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Quality Characteristics of Processed Low-Fat Beef Sausage as Affected by Chickpea Protein Isolates Prolonged Cold Storage

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ABSTRACT

Beef sausage was prepared by the incorporation of chickpea protein isolate (CPI) at the ratio levels 10, 15 and 20% of beef meat was studied in order to assess the yield, quality characteristics and shelf life of beef Sausage (BS). BS quality characteristics was assessed by pH, moisture content, Thiobarbituric acid (TBA) value, total plate count (TPC), mold and yeast count, organoleptic properties, and physical properties. Obtained results found that the pH values and moisture content are affected slightly prolonged storage period, However, TBA values were not affected by the addition of CPI at zero day of cold storage. The TPC of the fresh BS samples were found in the range of 3.7-4.3 logarithm cfu/g. BS samples were acceptable to Committee members and integration of CPI did not influence admissibility. Integration of CPI increased the values of Hunter L and b but reduced the other values and hardness. During refrigerated storage at (0 °C) the values for L, a, b was fluctuating irregularly. It was summarized that the integration of CPI prepared sausage could improves slightly sensory properties namely texture, color, and juiciness. Also, the shelf life of the emulsified sausages was not significantly changed by the incorporating CPI into the formula.

Keywords: Chickpea protein isolate, beef, texture, Emulsified Sausage Total Plate Count, Thiobarbituric acid.

INTRODUCTION

Emulsified meat products containing high amount of animal fats have a significant amount of saturated fatty acids and cholesterol that may directly affect consumer health (Jim´enez-Colmenero *et al*., 2001). Also, due to the fact that processed meats are highly linked to cardiovascular diseases and cancer, most consumers avoid eating meat (Carvalho *et al*., 2019). Various types of sausages are widely consumed all around the world. However, these products contain a high amount of fat (18–34%) with considerable ratios of cholesterol and saturated fatty acids (Feng and Xiong, 2002; Jimenez-Colmenero *et al*., 2010; Zhao *et al*., 2014; Ogawa and Adachi, 2017). In recent years, a major increasing interest in the food industry is to produce healthier low-fat meat products by incorporating healthy ingredient such as legume protein isolates since they contain 16-26% protein (Euston *et al*., 2000). Chickpea seeds are a good, cheap and concentrated source of protein compared with animal origin sources of protein. Plant protein products such as protein isolates and concentrates have been successfully used in food products due to their functional properties. Pakhare *et al*., (2018) studied the functional properties and chemical composition of protein isolates extracted from chickpea seeds and investigated their possible use in the food industry. They stated that protein isolates with high fat and water absorption are suitable for meat, cheese, and bakery products. Moreover, isolates with high emulsion capacity are more favourable for frankfurters or creamy products. Emulsifiers expedite the development of fine and smooth dispersions. These emulsions can be stabilized against coalescence, creaming and flocculation by various methods. The ability of an emulsifier to expedite formation of an emulsion is associated to its ability to adsorb onto and stabilize the oil–water interface. Emulsifiers decrease interfacial tension of liquids and the amount of work that is necessary to create new surfaces. Emulsifiers enhance formation of small droplets and decrease the rate at which droplets coalesce (Duda *et al*., 2019). Filip and Vidrih, (2015) investigated the relation between some functional properties of protein isolates and their amino acid composition. The net charge of amino acids is basically affected by pH and solubility characteristics, water-binding potential and surfactant properties of proteins. This was determined due to the quantities of acidic/basic and hydrophobic /hydrophilic amino acids.

Furthermore, the free sulfhydryl amino acids, which affect foaming and emulsifying properties, play an important act during thermal processing in inter- and intra-molecular disulphide bond formation. Therefore, this study aims to evaluate the effect of chickpea protein isolate as fat-replacer on characteristics, shelf life and sensory characteristics of low-fat emulsion sausage.

MATERIALS AND METHODS

Material

Beef meat was cut from a round portion of an adult female cow (2 years old) within 4 hours of slaughter time. Cow fat was obtained from thigh and rips area of the carcass. Beef meat and cow fat were obtained from the local market., Shebin El-Kom. Menofiya Gov. Other ingredients (non-meat) such as salt, spices, condiments, and clean intestinal casing (30-50 mm dia) were purchased from the local market., Shebin El-Kom. Menofiya Gov. Chickpea seeds (*Cicer arietinum L.*) (5 kg) were obtained from local market Shebin El-Kom. Menofiya Gov. Nutrient agar and potato dextrose agar procured from Al-Gomhoria Co., Tanta, Egypt.

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Methods:

Chickpea protein isolate (CPI):

Protein isolate (PI) from chickpea seeds was prepared as illustrated by El-Adawy (1996). Defatted chickpea flour was dispersed in distilled water (5%, w/v) and adjusted, at room temperature (30 C), to pH 9.5 with 0.1 N NaOH. The dispersion was mixed for 1 h and then centrifuged at 7000g for 20 min. For maximization of the PI yield, the extraction and centrifugation procedures were repeated three times on the residue. The solutions from all extractions were combined, and the pH was adjusted to 4.5 (isoelectric point) using 0.5 N HCl to precipitate the protein. The proteins were recovered by centrifugation at 7000g for 20 min, followed by discarding the supernatant. Protein curd was washed three times with distilled water and centrifuged at 7000g for 15 min. The precipitate was then dried at 40 °C and then ground as protein isolate.

Preparation of Beef sausage:

Emulsion beef sausage (BS) was prepared from a ground mixture of CPI, meat, fat and spices. Meat and fat were cooled at -2 ˚C : 4 ˚C for 30 hours, as follows for the control sample: Meat (1kg), fat (100g), salt (15g), commercial spice mixture (18g), garlic paste (30g), while, Chickpea protein isolate was used as a fat replacer to the meat with percentages of 0, 10, 15 and 20 (w/w) as mentioned in Table (A) where, C1 is the control beef sausage with 0% CPI; C2 is beef sausage with 10% CPI, C3 is beef sausage with 15% CPI and C4 is beef sausage with 20% CPI. Beef meat and fat were ground in a mill (Model 3510, Jenway Technology, Italy). The meat and fat mixture were transferred into a big bowl and ice cubes (200 g) were added to cool down the mixture and to help in the formation of the emulsion. The remaining ingredients and CPI portions (10, 15 and 20 replacement percentage) were added at the end of the process with the remaining of ice cubes (250 grams). The mixtures were carefully packed into the casings by a stuffing machine (Braun multi-quick 3 meat mincer G 1300, Germany). Emulsion sausage was packed, stored in a refrigerator at 0 °C for 0, 5, 10, 15, 20, 25 and 30 days and assessed for quality characteristics (Lei Sha *et al*., 2020).

Table A. Formula used in beef sausage preparing (g):

Ingredients	Sausage sample			
	C1(Contorl)	C2	C3	C4
Beef meat	1000	1000	1000	1000
Cow fat	100	90	85	80
CPI	0	10	15	20
Salt	15	15	15	15
Spice mixture	18	18	18	18
Garlic paste	30	30	30	30

C1- control beef sausage with 0% CPI; C2-beef sausage with 10% CPI, C3-beef sausage with 15% CPI, C4-beef sausage with 20% CPI.
CPI: Chickpea protein isolate

Analytical methods:

Chemical analysis: A digital pH meter (Model 3510, Jenway Technology, Italy) was used to examine the pH values of sausage samples. Two buffer solutions (pH 4 and 7) were used to calibrate the electrode of the pH meter. In a Cyclo-Mixer (CM- Model 3000 USA), five g of finely ground sausage samples were blended in 25 ml of distilled water in a test tube. It was extracted filtration through Whatman filter paper No 1. The pH of the filtrates were then recorded using the electrode of pH meter.

Moisture content of beef sausages were determined as described in AOAC (1995), as given in Food Industry Manual by using hot air oven (Model E53, FN-500- Germany) thermo statistically controlled at 150 ± 5°C.

Thiobarbituric acid (TBA value):

TBA values of beef sausage samples at different storage ratios of CPI addition and different storage periods were determined as described by Strange *et al*. (1997). The absorbance of prepared samples was measured at a wavelength 530 nm in a Spectrophotometer (Model UV-VIS-2802PC, USA).

Physical properties:

Color: Beef sausage samples were flat pressed using a burger meat presser. Parameters were measured Hunter (a *, b * and L *) scale color difference using a scale color spectrophotometer (machine colors Tristimulus) with CIE Lab colorimeter (Hunter, Lab Scan XE - Restoen VR, USA) in reflection mode, and was kept over the samples at room temperature (25°C) (Müge U. Ö., *et al*., 2020).

Texture: Texture analysis of beef sausage samples were examined by Texture Analyzer (Texture Analyzer TA-HDplus, 750kg.f (7.5kN), UK) using Warner–Bratzler blade. The probe measured the hardness of the sausage. The setting conditions were as follow: pretest speed = 3 mm/sec, test speed = 2 mm/sec, posttest speed = 6 mm/sec, rupture test = 2 mm/sec, Distance = 50 mm, and Force = 50 g (Müge U. Ö., *et al*., 2020).

Microbiological examination: The beef sausage samples were examined for total plate count TPC at 37°C for 48 h using nutrient agar, yeast and mold count were tested using Potato dextrose agar media at 3°C for 48 h according to standard procedures by (APHA, 1992).

Sensory characteristics of BS: Sausage samples were grilled (Tornado grill, Menoufia, Egypt – Temp: 170 °C) and evaluated by ten staff members from Food Science and Technology Department, Faculty of Agriculture, Menofiya University. The panelists were asked to evaluate on 9-point Hedonic rating tests (Ranganna, 1994) for the appearance, color, taste, flavor, mouth feel, texture, juiciness and overall acceptability. A 9-point scale was used where 9= excellent and 1 = extremely poor. Accuracy and precision were evaluated statistically.

Statistical analysis:

Results were expressed as the mean ± standard deviation. Data for multiple variable comparisons were analyzed by one-way and two-way analysis of variance (ANOVA). For the comparison of significance between groups, Duncan's test was used as a post hoc test according to the statistical package program (Artimage and Berry, 1987; Kowalczewski *et al*., 2015; Laleg *et al*., 2019).

RESULTS AND DISCUSSION

Characteristics of prepared beef Emulsion sausage:

Chemical properties:

pH values of the beef sausage samples were ranged from 5.6-6.8 in the fresh sample. pH values of samples which fortified by CPI have higher values being 6.8 as mentioned in Table 1, of the control samples this may be due to the addition of beef meat which being slightly acidic. The obtained values of pH were mainly close to the pH values of fresh meat, which were approximately ranged between 5.5 to 6.5 (Swatland 2008). The pH values of BS samples were increased to 6.6 – 6.8 as affected by replaced of CPI in prepared sausage samples

C2, C3 and C4, respectively. Ahmad *et al.* (2010) stated that addition of SPI to buffalo meat sausages at level 25% significantly increased pH value (from 5.7 to 6.8), which is closer to the findings of the current study. Incorporating of CPI caused observed increase in pH values to 1.0 – 1.2 time which was agreed by (Flores-silva *et al.*, 2015)..

Table 1. Changes of pH of beef sausage containing various ratios of chickpea protein isolate (CPI) over refrigeration storage at 0 °C for 30 days

BS samples	Storage period/days						
	Zero	5	10	15	20	25	30
C1(Contorl)	5.6 ± 0.25^{ap}	5.6 ± 0.20^{ap}	5.5 ± 0.27^{ap}	5.5 ± 0.21^{ap}	5.5 ± 0.28^{ap}	5.4 ± 0.21^{ap}	5.4 ± 0.22^{aq}
C2	6.8 ± 0.24^{bp}	6.8 ± 0.21^{bp}	6.8 ± 0.29^{bp}	6.7 ± 0.22^{bp}	6.6 ± 0.25^{bp}	6.6 ± 0.27^{bp}	6.6 ± 0.24^{bq}
C3	6.8 ± 0.21^{bp}	6.8 ± 0.22^{bp}	6.7 ± 0.29^{bp}	6.7 ± 0.24^{bp}	6.6 ± 0.19^{bp}	6.6 ± 0.25^{bq}	6.6 ± 0.21^{bq}
C4	6.8 ± 0.22^{bp}	6.8 ± 0.25^{bp}	6.7 ± 0.23^{bp}	6.7 ± 0.27^{bp}	6.7 ± 0.20^{bp}	6.6 ± 0.25^{bq}	6.6 ± 0.29^{bq}

C1- control beef sausage with 0% CPI; C2-beef sausage with 10% CPI, C3-beef sausage with 15% CPI, C4-beef sausage with 20% CPI.
Values with different letters in a row (a,b,c ---) and in a column (p,q,r) are significantly (p <0.05) (n = 6) different. BS: Beef sausage sample

Moisture content was 66.8% in control BS samples. Incorporation of CPI was significantly (P <0.05) decreased the moisture content (Table 2). A gradual decrease in the moisture content of the BS during cold storage (0 °C) was observed. The rate of humidity reduction was higher in the BS control compared with the CPI-BS. It was indicated by the correlation coefficient that ranged from 0.8771 to 0.9989.

Zeinab et. Al (2019) mentioned that control sausage samples have the higher amounts of moisture. However, incorporation of flaxseeds and chickpeas protein isolates decreased the moisture content which resulted in increasing in other nutrients, these results are also in agreement with those of Mansour (2003) and Owon *et al*, (2014).

Table 2. Changes of moisture content (%) of beef sausage containing various ratios of chickpea protein isolate (CPI) over refrigeration storage at 0 °C for 30 days.

BS samples	Storage period/days						
	Zero	5	10	15	20	25	30
C1(Contorl)	66.8 ± 0.5^{ap}	62.7 ± 0.2^{ap}	61.4 ± 0.5^{aq}	60.2 ± 0.2^{bq}	58.6 ± 0.2^{bq}	57.8 ± 0.2^{cr}	56.7 ± 0.6^{cr}
C2	63.7 ± 0.2^{bp}	62.3 ± 0.4^{bp}	60.4 ± 0.5^{bp}	57.1 ± 0.1^{cp}	54.7 ± 0.7^{dq}	53.1 ± 0.2^{cr}	52.4 ± 0.4^{cr}
C3	60.1 ± 0.4^{cp}	58.4 ± 0.5^{cp}	57.1 ± 0.4^{cp}	56.3 ± 0.4^{cp}	54.1 ± 0.4^{dq}	52.7 ± 0.4^{cr}	51.2 ± 0.5^{dr}
C4	60.9 ± 0.6^{dp}	59.3 ± 0.1^{dp}	57.2 ± 0.3^{cp}	56.2 ± 0.3^{dp}	54.1 ± 0.4^{dq}	51.7 ± 0.3^{dr}	50.4 ± 0.3^{dr}

C1- control beef sausage with 0% CPI; C2-beef sausage with 10% CPI, C3-beef sausage with 15% CPI, C4-beef sausage with 20% CPI.
Values with different letters in a row (a,b,c ---) and in a column (p,q,r) are significantly (p <0.05) (n = 6) different. BS: Beef sausage sample

TBA value: Obtained results showed that increased CPI ratio followed by the increase of TBA value of BS (Table 3). The rate of increase in the values of TBA in the control BS was lower than those of CPI incorporated BS.

All samples to be in sausage are the legal limit to prolonged storage period. Previous reports indicated that meat products containing a TBA value from 0.5 to 1.0 mg MA/Kg possessed no off flavor (Gallegos-Infante *et al.*, 2015). Gopalakrishnan *et al*., 2011 indicates that the values of TBA in the range of 1 to 2 mg MA/ kg was the lowest level can be detected for oxidized flavor in beef and its products.

Table 3. Changes of TBA value, mg MA/kg of beef sausage containing various ratios of chickpea protein isolate (CPI) over refrigeration storage at 0 °C for 30 days.

BS samples	Storage period/days						
	Zero	5	10	15	20	25	30
C1(Contorl)	0.28 ± 0.5^{ap}	0.29 ± 0.3^{ap}	0.31 ± 0.4^{ap}	0.32 ± 0.4^{bp}	0.34 ± 0.6^{ap}	0.36 ± 0.3^{bq}	0.39 ± 0.7^{bq}
C2	0.28 ± 0.4^{ap}	0.31 ± 0.8^{ap}	0.34 ± 0.4^{ap}	0.35 ± 0.4^{bp}	0.37 ± 0.7^{bq}	0.39 ± 0.1^{bq}	0.40 ± 0.4^{bq}
C3	0.29 ± 0.6^{ap}	0.30 ± 0.5^{ap}	0.33 ± 0.8^{ap}	0.35 ± 0.5^{bq}	0.36 ± 0.2^{bq}	0.38 ± 0.7^{bq}	0.41 ± 0.1^{bq}
C4	0.27 ± 0.7^{ap}	0.29 ± 0.4^{ap}	0.31 ± 0.6^{aq}	0.33 ± 0.6^{bq}	0.37 ± 0.5^{bq}	0.39 ± 0.8^{bq}	0.42 ± 0.3^{bq}

C1- control beef sausage with 0% CPI; C2-beef sausage with 10% CPI, C3-beef sausage with 15% CPI, C4-beef sausage with 20% CPI.
Values with different letters in a row (a,b,c ---) and in a column (p,q,r) are significantly (p <0.05) (n = 6) different. BS: Beef sausage sample

So, the addition of different ratios of CPI could improve chemical characteristics of BS in compared with the control one.

Physical properties:

Hardness: During refrigerated storage (0 °C), values of hardness were increased up to 15 days of storage then decreased. The increase in strength is may be due to increase in the compactness during sausage maturity process within 15 storage days (Howard *et al.* 2011) and observed decrease in stiffness after 15 days may be because of the microbial activity (Table 4).

Table 4. Changes of hardness, of beef sausage containing various ratios of chickpea protein isolate (CPI) over refrigeration storage at 0 °C for 30 days.

BS samples	Storage period/days						
	Zero	5	10	15	20	25	30
C1(Contorl)	3144.4^{ap}	3277.4^{bp}	3290.4^{bp}	3300.1^{cp}	3230.5^{dq}	3188.4^{dq}	3157.6^{dq}
C2	3099.2^{bp}	3154.8^{bp}	3177.5^{bp}	3230.8^{cp}	3204.8^{cp}	3186.2^{dq}	3110.6^{dq}
C3	3030.9^{bp}	3170.4^{bp}	3250.4^{cp}	3280.1^{cp}	3250.7^{dq}	3.128^{dq}	3050.6^{cq}
C4	3020.1^{bp}	3195.5^{bp}	3246.8^{cp}	3261.4^{cq}	3210.8^{cq}	3148.2^{dq}	3115.8^{dq}

C1- control beef sausage with 0% CPI; C2-beef sausage with 10% CPI, C3-beef sausage with 15% CPI, C4-beef sausage with 20% CPI.
Values with different letters in a row (a,b,c ---) and in a column (p,q,r) are significantly (p <0.05) (n = 6) different. BS: Beef sausage sample

Nevertheless, the present results are agreed with those which showed that levels of fat from beef (Ulu, 2006). Protein isolate and values of hardness had a direct relationship on physical properties (Ulu, 2006). Unfortunately, there is no consensus from literature about the effect of chickpea protein

isolate on texture of processed meat products (Danowska-Oziewicz, 2014; Padalino *et al*., 2014).

Hunter color measurement: Color is considered as one of the most important characteristics in meat production so, Hunter L and b values were increased ($p < 0.05$) and a value decreased ($p < 0.05$) after incorporation of CPI (Table 5, 6 and 7). It is clear that the addition of CPI improved brightness and reduced darkness. During storage, marginal fluctuations in L, a and b values were observed indicating no different trend. CPI fortified samples were darker than control samples. These latter differences were also significant ($p < 0.05$) in which was agreed by Paredes-Lopez and Ordorica-Falomir (1986). If necessary, a technique of this type might be used to decrease pigmentation level of chickpea proteins.

Table 5. Changes of Hunter color (L) of beef sausage containing various ratios of chickpea protein isolate (CPI) over refrigeration storage at 0 °C for 30 days.

BS samples	Storage period/days						
	Zero	5	10	15	20	25	30
C1(Contorl)	40.2 ± 0.40^{ap}	39.8 ± 0.33^{ap}	39.1 ± 0.31^{ap}	38.5 ± 0.41^{aq}	39.8 ± 0.44^{aq}	41.2 ± 0.43^{aq}	41.9 ± 0.31^{aq}
C2	42.5 ± 0.42^{bq}	38.9 ± 0.34^{bq}	44.8 ± 0.34^{bq}	44.9 ± 0.45^{bq}	45.9 ± 0.42^{bq}	47.3 ± 0.38^{bq}	48.9 ± 0.39^{bq}
C3	44.8 ± 0.38^{cp}	42.8 ± 0.37^{cp}	41.9 ± 0.35^{cp}	40.7 ± 0.48^{cq}	44.7 ± 0.47^{cq}	45.3 ± 0.31^{cq}	46.1 ± 0.34^{cq}
C4	51.9 ± 0.34^{dp}	50.4 ± 0.41^{dp}	48.1 ± 0.39^{dp}	48.9 ± 0.37^{dq}	50.2 ± 0.47^{dq}	51.7 ± 0.45^{dq}	52.6 ± 0.41^{dq}

C1- control beef sausage with 0% CPI; C2-beef sausage with 10% CPI, C3-beef sausage with 15% CPI, C4-beef sausage with 20% CPI.
Values with different letters in a row (a,b,c ---) and in a column (p,q,r) are significantly ($p < 0.05$) ($n = 6$) different BS: Beef sausage sample.

Table 6. Changes of Hunter color (a) of beef sausage containing various ratios of chickpea protein isolate (CPI) over refrigeration storage at 0 °C for 30 days.

BS samples	Storage period/days						
	Zero	5	10	15	20	25	30
C1(Contorl)	14.3 ± 0.27^{ap}	12.4 ± 0.39^{ap}	13.2 ± 0.42^{aq}	16.7 ± 0.44^{aq}	11.2 ± 0.44^{aq}	10.9 ± 0.41^{aq}	10.1 ± 0.41^{aq}
C2	10.2 ± 0.24^{bq}	14.2 ± 0.35^{bq}	12.5 ± 0.48^{bp}	10.4 ± 0.42^{bp}	11.7 ± 0.38^{bp}	12.0 ± 0.38^{bp}	12.9 ± 0.35^{bp}
C3	7.9 ± 0.44^{cp}	11.2 ± 0.37^{cp}	9.9 ± 0.39^{cq}	9.2 ± 0.34^{cq}	9.8 ± 0.44^{cr}	8.4 ± 0.31^{cr}	7.6 ± 0.34^{cr}
C4	7.6 ± 0.28^{dp}	10.4 ± 0.31^{dp}	11.9 ± 0.34^{dq}	10.8 ± 0.37^{dq}	9.4 ± 0.41^{dr}	9.2 ± 0.45^{dr}	8.7 ± 0.34^{dr}

C1- control beef sausage with 0% CPI; C2-beef sausage with 10% CPI, C3-beef sausage with 15% CPI, C4-beef sausage with 20% CPI.
Values with different letters in a row (a,b,c ---) and in a column (p,q,r) are significantly ($p < 0.05$) ($n = 6$) different. BS: Beef sausage sample

Table 7. Changes of Hunter color (b) of beef sausage containing various ratios of chickpea protein isolate (CPI) over refrigeration storage at 0 °C for 30 days.

BS samples	Storage period/days						
	Zero	5	10	15	20	25	30
C1(Contorl)	19.4 ± 0.34^{ap}	18.5 ± 0.38^{ap}	16.4 ± 0.33^{aq}	16.8 ± 0.33^{aq}	14.1 ± 0.32^{aq}	12.1 ± 0.33^{aq}	12.8 ± 0.33^{aq}
C2	18.8 ± 0.39^{ap}	18.9 ± 0.41^{ap}	17.4 ± 0.41^{aq}	19.7 ± 0.41^{aq}	14.7 ± 0.35^{bq}	13.1 ± 0.41^{bq}	12.8 ± 0.37^{bq}
C3	21.1 ± 0.33^{bp}	20.8 ± 0.41^{bp}	17.9 ± 0.42^{bp}	15.6 ± 0.41^{bq}	13.1 ± 0.36^{bq}	12.6 ± 0.45^{bq}	10.5 ± 0.41^{bq}
C4	22.1 ± 0.31^{bp}	20.6 ± 0.35^{bp}	16.1 ± 0.39^{bp}	15.7 ± 0.34^{bq}	14.9 ± 0.37^{bq}	12.9 ± 0.39^{bq}	9.4 ± 0.38^{bq}

C1- control beef sausage with 0% CPI; C2-beef sausage with 10% CPI, C3-beef sausage with 15% CPI, C4-beef sausage with 20% CPI.
Values with different letters in a row (a,b,c ---) and in a column (p,q,r) are significantly ($p < 0.05$) ($n = 6$) different BS: Beef sausage sample.

Microbiological properties:

The total bacterial, mold and yeast count TPC (log cfu / g) for BS sample was found in the range of 3.7–4.4 in the novel condition (Table 8). TPC for BS samples was not significantly different ($p < 0.05$) as a result of CPI inclusion. During refrigerated storage at 0 °C for 30 days the TPC significantly increased and ranged between 6.4–6.7 log cfu/g at day 30 of storage. Ranaken and Kiill (1993) illustrated that spoilage condition at 10^7/g of bacteria count. The results are also in agreement with Laleg *et al*. (2016). Yeast and mold were not detected in the sausage samples up to 20 days of cold storage at 0 °C, while, at 25-30 days, the yeast and mold counts were in the range of 2.2 to 2.4 log cfu/g.

Table 8. Changes of Standard plate count, cfu/g of beef sausage containing various ratios of chickpea protein isolate (CPI) over refrigeration storage at 0 °C for 30 days.

BS samples	Storage period/days						
	Zero	5	10	15	20	25	30
C1(Contorl)	3.9 ± 0.01^{ap}	4.2 ± 0.2^{ap}	5.1 ± 0.04^{ap}	5.8 ± 0.04^{aq}	5.9 ± 0.02^{aq}	6.2 ± 0.01^{aq}	6.3 ± 0.2^{aq}
C2	3.6 ± 0.08^{ap}	4.2 ± 0.09^{ap}	4.8 ± 0.09^{bq}	5.2 ± 0.09^{bq}	6.1 ± 0.08^{bq}	6.3 ± 0.2^{bq}	6.4 ± 0.09^{bq}
C3	4.1 ± 0.09^{ap}	5.1 ± 0.08^{ap}	5.4 ± 0.1^{bq}	6.1 ± 0.3^{bq}	6.6 ± 0.07^{bq}	6.7 ± 0.3^{bq}	6.9 ± 0.07^{bq}
C4	4.5 ± 0.1^{ap}	5.3 ± 0.2^{ap}	5.7 ± 0.5^{bq}	6.2 ± 0.08^{bq}	6.4 ± 0.02^{bq}	6.6 ± 0.07^{bq}	6.8 ± 0.2^{bq}

C1- control beef sausage with 0% CPI; C2-beef sausage with 10% CPI, C3-beef sausage with 15% CPI, C4-beef sausage with 20% CPI.
Values with different letters in a row (a,b,c ---) and in a column (p,q,r) are significantly ($p < 0.05$) ($n = 6$) different. BS: Beef sausage sample

Sensory characteristics: Sensory scores for studied parameters such as color, flavor, taste, texture, juiciness and overall acceptability were varied and affected significantly by the addition of chickpea protein isolate ($p < 0.05$). Sensory properties values were ranged between 7 and 8 in all prepared sausage samples, which represented between moderately and very much liked ones as presented in Table (9). In addition, the means values were given by the panelists of grilled sausage samples at zero-time. Results also revealed that there was a significant difference ($p < 0.05$) in over-all acceptability between the control sausage samples and the other three treatments. Values of various attributes were reduced during cold storage at 0 °C. sausage samples were organically, sensorial acceptable up to 30 days of storage. Color, juiciness, texture, and taste have the highest values by the inclusion of CPI. Incorporation of CPI also improved texture, juiciness and flavor of the prepared samples (Mansour and Khalil 1999; Marti *et al*., 2013; Megazyme, 2017; Laleg *et al*. 2019). The control sausage sample had the highest taste, and color values while had similar texture value with chickpea protein isolate incorporation which is in line with what Bilek and Turhan (2009) reported, that the addition of flaxseed flour significantly affected the appearance, flavor, tenderness, juiciness and overall acceptability of beef patties. The results indicated that CPI was better emulsifiers at higher pH values than that at acidic pH volumes in control samples.

Table 9. Sensory characteristics of emulsified beef sausage containing various ratios of CPI over refrigeration storage at 0 °C for 30 days.

BS samples	Storage period/days						
	Zero	5	10	15	20	25	30
				Color			
C1(Contorl)	7.7 ± 0.52 ^{ap}	7.2 ± 0. 23 ^{ap}	7.2 ± 0.15 ^{ap}	7.0 ± 0.44 ^{aq}	6.8 ± 0.34 ^{aq}	6.8 ± 0.33 ^{aq}	6.7 ± 0.14 ^{aq}
C2	8.0 ± 0.51 ^{ap}	7.9 ± 0.25 ^{bp}	7.8 ± 0.25 ^{bp}	7.6 ± 0.23 ^{bp}	7.4 ± 0.45 ^{bp}	7.2 ± 0.38 ^{bq}	7.0 ± 0.24 ^{bq}
C3	8.1 ± 0.40 ^{ap}	7.8 ± 0.29 ^{bp}	7.7 ± 0.17 ^{bq}	7.6 ± 0.33 ^{bq}	7.4 ± 0.27 ^{bq}	7.3 ± 0.39 ^{bq}	7.1 ± 0.23 ^{bq}
C4	8.2 ± 0.22 ^{ap}	8.0 ± 0.42 ^{bp}	8.1 ± 0.22 ^{bp}	8.1 ± 0.38 ^{bp}	7.6 ± 0.24 ^{bq}	7.4 ± 0.41 ^{bq}	6.9 ± 0.37 ^{bq}
				Flavor			
C1(Contorl)	7.8 ± 0.55 ^{ap}	7.6 ± 0.43 ^{ap}	7.3 ± 0.27 ^{ap}	7.3 ± 0.31 ^{ap}	7.1 ± 0.37 ^{bp}	7.0 ± 0.37 ^{bq}	6.4 ± 0.34 ^{bq}
C2	7.8 ± 0.54 ^{ap}	7.6 ± 0.41 ^{ap}	7.4 ± 0.21 ^{ap}	7.4 ± 0.34 ^{bp}	7.2 ± 0.34 ^{bq}	7.1 ± 0.36 ^{bq}	6.9 ± 0.35 ^{bq}
C3	7.8 ± 0.27 ^{ap}	7.7 ± 0.49 ^{ap}	7.5 ± 0.45 ^{ap}	7.3 ± 0.39 ^{bp}	7.1 ± 0.36 ^{bq}	7.1 ± 0.34 ^{bq}	6.8 ± 0.38 ^{bq}
C4	7.9 ± 0.29 ^{ap}	7.8 ± 0.47 ^{ap}	7.4 ± 0.41 ^{ap}	7.3 ± 0.35 ^{bp}	7.2 ± 0.32 ^{bq}	7.0 ± 0.37 ^{bq}	6.9 ± 0.34 ^{bq}
				Texture			
C1(Contorl)	8.1 ± 0.28 ^{ap}	7.9 ± 0.28 ^{ap}	7.8 ± 0.24 ^{ap}	7.7 ± 0.61 ^{ap}	7.5 ± 0.24 ^{ap}	7.4 ± 0.25 ^{bq}	7.2 ± 0.42 ^{bq}
C2	8.2 ± 0.26 ^{ap}	7.8 ± 0.24 ^{ap}	7.6 ± 0.28 ^{ap}	7.5 ± 0.55 ^{ap}	7.3 ± 0.51 ^{bp}	7.2 ± 0.28 ^{bp}	6.8 ± 0.27 ^{bq}
C3	8.1 ± 0.27 ^{ap}	7.9 ± 0.25 ^{ap}	7.6 ± 0.41 ^{ap}	7.4 ± 0.54 ^{bp}	7.3 ± 0.42 ^{bp}	7.1 ± 0.29 ^{cp}	6.9 ± 0.42 ^{cq}
C4	8.6 ± 0.43 ^{ap}	8.2 ± 0.27 ^{ap}	7.8 ± 0.51 ^{ap}	7.7 ± 0.47 ^{cp}	7.6 ± 0.61 ^{cp}	7.4 ± 0.27 ^{cp}	7.2 ± 0.52 ^{cp}
				Taste			
C1(Contorl)	8.0 ± 0.32 ^{ap}	7.9 ± 0.41 ^{ap}	7.8 ± 0.47 ^{ap}	7.6 ± 0.36 ^{ap}	7.4 ± 0.45 ^{ap}	7.2 ± 0.24 ^{ap}	7.0 ± 0.25 ^{ap}
C2	8.1 ± 0.33 ^{ap}	7.8 ± 0.52 ^{ap}	7.7 ± 0.27 ^{ap}	7.6 ± 0.46 ^{ap}	7.4 ± 0.63 ^{ap}	7.3 ± 0.19 ^{ap}	7.1 ± 0.28 ^{ap}
C3	8.2 ± 0.36 ^{ap}	7.8 ± 0.61 ^{ap}	7.6 ± 0.28 ^{ap}	7.5 ± 0.49 ^{ap}	7.3 ± 0.42 ^{ap}	7.2 ± 0.22 ^{ap}	6.8 ± 0.27 ^{ap}
C4	8.1 ± 0.33 ^{ap}	7.9 ± 0.55 ^{ap}	7.6 ± 0.22 ^{ap}	7.4 ± 0.37 ^{ap}	7.3 ± 0.41 ^{ap}	7.1 ± 0.14 ^{ap}	6.9 ± 0.24 ^{ap}
				Juiciness			
C1(Contorl)	7.8 ± 0.55 ^{ap}	7.7 ± 0.49 ^{ap}	7.5 ± 0.33 ^{ap}	7.3 ± 0.22 ^{bp}	7.1 ± 0.24 ^{bp}	7.1 ± 0.22 ^{bp}	6.8 ± 0.24 ^{bp}
C2	7.9 ± 0.45 ^{ap}	7.8 ± 0.51 ^{bp}	7.4 ± 0.35 ^{bp}	7.3 ± 0.29 ^{bp}	7.2 ± 0.28 ^{bp}	7.0 ± 0.22 ^{bp}	6.9± 0.28 ^{bp}
C3	8.2 ± 0.48 ^{ap}	7.8 ± 0.58 ^{bp}	7.6 ± 0.39 ^{bp}	7.5 ± 0.24 ^{bp}	7.3 ± 0.65 ^{bp}	7.2 ± 0.29 ^{bp}	6.8 ± 0.29 ^{bp}
C4	8.1 ± 0.55 ^{ap}	7.9 ± 0.55 ^{bp}	7.6 ± 0.34 ^{bp}	7.4 ± 0.45 ^{bp}	7.3 ± 0.22 ^{bp}	7.1 ± 0.27 ^{bp}	6.9 ± 0.23 ^{bp}
				Overall acceptability			
C1(Contorl)	7.9 ± 0.28 ^{ap}	7.8 ± 0.25 ^{ap}	7.4 ± 0.33^{ap}	7.3 ± 0.22 ^{ap}	7.2 ± 0.41 ^{bq}	7.0 ± 0.44 ^{bq}	6.9 ± 0.55 ^{bq}
C2	7.8 ± 0.26 ^{bp}	7.7 ± 0.26 ^{bp}	7.5 ± 0.31 ^{bp}	7.3 ± 0.22 ^{bp}	7.1 ± 0.40 ^{bp}	7.1 ± 0.40 ^{bq}	6.8 ± 0.45 ^{bq}
C3	7.9 ± 0.28 ^{bp}	7.8 ± 0.41 ^{bp}	7.4 ± 0.35 ^{bp}	7.3 ± 0.28 ^{bp}	7.2 ± 0.25 ^{bp}	7.0 ± 0.40 ^{bp}	6.9 ± 0.41 ^{bq}
C4	8.0 ± 0.24 ^{bp}	7.9 ± 0.42 ^{bp}	7.8 ± 0.30 ^{bp}	7.6 ± 0.31 ^{bp}	7.4 ± 0.29 ^{bp}	7.2 ± 0.41 ^{bp}	7.0 ± 0.43 ^{bp}

C1- control beef sausage with 0% CPI; C2-beef sausage with 10% CPI, C3-beef sausage with 15% CPI, C4-beef sausage with 20% CPI.
Values with different letters in a row (a,b,c ---) and in a column (p,q,r) are significantly (p <0.05) (n = 6) different. BS: Beef sausage sample

CONCLUSION

Incorporation of CPI significantly altered the physical, chemical characteristics, microbiological count, sensory properties, and structural properties of the low-fat sausage. Organoleptic properties and sensory characteristics, the quality of color, texture and juiciness are improved. Moreover, automated colorimetry by, L and b values were increased while some values reduced after CPI inclusion. Values of hardness were reduced because of the addition of CPI. To be concluded, chickpea protein isolate can be recommended for application in the low-fat beef sausage inclusion targeting high nutritional value and high-quality functional sausage products with high physical, chemical, texture and sensory characteristics. This could encourage the acclimation of the large-scale production for manufacturing the protein rich sausage on an industrial scale to be available in the market for consumers in which the results were agreeable with that recorded by the Egyptian Organization for Standards & Quality No. 3602/2013 for "chilled meat and meat products".

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خصائص جودة السجق البقري قليل الدهن المصنع بتأثير إضافة معزول بروتين حمص الشام خلال التخزين المبرد

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تمت دراسة تأثير إضافة البروتين المعزول المستخلص من حمص الشام بمستويات ٠ و ١٠ و ١٥ و ٢٠٪ مع لحم البقر لتقييم إنتاجية وجودة ومدة صلاحية السجق المستحلب. تم تقييم جودة السجق المستحلب من خلال تقدير كل من الرقم الهيدروجيني ، ومحتوى الرطوبة ، والعد الكلي الميكروبي ، ورقم حامض الثيوباربيتوريك ، وعدد الفطريات والخميرة ، والخصائص الحسية ، والقوام واللون. أظهرت النتائج حدوث تأثيرات طفيفة في محتوى الأس الهيدروجيني والرطوبة ، ومع ذلك ، لم يتأثر قيم رقم حامض الثيوباربيتوريك نتيجة إضافة معزول بروتين حمص الشام ، كما أظهرت نتائج العد الكلي الميكروبي لعينات السجق المستحلب الطازجة أن العدد كان ٣,٧-٤,٣ لوغاريتم cfu / g. و أظهرت نتائج التقييم الحسي لعينات السجق المستحلب قبول لأعضاء لجنة التقييم ولم يؤثر إضافة بروتين حمص الشام المعزول على درجة القبول الكلية لعينات السجق. كما أدي إضافة معزول بروتين الحمص إلى زيادة القيم اللونية L و b لجهاز هانتر ولكنه قلل من قيمة a و قيم الصلابة للسجق المستحلب. وخلال فترة التخزين المبرد على درجة صفر درجة مئوية حدث تغير في قيم L، a، b بشكل غير منتظم. وتشير النتائج المتحصل عليها إلى أن إضافة معزول بروتين حمص الشام يحسن بشكل ملموس من خواص الملمس واللون وعصيرية للسجق المستحلب كما لم تتأثر فترة صلاحية السجق نتيجة إضافته.