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ABSTRACT

The current research aimed to evaluate the effect of using red kidney bean powder (RKBP) as a fat replacer into beef burger formula on the chemical, physiochemical, microbiological and sensory characteristics during frozen storage periods for 60 days. Also, the impact of RKBP on the oxidative stability of manufactured burgers was studied. Fat was partly substituted with different levels of RKBP (2.5, 5,7.5 and 10%), it was found that 5% was the best for overall acceptance, so this percentage was applied in different measurements. The finding revealed that the RKBP contained high protein, ash and fibers contents, where the values were 26.78, 4.25and 6.79 g/100g, respectively. Therefore, the content of the burger increased after adding RKBP of protein, ash and fiber, but it contained lower values of fat and calories compared to the control samples. Moreover, it was found that RKBP is rich in phenolic compounds, flavonoids and DPPH% (3.54mg GAE/g DW,3.25 mg CE/g DW and 13.97%, respectively). Also, the addition of RKBP to the beef burger improved cooking properties such as raised water holding capacity (WHC), reduced cooking loss and shrinkage. Moreover, the oxidative stability of beef burger was improved, as the thiobarbituric acid values in the burger which stored at -18 °C for 60 days were reduced. Additionally, RKBP helped reduce the microbial growth of the burger during storage. Based on these finding, we can conclude that, the using of RKBP as a fat replacer in beef burger can improve its nutritional and functional properties.

Keywords: Legumes, Fat replacer, Phenolic compounds, Antioxidant activity, Lipid oxidation, Quality attributes, Sensory evaluation.

INTRODUCTION

Meat and meat products are excellent source of essential nutrients with high-quality proteins, fat, trace elements and minerals **(Mehta *et al.*, 2013)**. There are a wide variety of meat products including cured meats, patties, nuggets, meatballs, … etc**(Aminzare *et al.*, 2016)**. Beef burger is one of the most popular fast food in Egypt and all over the world with high nutritional value and highly desirable for humans (**Eldemery, 2010**). It has a high degree of general acceptability and is consumed in large quantities because it is a fast and cheap meal (**Colmenero, 2000**), but it contains a large amount of fats with saturated acids (20-30%) (**Selani *et al.*, 2016**). Fats play an important role in processed meat products such as reducing loss during the cooking process, increasing the stability of the meat emulsion, improving organoleptic properties and providing water holding capacity(**Rather *et al.*, 2015**). However, adding fats to meat products leads to higher cholesterol and saturated fatty acids (**Pappa *et al*., 2000**). Increased levels of saturated fat intake lead to harmful diseases such as obesity, some types of cancer, coronary heart disease, obesity, cardiovascular disease and high blood pressure, in addition to the added synthetic antioxidants and antimicrobials that are added during manufacturing, which poses a risk to human health **(Cofrades *et al.*, 2016)**. The high fat content in meat and lack of water activity are among the main causes of degradation of meat and meat products, as they lead to lipid oxidation, that decrease the shelf life of meat, meat products and affects the nutritional value, texture, water holding capacity and flavor of these products **(Guyon *et al.*, 2016)**.

The WHO states that fats should contribute about 15% to 30% of daily calories. Saturated fat should be no more than 10% of the daily supplemented calories **(Sullivan *et al*., 2014)**. According to these recommendations, several investigations have been carried out to produce healthy, low-fat meat products **(Weiss *et al*., 2010)**. However, reducing fat percentages may cause some problems related to product acceptance, since fat is an important component that affects properties of meat products such as flavor, sensory attributes and texture **(Youssef and Barbut, 2011)**.

Replacing fats by adding substances that contain carbohydrates, non-meat proteins, or dietary fiber is a practical approach to solving these problems **(Brewer, 2012)**.Despite the importance of dietary fiber in human nutrition, its consumption is less than recommended by FAO (23-38 g / day) **(McGill *et al*., 2015)**. Fiber is the most common functional ingredient because it is used in food processing as a fat replacer, which reduces fat absorption by frying, volume improvement, stabilizer, bulking and binding agent **(Verma and Banerjee, 2010)**. In meat processing, fiber has successful applications in improving cooking yield, improving texture, and reducing formula cost **(Shariati-Oevari *et al*., 2016)**. Besides, many clinical, biochemical and epidemiological investigations have reported that fiber plays a positive role in human health by reducing cholesterol levels, reducing blood pressure and hyperlipidemia, improving digestive health and preventing some types of cancers such as colon cancer **(Slavin, 2013)**.

Legumes are the third largest family of angiosperms belong to *Fabaceae / Leguminosae*. They are a component of a traditional healthy diet worldwide **(Malaguti *et al*., 2014)**. They contain many essential nutrients, such as protein, dietary fiber, low-glycemic index carbohydrates, vitamins and minerals. Legumes are particularly high in protein as well as dietary fiber. Legume plants' protein-derived bioactive peptides have many important roles as health-enhancing compounds (especially interact with amino acids of enzymes related with diseases). The presence of these bioactive peptides in legumes can lead to the quality of food **(Ortiz-Martinez *et al*., 2014)**. Plant materials are rich sources of bioactive phenolic compounds; hence they can be an effective alternative to synthetic antioxidants **(Minzare *et al*., 2019)**. Red kidney bean (*Phaseolus vulgaris, L*.) known as navy, pinto red kidney, or French beans, are a valuable food source for humans around the world. They are recognized as an essential component of balanced diets, not only because of their high nutritional value (rich in protein and low in fat), but also because of their functional properties. Carbohydrates, lectins, vitamins, phytates, phenolics and soluble fiber are the main functional components of popular beans. Phenolics, which include phenolic acids, proanthocyanidins and flavonoids, are particularly notable because of their potent antioxidant properties**(Garcia-Lafuente *et al.*, 2012)**. Red kidney bean is known to be high in protein (29.1%), fiber (4%), vitamin E and unsaturated fatty acids **(Hayat *et al.*, 2013)**. It also contains a large number of phytochemical compounds including phenolic, isovitexin, flavonoids and vitexin. It has the highest antioxidant activity compared to other types of legumes **(Luo *et al.*, 2016)**. Moreover, they were reported to show chemopreventive, antioxidant, antimutagenic, anti inflammatory and antibacterial effects **(Gan *et al.*, 2016)**. Therefore, as a dietary component, common beans have considerable health benefits.

The current study aimed to estimate the effect of using RKBP (as the source of antioxidants and dietary fiber) in functional beef burger by partial replacement of fat on the physical, chemical, sensorial properties, shelf life and quality.

Material and Methods:

Material:

Fresh lean beef, kidney fat, red kidney beans and others materials (i.e salt, onion, garlic and herbal spices) was purchased from local market in Alexandria, Egypt.

Methods:

Preparation of red kidney beans powder:

The method described by **Onwuka (2005)** was employed. First, the red kidney bean seeds were sorted manually to remove extraneous materials like dirt, residue, shriveled and diseased seeds. The healthy bean seeds were then used. The powder used for this study was gotten from the whole seeds, grinded without dehulling. It was then divided into two equal portions, with one portion sieved to a particle size of 1mm to separate the hulls from the powder.

Preparation of beef burger :

The beef burger were manufactured according to the **Aleson-Carbonell *et al*. (2005)**. The lean beef and kidney fat were separately ground in meat grinder (Moulinex 505, France). Fat content of the lean and fat portions were determined prior to the manufacture of beef burger. The lean beef (4% fat), kidney fat (90% fat), RKBP and water were used to formulate the beef burger (Table 1). The control burger was formulated to contain 65 % lean beef and 20% kidney fat, 10% water, 5% salts and spices mixtures. Different levels of kidney fat (2,5, 5, 7,5 and 10%) were replaced by equal amounts of RKBP. Appropriate amounts of each formulation were mixed by hand, subjected to final grinding (0.5 cm plate) and processed into patties (70 g, 1.2 cm thick and 12 cm diameter). Burger were placed on plastic foam meat trays, wrapped with polyethylene film and kept frozen at -18°C until further analysis.

Cooking procedure:

Beef burgers were cooked in a preheated (200ºC) electric grilled (Genwex GW-066) which was standardized for temperature. The burger were cooked 6 min, turned over, cooked 6 min, turned again and cooked 4 min according to **Saba (1991)** methods. The burger were weighed before and after cooking.

Table (1): Beef burger formulation containing red kidney beans powder (RKBP)

Ingredients	Fat replacers (red kidney beans powder)(g)				
	Control	**2.5%**	**5%**	**7.5%**	**10%**
Lean beef (g)	65	65	65	65	65
Kidney fat(g)	20	19.5	19	18.5	18
Water (g)	10	10	10	10	10
Salts and * Spices mixture (g)	5	5	5	5	5
RKB powder (g)	0	0.5	1	1.5	2

Spices mixture: Thyme, turmeric, curry, black pepper, cumin, ginger, cinnamon, nutmeg, chili, parsley and celery.

Gross chemical composition

The gross chemical composition of RKBP and burger sample was performed with method adopted by Association of Official Analytical Chemists **(A.O.A.C., 2010)**. The procedure is for moisture, crude protein, crude fat, crude fiber, ash content and N-free extract. The protein content was determined using the micro kjeldhal method (N x 6.25) and (N-free extract)was calculated on moisture free basis by difference according to the sum NFE % = 100 - (crude protein % + crude fat % + crude fiber + ash). Calories were calculated from the sum of the percentages of crude protein and total carbohydrates (N-free extract) multiplied by a factor of 4 ($Kcal.g^{-1}$) plus the crude fat content multiplied by a factor of 9 ($Kcal.g^{-1}$) according to **Zambrano *et al*.(2004).**Total dietary fiber content in the RKBP was estimated according to the method described by **Asp *et al*. (1983)**.

Mineral analysis:

The following mineral elements: Mg, Ca, Fe, Mn, Ni, Zn and Cu were measured. The digestion of seed powder sample was carried out by using concentrated nitric acid and perchloric acid at 180-200^{o}C, while the digested sample was subjected to atomic absorption spectrophotometer (GBC-932, Australia) by following the standard procedure of **AOAC (2000)**. Whereas Na and K was determined using Flame Photometer Model PEP7 as described by the **AOAC (2000)**. Total phosphorus was assayed colorimetrically at 630 nm using Carlzeiss Spekol colorimeter **(AOAC, 1990)**.

Determination of polyphenols and antioxidants:

Total Phenols

The quantification of the total phenols content of RKBP extract was determined according to the method of **Mena *et al*. (2011)**, with minor modifications. The results expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

Flavonoids

The flavonoids content of RKBP extracts was determined using the method described by **Zhishen *et al.* (1999)**. The results were expressed as milligrams of catechin equivalents per gram of dry weight (mg CE/g DW).

Individual Phenolic Compounds

The individual phenolic compounds were determined following the method described by **Lin *et al.*(2008),** with slight modifications.

Antioxidant activity

The free radical scavenging DPPH assay and ferric reducing antioxidant power (FRAP) assay were performed according to the methods described by **Espín *et al.*(2000)**, with slight modifications. The results of all procedures were measured as µM of trolox equivalents per gram of dry weight (µM TE/g DW).

Sensory evaluation

Sensory evaluation was organized with thirty panelists from staff and students of faculty of Specific Education, Alexandria University. The samples were evaluated for color, odor, taste, texture, tenderness and overall acceptability using a hedonic scale of 1-10 according to the method of **Badr and El-Waseif (2017).** Plain water was provided to rinse the mouth between the samples. The statistical analysis of the results showed that the best percentage in terms of general acceptance was 5%, as this percentage was used in various analyzes.

All the analyses were undertaken to the cooked and uncooked beef burger samples except that of sensory evaluation that was made to the cooked beef burger only.

Cooking properties

Cooking yield (%), cooking loss (%), shrinkage (%), fat retention (%) and moisture retention (%) values were measured using the procedure explained by **Kılıncceker and Kurt (2018)**. The results were calculated from the following equations:

Cooking yield (%) = (Weight of cooked burger/Weight of uncooked burger) × 100

Cooking loss (%) = (Raw weight - Cooking weight/ Raw weight)× 100

Shrinkage (%) =(Raw diameter-Cooking diameter / Raw diameter) × 100

Fat retention (%) = (Cooked weight **(g)** x % fat in cooked burger) / (Raw weight **(g)** x % fat in raw burger) x 100

Moisture retention (%) = (cooked weight (g) × % moisture in cooked samples) /(raw weight (g) ×% moisture in raw samples**)** x 100

Water holding capacity (WHC):

Press technique was used to measure the water holding capacity of beef burgers **(Tsai and Ockerman, 1981).** Raw beef burger (0.5 g) was placed on filter paper (Whatman No. 1, stored in saturated KCl) which was placed between two glass sheets and pressed for 20 min by a 1 kg weight. The area of free water was measured using a compensating polar planimeter and the WHC was calculated as follows:

Free water (%) = (Total surface area - meat film area, mm) (6.11) / (Total moisture (mg) in meat sample) × 100

WHC (%) = 100- free water.

oxidation of stored burger:

Thiobarbituric acid reactive substances of samples was quantified using a colorimetric method as mentioned by **Kryževičūtė *et al.* (2017)**.

Microbiological analysis :

Total count and *Coliform* group were determined according to method described by **EL-Shawaf (1990).** Molds and yeasts count were determined according to the **Oxoid Manual (1982).** The culture was incubated at 37^0C for 48 hours.

Statistical analysis:

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. **(**Armonk, NY: IBM Corp**)** **(Kirkpatrick and Feeney 2013**). The used tests were Student t-test, ANOVA, Post Hoc test (LSD). Significance of the obtained results was judged at the 5% level.

Results and Discussion

Gross Chemical composition of red kidney bean powder:

The results regarding the gross chemical composition of RKBP are depicted in Table (2-a).Moisture content of RKBP was found to be 7.05 g/100g. The protein content results showed that RKBP are an excellent source containing 26.78 g/100g protein. These results are consistent with the findings of **Rui *et al*. (2011)**who reported the protein content in different types of beans at 22.36-28.50 g/100g. Beans contain between 21 to 25 **%** protein by weight, which is much higher than other sources of vegetable protein (**USDA, 2015**).Concerning the crude fat content of RKBP was found to be fairly low 1.72 g/100g.This value was dissimilar to **Sarker *et al*. (2020)** who found that the crude fat content was3.07%**.** It may be due to the variation of cultivar and environmental impacts of diverse regions. The ash content was found to be 4.25g/100g**,** which falls within the range of 4-5% as recorded for various bean varieties (**Rui *et al*., 2011**). The crude fiber content of RKBP was observed to be 6.79 g/100g. These results were close to **Pathak and Kulshrestha (2017)** who observed that the crude fiber content in small and large RKB was 7.87-8.16%,respectively. With regard to the carbohydrate content, which was calculated as nitrogen free extract by difference accounted for 60.46 g/100g. The carbohydrate value observed in this study is in conformity with the value of (58.64g /100g) for dark RKB **(Sarker *et al*., 2020)**. As for, the total dietary fiber content was 13.47g/100g.This is consistent with **Tosh and Yada (2010)** who found that dry beans are rich in both soluble and insoluble fibers. The calories of RKBP were found to be 364.44 Kcal/100g indicating these to be a fairly good source of energy. Previously, **Khattab *et al*. (2009)** reported a caloric content range of 383.91 - 375.32 Kcal/100g for Egyptian and Canadian cowpea and kidney beans.

Table (2-a): Gross Chemical composition of RKBP

Parameters	(g/100g)
Moisture	7.05 ± 0.48
Crude protein	26.78 ± 0.59
Crude fat	1.72 ± 0.41
Ash	4.25 ± 0.61
Crude fiber	6.79 ± 0.27
* N-free extract	60.46 ± 0.35
Calories (Kcal / 100g)	364.44 ± 0.88
Total dietary fiber	13.47 ± 0.02

Data was expressed using Mean ±SD. of three replicate

The results regarding mineral composition of RKBP are depicted in Table (2-b). These findings elucidated that RKBP to be a good source of minerals containing magnesium, calcium, sodium, potassium and iron in relatively higher amounts(765.7, 52.4, 40.2, 20.6 and 16.2 mg/100g, respectively), whereas phosphorus, nickel, zinc, manganese and copper in smaller quantities (4.9, 3.89, 3.85, 2.99 and0.98 mg/100g,respectively). Magnesium was found to be most abundant mineral in the raw powder (765.7 mg/100g) which contradicts the findings of **Margier *et al*. (2018)** who reported potassium to be the abundant element in Kidney Beans. The results on mineral analysis corroborate those of **Audu and Aremu (2011)**, however, there may be some slight differences due to different taxa and geographical conditions. From the previous results, it became clear that RKBP are rich in nutrients such as protein, fiber and many minerals.

Table (2-b): Mineral composition of RKBP

Minerals	(mg/100g)
Potassium	20.60 ± 0.56
Magnesium	765.70 ± 0.27
Phosphorous	4.90 ± 0.11
Sodium	40.20 ± 0.32
Calcium	52.40 ± 0.27
Iron	16.20 ± 0.26
Manganese	2.99 ± 0.09
Nickel	3.89 ± 0.08
Zink	3.85 ± 0.28
Copper	0.98 ± 0.06

Data was expressed using Mean ±SD. of three replicate

Bioactive compounds and antioxidant activity of red kidney been powder:

The results of bioactive compounds and antioxidant activity of RKBP are presented in Table (3). The findings obtained for the total phenolic contents (TPC) of RKBP showed that the powder contained (3.54mg GAE/g DW**)**. **Giusti *et al*. (2017)** reported similar amounts for RKBP (3.00 mg GAE/g DW), although lower amounts were found (0.96 mg GAE/g DW) by **Rocchetti *et al*. (2019).** Also, these results were consistent with **Gujral *et al*. (2013)** who found that the highest TPC was exhibited by kidney beans while it was the lowest for chickpea. The results indicated that the total flavonoids content (TFC) was high (3.25 mg CE/g DW).This is in agreement with **Ombra *et al*. (2016).Lopez *et al*. (2013)** found that the anthocyanins are important type of flavonoids in pigmented common beans. Mostly, anthocyanins occur as glycosides in nature. Twenty anthocyanins have been identified from common beans, the most common of which are the glycosides of cyanidin, delphinidin, malvidin, and petunidin. Pelargonidin glycosides such as pelargonidin 3-O-glucoside, pelargonidin 3, 5-O-diglucoside and pelargonium 3- O-(6"-malonyl) glucoside are also found in common beans. The TFC, TPC and chemical composition of common beans are influenced by multiple factors, such as storage, environmental conditions, genotype and processing methods (**Nayak *et al*., 2015**) .

Antioxidant activity of RKBP is also presented in Table (3). Results showed that RKBP powder has highly values for DPPH (13.97 µmol TE/g DW) and FRAP (14.55 (µmol TE/g DW). Similar findings were reported by **Waniab *et al*. (2013)**. Also, several studies have proven the high antioxidant activity of RKBP, such as **Chutipanyaporn *et al*. (2014)** who determined the DPPH in RKBP and found that the value was 719 (µM trolox equivalent /100g DW).

Table (3) :Bioactive compounds and antioxidant activity of RKBP

Parameters	Amount
Total phenolic content (mg GAE/g DW**)**	3.54 ± 0.03
Flavonoids(mg CE/g DW)	3.25 ± 0.03
DPPH (µmol TE/g DW)	13.97 ± 0.48
Ferric reducing antioxidant power (FRAP)(µmol TE/g DW)	14.55 ± 0.01

Data was expressed using Mean ±SD. of three replicates

Table (4) illustrates the concentration of individual phenolic compounds in RKBP. It was obvious that catechin exhibited the highest content among other individual phenolic compounds as it was 373.64 µg/g, followed by kaempferol 3-O-glucoside (56.43 µg/g), Quercetin 3-O-glucoside (45.7 µg/g), myricetin (19.35 µg/g) and kaempferol 3-O-(6´´-O-manolyl) glucoside (12.21 µg/g). On the other hand, RKBP contained a low amount of Quercetin 3-O-(6´´-O-manolyl) glucoside (3.38 µg/g). While, kaempferol 3-O- (malonyl) glucoside, kaempferol, protocatechuic acid and Gallic acid were not detectable.

Many studies have estimated the individual phenolic compounds, as verified by **Mecha *et al*. (2019)** who found that flavonols, namely catechin, is the main phenolic compound found in the coat of beans. **Gan *et al*. (2016)** found that catechin, epicatechin, epigallocatechin, and epicatechingallate are the most abundant flavonoids detected in common beans with the range of ND to 611 µg/g DW. Also, **Choung *et al*. (2003)** found that RKB contains Delphinidin 3-O-glucoside, pelargonidin 3-O-glucoside, cyanidin 3-O-(6"-malonyl) glucoside and cyanidin 3, 5-diglucoside.**Giusti *et al*. (2017)** did not find Kaempferol 3-glucosideit in RKBP, but in this present study kaempferol3-O-glucoside was found at 56.43 µg / g DW. Altogether, these results suggest that RKBP could be a particularly good source of phenolic compounds. Furthermore, their food industry application would not be compromised by the cooking method, since these beans were shown to retain over 83% of total phenolic acids after cooking, and they even increase the antioxidant activity (**Gallegos-Infante *et al*., 2010**).

Table (4): Individual phenolic compounds of RKBP

Phenolic compounds	µg/g
Myricetin 3-O-glucoside	ND
Quercetin 3-O-glucoside	45.7
Quercetin 3-O-(6´´-O-manolyl) glucoside	3.38
Kaempferol 3-O-glucoside	56.43
Myricetin	19.35
Kaempferol 3-O-(6´´-O-manolyl) glucoside	12.21
Kaempferol 3-O- (malonyl) glucoside	ND
Kaempferol	ND
Protocatechuic acid	ND
Gallic acid	ND
Catechin	373.64

ND —**Not detectable**

Sensory evaluation of beef burger

The sensory evaluation of cooked beef burgers containing RKBP with 2.5%, 5%, 7.5% and 10% are shown in Table (5). Mean sensory scores for color, odor, taste, texture, tenderness and overall acceptability showed a significant decreasing trend ($P \leq 0.05$) with a gradual increase in RKBP level.

Sensory scores were significantly lower ($P \leq 0.05$) than control for color, odor, taste texture, tenderness in burgers containing 2.5, 5, 7.5% and 10% RKBP, but at the 2.5and 5% RKBP level the score was comparable to control. A decrease in color score with an increase in the RKBP level may be due to an increase in dark red color. Decrease in taste scores with increasing RKBP level could be due to decrease in fat content and/or the RKBP flavor.

Overall acceptability scores for burgers with7.5 and 10% RKB powder were significantly lower ($P \leq 0.05$) than control, but the score at 2.5 and 5 % level was comparable to control. Since fats generally in meat products contain a high amount of saturated fatty acids and cholesterol, substitution of saturated fats in the processed meat industry is a problem as it plays an essential role in terms of texture, juiciness, mouth feel and general product acceptance by consumers (**Siraj *et al*., 2015**).

The sensory scores for all the attributes at 2.5 and 5% level had a very good acceptance and were comparable to control. Hence, the optimum incorporation level of RKBP in cooked burgers was adjudged as 2.5 and 5%. One percentage (5%) was chosen to conduct the rest of the tests, because they obtained the highest percentage of acceptance after the control sample.

Table(5): Sensory evaluation of cooked beef burgers

Treatments	Color	Odor	Taste	Texture	Tenderness	Overall acceptability
Control	7.20 ± 0.06 [a]	7.22± 0.01 [a]	7.20 ± 0.04 [a]	7.18 ± 0.02 [a]	7.20 ± 0.02 [a]	7.26 ± 0.02 [a]
2.5%	7.14 ± 0.01 [b]	7.10 ± 0.01 [c]	7.15 ± 0.05 [b]	7.14 ± 0.09 [b]	7.17 ± 0.03 [b]	6.83 ± 0.01 [c]
5%	7.15 ± 0.03 [b]	7.18± 0.03 [b]	7.14 ± 0.02 [b]	7.11 ± 0.08 [b]	6.69 ± 0.07 [d]	7.11± 0.02 [b]
7.5%	6.89± 0.03 [c]	7.06 ± 0.03 [d]	6.73 ± 0.09 [c]	6.82± 0.01 [c]	6.73 ± 0.05 [c]	6.65 ± 0.04 [d]
10%	6.75 ± 0.06 [d]	7.01 ± 0.02 [e]	6.67 ± 0.03 [d]	6.61 ± 0.02 [d]	6.42± 0.02 [e]	6.50± 0.07 [e]
F	**680.87***	**518.42***	**688.58***	**597.67***	**1877.34***	**2254.50***
p	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***
LSD	**0.021**	**0.0107**	**0.0271**	**0.028**	**0.0217**	**0.0186**

F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (LSD)**

Values are expressed as mean ± SD Mean value with different letters in the same column are significantly different ($P \leq 0.05$), and vice versa.

Chemical composition of beef burger

The data in Table (6) shows the chemical composition of uncooked and cooked beef burger after replacing the fat with 5% RKBP during storage by freezing for two month. From the tabulated data presented in Table (6) it could be noticed that the uncooked RKBP burger had a higher moisture content at zero time and at any time of frozen storage (65.95, 65.63,64.78, 64.55 and 63.68 g/100g, respectively) with a significant difference ($P \leq 0.05$) for uncooked control samples (65.75, 64.58, 64.52, 64.20 and 64.08 g/100g, respectively). Also, cooked RKBP burger has higher moisture content (53.35, 53.23, 53.17, 53.09 and 52.98 g/100g, respectively) compared to cooked control samples (52.85, 52.68, 52.45, 52.32 and 52.18 g/100g, respectively).The increment in moisture content may be due to the capability of RKBP which rich with fiber to hold more water via preparation and cooking process. These results agree with **Choi *et al*. (2016)** who stated that, dietary fiber sources have the capacity to hold three or four times its weight of water. These findings also coincide with **Jeong *et al*. (2004)** who reported that high-fat patties had lower moisture content than low-fat treatments. **Turhan *et al*. (2005)** found that the lowest moisture content of control beef burgers was 59.43%, the lowest moisture content of control beef burger was due to the adjustment of fat to 20%. Furthermore, moisture content of all samples under investigation decreased continuously after cooking and after storage for 60 days. These results are consistent with **El-Sayed *et al*.(2020)** who found that the moisture content of beef burger samples after cooking process were lower than that of a raw beef burger. The decrease during storage might be due to the drip loss and partially evaporation of moisture through the polyethylene bags used for packing. The results are in agree with those previously by **Ali (2008)**.

From the results given in same Table (6), it could be observed that protein content was higher in RKBP burger compared to the control sample whether at zero time or at any time of frozen storage by (23.85, 23.55, 22.80, 21.65, 21.50*vs.* 21.75, 21.32, 20.82, 20.71 and 20.52 g/100g, respectively) in uncooked samples, or (20.85, 20.55, 19.80, 19.05, 18.95 *vs.* 18.75, 18.32,18.17, 18.09 and17.82 g/100g, respectively)in cooked samples. Such results may be due to the highest original crude protein content of RKBP. This agrees with **Sarker *et al*. (2020)** who found that RKBP contains 22.1% protein. Also, **Roy *et al*. (2020)** found that RKBP contains 28.3% protein.

Significant differences ($P \leq 0.05$) were found among protein contents for raw and cooked beef burger in control and RKBP burger. The data concluded that protein content of cooked beef burger (on dry weight basis) was less than that of uncooked. This may be due to cooking, which causes decrement in protein content. This interpretation was confirmed by **Dreeling *et al*. (2000)** who concluded that grilling causes a reduction in the protein content of the beef burger by 18%.Nevertheless, by advancement of frozen storage, the protein content of all treatments was decreased with a significant statistical difference when compared treatments with time of storage. The results agreed with **Ali (2008)** who found that protein content of the uncooked low-fat beef burger samples decreased with the progression of frozen storage(on dry weight basis). Also, **Hammad *et al*. (2019)** reported that throughout the storage, protein content decreased with increased storage period. The decrease in protein content observed during the study could related to denaturation of meat protein that is associated with frozen meats, this agrees with **Arannilewa *et al*. (2005**).

Results given in the same Table (6) showed that the fat content of uncooked and cooked control burger was (16.25, 16.32, 16.54, 16.68 and16.73 g/100g, respectively) and (14.25, 14.10, 13.80, 13.68 and 13.43 g/100g, respectively). While, The fat content decreased when replacing it with RKBP by 5% to (15.02, 14.75, 14.52, 14.30 and 14.10 g/100g, respectively for uncooked burger) and (13.82, 13.65, 13.42, 13.21 and 13.10, respectively for cooked burger) during frozen storage. Partial fat replacement with RKBP reduced the fat content. This is similar to **Gad (2019)** who found that replacing fat with wheat dietary fiber led to a decrease in the fat content, also **El-Sayed *et al*. (2020)**who found that partial substitution of fat with gum arabic led to a decrease in the fat content of the burger as well. The data also revealed that fat content of all samples was decreased after cooking which might be due to fat loss from beef burger by grilling. The results are in agreement with that previously obtained by **Ali (2008**) who showed that grilling or microwave-grilling caused a clear reduction in the fat content of beef burger samples from ~ 12.7% (on dry weight basis) in uncooked beef burger to ~ 8% (on dry weight basis) in the grilled burger.

From the same Table (6), it could be observed that a significantly ($P \leq 0.05$) higher amount of ash content was found in the uncooked RKBP burger (4.11, 4.21, 4.30, 4.42 and 4.53 g/100g, respectively) compared to the uncooked control burger (3.25, 3.42, 3.55, 3.60 and 3.67 g/100g, respectively). Also, the ash content of cooked RKBP burger were significantly ($P \leq 0.05$) higher (4.22, 4.38, 4.55, 4.62 and 4.79 g/100g, respectively) than cooked control burger (3.53, 3.59, 3.71, 3.84 and 3.90 g/100g, respectively).This increase is attributed to the higher RKBP ash content. **Sarker *et al*. (2020)** found that RKBP contains 4.19% ash. With advancement of frozen storage, the percentage of ash content slight increased for all samples whether control or RKBP burger samples. Data also revealed that after cooking, it could be noticed that all samples recorded a significant ($P \leq 0.05$) increase for ash content than raw beef burgers. This increase might be due to the moisture loss during cooking. This explanation coincided with **Mohamed (2005**) findings.

Regarding to the crude fiber, the finding indicated that uncooked RKBP burger has a significantly (*P*≤0.05) higher amounts of crude fiber(5.85, 5.79, 5.74, 5.68 and 5.61 g/100g, respectively) than uncooked control burger (2.72, 2.68, 2.64, 2.58 and 2.53 g/100g, respectively). On the other hand, cooked RKBP burger contained a significantly (*P*≤0.05) higher amounts of crude fiber(4.95, 4.87, 4.79, 4.70 and 4.64g/100g, respectively) than cooked control burger (1.82, 1.76, 1.68, 1.62 and 1.58 g/100g, respectively)during storage period. This is due to the high fiber content in RKBP, and this is in agreement with **Pathak and Kulshrestha (2017**) who found a high fiber content in both large and small RKBP (8.16% and 7.87%, respectively).

With regard to the N-free extract, it is obvious that the uncooked RKBP burger contained less amount of N-free extract (51.17, 51.70, 52.64,53.95 and 54.26g/100g, respectively) compared to the uncooked control burger (56.03, 56.26, 56.45, 56.43 and 56.55g/100g, respectively) during storage period. In addition to that the cooked RKBP burger has insignificant (*P*≤0.05) decrease in N-free extract (56.16, 56.55, 57.44, 58.42 and 58.52g/100g) compared to cooked control burger (61.65, 62.23, 62.64,62.77 and 63.27g/100g, respectively). There was an increase in the N-free extract content after cooking in all samples compared to uncooked burgers, this probably due to the moisture loss during the cooking process. Similar finding was reported by **Mohamed (2005**) who found that after cooking total carbohydrates content increased for all studied low-fat sausage samples. The N-free extract content increased for all samples as the frozen storage duration was extended, with a significant (*P*≤0.05) difference between samples and frozen storage time. This in accordance with **El-Nashi *et al*. (2015)** who found during storage the total carbohydrates content in all sausage samples increased continuously as the time of storage period was increased, possibly due to the loss in either moisture or protein or both contents.

As for the caloric value, the data represented in Table (6) also indicated that the uncooked control sample had the highest caloric value during storage period(457.37, 457.20, 457.94, 458.7 and 458.85 k.cal/100g, respectively)with high significant difference (*P*≤0.05) when compared with uncooked RKBP burgers (435.26, 433.75, 432.44, 431.1 and 429.94 k. cal / 100g,respectively). Also, the control cooked sample had the highest caloric value (449.85, 449.10, 447.44, 446.56 and 445.23 K. cal / 100g, respectively).While, cooked RKBP burger contain (432.42, 431.25, 429.74, 428.77 and 427.78K. cal / 100g, respectively).It was found that the caloric content of the uncooked and cooked control burgers was higher than that of RKBP burger samples. These results may be due to the higher fat content in the control sample, as expected, because fats are the most concentrated source of dietary energy, providing 9 K. cal / 100 g, which is more than double the amount provided by protein or carbohydrates**(Giese, 1996)**. The caloric values of the uncooked and cooked RKBP burgers were lower than the control sample may be due to the higher fiber content of the RKBP. This agrees with **Hygreeva *et al*. (2014)** who found that fibers decrease the caloric content, therefore may be regarded as functional ingredients in meat products. Additionally, reduced fat content resulted in lower caloric values in low-fat beef burgers. These results agree with **El-Beltagy *et al*. (2007)** who found that caloric reduction positively correlated with fat reduction. According to the data in Table (6), the cooking process resulted in a significant reduction (*P*≤0.05) in caloric values compared to a raw beef burger due to lost liquid and melting of fat during grilling. With advancement of frozen storage time, the caloric value of most samples decreased with a significant statistical difference (*P*≤0.05). These results indicated that using RKBP in burger considered a good method for caloric reduction which is very important for consumers restricted for their fat intake. From the previous results, it can be concluded that the chemical composition of the beef burger resulting from the substitution of the fat content with RKBP showed an increase in the protein, ash and dietary fiber content, while the fat and calories content were decreased.

Table (6): Chemical composition of beef burger samples during storage period at – 18 °C for two months

Storage period (day)	Uncooked		Cooked	
	Control beef burger	Beef burger with 5% RKBP	Control beef burger	Beef burger with 5% RKBP
	Moisture			
Zero	65.75±0.02 ^{aA}	65.95±0.02 ^{bA}	52.85±0.16 ^{aB}	53.35±0.24 ^{bB}
15	64.58±0.06 ^{aAx}	65.63±0.04 ^{bAx}	52.68±0.04 ^{aB}	53.23±0.03 ^{bB}
30	64.52±0.04 ^{aAx}	64.78±0.07 ^{bAxy}	52.45±0.01 ^{aB}	53.17±0.01 ^{bB}
45	64.20±0.03 ^{aAxyw}	64.55±0.02 ^{bAxy}	52.32±0.03 ^{aBy}	53.09±0.01 ^{bBw}
60	64.08±0.03 ^{aAxywz}	63.68±0.04 ^{bAxywz}	52.18±0.19 ^{aBx}	52.98±0.04 ^{bBy}
	Crude Protein			
Zero	21.75±0.06 ^{aA}	23.85±0.07 ^{bA}	18.75±0.07 ^{aB}	20.85±0.08 ^{bB}
15	21.32±0.02 ^{aAx}	23.55±0.02 ^{bA}	18.32±0.02 ^{aBx}	20.55±0.05 ^{bBx}
30	20.82±0.39 ^{aA}	22.80±0.08 ^{bAxy}	18.17±0.09 ^{aBx}	19.80±0.07 ^{bBxy}
45	20.71±0.04 ^{aAxy}	21.65±0.07 ^{bAxyw}	18.09±0.02 ^{aBxy}	19.05±0.01 ^{bBxyw}
60	20.52±0.04 ^{aAxyz}	21.50±0.07 ^{bAxywz}	17.82±0.08 ^{aBxyw}	18.95±0.09 ^{bBxyw}
	Crude Fat			
Zero	16.25±0.39 ^{aA}	15.02±0.10 ^{bA}	14.25±0.06 ^{aB}	13.82±0.20 ^{bB}
15	16.32±0.55 ^{aA}	14.75±0.07 ^{bAx}	14.10±0.06 ^{aBx}	13.65±0.01 ^{bB}
30	16.54±0.10 ^{aA}	14.52±0.16 ^{bA}	13.80±0.01 ^{aBx}	13.42±0.03 ^{bB}
45	16.68±0.05 ^{aA}	14.30±0.06 ^{bAxy}	13.68±0.13 ^{aBx}	13.21±0.01 ^{bByw}
60	16.73±0.07 ^{aA}	14.10±0.19 ^{bAxw}	13.43±0.17 ^{aB}	13.10±0.09 ^{bB}
	Ash			
Zero	3.25±0.02 ^{aA}	4.11±0.01 ^{bA}	3.53±0.01 ^{aB}	4.22±0.03 ^{bB}
15	3.42±0.04 ^{aAx}	4.21±0.01 ^{bAx}	3.59±0.03 ^{aB}	4.38±0.03 ^{bBx}
30	3.55±0.05 ^{aAxy}	4.30±0.04 ^{bA}	3.71±0.07 ^{aB}	4.55±0.08 ^{bB}
45	3.60±0.01 ^{aAxy}	4.42±0.02 ^{bAxy}	3.84±0.06 ^{aBw}	4.62±0.02 ^{bBxy}
60	3.67±0.09 ^{aA}	4.53±0.02 ^{bAxywz}	3.90±0.05 ^{aBxyw}	4.79±0.03 ^{bBxyz}
	Crude fiber			
Zero	2.72±0.07 ^{aA}	5.85±0.06 ^{bA}	1.82±0.05 ^{aB}	4.95±0.16 ^{bB}
15	2.68±0.09 ^{aA}	5.79±0.01 ^{bA}	1.76±0.05 ^{aBx}	4.87±0.26 ^{bB}
30	2.64±0.08 ^{aA}	5.74±0.09 ^{bA}	1.68±0.06 ^{aBxy}	4.79±0.09 ^{bB}
45	2.58±0.01 ^{aA}	5.68±0.08 ^{bA}	1.62±0.07 ^{aBx}	4.70±0.04 ^{bB}
60	2.53±0.04 ^{aA}	5.61±0.06 ^{bAx}	1.58±0.08 ^{aBx}	4.64±0.02 ^{bB}
	N-free extract			
Zero	56.03±0.07 ^{aA}	51.17±0.17 ^{bA}	61.65±0.07 ^{aB}	56.16±0.27 ^{bB}
15	56.26±0.48 ^{aA}	51.70±0.21 ^{bAx}	62.23±0.10 ^{aBx}	56.55±0.19 ^{bB}
30	56.45±0.13 ^{aA}	52.64±0.09 ^{bAxy}	62.64±0.48 ^{aB}	57.44±0.02 ^{bB}
45	56.43±0.40 ^{aA}	53.95±0.01 ^{bAxyw}	62.77±0.37 ^{aB}	58.42±0.35 ^{bBxy}
60	56.55±0.23 ^{aA}	54.26±0.58 ^{bA}	63.27±0.22 ^{aBxy}	58.52±0.03 ^{bBxyw}
	Calories			
Zero	457.37±0.28 ^{aA}	435.26±0.22 ^{bA}	449.85±0.49 ^{aB}	432.42±0.77 ^{bB}
15	457.20±0.04 ^{aA}	433.75±0.25 ^{bAx}	449.10±0.08 ^{aB}	431.25±0.68 ^{bBx}
30	457.94±0.25 ^{aAx}	432.44±0.51 ^{bAx}	447.44±0.33 ^{aBx}	429.74±0.87 ^{bBx}
45	458.7±0.18 ^{aAxyw}	431.1±0.53 ^{bAxyw}	446.56±0.69 ^{aBx}	428.77±0.37 ^{bBx}
60	458.85±0.23 ^{aAxyw}	429.94±0.06 ^{bAxy}	445.23±0.52 ^{aBxyw}	427.78±0.44 ^{bBxyz}

Comparison between groups was done by **Student t-test** **RKBP:** Red kidney bean powder

Comparison between periods was done by **F test (ANOVA) with repeated measures**, Sig. bet. periods was done using Post Hoc Test (**adjusted Bonferroni**)

Values are expressed as mean ± SD

Small letters for Comparing between **Control** and **Beef burger with 5% RKBP** in each process

Capital letters for Comparing between **Uncooked** and **Cooked** in each process

x: sig. with **0** **y**: sig. with **15 d** **w**: sig. with **30 d** **z**: sig. with **45 d**

Cooking properties of beef burger:

Data given in Table (7) showed that, the addition of RKBP affected the cooking yield of the burgers. As the amount of RKBP added, the cooking yield increases. This tendency was observed during all storage period (84.29, 80.0, 77.14, 74.29 and 72.44%, respectively) compared to control burger (80.0, 77.14, 74.29, 72.86 and 71.43%, respectively). This higher yield of burgers with RKBP, could be related to the fiber content and water holding capacity (WHC) of the RKBP. These findings are consistent with **Borderías *et al*. (2005)** who mentioned that plant dietary fibers has been successfully used as a partial fat in many studies, and was concluded that utilization of fiber for obtaining meat products can improve binding properties, textural characteristics and cooking yield.

When grinding meat during making burger, this leads to the breakdown of myofibrils and connective tissues, which results in weight loss during the cooking process, as a result of the release of fluids, such as water, water-soluble nutrients, color pigments and compounds responsible for flavor and odor (**Meira, 2013**). Data revealed that RKBP burger samples have cooking loss percentages lower than control during storage period at -18°C (15.71, 20.0, 22.85, 25.7 and 26.77 *vs.* 20.0, 22.86, 25.71, 27.14 and 28.57%, respectively).This decrease is due to the ability of RKBP fiber to hold a large amount of water. These results agree with **Namir *et al.* (2015)** who stated that there was a decrement in the cooking loss values of low fat burger when the levels of high fiber substances were increased.

Table (7) also shows that, control beef burger sample had a high percentage of shrinkage after cooking process during storage period at -18°C (16.67, 16.59, 16.52, 16.48 and16.44%, respectively) in a comparison with burger integrated with RKBP(8.33, 8.28, 8.23, 8.19 and 8.15%, respectively). During cooking, meat products shrink due to evaporated water, protein denaturation and fat loss. The addition of RKBP reduced the diameter reduction in all samples in comparison with the control. These lower values could be related to a higher retention of moisture and fat in the meat matrix caused by the RKBP fiber as reported by **Pathak and Kulshrestha (2017**). These effects were similar to other studies with fiber-rich ingredients (**Sánchez-Zapata *et al*., 2010** and **Essa and Elsebaie 2018**). Therefore, RKBP could avoid cooking loss and shrinkage in the burger, which contribute to a bad reaction from consumers.

Data given in Table (7) also shows that, the fat and moisture retention percentages were also influenced by RKBP addition. The results indicated that the RKBP beef burger had higher percentages of fat retention during storage period (77.55, 74.03, 71.29, 68.62 and 66.36%, respectively) compared to the control sample (70.15, 66.65, 61.98, 59.75 and 57.34%, respectively). Also, moisture retention in the RKBP beef burgers was higher as the values were (68.18, 64.88, 63.32, 61.09 and 59.43 %, respectively) compared to the control burger (64.30, 62.93, 60.39, 59.38 and 58.16%, respectively).

The results observed for moisture retention can be explained by the property of the fiber to hold water. Since RKBP has the higher content of fiber, consequently it showed a higher water holding capacity as when used as a food ingredient in beef burger, it resulted in products with higher percentages of water retention. This result is important since high retention of both water and fat, positively influences characteristics such as texture and juiciness of meat products. These findings were in accordance with **Ammar *et al*. (2014)**.

Table (7): Cooking properties of beef burger samples during storage period at – 18 °C for two month

Storage period (day)	Control beef burger	Beef burger with 5% RKBP
Cooking yield (%)		
Zero	80.0±0.03 [a]	84.29±0.24 [b]
15	77.14±0.07 [ax]	80.0±0.59 [bx]
30	74.29±0.46 [ax]	77.14±0.46 [bxy]
45	72.86±0.07 [axy]	74.29±0.57 [bxyw]
60	71.43±0.06 [axyz]	72.44±0.10 [axyw]
Cooking loss (%)		
Zero	20.0±0.24 [a]	15.71±0.50 [b]
15	22.86±0.24 [ax]	20.0±0.71 [bx]
30	25.71±0.34 [axy]	22.85±0.23 [bx]
45	27.14±0.09 [axy]	25.7±0.32 [bxyw]
60	28.57±0.60 [axyw]	26.77±0.24 [axywz]
Shrinkage (%)		
Zero	16.67±0.12 [a]	8.33±0.69 [b]
15	16.59±0.02 [a]	8.28±0.20 [b]
30	16.52±0.51 [a]	8.23±0.03 [b]
45	16.48±0.02 [ay]	8.19±0.06 [b]
60	16.44±0.20 [a]	8.15±0.04 [bw]
Fat retention(%)		
Zero	70.15±0.54 [a]	77.55±0.02 [b]
15	66.65±0.65 [ax]	74.03±0.16 [bx]
30	61.98±0.15 [axy]	71.29±0.06 [bxy]
45	59.75±0.08 [axyw]	68.62±0.29 [bxyw]
60	57.34±0.23 [axywz]	66.36±0.39 [bxywz]
Moisture retention (%)		
Zero	64.30±0.28 [a]	68.18±0.10 [b]
15	62.93±0.19 [ax]	64.88±0.26 [bx]
30	60.39±0.13 [axy]	63.32±0.04 [bx]
45	59.38±0.16 [axyw]	61.09±0.08 [bxyw]
60	58.16±0.04 [axywz]	59.43±0.12 [bxywz]

Comparison between groups was done by **Student t-test** **RKBP:** Red kidney bean powder
Comparison between periods was done by **F test (ANOVA) with repeated measures**, Sig. bet. periods was done using Post Hoc Test (**adjusted Bonferroni**)
Values are expressed as mean ± SD
Small lettersfor Comparing between **Control** and **Beef burger with 5% RKBP** in each process

x: sig. with **0** **y**: sig. with **15 d**
w: sig. with **30 d** **z**: sig. with **45 d**

Water holding capacity (WHC):

Results of WHC of uncooked and cooked beef burger samples which prepared by replacing fat with 5 % RKBP are illustrated in table (8). It could be observed that, the control beef burger had low water holding capacity during storage period at -18°C which found to be (80.1, 78.82, 76.40, 74.30 and 70.19%, respectively for uncooked burger) and (76.9, 73.30, 71.93, 69.30 and 68.50 %, respectively for cooked burger**)**with a significant difference ($P \leq 0.05$). On the contrary, RKBP beef burger samples showed the highest values of WHC during storage which found to be (99.1, 95.2, 90.4, 89.5 and 85.98%, respectively for uncooked burger) and (93.5, 91.8, 88.6, 86.3 and 84.50 %, respectively for cooked burger). A significant decrease ($P \leq 0.05$) in WHC was observed during storage period in all samples, possibly due to water loss through evaporation, protein denaturation or aggregation which caused a decrease in the ability to bind water, or to biochemical changes associated with storage of meat products, as reported by **Qin *et al*. (2013)**.

There was an improvement in WHC in uncooked and cooked burger after using RKBP which reflect the increased ability of meat to holding water. This improvement is due to the higher RKBP content of protein as it is responsible for the formation of hydrogen bonds between water molecules and the overall chemical components of the beef burger. **Boye *et al*. (2010)** stated that RKBP contains many amino acids such as leucine, lysine, aspartic acid, glutamic acid and arginine. Also, **Tounkara *et al*. (2013)** found a relation between peptides and WHC during the hydrolysis process. Additionally, the improvement in WHC in cooked burger samples after using RKBP may be due to starch gelatinization at high temperatures, which leads to water absorption into the granules as well as could be due to the swelling of the fibers, as **Ali *et al*. (2011)** interpreted it.

It was evident from the previous results and discussion that the RKBP improved WHC which is related to many organoleptic properties (such as tenderness, juiciness, thawing drip and cooking loss) of meat and meat products.

Table(8): Water holding capacity (WHC) of beef burger samples during storage period at – 18 °C for two months

Storage period (day)	Uncooked		Cooked	
	Control beef burger	Beef burger with 5% RKBP	Control beef burger	Beef burger with 5% RKBP
	WHC%			
Zero	80.1±0.03 aA	99.1±0.18 Ab	76.9±0.01 aB	93.5±0.07 bB
15	78.8±0.07 aAx	95.2±0.20 Abx	73.30±0.32 aBx	91.8±0.02 bBx
30	76.40±0.34 aAxy	90.4±0.23 Abxy	71.93±0.08 aBx	88.6±0.05 bBxy
45	74.30±0.08 aAxy	89.5±0.12 Abxyw	69.30±0.19 aBxyw	86.3±0.12 bBxyw
60	70.19±0.15 aAxywz	85.98±0.05 Abxywz	68.50±0.09 aBxywz	84.50±0.12 bBxywz

TBA: Thiobarbituric acid **RKBP:** Red kidney bean powder
Comparison between groups was done by **Student t-test**
Comparison between periods was done by **F test (ANOVA) with repeated measures**, Sig. bet. periods was done using Post Hoc Test (**adjusted Bonferroni**)
Values are expressed as mean ± SD
Small lettersfor Comparing between **Control** and **Beef burger with 5% RKBP** in each process
Capital lettersfor Comparing between **Uncooked** and **Cooked** in each process

x: sig. with **0** **y**: sig. with **15 d**
w: sig. with **30 d** **z**: sig. with **45 d**

Burger oxidation:

Thiobarbituric acid (TBA) test value is considered as one of the most popular test used to measure lipid oxidation in meat and meat products. Changes in TBA values in uncooked and cooked beef burger during storage by freezing for two month were described in Table (9). Results given in Table (9)indicated that the TBA of the control samples had the highest value during storage period, as it was0.38,0.45, 0.51, 0.60 and 0.75 mg malonaldehyde / kg sample, respectively for uncooked burger and0.85, 0.93, 1.01, 1.11,1.25 mg malonaldehyde / kg sample, respectively for cooked burger. On the other hand, it was found that the RKBP burger had a significantly ($P \leq 0.05$) lower TBA value during the storage period, being (0.15, 0.19, 0.23, 0.29 and 0.31 mg malonaldehyde / kg sample, respectively for uncooked burger and0.32, 0.40, 0.52, 0.57, 0.60 mg malonaldehyde / kg sample, respectively for cooked burger). The lower TBA values recorded for RKBP beef burger were a result of the partial replacement of fat content with RKBP in the formulation. These results agree with **Malav *et al.* (2016).**

This improvement is probably due to the presence of natural antioxidants in RKBP that can retard lipids oxidation (**Chutipanyaporn *et al*., 2014)**.

It was clear that thiobarbturic acid in all samples was increased during frozen storage with a significant difference ($P \leq 0.05$), indicating continuous oxidation of lipids and consequently production of oxidative by samples. But this increase decreased when using RKBP as a fat replacer. These results agree with **Ali (2008)** who found that extending storage time of uncooked prepared low-fat beef burger for 3 months at -20°Cwas accompanied by an increase in TBA level to 0.17 - 0.21 mg malonaldehyde / kg sample regardless of the type of additive used. Also, **Bağdatli and Kayaardi (2015**) reported that TBA of all meat samples increased as storage period increased. It could be noticed that cooked beef burger samples had higher TBA values than that obtained for uncooked beef burger. Increasing TBA values after cooking indicated continuous oxidation of lipids and consequently production of oxidative by samples. Similar findings were reported by **Essa and Elsebaie (2018).**

Table(9): TBA (mg malonaldehyde /1 kg) of beef burger samples during storage period at – 18 °C for two months

Storage period (day)	Uncooked		Cooked	
	Control beef burger	Beef burger with 5% RKBP	Control beef burger	Beef burger with 5% RKBP
		TBA		
Zero	0.38±0.08 ^{aA}	0.15±0.04 ^{bA}	0.85±0.03 ^{aB}	0.32±0.06 ^{bB}
15	0.45±0.01 ^{aA}	0.19±0.05 ^{bA}	0.93±0.02 ^{aB}	0.40±0.06 ^{bBx}
30	0.51±0.05 ^{aA}	0.23±0.08 ^{bA}	1.01±0.05 ^{aBx}	0.52±0.07 ^{bBxy}
45	0.60±0.02 ^{aA}	0.29±0.06 ^{bA}	1.11±0.07 ^{aB}	0.57±0.02 ^{bB}
60	0.75±0.06 ^{aAxw}	0.31±0.07 ^{bA}	1.25±0.08 ^{aBxz}	0.60±0.08 ^{bBxyw}

TBA: Thiobarbituric acid **RKBP:** Red kidney bean powder
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Capital lettersfor Comparing between **Uncooked** and **Cooked** in each process
x: sig. with **0** **y**: sig. with **15 d**
w: sig. with **30 d** **z**: sig. with **45 d**

Changes in microbiological load of beef burger:

Data tabulated in Table (10) shows the microbiological load of uncooked and cooked beef burger during frozen storage at -18 °C for 2 months as (log CFU /g). The initial counts of total bacterial, coliform group and mold& yeast for the uncooked control burger were high ($7.4x10^6$, $4.8x10^4$ and $5.3x10^3$, respectively) , while adding RKBP to burger formulas lowered the counts of microbial load to ($6.8x10^6$, $3.7x10^4$ and $4.8x10^3$, respectively). Also, The initial counts of total bacterial and coliform group for the cooked control burger were high ($6.9x10^1$ and $3.5x10^1$, respectively), but there was a significant (($P \leq 0.05$) decrease in the count after using RKBP as the values were as follows ($5.8x10^1$ and $2.9x10^1$, respectively). The Mold & Yeast were not detected in cooked burger. With the increase in the storage period, the count of total bacteria, coliform and mold & yeast decreased as the count at the end of the storage period was as follows ($4.0x10^4$, $6.5x10^2$ and $4.1x10^2$, respectively for uncooked control burger)($0.5x10^1$, $0.7x10^1$ and ND, respectively for cooked control burger), ($0.9x10^2$, $3.4x10^1$ and $2.8x10^1$, respectively for uncooked RKBP burger) and ($0.3x10^1$, $0.4x10^1$, and ND, respectively for uncooked RKBP burger).

It is noted from the results that the total plate count does not increase, but decreases in all samples during the storage period. This may be related to the stability of growth of the microorganism during frozen storage. These results are in agree with **Gomaa, (2002)** who reported that frozen storage at -18 to -20 $^{\circ}$C caused a decrease in total bacterial count of all beef patties. This reducing might be explained by the mechanical effect of freezing, due to the action of ice crystals and protein denaturation. Additionally, the results showed that the cooked burger in all samples had a lower microbial count than the uncooked burger. This may be due to the effect of the temperature used in cooking. These results agree with **Rodrigo *et al*. (2016)**.

The **E.O.S. (2005)** recommended that the total bacterial and coliform bacterium group counts should not exceed 5 and 3 log cfu/g, respectively for frozen beef burger and be free of *Staphylococcus aureus*. The results indicated that the microbial count for all RKBP beef burger samples was within the permissible numbers. Conversely, the bacterial count for all control samples for the beef patties was more than 10^6cfu / g at zero time, which is the limit indicating contamination of the meat product. It was noted from the previous discussion of the results that cooked and uncooked RKBP burger are less in microbial gross, this may be due to the antioxidant content of RKBP, which has a role in inhibiting the microbial growth of the stored burger. **Sarker *et al*. (2020)** found that RKBP rich in total polyphenol content. Also, **Chibane *et al*. (2019)** reported that foods rich in polyphenols relate with a wide range of biological properties such as antimicrobial activity.

Table (10): Microbiological load of uncooked and cooked beef burger during frozen storage at -18 °C for 2months as (CFU /g)

Storage period (day)		Uncooked		Cooked	
		Control beef burger	**Beef burger with 5% RKB**	**Control beef burger**	**Beef burger with 5% RKB**
		T.B.C			
Zero	CFU /g	7.4×10^{6}	6.8×10^{6}	6.9×10^{1}	5.8×10^{1}
	Log /g	6.87±3.15 ^{aA}	6.83±3.24 ^{aA}	1.84±0.90 ^{aA}	1.76±0.99 ^{aA}
15	CFU /g	3.2×10^{6}	2.9×10^{5}	4.2×10^{1}	3.6×10^{1}
	Log /g	6.51±0.01 ^{aA}	5.46±0.15 ^{bA}	1.62±0.01 ^{aB}	1.56±0.02 ^{bB}
30	CFU /g	6.2×10^{5}	5.8×10^{4}	1.1×10^{1}	0.8×10^{1}
	Log /g	5.79±0.24 ^{aA}	4.76±0.14 ^{bAy}	1.04±0.05 ^{aBy}	0.90±0.01 ^{bBy}
45	CFU /g	4.0×10^{5}	3.7×10^{2}	0.8×10^{1}	0.5×10^{1}
	Log /g	5.60±0.40 ^{aA}	2.57±0.40 ^{bAyw}	0.90±0.08 ^{aBy}	0.70±0.08 ^{bBy}
60	CFU /g	4.0×10^{4}	0.9×10^{2}	0.5×10^{1}	0.3×10^{1}
	Log /g	4.60±0.26 ^{aAw}	1.95±0.32 ^{bAyw}	0.70±0.05 ^{aByw}	0.48±0.03 ^{bByw}
		Coliform group			
Zero	CFU /g	4.8×10^{4}	3.7×10^{4}	3.5×10^{1}	2.9×10^{1}
	Log /g	4.68±2.79 ^{aA}	4.57±2.25 ^{aA}	1.54±1.08 ^{aA}	1.46 ± 0.90 ^{aA}
15	CFU /g	2.9×10^{3}	2.5×10^{2}	2.1×10^{1}	1.6×10^{1}
	Log /g	3.46±0.01 ^{aA}	2.40±0.04 ^{bA}	1.32±0.03 ^{aB}	1.20±0.00 ^{bB}
30	CFU /g	1.5×10^{3}	1.2×10^{2}	1.4×10^{1}	1.0×10^{1}
	Log /g	3.18±0.02 ^{aAy}	2.08±0.11 ^{bA}	1.15±0.01 ^{aBy}	1.00±0.06 ^{bB}
45	CFU /g	7.3×10^{2}	5.7×10^{1}	1.0×10^{1}	0.8×10^{1}
	Log /g	2.86±0.03 ^{aAyw}	1.76±0.03 ^{bAy}	1.00±0.00 ^{aByw}	0.90±0.01 ^{bBy}
60	CFU /g	6.5×10^{2}	3.4×10^{1}	0.7×10^{1}	0.4×10^{1}
	Log /g	2.81 ±0.02 ^{aAyw}	1.53±0.08 ^{bAyw}	0.85±0.02 ^{aBywz}	0.60±0.02 ^{bByz}
		Mold & Yeast			
Zero	CFU /g	5.3×10^{3}	4.8×10^{3}	ND	ND
	Log /g	3.72±1.71 ^{a}	3.68±2.25 ^{a}		
15	CFU /g	3.8×10^{3}	3.3×10^{2}	ND	ND
	Log /g	3.58±0.09 ^{a}	2.52±0.30 ^{b}		
30	CFU /g	1.1×10^{3}	0.9×10^{2}	ND	ND
	Log /g	3.04±0.23 ^{a}	1.95±0.34 ^{by}		
45	CFU /g	6.2×10^{2}	4.8×10^{1}	ND	ND
	Log /g	2.79±0.33 ^{a}	1.68±0.10 ^{b}		
60	CFU /g	4.1×10^{2}	2.8×10^{1}	ND	ND
	Log /g	2.61±0.13 ^{ay}	1.45±0.03 ^{b}		

Comparison between groups was done by **Student t-test**
Comparison between periods was done by **F test (ANOVA) with repeated measures**, Sig. bet. periods was done using Post Hoc Test (**adjusted Bonferroni**)
Values are expressed as mean ± SD
Small lettersfor Comparing between **Control** and **Beef burger with 5% RKBP** in each process
Capital lettersfor Comparing between **Uncooked** and **Cooked** in each process
x: sig. with **0** **y**: sig. with **15 d**
w: sig. with **30 d** **z**: sig. with **45 d**
ND= Not detectable

Conclusions

The results obtained during this study conclude that fat can be partially substituted with RKBP in the production of beef burger to produce a healthy burger that contains many nutrients, dietary fiber and phytochemicals such as antioxidants compounds. Moreover, RKBP help to avoid pigment and lipid oxidation, reduce microbial count, improve physical, nutritional, chemical and cooking properties, storage stability and consumer acceptance beef burger. Therefore, the use of RKBP is a new method that contributes to the manufacture of healthier meat products.

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الملخص العربي

تأثير استخدام مسحوق الفاصوليا الحمراء كبديل للدهون على جودة برجر لحم البقر

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يهدف البحث الحالي إلى التعرف على تأثير استخدام مسحوق الفاصوليا الحمراء (RKBP) كبديل للدهن في تصنيع برجر اللحم البقري على الخصائص الكيميائية و الفيزيوكيميائية و الميكروبيولوجية والحسية أثناء فترات التخزين بالتجميد لمدة 60 يومًا. كما تم دراسة تأثير مسحوق الفاصوليا الحمراء على الثبات التأكسدي للبرجر المعد. تم استبدال الدهن جزئيًا بمستويات مختلفة من مسحوق الفاصوليا الحمراء (2.5 و5 و 7.5 و 10٪) ، ووجد أن 5٪ كانت الأفضل من حيث القبول العام ، لذلك تم تطبيق هذه النسبة في القياسات المختلفة. أظهرت النتائج أن مسحوق الفاصوليا الحمراء يحتوي على نسبة عالية من البروتين والرماد والألياف حيث كانت القيم 26.78 و 4.25 و 6.79 جم / 100 جم على التوالي، لذلك ارتفع محتوى البرجر بعد إضافة مسحوق الفاصوليا الحمراء من البروتين والرماد والألياف و لكنه احتوى على قيم أقل من الدهون والسعرات الحرارية مقارنة بالعينات الكنترول.علاوة على ذلك ، وجد أن مسحوق الفاصوليا الحمراء غني بالمركبات الفينولية و الفلافونويد و DPPH٪ (3.54ملجم جاليك/ جرام وزن جاف، 3.25ملجم كاتشين / جرام وزن جاف و 13.97% على التوالي). أيضًا ، أدت إضافة مسحوق الفاصوليا الحمراء إلى برجر اللحم إلى تحسين خصائص الطهي مثل زيادة قدرة الاحتفاظ بالمياه (WHC) ، وتقليل الفقد والانكماش. علاوة على ذلك ، تحسن الثبات التأكسدي لبرجر اللحم البقري ، حيث قلت قيم حمض الثيوباربيتوريك في البرجر المخزن عند -18 درجة مئوية لمدة 60 يومًا. بالإضافة إلى ذلك ، ساعدت الفاصوليا الحمراء على تقليل النمو الميكروبي للبرجر أثناء التخزين ، وبناءً على هذه النتائج ، يمكننا أن نستنتج أن استخدام مسحوق الفاصوليا الحمراء كبديل للدهون في البرجر يمكن أن يحسن خصائصه الغذائية والوظيفية.

الكلمات المفتاحية: البقوليات، بديل الدهن، المركبات الفينولية ، النشاط المضاد للأكسدة ، أكسدة الدهون ، خصائص الطهي، الاختبارات الحسية.