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A Pilot Trial for Production of Healthy Rabbit Frankfurter in Egypt

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Abstract: Rabbit meat has very good nutritional value of high quality protein, minerals, essential amino acids and it is low in fat, cholesterol and sodium. This study was carried out to evaluate rabbit meat and the possibility of using it in manufactured some products such as frankfurter and to study the effect of replaced rabbit meat with defatted soy flour (DSF) as meat extenders at different ratios (0, 15, 30 and 45%). Chemical composition, physical, minerals content, amino acids composition, fatty acids profile, sensory evaluation as well as microbiological aspects were studied. Rabbit frankfurters were stored at frozen storage -18C° for 3 months. Results showed that rabbit meat is a valuable source of protein (92%, DW), minerals such as K (307.93 mg/100g) and P (211.37 mg/100g), respectively. Rabbit meat had the highest total essential amino acids compared to FAO/WHO pattern. As the substitution level of DSF unsaturated fatty acids were raised in rabbit frankfurter total fatty acids. Moreover, the storage stability of the frozen rabbit frankfurter was investigated by monitoring changes occurring in TVN, TBA and PV. The obtained results showed that partial substitution of rabbit meat with DSF caused decrease in TVN, TBA and PV followed by a slight increase in these values for all the samples during the frozen storage. Results of microbiological examination showed that total viable count, coliform bacterial count as well as moulds and yeast count of frankfurter stored under frozen condition decreased gradually as the storage time increased. Whereas, moulds and yeasts were disappeared after one month of storage and psychrophilic bacterial count increased. It could be concluded that rabbit meat can be successfully used for production of good quality safe products such as frankfurter.

Key words: Rabbit Meat · Frankfurter · Proximate Analysis · Amino Acids · Minerals · Fatty acids · Sensory Evaluation

INTRODUCTION

The world production of rabbit meat has increased by 2.5-fold to 1.6 million tons in 2009. China is currently the world's leading producer of rabbit meat (700,000t/year). Italy (230,000 t/year), Spain (74,161 t/year) and France (51,400 t/year) are the main rabbit-meat producers in Europe (FAOSTAT) [1].

Rabbits are good source of meat and have better nutritional and dietary properties than have red meats. On average, protein content is higher and fat and cholesterol contents are low [2, 3]. Rabbit meat is mostly recommended by dietitians because of its low calories compared to other traditional meats [4]. Nowadays, rabbit meat is increasingly suitable for contemporary consumers because of its low cholesterol contents, high digestibility, high levels of essential amino acids and polyunsaturated fatty acids and high contents of vitamin B family (B2, B3, B5, B6 and B12) as compared with other meats [5].

The meat processing industry is driven by increasing consumer demand for healthy meat products including products with reduced level of fat, cholesterol, sodium and nitrite, improved fatty acid composition including omega-3 fatty acids enriched meat products [6]. Healthy meat must be rich in protein, low in fat, higher in n-3 fatty acids and lower in n-6 fatty acids. In this respect, it was reported that rabbit meat offers excellent nutritive and dietetic properties [7].

Introduction and success of novel rabbit meat products bring new challenges for meat processors because of more dynamic, complex, and differentiated demands of the present consumers along with emphasis on quality foods that are rich in sensory and organoleptic properties and also convenient [8]. Despite of the

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potential to introduce different products, only a few meat processors have focused on introducing processed rabbit meat products for the consumers [9]. The processed rabbit meat products (e.g., meat patties and sausages) are currently available and made from coarsely ground meat [10].

Rabbit meat is appreciated due to its high nutritional and dietetic properties: it is lean, contains highly unsaturated lipids (60% of total fatty acids are unsaturated), is rich in proteins (20–21%) and has amino acids of high biological value, while it is poor in cholesterol and sodium and rich in potassium, phosphorus and magnesium [11]. Rabbit meat offers excellent nutritive and dietetic properties [7] it is a lean meat with a low-fat content and less saturated fatty acids and cholesterol than other meats [12].

Traditionally frankfurters are made from beef, pork and veal. Other meat animals, however, such as chicken and rabbit are excellent sources of protein and should make acceptable products [13]. However, the tradition of use of rabbit meat has not been established in our regions, particularly as regards the preparation of certain special local meals. Since rabbit meat has a nutritional profile and suitable high technological traits for inclusion as added value products to meet modern consumer demands regarding food health, convenience and industrial requirements, this must stimulate food science personals to explore the potential of rabbit meat as functional food such as frankfurters.

Value addition in minced meat either through incorporation of meat of other species or inclusion of non-meat ingredients such as binders, spices and condiments has an added advantage of improved palatability and reduced cost of production [14]. Development of other meat products includes frankfurter from chicken and mutton blends and sausages from chicken, pork and rabbit meat [15]. Some of binders such as cereals, starches, soy protein products and milk protein derivatives act as emulsifiers in the formulation of several processed meat products [16].

Soy protein is one of the most widely used vegetable proteins in meat industry as extenders due to cheaper source and for its various technological benefits like improve the cooking yield, viscosity, texture, firmness, emulsion stability, improve nutritional content, sensory properties, storage stability and water binding properties, whereas it plays a significant role in the modification of the functional characteristics of the meat products, also, it reduces the cost of meat products [17-19].The increasing use of soy proteins in meat product is also due to the availability and low cost of soybean relative to other extenders such as cotton, sunflower, wheat flour, sodium caseinate, egg protein and non-fat dry milk [20]. They are also used in processing frankfurters, bologna, meat loaves, meatballs, meat patties, and luncheon meats [21]. Non-meat ingredients are used as binders, fillers and extenders in frankfurter formulations to reduce the cost and shrinkage during cooking [22]. There are few processed rabbit meat products, most rabbit meat worldwide is still sold as whole carcass, whereas very low quantities as processed products which make it easier for consumer.

The present study was carried out to test the suitability of rabbit meat for frankfurter manufacture to increase the nutritional value of rabbit meat products as new healthy products by using different ratios of defatted soy flour in formulation with assessment of their chemical, physical, amino acid composition, fatty acids profile and sensory properties of rabbit frankfurter and study frozen stability on quality parameters and microbiological aspects.

MATERIALS AND METHODS

Materials: Defatted soy flour was purchased from the Food Technology Institute, Agriculture Research Center-Giza, Egypt.

Ten New Zealand white rabbits obtained from a farm located in Kafr El-Sheik government at live weight of 2.0 – 2.5 Kg were slaughtered, then the head, viscera and skin were immediately removed according to Cheeke *et al.* [23]. Carcasses were washed in running tap water and drained for 10 minutes to drip-dry. The dressed carcasses were kept overnight for chilling in refrigerator at a temperature of 5±1°C. Each carcass was taken out from the cold store to study the different parameters and the meat was separated from the bone, well minced and mixed to form a composed of meat homogenate.

Beef fat (Tallow) and spices (White paper, nutmeg, black paper, cumin, red paper and ginger were obtained from local market of Tanta city, Egypt). Whereas, dried onion and garlic were purchased from supermarket.

Chemicals such as sodium nitrite and ascorbic acid were purchased El-Nasr Company for pharmaceutical Chemicals, Egypt.

Preparation of Rabbit Meat Frankfurter: The formulations of rabbit meat frankfurter are shown in Table 1. Rabbit meat and beef fat were separately minced three times using an electrical meat mincer. About half of

Table 1: Rabbit frankfurter formulations with different levels of defatted soy flour

Ingredients	Control	Defatted soy flour (%) 15	30	45
FRM	61.7	52.46	43.22	33.98
Beef Fat	18.34	18.34	18.34	18.34
DSF	-	9.24	18.48	27.72
Salt (NaCl)	2.07	2.07	2.07	2.07
Sucrose	0.1	0.1	0.1	0.1
STPP	0.3	0.3	0.3	0.3
NaNO3	0.01	0.01	0.01	0.01
Ascorbic acid	0.1	0.1	0.1	0.1
Garlic	1.03	1.03	1.03	1.03
Onion	1.03	1.03	1.03	1.03
Spices	1.57	1.57	1.57	1.57
Ginger	0.02	0.02	0.02	0.02
Dextrose	0.1	0.1	0.1	0.1
Crushed Ice	13.63	13.63	13.63	13.63

FRM: Fresh rabbit meat
DSF: Defatted soy flour
STPP: Sodium tripolyphosphate

iced water containing 2.07% salt was mixed and added. Another ingredient such as sodium nitrite and ascorbic acid were dissolved in remaining half of iced water into solution and added to minced meat bulk. The rabbit meat /fat mixture was reground for 2 – 3 min at high speed in electrical mincer and mixed with spices and continuously mixed for another 2 min. The standard formulation was used as a control frankfurter (No added defatted soy flour) and for the other formulations defatted soy flour was substituted at three ratios (15, 30 and 45%). The batter was stuffed into the natural cleaned sheep casing and formed into links of 15 cm. Frankfurter was cooked in boiling water for 5 min and then chilled with cold water before peeling. Finally, finished frankfurters were packed and stored under refrigeration at 4°C until their analysis.

Analytical Methods

Proximate Chemical Composition: Chemical analysis including: moisture content by drying to constant weight, ether extract content by extraction method after Grossfeld, protein content after Kjeldahl's method and ash content by ignition in a furnace at 500°C [24].Total lipids were extracted from fresh rabbit meat and rabbit meat frankfurter samples with chloroform / methanol (2: 1 v/v) according to the method of Bligh and Dyer [25] and the extracts were dried under vacuum by a rotary evaporator.

Total Volatile Nitrogen (TVN): Total volatile nitrogen content of different rabbit frankfurter samples was performed according to the method of Harold *et al*. [26].

Thiobarbituric Acid Value (TBA): The thiobarbituric acid values (As mg malonaldehyde/Kg sample) of different rabbit frankfurter samples were determined by the distillation method outlined by Harold *et al*. [26].

Peroxide Value (PV): Peroxide value was determined as milliequivelant peroxides per kilogram of sample according to the method described by AOAC [27].

pH Measurement: pH value of rabbit meat frankfurter was measured using Digital pH-meter (JENWAY model 333 Research pH meter) after blending 10g rabbit meat frankfurters with 100 ml deionized water for 2 min [28].

Physical Properties

Water Holding Capacity (WHC) and Plasticity: WHC and plasticity of rabbit meat and frankfurter were measured using the method of Soloviuskaia and Merkodlovia [29].

Cooking Loss and Cooking Yield: Prepared rabbit frankfurter samples were weighted before cooking and then allowed to cool after cooking to room temperature. After cooling, the cooked rabbit frankfurter samples were reweighted and the cooking loss was calculated according to Lee *et al*. [30] as follows:

Cooking loss (g/100 g) =Wr–Wc× 100 /Wr

whereas:
Wr: the weight of raw rabbit frankfurter (g).
Wc: the weight of cooked rabbit frankfurter (g)
Cooking yield of different frankfurter samples was measured by subtracting cooking loss from 100.

Determination of Minerals Content of Rabbit Meat: Minerals content (Ca, Mg, Fe, Cu, Zn and Mn) were determined by using Atomic Absorption Spectrophotometer (Pyeunican SP 1900) according to Brandifeld and Spincer [31] while (Na and K were determined using Flame photometer apparatus according to Brown and Lilleland [32]. Phosphorus content was determined colorimetrically according to the method of Jackson [33].

Determination of Amino Acids Composition of Rabbit Meat: Amino acids composition was determination according to the method of Duranti and Cerletti [34]. The amino acids composition was determined by subjecting 100 mg of sample to acid hydrolysis using 10 ml of 6N HCL with 0.1% mercaptoethanol in an evacuated tube of

110°C for 24 h. After cooling at room temperature, the hydrolyzed samples were filtered through Whatman No. 1 filter paper and the filtrate was diluted with distilled water to 25 ml in a volumetric flask. Five ml of the diluted filtrate was dried in a vacuum desiccator in the presence of potassium hydroxide. The resultant dried residue was dissolved in 1 ml of sodium citrate buffer (pH 2.2) and stored at 4°C until analysis by using amino acid analyzer (Beckman amino acid analyzer, Model 119CL).

Fatty Acids Composition: Preparation of fatty acid methyl esters from total lipids was performed according to the procedure of Radwan [35] using 1% sulphuric acid in absolute methanol. The fatty acid methyl esters obtained were separated by Shimadzu gas chromatography (GC-4CM, PFE) with a flame ionization detector (FID). Standard fatty acid methyl esters were used for identification. The area under each peak was measured by the triangulation method and percentage of each fatty acid was expressed with regard to the total area.

Total Cholesterol Content: The contents of total cholesterol were determined in the extracted lipids by direct saponification and gas chromatography as described by Hwang *et al*. [36] and expressed as % of total lipids.

Sensory Evaluation: Sensory evaluation of cooked rabbit frankfurters was carried out by 10 panelists from Food Science and Technology Department, Faculty of Home Economic, El-Azhar University. The panelists were asked to evaluate juiciness, firmness, texture, flavor, tenderness, taste and overall acceptability on a 1 to 10 hedonic scale as described by Watt *et al*. [37]. A score of 1 being dislike extremely and 10 being like extremely.

Microbial Analyses: Ten grams of rabbit meat or rabbit frankfurter were blended with 90 ml of sterilized peptone water for 5 min in sterilized glass jar of a blender. Appropriate dilution was prepared for enumeration using standard microbiological pour plate technique and the recommended culture media of Oxoid [38]. Plate count agar medium was used for enumerating the Total Viable Count (TVC) and Psychrotrophic count (PC) bacteria after incubating at 35 - 37°C for 48 hrs and 7°Cfor 10 days, respectively. Moulds and yeasts count was carried out using potato dextrose agar media at 25°C for 3 – 5 days according to the method described by Oztekin *et al*. [39]. Coliform bacterial counts were determined according to Harrigan and Margaret [40] using violet red bile agar medium. *Staphylococci* were determined according to Baird-Parker [41] using Baird-Parker's medium. *Salmonella* and *Shigell*a were detected according FAO/WHO [42].

Statistical Analysis: Statistical analyses for obtained data were carried out using analysis of variance and SPSS [43]. Significant differences among individual means were analyzed by Duncan's multiple range test [44].

RESULTS AND DISCUSSION

Chemical Analysis

Proximate Chemical Composition: The chemical composition of fresh rabbit meat and defatted soy flour was determined (Table 2). Results indicated that rabbit meat had 92.49% crude protein, 4.54% ether extract, 2.86% ash, 0.109% total carbohydrate (Dry weight basis) and 57.33 mg/100g cholesterol. Findings are in agreement with those obtained by Rafay *et al*. [45] and Baiomy and Hassanien [46] who give figures of 65.93 – 77.34% water, 19.43 – 24.40% protein, 0.90 – 4.10% fat and 0.99 – 2.08% ash for rabbit meat. Also, Nistor *et al*. [47] showed that rabbit meat was richer in calcium (21.4 mg/100 g) and phosphorus (347 mg/100 g) than other types of meat and low in fat (9.2 g/100 g) and cholesterol (56.4 mg/100 g). Rabbit meat is considered as a lean meat [48] and its fat percentage (7 %) is very low compared with other meats such as pork, beef or lamb [49]. Optimum indicator of rabbit meat quality is its exceptionally low cholesterol level that ranges between 45 and 85 mg/100 g of fresh meat [2, 50].

Rabbit meat is characterized by its low energetic value due to low fat meat as compared with red meats [51]. Moreover**,** Fernandez-Espla and O'Neill [52] showed that the lipids of rabbit meat would be more susceptible to the development of rancidity than other meats this due to its high content of polyunsaturated fatty acids, thus lipid oxidation may have an important influence on the stability of products containing rabbit meat.

Minerals Content of Rabbit Meat: Data presented in Table 3 show some minerals of rabbit meat. The data indicated that the highest minerals concentration in rabbit meat were potassium (K) and phosphorus (P) which were 307.93 and 211.07 mg/100g, respectively, followed by sodium (Na), magnesium (Mg) and calcium (Ca) in decreasing order. At the same trend results confirmed by Dalle Zotte [2] and Dierenfeld *et al*.[53]who indicated that rabbit meat is low in Na, Ca and Fe but rich in K, P and Mg. These values showed that rabbit meat with its lower

Table 2: Gross compositional analysis of rabbit meat and defatted soy flour

Constituent (%)	Rabbit meat	Defatted soy flour
Moisture	74.77±0.121[a]	6.88±0.005[b]
Crude protein	92.49±0.001[a]	52.81±0.001[b]
Ether extract	4.54±0.006[a]	2.97±0.008[b]
Ash	2.86±0.000[b]	7.33±0.003[a]
*Total carbohydrates	0.109±0.001[b]	36.89±0.000[a]
Cholesterol (mg/100g)	57.33	ND

* Total carbohydrate was calculated by difference, ND= not determined
M±SD = means and Standard division
In a row means having the same superscript letters are not significantly different at 5% level

Table 3: Minerals content of rabbit meat

Mineral	Value (mg/100g)
Phosphorus (P)	211.37
Potassium (K)	307.93
Calcium (Ca)	10.27
Magnesium (Mg)	21.44
Sodium (Na)	42.35
Ion (Fe)	2.53
Cupper (Cu)	0.787
Zinc (Zn)	6.28
Manganese (Mn)	0.27

Na content is more suitable for individuals with heart diseases. Also, Combes [7] reported that the mineral fraction of rabbit meat is characterized by its low contents in sodium (49 and 37 mg/100 g for hind leg and loin, respectively) and iron (1.3 and 1.1 mg/100 g for hind leg and loin, respectively), while the phosphorus level is high (230 and 222 mg/100 g for hind leg and loin, respectively. Potassium content of rabbit meat is the highest as compared with other animals, 150 - 171 mg/100g in cattle, 172 – 175 mg/100g in pigs and were between 248 – 259 mg/100g in birds and 295 – 350 mg/100g in sheep [7, 54].

Amino Acids Composition: The nutritional protein quality of rabbit meat and frankfurter containing different levels of defatted soy flour (0, 15, 30 and 45%) was evaluated according to their content of essential amino acids (As g/100g protein) in comparison to the reference protein pattern FAO/WHO [55] as shown in Table 4. Results clearly indicated that rabbit meat is very rich in both essential and non-essential amino acids with lysine (9.55), leucine (8.77), valine (5.63), glutamic acid (18.26), aspartic

Table 4: Amino acids composition of rabbit meat, DSF and rabbit frankfurter (g /100g protein)

			Supplementation levels (%)					
Amino acids	FRM	DSF	T0	T1	T2	T3	FAO / WHO *	Whole egg protein**
EAA								
Methionine	2.28	1.15	2.23	2.19	1.87	1.62	2.2	3.4
Isoleucine	4.98	3.94	4.93	4.90	4.85	4.76	4.2	6.3
Leucine	8.77	7.18	8.64	8.57	8.37	8.11	4.8	8.8
Lysine	9.55	6.61	9.48	9.46	9.42	9.40	4.2	7.00
Histidine	4.42	2.67	4.31	4.30	4.36	4.33	1.90	2.40
Threonine	4.18	3.73	4.14	4.11	4.07	4.09	2.8	5.1
Tyrosine	3.26	3.15	3.24	3.22	3.23	3.19	2.80	4.2
Valine	5.63	4.35	5.58	5.51	5.50	5.50	4.2	6.9
Phenylalanine	3.89	4.57	3.82	3.89	3.90	3.92	2.8	5.7
Total EAA	46.96	37.35	46.43	46.21	45.57	44.92	29.9	49.8
NEAA								
Alanine	5.89	4.27	5.75	5.83	5.80	5.81	-	5.9
Arginine	5.04	7.04	4.89	5.11	5.18	5.32	-	6.1
Aspartic acid	9.35	11.19	9.40	9.47	9.70	9.82	-	9.6
Glutamic acid	16.26	17.81	16.38	16.39	17.25	17.60	-	12.7
Proline	4.96	4.63	4.92	4.90	4.84	4.81	-	4.2
Serine	5.46	4.96	5.63	5.60	5.63	5.60	-	7.6
Glycine	4.89	4.25	4.82	4.80	4.78	4.77	-	3.3
Cysteine	1.13	1.44	1.12	1.22	1.20	1.21	-	5.9
Total NEAA	52.98	55.59	52.91	53.32	54.38	54.94	-	55.3
Total AA	99.94	92.94	99.34	99.53	99.95	99.86		105.1

Tryptophan was not determined
* FAO / WHO pattern (FAO, 1990) [55]
** Whole egg protein (FAO, 1968), EAA: Essential amino acids, NESS: Non-essential amino acids, T0 = Control rabbit frankfurter; T1 = Rabbit frankfurter containing 15% defatted soy flour; T2=Rabbit frankfurter containing 30% defatted soy flour; T3 = Rabbit frankfurter containing 45% defatted soy flour

Table 5: Fatty acids composition (%) of rabbit meat and frankfurter

	Treatments					
Fatty acid (%)	Symbol	Rabbit meat	T0	T1	T2	T3
Lauric	C12:0	0.29	0.075	0.13	0.14	0.037
Myristic	C14:0	2.50	1.18	0.14	0.15	0.16
Palmitic	C16:0	27.36	23.40	28.17	29.16	29.42
Stearic	C18:0	6.21	13.17	13.25	14.23	13.51
Arachidic	C20:0	0.16	0.21	0.24	0.22	0.19
Total SFA	-	36.52	38.04	41.93	43.90	43.32
Palmitolic	C16:1	3.51	3.68	4.52	3.98	4.06
Oleic	C18:1	24.96	38.33	35.66	32.54	33.25
Eicosaenoic	C20:1	0.33	0.23	0.32	0.68	0.79
Total MUFA	-	28.80	42.24	40.50	37.20	38.10
Linoleic	C18:2	26.19	10.67	10.76	11.73	11.66
Linolenic	C18:3	3.17	2.43	2.28	2.55	2.73
Eicosadienoic	C20:2	0.29	0.33	0.61	0.27	0.21
Eicosatrienoic	C20:3	0.42	0.08	0.07	0.07	0.03
Arachidonic	C20:4	0.24	0.45	0.25	0.61	0.45
Eicosapentaenoic	C20:5	0.22	0.38	0.39	0.38	0.31
Eurcic	C22:1	0.042	0.14	0.11	0.12	0.11
Docosatetraenic	C22:4	0.24	0.06	0.08	0.07	0.09
Docosapentanoic	C22:5	0.18	0.04	0.12	0.13	0.12
Docosahexanoic	C22:6	0.15	0.23	0.19	0.18	0.21
Unknown	-	3.53	4.94	2.71	2.79	2.66
Total PUFA	-	31.15	14.81	14.86	16.11	15.92
Total UFA	-	59.95	57.05	55.36	53.31	54.02
Total FA		96.47	95.06	97.29	97.21	97.34
PUS / SFA *	-	0.853	0.389	0.354	0.367	0.367
U/S**		1.642	1.399	1.320	1.214	1.247

*Ratio of polyunsaturated to saturated

** Ratio of unsaturated to saturated

*T0 = Control rabbit frankfurter; T1 = Rabbit frankfurter containing 15% defatted soy flour;

T2= Rabbit frankfurter containing 30% defatted soy flour; T3 = Rabbit frankfurter containing 45% defatted soy flour

acid (9.45) and arginine (6.04). Also, all essential amino acids of rabbit meat were higher than those of FAO/WHO [55] pattern. This indicates that rabbit meat is an excellent source of essential amino acids. These results are in accordance with Sales and Hayes [56] and Straková *et al*. [57] who showed that rabbit meat contains high amounts of threonine, histidine, lysine, serine, glutamic acid and glycine than ostrich meat and also high amounts of threonine, lysine, glutamic acid, and glycine as compared to chicken meat Rabbit meat had the highest total essential amino acids as compared to recommend by FAO/WHO [55] Also, all rabbit frankfurters are higher total essential amino acids as FAO/WHO provisional amino acid pattern.

Amino acid content of frankfurters extended with nonmeat additives is one of the important criteria for nutritional quality [58].Complete meat proteins contain sufficient amounts of essential amino acids, yet the quality of a protein, as a primary food component depends on its amino acid composition [59, 60].

High level of essential amino acid (EAA) are present in the meat of rabbits as compared to other meats, it is actually richer within lysine (2.12 g/100 grams), sulfur-containing proteins (1.10 g/100 grams), threonine (2.01 g/100 grams), valine (1.19 g/100 grams), isoleucine (1.15 g/100 grams), leucine (1.73 g/100 grams) as well as phenylalanine (1.04 g/100 grams) [61]. These prominent as well as balanced essential amino acids content provides rabbit meat proteins higher biological worth due to simple digestibility.

Fatty Acids Composition: Table 5 shows the fatty acid composition of rabbit meat and frankfurter produced from rabbit meat and defatted soy flour. According to FA profile results, rabbit meat contained of 36.52% of saturated fatty acids (SFA), 28.80% of monounsaturated fatty acids (MUFA) and 31.15% of polyunsaturated fatty acids (PUFA).These results agree with those reported by Hernandez [62] and Mattioli *et al*. [63] who reported that monounsaturated fatty acids in rabbit meat fat are less represented (About 28.5%). Rabbit meat presented the higher polyunsaturated fatty acid concentration (59.95%). Also, the same author showed that fatty acid composition of rabbit meat is characterized by high polyunsaturated fatty acid content. Dalle Zotte [2] reported that rabbit lipids contain lower concentrations of stearic and oleic acids and higher proportions of polyunsaturated fatty acids. Also, Dalle Zotte and Syendro [64] showed that rabbit meat may be recommended, as the unsaturated fatty acids represent around 60% of the total FA, while the content of polyunsaturated FA (PUFA) is much greater than in other meats, including poultry. Rabbit meat is characterized by excellent nutritive and dietetic properties associated with high protein content, high essential amino acid levels, low lipid content and high (60% of the total) unsaturated fatty acid (UFA) and polyunsaturated fatty acid (PUFA) contents [2] therefore, it is a useful food in human diets.

Palmitic acid (16:0) was the major saturated fatty acid found, ranging of 23.17% for control frankfurter to 29.42%for frankfurter containing 45% defatted soy flour, respectively, followed by stearic acid and myristic acid. SFA are considered to raise plasma cholesterol, except for stearic acid which reduces total and LDL cholesterol; therefore, the content of this fatty acid (Stearic) is subtracted from the SFA fraction when the association between food saturated fatty acids and risk of heart diseases is studied. Moreover, MUFA have hypocholesterolemic effect, but they do not decrease HDL cholesterol, which protects against cardiovascular diseases [65].

The fatty acid present in greater percentage was oleic acid in rabbit frankfurter which varying from 32.54% to 38.33% for frankfurter containing 30% and 15% defatted soy flour, respectively.

The PUFA/SFA ratio is one of the major parameters currently used to assess the nutritional quality of the lipid fraction of foods. Nutritional guidelines recommend a PUFA/ SFA ratio above 0.4 [66].

Values for PUFA / SFA ratio in rabbit frankfurter supplemented with defatted soy flour (DSF) varied from 1.64 for rabbit meat to 1.21 for frankfurter with 30% DSF, whereas rabbit meat fat had the highest ratio which recorded o.853. Similar results obtained by Ramirez *et al.* [67] who reported that rabbit meat has a high ratio of PUFA to SAT fatty acids (0.75 and 0.85 for the loin and the meat of hind leg, respectively. From this aspect, all examined frankfurter samples have had favorable PUFA / SFA ratio.

Furthermore, according to some recently conducted studies the composition of fat, fatty acids respectively, in rabbit meat is as the following: monounsaturated acids 34.15%, polyunsaturated acids25.10%and saturated fatty acids 40.9% [50].

The fatty acids of the rabbit burgers were composed mainly of linoleic acid (C18:2n-6) followed by palmitic (C16:0) and oleic (C18:1) acids at contents of approximately 30, 28, and 21%, respectively [68].

Rabbit meat, as it has been previously discussed, is a lean meat rich in proteins of high biological values, with highly unsaturated lipids, low cholesterol content and noticeable quantities of linolenic fatty acid (C18:3 ù3). Also, it displays a low content of sodium and a high content of phosphorus and can be a good source of B vitamins [69].

The presence of high amounts of oleic and linoleic acids in both rabbit meat and frankfurter which produced from rabbit meat and defatted soy flour suggests that they are highly recommended to be rich in the unsaturated fatty acids and can hence they can be considered as valuable healthy foods.

Chemical Composition of Rabbit Meat Frankfurter: Table 6 displayed that moisture content of control fresh prepared rabbit frankfurter was 60.62% which decreased to 59.46% in rabbit frankfurter contained 15% defatted soy flour (DSF) and then increased to 62.14% and 63.57% in rabbit frankfurter contained 30 and 45% DSF, respectively. These results are in full agreement with those reported by El-Wakeil *et al*. [70] who found that replacement of 20% hen meat with soy protein flour in sausage reduced moisture content of the final products. Whereas soy protein could be bind up to seven times its weight of water [71]. It is obvious from the results that protein content of fresh prepared rabbit frankfurter was decreased as the replacement of plant protein increased. This may be related to the property of soy protein since it contains more carbohydrate and ash rather than rabbit meat. Also, increment of defatted soy flour in rabbit frankfurter during preparation to 45%, led to decrease the fat content and increased in moisture content.

Cholesterol content of rabbit frankfurter was markedly influenced by substitution level of DSF. For example, cholesterol content of control frankfurter (With DSF) was 181.03 mg/100g significantly decreased ($p \leq 0.05$) to 180.51, 147.69 and 89.74 mg/100g in rabbit frankfurter samples contained 15%, 30% and 45% DSF, respectively.

Table 6: Chemical composition of rabbit frankfurter as affected by added defatted soy flour

	Treatments			
Compound	T0	T1	T2	T3
Moisture	60.62±.002[c]	59.46±.00[d]	62.14±1.0[b]	63.57±.001[a]
Crude protein	74.23±.003[a]	72.03±.005[b]	69.89±.00[c]	66.61±.001[d]
Crude lipids	18.92±.010[a]	17.73±.003[b]	16.36±.001[c]	16.27±.002[d]
Total ash	3.79±.00[d]	4.56±.10[c]	5.61±.001[b]	6.42±.22[a]
*Carbohydrates	3.17±.12[d]	5.68±.002[c]	8.14±.12[b]	10.70±.23[a]
Cholesterol	181.03±.005[a]	180.51±.27[b]	147.69±.19[c]	89.74±.24[d]
**TBA value	1.67±.001[a]	1.61±.001[b]	1.43±.021[c]	1.36±.002[d]

T0 = Control rabbit frankfurter; T1 = Rabbit frankfurter containing 15% defatted soy flour;

T2 = Rabbit frankfurter containing 30% defatted soy flour; T3 = Rabbit frankfurter containing 45% defatted soy flour

* Carbohydrate was calculated by difference

** TBA (Thiobarbituric acids as mg malonaldehyde / Kg fat)

M±SD means and Standard division

In a row means having the same superscript letters are not significantly different at 5% level

Table 7: Physical properties of rabbit meat and rabbit frankfurter as affected by supplementation of defatted soy flour

Samples	pH value	WHC (%)	Plasticity (cm^2)	Cooking yield (%)	Cooking loss (%)
FRM: fresh rabbit meat	5.96±0.147	88.54±0.004[a]	3.72±0.003[e]	70.29±0.006[e]	29.71±0.104[a]
T0	6.18±0.006	82.24±0.001[e]	4.71±0.001[d]	78.87±0.113[d]	21.14±0.006[b]
T1	6.22±0.002	84.35±0.008[d]	5.88±0.231[a]	88.20±0.005[b]	11.80±0.000[d]
T2	6.36±1.706	86.21±0.001[c]	5.45±0.008[b]	87.25±0.005[c]	12.75±0.000[c]
T3	6.48±0.00	87.79±0.130[b]	5.03±0.005[c]	90.48±0.106[a]	9.52±0.002[e]

M±SD = means and standard division

In a column means having the same superscript letters are not significantly different at 5% level

T0 = Control rabbit frankfurter; T1 = Rabbit frankfurter containing 15% defatted soy flour;

T2 = Rabbit frankfurter containing 30% defatted soy flour; T3 = Rabbit frankfurter containing 45% defatted soy flour

Thiobarbituric acid number (TBA) is used as an index for measuring oxidative rancidity which takes place of meat products. The obtained results in Table 6 show that TBA value of fresh prepared rabbit frankfurter was affected by soy protein substitution level. A substitution of rabbit meat defatted soy flour caused reduction in the TBA value of rabbit frankfurter, especially at 45% replacement. TBA value of control fresh rabbit frankfurter was 1.67 mg malonaldehyde /Kg which decreased to 1.61, 1.43 and 1.36 mg malonaldehyde /Kg in rabbit frankfurter containing 15, 30 and 45% soy flour, respectively. These results agree with those reported by Awad Allah [72] who found that TBA of fresh frankfurter made of chicken was significantly higher than that of chicken frankfurter containing soybean flour.

Data in the same Table 6 indicated that fresh rabbit meat had the highest TBA value. This result was in accordance with Kim and Kim [73] who reported that rabbit meat had higher content of phospholipids and polyunsaturated fatty acids than chicken meat. The decrement in TBA value of frankfurter containing plant protein may be related to the presence of polyphenolic compounds and other substances such as polypeptides in the substituted plant proteins, which act as natural antioxidants and caused reduction of lipid oxidation and consequently reduced TBA number [74].

Physical Properties of Rabbit Meat Frankfurter: As shown in Table 7, pH value of fresh prepared rabbit frankfurter (Control) was 6.18. The pH value increased to 6.22, 6.36 and 6.48 in rabbit frankfurter contained 15, 30 and 45% defatted soy flour, respectively. pH value of frankfurter increases by increasing substitution percentage of plant protein. This may be due to the specificity of plant protein and its alkaline ash [75].

Water holding capacity (WHC) influences economic viability of poultry processors and retailers [76] and affects qualitative and quantitative quality aspects of meat and meat products [77]. As shown in Table 7 WHC of fresh rabbit meat was 88.54%.

From the obtained results, it could be noted that values of water holding capacity of rabbit frankfurter of control, 15, 30 and 45% defatted soy flour formulations were 82.24, 84.35, 86.21 and 87.79%, respectively. It is clear that water holding capacity (WHC) of the rabbit frankfurter increase as the substitutions level of DSF

Table 8: Sensory characteristics of rabbit frankfurter as affected by addition of defatted soy flour

Samples	Juiciness	Firmness	Texture	Flavor	Tenderness	Taste	Overall acceptability
T0	9.79±.41[a]	9.45±.64a	9.33±.79[a]	9.83±.41[a]	9.70±.47a	9.58±.64[a]	9.60±.51[a]
T1	8.54±.67[b]	8.45±.69b	8.70±.809[b]	8.41±.52[b]	8.50±.52b	8.33±.78[b]	8.50±.69[b]
T2	8.50±.67[b]	8.33±.81b	8.66±.46[ab]	8.75±1.12[b]	8.50±.93b	8.58±1.027[b]	8.60±.52[b]
T3	8.66±.93[b]	8.58±1.03b	8.41±.67[b]	8.58±.92[b]	8.54±.69b	8.33±.67[b]	8.50±.405[b]

M±SD means and Standard division

In a column means having the same superscript letters are not significantly different at 5% level

T0 = Control rabbit frankfurter; T1 = Rabbit frankfurter containing 15% defatted soy flour;

T2 = Rabbit frankfurter containing 30% defatted soy flour; T3 = Rabbit frankfurter containing 45% defatted soy flour

increased compared to control frankfurter. Detienne and Wicker [78] reported that non-meat ingredients affect the WHC and WBA values due to electrostatic repulsion, as well as the pH of meat products.

The plasticity of rabbit meat and frankfurter samples under investigation was measured as an index of tenderness. The plasticity of beef and chicken meat is one of the most important properties affecting the eating quality of such products [79]. Replacement rabbit meat with soy flour was found to increase the plasticity of prepared rabbit frankfurter as compared with the control and fresh rabbit meat. Meanwhile, replacement of 20% hen meat with soy flour was found to reduce the plasticity of the prepared sausages as compared with the control and the other sausages formulated with soy-sunflower mixtures [80].

Referring to Table 7, it could be noted that cooking loss (%) of fresh prepared rabbit frankfurter gradually decreased and cooking yield increased by augmented the percentage of incorporated soy protein, whereas rabbit frankfurter made of ground rabbit meat only without addition soy flour (Control) had the highest value (21.14%) of cooking loss and lowest value of cooking yield (78.87%) among the other samples contained soy flour. The increment in cooking yield could be attributed to capability of plant protein (Soybean) to bind higher moisture content rather than rabbit protein. In this respect, Rhee [81] indicated that the isolated soy protein acts as a binder for moisture and fat in finely ground meat.

Sensory Evaluation of: Sensory attributes such as colour, taste and firmness are important for consumer acceptance [82, 83]. Sensory characteristics of fresh rabbit frankfurter samples as affected by defatted soy flour substitution at different levels were given in Table 8, substitution of defatted soy flour at different proportion of rabbit frankfurter showed no significant differences among samples for firmness, texture, flavor, tenderness, taste and overall acceptability, whereas substitution of 45% defatted soy flour had higher than juiciness comparing with another treatment. The consumer acceptance of quality elements such as tenderness, juiciness, and flavour of meat is the major determinant of meat quality [84].

Panelist's overall acceptability scores of fresh prepared rabbit frankfurter were insignificant influenced by addition of defatted soy flour in its formula. Since the control rabbit frankfurter had the highest panelist's score (9.69) compared with other samples containing soy flour (Table 8). In this respect, Baker *et al*. [85] compared frankfurter which made from chicken, pork, beef and rabbit using sensory analysis and found rabbit or rabbit-chicken combinations to be better in flavor, juiciness and tenderness than all beef frankfurter. Also, Rao *et al*. [86] briefly reported that sensory panelists found the juiciness, tenderness, flavor and general acceptability of rabbit frankfurter to be the same as all beef or commercial frankfurters. Sensory evaluations for flavor, texture and overall acceptability demonstrated that frankfurters made from rabbit meat were equal to those from beef and slightly superior to those from chicken [87]. Rabbit meat is one of the best white lean meats available markets, very tender and juicy [88, 89].

Considering the taste and appearance of rabbit meat and relatively easy preparation for various meals [90] it stands to reason why this type of meat is popular and widely represented in human diet in many countries all over the world.

Quality Attributes of Rabbit Frankfurter Supplemented with Defatted Soy Flour During Storage Period at - 18°C for 3 Months: The changes occurred in total volatile nitrogen (TVN), thiobarbituric acid number (TBA) and peroxide value (PV) of rabbit frankfurter containing defatted soy flour during storage at - 18°C for three months are presented in Table 9. According to the obtained results, it could be noticed that TVN contents of rabbit frankfurter containing defatted soy flour (At different ratios) were lower than that recorder for control. There was a gradual increase in TVN with the

Table 9: Quality characteristics of rabbit frankfurter during frozen storage at - 18°C for 3 months

	Storage periods (months)											
	Zero			One			Two			Three		
Samples	TVN*	TBA**	PV ***	TVN	TBA	PV	TVN	TBA	PV	TVN	TBA	PV
T0	18.66	1.13	13.56	19.87	1.18	15.73	20.65	1.23	20.35	22.16	1.30	24.26
T1	15.36	1.11	11.96	17.22	1.13	13.22	19.16	1.16	15.16	21.47	1.18	17.31
T2	13.72	1.09	11.21	14.86	1.12	11.89	15.56	1.14	13.67	17.86	1.15	15.42
T3	11.29	1.05	10.66	12.16	1.09	11.38	13.72	1.12	12.13	14.98	1.14	13.64

* TVN: mg/100g sample

** TBA: mg malonaldehyde/Kg sample

*** PV: meq. O_2/kg sample

T0 = Control rabbit frankfurter; T1 = Rabbit frankfurter containing 15% defatted soy flour;

T2 = Rabbit frankfurter containing 30% defatted soy flour; T3 = Rabbit frankfurter containing 45% defatted soy flour

Table 10: Changes in microbial count of rabbit frankfurter during storage

	Treatments							
	T0		T1		T2		T3	
Storage period	TVC	M&Y	TVC	M&Y	TVC	M&Y	TVC	M&Y
Fresh	1.6×10^5	2.2×10^2	3.1×10^4	4.1×10^2	3.1×10^4	3.6×10^2	8.3×10^3	3.74×10^2
One month	8.4×10^4	None	2.8×10^3	None	2.8×10^3	None	2.4×10^3	None
Two month	5.0×10^3	None	3.5×10^2	None	3.5×10^2	None	4.8×10^2	None
Three month	3.0×10^3	None	2.7×10^2	None	2.7×10^2	None	2.8×10^2	None

Fresh rabbit meat contain TVC = 2.8×10^5, M&Y= 1.9×10^3

DSF contain TVC = 1.7×10^2, M&Y = none

T0 = Control rabbit frankfurter; T1 = Rabbit frankfurter containing 15% defatted soy flour;

T2 = Rabbit frankfurter containing 30% defatted soy flour; T3 = Rabbit frankfurter containing 45% defatted soy flour

prolongation of frozen storage period. In this respect, Brake and Fennema [91] reported that TVN increased in meat products during storage due to the effect of microorganisms as well as autolysis processes indicated some protein breakdown by enzymes, which were not completely inactivated during frozen storage. Also, Zayas and Lin, 1989a [92] and Zayas and Lin, 1989b [93] reported that TVN increased in frankfurter by increasing storage time.

Thiobarbituric acid number (TBA) is used as an index for measuring oxidative rancidity which takes place during storage. Substitution of rabbit meat with defatted soy flour caused reduction in the TBA number of the prepared frankfurter especially 45% replacement, therefore, it seemed that the positive effect of the addition of DSF with different percentage was observed for all frankfurter samples, this effect might be due to the presence of some natural antioxidants such as phenolic compounds, flavonoids and glycosides in DSF which caused reduction of lipid oxidation and consequently reduced TBA number [94].

During the storage period, a significant difference was observed for TBA values of prepared rabbit frankfurter samples with a gradual significant increase in TBA values for all prepared rabbit frankfurter samples.

Peroxide value, expressed as meq. O_2 per Kg, control rabbit frankfurter was 13.56 decreased to 11.96, 11.21 and 10.66 meq. O_2/Kg sample in rabbit frankfurter containing 15, 30 and 45% defatted soy flour, respectively. Also, PV of rabbit frankfurter increased gradually by increasing the period of frozen storage with lower rate comparing with control samples. Different research works indicated that peroxide value increased gradually in control beef patties by increasing storage period (4°C / 0 – 7 days) [95].

Effect of Storage at - 18°C for Three Months on Microbiological Aspects:

Total Viable Count and Moulds & Yeasts: Safety and shelf life of meat are limited by microbial growth [64]. Data in Table 10 show total viable count (TVC) and moulds and yeasts in rabbit frankfurter contained different levels of DSF (0, 15, 30 and 45%) during storage at -18°C for three month. The data indicated that TVC in fresh rabbit frankfurter was 1.6×10^5 decreased to 3.0×10^3 after 3 month of storage. The decrement could be explained by death of microorganisms during freezing via mechanical action of ice crystals and denaturation of protein intercellular microbes or the death of germinated spores at low temperature [96]. Also the addition of non-

Table 11: Psychrophilic and coliform bacterial counts of rabbit meat and rabbit frankfurter prepared with DSF during frozen storage at -18°C for 3 months

	Treatments			
Storage period	T0	T1	T2	T3
	Psychrophilic Bacterial Count			
Fresh	9.4×10^2	8.3×10^2	7.2×10^2	6.5×10^2
One month	10.5×10^2	9.8×10^2	8.6×10^2	7.8×10^2
Two month	12.7×10^2	13.7×10^2	12.9×10^2	12.6×10^2
Three month	13.9×10^2	14.6×10^2	13.8×10^2	13.8×10^2
	Coliform Bacterial Count			
Fresh	2.5×10^3	4.3×10^3	8.4×10^3	8.7×10^3
One month	1.8×10^2	3.2×10^2	6.9×10^2	6.33×10^2
Two month	1.2×10	2.4×10	3.5×10	3.6×10
Three month	None	None	None	None

T1 = Rabbit frankfurter containing 15% defatted soy flour; T2= Rabbit frankfurter containing 30% defatted soy flour; T3 = Rabbit frankfurter containing 45% defatted soy flour

meat ingredient (Spice mix, water, phosphate, corn starch) may have reduced microbial population in raw rabbit frankfurter.

From the tabulated data it could be observed that TVC were all within acceptable limits, since 10^6 CFU /g is maximum permissible level for aerobic plate counts in meat products [97].

Thus, all the frankfurters analyzed over the three month of frozen storage can be classified as acceptable for human consumption.

Referring to Table 10, moulds and yeasts of fresh frankfurter (Control), was 2.2×10^2 CFU/g at zero time, whereas moulds and yeasts of fresh rabbit frankfurter contained 15, 30 and 45% DSF were 4.1×10^2, 3.6×102 and 3.73×10^2 CFU/g, respectively. After one month of frozen storage, moulds and yeasts in either control or contained different levels of DSF were not detected.

Sachindra *et al.* [98] reported that microorganisms could gain access into sausage from meat, spices and other ingredients, from environment, equipment, and handlers during processing.

Psychrophilic and Coliform Bacterial Counts: As shown in Table 11, psychrophilic bacterial count of fresh rabbit frankfurter was 9.4×10^2 CFU/g which decreased to 8.3×10^3, 7.2×10^2 and 6.5×10^2 for frankfurter containing 15, 30 and 45% defatted soy flour, respectively. The same trend was observed for coli form bacterial count which was observed for coliform bacterial count which was 2.5×10^3 CFU/g and 8.7×10^3 CFU/g for rabbit frankfurter containing 0, 15, 30 and 45% defatted soy flour, respectively. From the obtained results, it could be noted that psychrophilic bacteria increased as the storage time prolonged. Kishanrao [99] stated that psychrophilic count of chicken sausage increased significantly throughout refrigerated storage of 20 days. This may be attributed to growth preference of psychrophilic organisms during storage at refrigeration temperature. Increase in psychrophilic count during storage of low fat chevon rolls was also reported by Yadav and Sharma [100]. On the other hand, after three months coliform bacterial count of all frankfurter samples was not detected. Sachindra *et al*. [98] observed coliform count 10^1-10^3 CFU per gram in cooked frankfurters and there was no significant change in the number of coli forms during storage at 30°C. Also, Andres *et al*. [101] studied storage stability of low fat chicken sausage and recorded absence of coli form bacteria during storage 28 days sample.

Staphylococcus aureus, *Salmonella* and *Shigella* were absents in all studied frankfurter samples either the fresh (Zero time) or during storage at -18°C as a result of the strict hygienic conditions followed during the preparation and storage.

CONCLUSIONS

It can be concluded that using rabbit meat in the formulation of meat products such as frankfurter could contribute to healthy foods with improved nutritive value due to, high protein and amounts of essential amino acids, low fat, cholesterol and biological value. The results showed good acceptance of rabbit meat frankfurter

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