20 ± 0.02^{Bb}	7.80 ± 0.01^{Cd}	7.52 ± 0.03^{Db}
	2	8.60 ± 0.02^{Ad}	8.25 ± 0.04^{Bb}	7.64 ± 0.03^{Cb}	7.31 ± 0.05^{Cb}
	3	8.20 ± 0.03^{Aa}	8.00 ± 0.05^{Ba}	7.40 ± 0.04^{Ca}	7.20 ± 0.04^{Dc}

Mean ± Standard deviation

In a row, means have the same capital superscript letter are not significantly different by Danken's Test at 5% level

In a column, means have the same small superscript letter are not significantly different by Danken's Test at 5% level

minimum chewiness value (5.90N) and the control sausage had the highest chewiness value (14.20N). The decrease of chewiness could be meaning the product is easier to chew [51]. These results were in disagreement with AL-Juahimi *et al*. [48] who reported that the chewiness of uncooked meatballs increased with increment of moringa seed flour. Some researchers reported that carbohydrates such as starch and dietary fiber component in the added vegetable sources may interact with water and fat of meat products to form a softer texture thus leading to a change in textural properties [52]. Also, Feng and Xiong [53] reported that the interactions between meat and nonmeat additives may effectively affect the gel properties in emulsified meat products through modifying the product texture.

Sensory Characteristics of Beef Sausage Prepared with Different Ratios of Quinoa Flour During Storage at - 18°C for 3 Months: According to the means given by the panelists of grilled samples at zero time, sensory scores for studied parameters such as, texture, odor, color, taste and overall acceptability were varied and affected significantly by the addition of QF Table (8). The obtained data shows that there was a significant difference in

Table 9: Microbiological criteria (x 10^3 cfu/g) of beef sausage prepared with different ratio of quinoa powder during freezing storage for 3 months

		Quinoa flour ratios (%)			
Type of Microorganisms	Frozen storage time (months)	0 (Control)	5	10	15
Total bacterial count	0	66.50±0.02^{Aa}	63.60±0.03^{Ba}	61.00±0.01^{Ca}	58.65±0.05^{Da}
	1	65.00±0.04^{Ab}	60.51±0.02^{Bb}	59.55±0.03^{Cb}	57.00±0.01^{Db}
	2	60.12±0.01^{Ac}	57.44±0.05^{Bc}	55.24±0.02^{Cc}	53.78±0.04^{Dc}
	3	55.73±0.03^{Ad}	53.46±0.01^{Bd}	50.22±0.04^{Cd}	49.50±0.02^{Dd}
Molds and Yeasts count	0	39.00±0.01^{Aa}	38.00±0.04^{Ba}	33.34±0.02^{Ca}	30.12±0.03^{Da}
	1	15.11±0.05^{Ab}	9.00±0.03^{Bb}	8.50±0.02^{Cb}	6.00±0.04^{Db}
	2	ND	ND	ND	ND
	3	ND	ND	ND	ND
Coliform group	0	7.00±0.01^{Aa}	2.00±0.04^{Ba}	1.00±0.01^{Ca}	0.50±0.04^{Da}
	1	6.50±0.03^{Ab}	1.50±0.02^{Bb}	ND	ND
	2	ND	ND	ND	ND
	3	ND	ND	ND	ND

Mean ± Standard deviation ND = not detected

In a row, means have the same capital superscript letter are not significantly different by Danken's Test at 5% level

In a column, means have the same small superscript letter are not significantly different by Danken's Test at 5% level

texture between the control beef sausage samples and samples prepared with different ratios of QF. Formula with 5% QF had the highest value of texture compared with the control sample or samples contain QF. The data in the same table indicated that there are significant differences ($P<0.05$) among odor, color and taste of beef sausage substituted with 0 and 5, 10 or 15 % QF. With regard to the Overall acceptability, the sample containing 15 % of QF was the lowest acceptable sample. On the other hand, all the parameters of sensory evaluation decreased as the frozen storage time increased.

Microbiological Criteria of Beef Sausage Prepared with Different Ratios of QF During Storage at -18°C for 3 Months: All beef sausage samples were subjected to microbiological analysis at zero time and during storage at - 18°C. The results given in Table (9) show that the total bacterial counts were gradually decreased with increasing ratio of QF, whereas TBC of control beef sausage was 66.5 × 103 cfu/g decreased to 63.60 ×10^3, 61.00 × 10^3 and 58.65 × 10^3 cfu/g for beef sausage contained 5, 10 and 15% QF. This may be due to the presence of bioactive compounds which act as antimicrobial agents such as phenolic compounds. Also, TBC decreased during frozen storage to reach their lowest number at the end of storage period which could be due to the lethal effect of frozen storage [54].

At the same trend, counts of moulds and yeasts decreased and reach to 15.11, 9.00, 8.50 and 6.00 × 10^3 cfu/g for the control sausage sample and prepared with 5, 10 and 15% QF after month of frozen storage, respectively. After one month of storage moulds and yeasts in either control or supplemented with different ratio of QF were not detected. This may be due to destroyed effect of freezing on moulds & yeasts [55]. Also, coliform group was 7.00 × 10^3 cfu/g for the control beef sausage decreased to 6.50 × 10^3 cfu/g after one month of frozen storage and then disappeared at the end of storage. The same trend was also observed for beef sausage contained QF. *Salmonella*, *Shigella* and psychrophilic bacteria were absent in all the studied beef sausage samples either at zero time or during storage at - 18°C. This may be due to the hygienic conditions followed during the preparation or frozen storage.

Sharaf *et al*. [56] indicated that TBC, Psychrophilic bacteria, coliform bacteria group and mold and yeast counts decreased with increasing level of moringa meal flour, which may be due to the reducing of free water resulting from the high water binding capacity of moringa meal flour.

CONCLUSION

It could be concluded that using quinoa seed flour partial replacement of meat improved the nutritional quality of product and can be produced healthy foods and reduced the costs, especially after high price of red meat.

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