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Characterization and microbiological quality of low-fat chicken burger containing defatted peanut flour

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ABSTRACT

Fast foods are rich in fats affecting human health; therefore there is a great demand for non-meat protein sources leading to the growing interest in using legumes in food industry. Thus, the aims of this study were to include defatted peanut flour (DPF) in chicken burger and to evaluate the organoleptic, physiochemical and microbial characteristics of the end product. Chicken burger supplemented with 20% roasted defatted peanut flour (R-DPF) showed that the taste was the most affected parameter of cooked samples followed by texture, colour and flavour. These samples registered a remarkable increase in fat (8.87%) and protein (32.56%) contents as well as water holding capacity (WHC) accompanied with a decrease in cooking loss (19.0%) leading to improvement of the final product. *Escherichia coli* and *Staphylococcus aureus* counts were detected in the control samples (5.00 and 6.20 log₁₀ CFU/g respectively); however, only *E coli* was decreased significantly in burger supplemented with 10% unroasted DPF and 20% R-DPF. Moreover, total fungal count was only decreased in burger supplemented with 10% R-DPF. A decrease was observed in both total bacterial and fungal counts after storage at -20°C for 60 d. Supplementation with 20% DPF to low fat chicken burger exhibited good quality attributes and was the most acceptable. Thus the use of DPF could be considered a good source of protein which could increase nutritional value, minimize the product cost and microbiological contamination.

Key words: low fat; chicken burger; peanut flour; characteristics; microbiological; aflatoxins.

Introduction

Peanut (*Arachis hypogaea* L.) has traditionally been used as a source of oil as well as for peanut butter, confectionaries and snack products (Hind, 1995). However, its worldwide annual protein harvest has increased tremendously in recent years (Kain and Chen, 2008). Vegetable oil extraction from peanut yields defatted peanut flour (DPF). DPF is a protein-rich, low fat, inexpensive and under-utilized by-product of the peanut industry that offers the same health and dietary benefits as peanut, and are considered important in food processing and food product formulation (Ahmed and Schmidt, 1979). The properties of DPF are affected by many environmental factors including the processing, the production methods and the presence of other components in the food system (Bland and Lax, 2000). The importance of these properties varies with the type of food products in which the flour is used. Non-meat ingredients can be added to meat and meat based products via many sources in the form of extenders, fillers, binders, to also improve the other characteristics of the products and increase the profit margins of the industry. Among the non-meat ingredients tried are fava beans, lentils, lupin and chickpeas in beef sausages (Abo Bakr *et al.*, 1986), wheat flour in chicken nuggets (Rao *at al.*, 1997) and cowpea in chicken nuggets (Prinyawiwatkul *et al.*, 1997).

Poultry meat is comprised of about 20 to 23% protein, which comminuted products, such as frankfurters, bologna and sausages that typically contain about 17 to 20% protein, 0 to 20% fat, and 60 to 80% water (Smith, 2001). Many efforts have been made to improve the quality and stability of burgers because consumer demand for fast food has been increasing rapidly in recent years. The microbiological safety and quality of poultry meat are equally important to producers, retailers and consumers, and both involve microbial contaminants on the processed product (Mead, 2004). Although *Salmonella* and *Campylobacter* species are the predominant food borne pathogens associated with poultry, other pathogens also may occur including *Clostridium perfringens*, *Escherichia coli* and *Listeria monocytogenes*, together with those recognized more recently such as *Acrobacter* and *Helicobacter* species (Corry and Atabay, 2001). The aims of this work were to evaluate the organoleptic, physiochemical and microbiological characteristics of chicken burgers containing different levels of roasted and un-roasted DPF to produce low fat chicken burger.

Materials and Methods

Materials:

Fresh chicken breasts (3 kg), mixed spices (equal weights of clove, black pepper, Chinese cubebs, paprika and nut-mug), white oat (Hahne, Germany), salt, onion, garlic, parsley and peanuts were obtained from local markets. Chicken meat was minced using a mincer (home mincer) and comminuted chicken meat was used for the processing of chicken burger. Oat was ground to powder using a mill then was used as a fat-replacer for preparing chicken burger to improve palatability.

Extraction and determination of aflatoxins:

Peanuts were analyzed for the presence of aflatoxins (AFs) before use (AOAC, 2000). Briefly 25 g of ground peanut samples were mixed with 125 ml of 60% methanol and 5 g sodium chloride in a blender at high speed for 1 min, after which the liquid supernatant was filtered through Whatman filter paper No. 4. Twenty mL of the filtered supernatant extract was added to 20 mL Phosphate Buffer Saline (pH 7.0) (PBS). The final extract (10 mL) was passed through an Immunoaffinity column (AflaTest®, VICAM, USA) for clean-up. After clean up, AFs were analyzed using HPLC. A Waters (Milford, MA) HPLC equipped with a model 600 pump, and a model 474-fluorescence detector and Millennium 2010 software (Waters) was used to quantify AFs. Separations were carried out at ambient temp on Phenomenex 4m ODS column, (250 x 4.6 mm). AFs were eluted with acetonitrile / methanol / water (1:3:6 v/v/v) as the mobile phase at a 1 mL/min flow rate. The detection wavelength for excitation and emission were set at 365 and 450 nm, respectively.

Preparation of peanut flour:

Peanuts samples (with AFs below detection limit) were divided into two parts; the first part (un-roasted) was ground to powder using a miller, whereas the second part was roasted in an oven at 166 °C for 20 min. Upon removal from the oven, roasted peanuts were cooled using forced ambient air, and skins were manually removed using gloved hands and then ground to powder. Ethanol was used to make the complete extraction of peanut oil, and the rate of agitation was kept constant to maintain a well-mixed fluid. At the end, the mixture was filtered and traces of solvent remaining were removed with nitrogen steam.

Preparation of peanut chicken burger:

Eight chicken burger formulae were prepared from un-roasted (U) and roasted (R) peanut at ratios of 10, 20, 30 and 50%. A sample without peanut was prepared as a control as shown in Table (1). Chicken burger formulae were prepared by well mixing minced chicken breasts with all ingredients. The burger formulae were formed and each sample (50 g) was packed in a polyethelene bag and stored at -20 °C until analysis.

Table 1: Low-fat chicken burger formulae (100 g).

Ingredients	DPF (%)				
	0 (Control)	10	20	30	50
Minced Chicken breasts (g)	83.25	74.92	66.59	58.27	41.62
Salt (g)	1	1	1	1	1
Oat (g)	2.5	2.5	2.5	2.5	2.5
Peanut (unroasted or roasted) (g)	-----	8.33	16.66	24.98	41.63
Onion (g)	0.5	0.5	0.5	0.5	0.5
Garlic (g)	0.25	0.25	0.25	0.25	0.25
Parsley (g)	0.25	0.25	0.25	0.25	0.25
Mixed spices (g)	0.25	0.25	0.25	0.25	0.25
Iced water (mL)	12.00	12.00	12.00	12.00	12.00

Cooking of chicken burger:

Samples under investigation were grilled on electrical heater in a non-sticky pan with no added fat for 4 min at 72 °C on each side.

Organoleptic evaluations:

Cooked chicken burger samples were evaluated organoleptically, immediately after cooking by ten members of Food Science and Technology Department, National Research Centre. Panellists were instructed to

evaluate colour, flavour, taste, appearance, texture and overall acceptability using 10 point scale for grading the quality of samples (Gelman and Benjamin, 1989).

Chemical analysis:

Moisture, protein, ash and fat content were determined for uncooked burger samples according to the AOAC (2000).

Physical analysis:

Water Holding Capacity (WHC): WHC was measured using the method of Wierbicki and Deatherage (1958). Peanut chicken burger samples (0.39g) were placed on ashless filter paper Whatman No. 42 and placed between two glass plates and pressed for 10 min by one kg weight; two zones were formed on the filter paper. Their surface area was measured by planimeter and WHC was calculated by subtracting the area of the internal zone from that of the outer zone, and presented as cm².

Colour: Colour of raw chicken burger samples was measured using a Spectrocolorimeter (Tristimulus colour machine) with the CIE lab colour scale (Hunter, lab scan XE-reston VA, USA) in the reflection mode (Hunter, 1958). Colour was described as follows: lightness (L*), redness (a*) red-green and yellowness (b*) yellow-blue. Nine replicate measurements were taken for each sample, following the guidelines for colour measurements from American Meat Science Association (Hunt *et al.*, 1991).

Cooking loss: Cooking loss was determined according to the AOAC (2000), and was calculated as follows:

$$\text{Cooking loss \%} = \frac{f-g}{f} \times 100$$

Where f: fresh burger sample weight
g: grilled burger sample weight

Shrinkage: Shrinkage percentage was calculated according to the AOAC (2000) as follows:

$$\text{Shrinkage \%} = \frac{(a-b)+(c-d)}{a+c} \times 100$$

Where a: thickness of un-cooked burger
b: thickness of cooked burger
c: diameter of un-cooked burger
d: diameter of cooked burger

Tenderness: The Warner Bratzler shear force apparatus (Ametek /Mansfield and Green div. Largo, Florida) was used to measure the tenderness of cooked samples (El-Naggar, 1999). Samples were cooked and then cooled to room temperature, sheared for three times at different position and average of the shear force test was presented in Newton (N).

Microbiological analysis:

Peanut chicken burger samples were microbiologically examined according to International Committee on Microbiological Specifications for Foods (ICMSF, 1996), before and after cooking at 72 °C. The samples were examined for total fungal count, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* count (log₁₀ CFU/g) according to American Public Health Association (APHA, 1992). Aseptically, chicken burger samples (25 g) were homogenized for 1 min in 225 mL of sterile solution of buffered peptone water. Serial decimal dilutions were made and the following analyses were carried out on agar plates in duplicates: (1) total fungal count on Potato Dextrose Agar (PDA, Oxoid) incubated at 28°C for 5-7 d, (2) *Salmonella* count on Brilliant Green Agar (BGA, Oxoid) incubated at 35°C for 24-48 h, (3) *E. coli* count on Eosin-Methylene Blue Agar (EMB, Oxoid) incubated at 37°C for 24 h, (4) *S. aureus* on Mannitol Salt Agar (MSA, Oxoid) incubated at 32°C for 3 d. Pure cultures of the microorganisms were identified using the standard procedures (Barrow and Feltham, 1993). The test was performed for the identification of isolates included gram stain, biochemical test, and colony morphology. The peanut chicken burger was stored for 60 d at -20°C after which samples were examined microbiologically to determine the effect of freezing storage on the microbial count.

Statistical analysis:

Statistical analysis was performed using SPSS statistical program for windows (Version 16) (SPSS Inc., Chicago, IL, USA). All data were statistically analyzed using analysis of variance and the results were considered significant at $P > 0.05$.

Results and discussion

Occurrence of aflatoxins in peanuts:

The contamination of peanut samples by AFs was determined and the results were presented in Table (2). It was noticed that aflatoxin G₁ (AFG₁) was absent in all peanut samples; whereas, trace amounts of aflatoxin G₂ (AFG₂) was detected and recorded 0.170, 0.905 and 0.760 µg/kg, respectively for three samples out of five. Concerning the contamination by aflatoxin B₁ (AFB₁), the results revealed that AFB₁ levels reached 1.736, 3.240, 1.040 and 0.407 µg/kg for the four tested samples, respectively. The presence of AFB₁ at a concentration of 3.240µg/kg is potential for damage and should be taken seriously into consideration as the International Agency for Research on Cancer of the World Health Organization has classified AFB₁ as human carcinogens.

Table 2: Aflatoxin concentration in peanut samples collected from Cairo Governorate.

No. of samples	Aflatoxin concentration (µg/kg)				
	AFB ₁	AFB ₂	AFG ₁	AFG ₂	Total aflatoxins
1	1.736	3.560	ND	0.905	6.201
2	3.240	1.730	ND	0.760	5.730
3	1.040	0.325	ND	ND	1.365
4	0.407	1.500	ND	0.170	2.077
5	ND	ND	ND	ND	ND

ND: Not determined

In agreement with the current results, Min *et al.* (2008) indicated that contaminated peanut samples recorded 1.57µg/kg for AFB₁ and 0.39µg/kg for AFB₂. Sangare-Tigori *et al.* (2006) examined 10 samples of peanuts from Côte d'Ivoire for AFB₁ and found that peanut samples contained concentrations of AFB₁ above the EU regulatory limits. On the other hand, Wang and Liu (2006) found that AFs detection rate in peanut samples was 24.24%, whereas the average level of AFs contamination was 80.27µg/kg. The authors also reported that there were 3.03% peanut samples exceeding Codex tolerance limit.

Evaluation of peanut chicken burger:

The current results indicated that there were significant differences between the control and DPF-supplemented burger in chemical and physical results as an outcome of the roasting process. Moreover, the organoleptic properties and microbiological quality also showed significant differences.

Organoleptic evaluation:

Organoleptic properties are among the major concerns for the utilization of plant proteins in foods. Data in Table (3) revealed that appearance showed a significant decrease in the burger supplemented with 30 and 50% of unroasted and roasted DPF.

Table 3: Organoleptic evaluation of peanut chicken burger.

Parameters	DPF (%)									
	0	10		20		30		50		
	Control	U	R	U	R	U	R	U	R	
Appearance	7.00 ± 1.33 ^a	7.30 ± 0.95 ^a	7.30 ± 1.16 ^a	7.50 ± 1.51 ^a	7.60 ± 1.26 ^a	6.30 ± 1.16 ^b	6.60 ± 0.84 ^b	6.50 ± 1.51 ^b	5.70 ± 1.34 ^c	
Colour	6.80 ± 1.32 ^a	7.30 ± 1.70 ^b	7.20 ± 1.23 ^b	7.25 ± 1.55 ^b	7.70 ± 1.34 ^c	5.80 ± 1.23 ^d	6.20 ± 1.23 ^e	5.90 ± 1.29 ^d	5.30 ± 1.49 ^f	
Flavour	7.00 ± 1.49 ^a	7.30 ± 1.06 ^a	6.60 ± 1.17 ^b	7.20 ± 1.40 ^a	7.40 ± 1.17 ^a	6.40 ± 1.35 ^b	6.40 ± 1.26 ^b	6.00 ± 1.83 ^d	5.40 ± 1.96 ^e	
Taste	6.70 ± 1.57 ^a	7.30 ± 1.25 ^b	7.10 ± 1.10 ^b	7.05 ± 1.64 ^b	7.90 ± 1.10 ^c	6.80 ± 1.69 ^a	6.90 ± 1.29 ^a	6.30 ± 1.42 ^d	5.20 ± 1.93 ^e	
Texture	7.30 ± 1.06 ^a	7.20 ± 1.32 ^a	7.10 ± 0.99 ^a	7.15 ± 1.11 ^a	7.40 ± 1.35 ^a	7.00 ± 1.56 ^a	6.80 ± 1.32 ^b	6.60 ± 1.26 ^b	5.80 ± 1.62 ^c	
Over all accessibility	7.40 ± 1.63 ^a	7.41 ± 1.04 ^a	7.15 ± 1.11 ^a	6.90 ± 0.84 ^a	7.75 ± 1.09 ^a	6.50 ± 1.05 ^b	6.70 ± 1.16 ^b	6.35 ± 1.60 ^b	5.55 ± 1.74 ^c	

Results are mean values ± SE obtained from 10 independent measurements

U: Unroasted peanut R: Roasted peanut

Within each row, means superscript with different letters are significantly different ($P > 0.05$).

The colour revealed significant changes in the prepared burger and showed high score in the samples supplemented with 10 and 20% for unroasted and roasted DPF; however, it showed low score in the samples supplemented with 30 and 50% DPF. The flavour did not show any significant difference with the control in the samples supplemented with unroasted DPF at concentration of 10% and the roasted and unroasted DPF at 20% whereas, the other ratios of DPF resulted in a significant decrease in the flavour. Only the samples supplemented with 50% unroasted and roasted DPF showed a significant decrease in taste score; however, the other ratios showed a significant improvement. A significant decrease was only found in the texture of burger supplemented with 30% roasted and 50% unroasted and roasted DPF respectively. The overall acceptability revealed that the addition of DPF was acceptable at concentration of 10 and 20% of the unroasted and roasted DPF. Therefore, DPF at a level of 10 and 20% were considered more favourable to use, and were chosen for further studies. The current results are in good harmony with previous studies who reported that none of the legumes had detrimental effect on sensory properties at the level used (Modi *et al.*, 2003; Serdaroğlu *et al.*, 2005).

Chemical analysis:

Data presented in Table (4) showed that moisture content was generally decreased as the percentage of DPF increased in all prepared burger, recording 57.48 and 51.18% for control and burger sample supplemented with 20% U-DPF. The decrease in moisture content of peanut chicken burger may be due to the increase of solid content (Serdaroğlu, 2006). However, in a previous work, the use of cereal and legume flours instead of fat increased the moisture of cooked meat patties (Kurt and Kılınççeker, 2012). Moreover, oat products such as oat bran and oat fibre were reported to increase moisture retention of low-fat meat products (Giese, 1992).

In the current study, protein content in DPF-supplemented burger was significantly higher than control (Table 4). A remarkable increase was noticed at the level of 10 and 20% R-DPF supplementations which recorded 32.71 and 32.56%, respectively. These findings are due to the higher protein content in DPF (Data not shown). Several researchers have found that protein content of comminuted meat products increased with the addition of different legumes (Kaya and Gökalp, 1990; Modi *et al.*, 2003); whereas, lower protein content was reported for beef patties extended with common-bean flour (Dzudie *et al.*, 2002), and in chicken patties formulated with different levels of Ragi millet flour (Naveena *et al.*, 2006). These differences may be due to the percentage of protein in different legumes or to the style of the product.

The current data also showed that fat content was remarkably increased by the addition of DPF (Table 4) compared to control, and recorded 8.91% for samples supplemented by 20% U-DPF which remained below the 10% levels (low fat). Moreover, these effects were higher in parallel to levels of DPF supplementation. This could be attributed to the percentage of fat still in DPF and are in agreement with Prinyawiwatkul *et al.* (1997) who indicated that nuggets containing different concentrations of Fermented Partially Defatted Peanut flour (FPDPF) had higher fat content than the control.

Table 4: Chemical compositions of peanut chicken burger.

Matrix %	DPF (%)				
	0	10		20	
	Control	U	R	U	R
Moisture	57.48 ± 1.51 ^a	51.79 ± 1.54 ^b	51.51 ± 1.27 ^b	51.18 ± 0.06 ^{ab}	51.38 ± 0.03 ^b
Protein	29.24 ± 0.07 ^a	31.96 ± 0.03 ^b	32.71 ± 0.03 ^b	31.16 ± 0.02 ^b	32.56 ± 0.01 ^b
Fat	2.72 ± 0.015 ^a	7.90 ± 0.01 ^b	8.93 ± 0.005 ^c	9.91 ± 0.01 ^d	10.88 ± 0.01 ^e
Ash	5.12 ± 0.02 ^a	5.53 ± 0.03 ^a	5.44 ± 0.07 ^a	5.25 ± 0.07 ^a	5.65 ± 0.22 ^a

Results are mean values ± SE obtained from three independent measurements

U: unroasted peanut R: Roasted peanut

Within each row, means superscript with different letters are significantly different ($P > 0.05$).

Ashes are the sum of the total minerals presented in food such as sodium, phosphorus and iron that can be contributed by the meat as raw material, salt and spices added (Fernández-López *et al.*, 2006). It was noticed that ash content of DPF-supplemented burger samples slightly increased with the increase in the level of DPF supplementation and ranged between 5.12 and 5.65 % in control and peanut chicken burger supplemented with 20% R-DPF samples respectively (Table 4). This was expected as the addition of DPF increased the total solids content and subsequently the ash content (moisture content of DPF was below 12%).

Physical analysis:

Cooking characteristics for experimentally produced chicken burger indicated that incorporation of DPF decreased the cooking loss (Table 5). Loss of weight occurring during cooking might be due to moisture

evaporation (Mansour and Khalil, 1997; Alakali *et al.*, 2010). Choi *et al.* (2007) reported that meat batters containing dietary fiber from rice bran have lower cooking loss than counterpart control. Similar effects were also reported on using black eye bean flour, chickpea flour and lentil flour which resulted in the highest cooking yields (Serdaroğlu *et al.*, 2005).

Shrinkage is considered as one of the most important physical quality changes that occur in burgers during cooking process due to protein denaturation. The present results revealed lower shrinkage in the samples supplemented with 10 and 20% R-DPF, which reached 9.40 and 9.43%, respectively. The same observation was recorded previously and reported that the addition of fibers and non-meat protein ingredients may reduce diameter shrinkage and weight loss (Gujral *et al.*, 2002; Turhan *et al.*, 2009). The shrinkage in burger during heating is explained to be caused by muscle protein denaturation and partly from the evaporation of water and/or drainage of melted fat and juices (Alakali *et al.*, 2010). Moreover, Ziegler and Acton (1984) reported that thermally denatured proteins formed irreversible strong gels and added that heat treatment allows protein-protein interactions, which cause a stronger protein matrix. Moreover, it was also reported that protein matrix may be responsible for the size and shape of the products (Serdaroğlu and Değirmencioğlu, 2004; Kurt and Kılınççeker, 2011).

An improvement in WHC was observed with increasing the percentage of DPF supplementation compared with control samples, recording 2.40 and 0.40 cm² (Table 5) for control and supplemented samples (20%R-DPF) respectively. The current results are in good harmony with Pietrasik and Shand (2003) who suggested that the addition of common bean flour and other legumes increased WHC of beef sausages. Earlier studies reported that non-meat proteins from sunflower protein and wild rice have been used as extenders in meat systems and serve to enhance water retention and structural formation of meat products (Dzudie *et al.*, 2002).

Shearing force is a method for measuring tenderness which increased by increasing DPF supplementation recording 0.92 and 1.32 N (Table 5) for control and samples supplemented with 20 % U-DPF, respectively. These results are in contrast with those obtained by Das *et al.* (2006) who found that full-fat soya paste (FFSP) used in the development of goat meat decreased the shear force values.

Table 5: Physical parameters of peanut chicken burger.

Parameters	DPF (%)				
	0	10		20	
	Control	U	R	U	R
Cooking loss (%)	42.27 ± 0.25 ^a	33.33 ± 0.12 ^b	30.71 ± 0.36 ^c	25.61 ± 0.13 ^d	19.0 ± 0.10 ^e
Shrinkage (%)	10.47 ± 0.25 ^a	11.32 ± 0.44 ^a	9.40 ± 0.10 ^b	10.57 ± 0.63 ^a	9.43 ± 0.15 ^b
WHC (cm ²)	2.40 ± 0.22 ^a	0.50 ± 0.24 ^b	0.58 ± 0.46 ^b	0.20 ± 0.26 ^c	0.40 ± 0.20 ^d
Tenderness (N)	0.92 ± 0.28 ^a	1.46 ± 0.3 ^b	1.21 ± 0.22 ^c	1.32 ± 0.50 ^c	1.06 ± 0.35 ^a

Results are mean values ± SE obtained from three independent measurements

U: unroasted peanut R: Roasted peanut

Within each row, means superscript with different letters are significantly different ($P > 0.05$).

Blouin *et al.* (1981) pointed out that colour is probably the first characteristic of a food to be observed by consumers. In many cases, the quality of a food is judged according to its colour based on consumers' expectations. The results presented in Table (6) revealed that the addition of DPF resulted in approximately equal values to control concerning L* values; meanwhile, there was significant differences in a* and b* values among the treatments. In a previous study, it was suggested that meatballs extended with chickpea flour were lighter and less red compared with the controls (Serdaroğlu *et al.*, 2005). On the other hand, Babji *et al.* (2000) reported that burgers made from chicken breast meat had less redness intensity compared with burgers made from thigh or mechanically deboned meat.

Table 6: Hunter colour parameters of peanut chicken burger.

Colour	DPF (%)				
	0	10		20	
	Control	U	R	U	R
L*	43.34 ± 0.05 ^a	39.78 ± 0.03 ^b	42.08 ± 0.01 ^a	39.80 ± 0.05 ^b	42.40 ± 0.04 ^a
a*	7.50 ± 0.05 ^a	9.05 ± 0.02 ^b	8.04 ± 0.03 ^c	7.73 ± 0.05 ^a	7.12 ± 0.05 ^a
b*	16.85 ± 0.03 ^a	15.95 ± 0.01 ^b	16.33 ± 0.01 ^a	13.49 ± 0.06 ^c	14.19 ± 0.03 ^d

Results are mean values ± SE obtained from nine independent measurements

U: unroasted peanut R: Roasted peanut

Within each row, means superscript with different letters are significantly different ($P > 0.05$).

Microbiological analysis:

Data presented in Table (7) revealed that some pathogenic microorganisms were associated with chicken burger samples before cooking. Of these microorganisms, *S. aureus* was detected in high counts, reaching 6.95 log₁₀ CFU/g for burger samples supplemented by 20% R-DPF compared to control samples (6.20 log₁₀ CFU/g). The results also demonstrated that samples supplemented by 10% U-DPF had the lowest counts for *E. coli*, and *S. aureus* compared to the other samples and the control. Moreover, total fungal count was detected in all samples but in lower counts, which recorded 1.48 log₁₀ CFU/g for control samples. It could be noticed that *E. coli*, counts significantly increased on the addition of DPF to chicken burger (10 and 20%), whereas total fungal count were slightly decreased. *Salmonella* was not detected in any of the chicken burger samples manufactured.

The current results revealed that the main source of microbial contamination in burger samples may be due to chicken meat and other non-meat ingredients, since chicken is rich in protein and easily spoiled. According to USDA, the bacteria associated with chicken include *S. aureus*, and *Listeria monocytogenes* (USDA, FSIS, 2000). On the other hand, the Egyptian Organization for Standardization and Quality for microbial levels of foodstuffs reported that the required microbial level in poultry meat varies according to the type of the product (EOS, 2005) whereas the acceptability level for *S. aureus* is 10² CFU/g and negative for *E. coli* and *Salmonella* in chicken burger.

The presence of *E. coli* in peanut chicken burger was confirmed recently by Waliullah and Ahsa (2011) who reported that all portions of chicken burger contained higher number of *E. coli*. Data illustrated in Table (7) also showed differences in total fungal count between burger samples. These results are in good harmony with those reported by Easa (2010) who isolated fungi such as *Aspergillus fumigates*, *Mucor* sp. *Alternaria* sp. *Penicillium* sp. and *Cladosporium* sp. from chicken shawarma and beef burger collected from local markets. Similarly, several investigators did not reveal the presence of the pathogen (*Salmonella* species) in collected hamburger samples (Tavares and Serafini, 2003; Bezerra *et al.*, 2010; Waliullah and Ahsa, 2011). Moreover, the current results are in contrast with the earlier investigators who detected *Salmonellae* in examined chicken burger and during burger manufacturing (Capita *et al.*, 2003; Narváez *et al.*, 2005). Recently, Fortuna *et al.* (2012) found that 27.5% of hamburger samples were contaminated by *Salmonella* species and thus were considered inappropriate for human consumption. These differences may be attributed to environmental conditions, quality assurance and/or quality management during processing.

After cooking of peanut chicken burger at 72 C, the microorganisms were absent in the samples (Table 7). In agreement with the current observations, Ogiehor *et al.* (2005) reported that the initial drastic reduction recorded in the total viable bacteria and total viable fungi counts may be attributed to the effects of processing (boiling, stirring, cooking at 78±2.5°C). Moreover, Abdalla *et al.* (2008) added that the adequate temperature in cooking of foods is important to minimize the growth of bacteria

Data presented in Table (8) and Fig (1) illustrated the effect of freezing on the microbial count and the results revealed that the most sensitive pathogen to freezing was *E. coli*, whereas the count was decreased from 6.20 to 2.00 log₁₀ CFU/g showing a 67.70% reduction in samples supplemented by 20% R-DPF. The data also showed a remarkable decrease in *S. aureus* (38.83%) for samples supplemented by 10% R-DPF.

Table 7: Microbiological characteristics (log₁₀ CFU/g) of peanut chicken burger at zero time before and after cooking.

Microorganisms		DPF (%)					
		0	10		20		
			Control	U	R	U	R
<i>Escherichia coli</i>	Before cooking	5.00 ± 0.70 ^a	4.16 ± 0.89 ^b	5.18 ± 0.76 ^a	5.60 ± 0.01 ^a	6.20 ± 0.01 ^c	
		6.20 ± 2.12 ^a	6.01 ± 1.41 ^a	6.54 ± 0.70 ^a	6.85 ± 1.39 ^a	6.95 ± 0.01 ^a	
							<i>Staphylococcus aureus</i>
<i>Salmonella</i>		ND	ND	ND	ND	ND	
Total fungal count		After cooking	1.48 ± 0.01 ^a	1.30 ± 0.05 ^a	1.00 ± 0.01 ^b	1.48 ± 0.05 ^a	1.30 ± 0.01 ^a
			<i>Escherichia coli</i>	ND	ND	ND	ND
<i>Staphylococcus aureus</i>	ND		ND	ND	ND	ND	
<i>Salmonella</i>	ND		ND	ND	ND	ND	
Total fungal count	ND		ND	ND	ND	ND	ND
			ND	ND	ND	ND	ND

Results are mean values ± SE obtained from three independent measurements

U: unroasted peanut R: Roasted peanut

ND: Not detected

Within each row, means superscript with different letters are significantly different ($P > 0.05$).

These results are in agreement with Al-Jasser (2012) who reported that cooling and freezing temperatures are considered two of the most efficient methods to delay or inhibit the growth of microorganisms in chicken meat and meat products during transportation or storage and it can help to improve the safety and prolong shelf life of such products.

Table 8: Microbiological characteristics (\log_{10} CFU/g) of peanut chicken burger after 60 days of freezing storage.

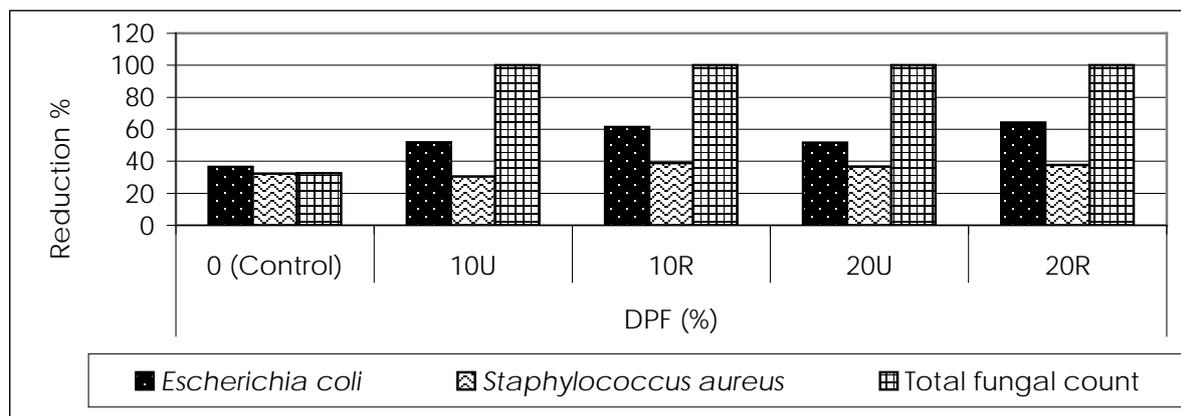
Microorganisms	DPF (%)				
	0	10		20	
	Control	U	R	U	R
<i>Escherichia coli</i>	3.18 ± 0.70 ^a	2.00 ± 0.31 ^b	2.00 ± 0.91 ^b	3.00 ± 1.12 ^a	2.00 ± 1.25 ^b
<i>Staphylococcus aureus</i>	4.20 ± 0.12 ^a	4.18 ± 0.13 ^a	4.00 ± 0.07 ^a	4.40 ± 1.27 ^a	4.27 ± 1.35 ^a
<i>Salmonella</i>	ND	ND	ND	ND	ND
Total fungal count	1.00 ± 0.50 ^a	ND	ND	ND	ND

Results are mean values ± SE obtained from three independent measurements

U: unroasted peanut R: Roasted peanut

ND: Not detected

Within each row, means superscript with different letters are significantly different ($P > 0.05$).

**Fig. 1:** Reduction (%) in the microbiological contamination of peanut chicken burger after freezing for 60 days.

Conclusion:

Supplementation of chicken burger with DPF especially at 20% exhibited good quality attributes and high acceptability. This new product is considered a very good source of protein which could increase the nutritional value and decrease the product cost. It is also recommended to evaluate aflatoxin contamination in DPF before use in the food industry to avoid any health hazards, since the International Agency for Research on Cancer has classified AFB₁ as human carcinogens.

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