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Utilization of Wheat Germ as Natural Antioxidant and Fat Mimetic to Increase Shelf-Life in Beef Sausage and as Lowering Cholesterol in Rats

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ABSTRACT

Wheat germ is one of the most potential and excellent sources of vitamins, antioxidant, minerals, fiber and proteins. Therefore, it could be used as an alternative of sheep fat at level 25, 50 and 75% from sheep fat to give three beef sausage formulae and compared with beef sausage as a control which contained from 20% sheep fat. Phenolic and flavonoids compounds were fractionated in wheat germ extract using HPLC and the results showed that the wheat germ had contained rich amounts from phenolic and flavonoids compounds. Moreover, physical and chemical were determined during storage period (three month) in beef sausage and its formulae and also sensory characteristics and texture profile analysis was evaluated. The results showed that the water holding capacity (WHC) and cooking loss were increased whilst, plasticity and cooking yield were decreased by increasing the wheat germ amounts in sausage formulae at zero time compared with control. After 3 months frozen storage, the WHC and cooking loss were decreased for sausage formulae, and also plasticity and cooking yield were increased during storage period. Changes in chemical properties of sausage formulae containing wheat germ and during frozen storage at-18ºC for up three months, the results showed that the thiobarbituric acid (TBA) values, total volatile nitrogen (TVN) content and trimethylamine-nitrogen (TMA-N) content were decreased with addition of wheat germ at zero time and until the end of frozen storage compared with control. Sensory properties (appearance, aroma, texture, taste and overall acceptability) were determined in beef sausage and its sausage formulae containing wheat germ. From the results it was observed that the addition of wheat germ improved the sensory characteristics at replacement sheep fat at levels 25, 50 and 75% wheat germ. Moreover, the addition of wheat germ was influenced the texture of sausages and its formulae may be wheat germ was high contained of total dietary fiber. At the end of biological investigation the total lipid, triglyceride, total cholesterol and cholesterol fractions were decreased in the hypercholesterolemia rats fed on sausage formulae than control negative hypercholesterolemia rats fed on basal diet. The best rats group fed on sausage formula prepared from 75% wheat germ and 25% sheep fat, the lipids pattern was decreased followed by the groups fed on the formulae made from 50 and 25 % wheat germ. From the obviously results it could recommended that the wheat germ had rich contained in natural antioxidant which improvement the sensory acceptability in sausage formulae and it was as alternative sheep fat. The high amounted from wheat germ in sausage formulae reported that decreased the lipid parameters in hypercholesterolemia rats fed on different sausage formulae.

Key words: Wheat germ, vitamins, natural antioxidant, fat, beef sausage

Introduction

Wheat germ (WG) is widely recognized as a nutritious raw material for incorporation into food product formulations or as a food in its own right. Typical applications are in germ-enriched bread, snack foods, and supplements to breakfast cereals, and for production of wheat-germ oil. Wheat germ, containing about 8% - 14% oil (average 10%), is mainly used in food, medical and cosmetic industries as a source of oil (Zhu *et al.*, 2006). Relatively a huge quantity of wheat germ is produced annually as a by-product of wheat milling industry in Egypt.

Wheat germ usually contains about 30% protein, 10% fat and 45% carbohydrate; it is known for its high content in minerals, trace elements and vitamins Nelson (1985). The total dietary fiber content was estimated at 7.8 and 10.6%, respectively, for English wheat and Canadian wheat Fisher (1985) and 23.9% on a dry weight basis for U.S. hard red spring wheat Saunders (1978).

Meat is still the most valuable food in the world. The quality aspect of meat is decided by the nutritional and sensory values. Consumers awareness on diet and health increased the demand for healthy food, specially, meat. Meat is high in saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). Replacing or reducing animal fat in meat products could create a better image for the industry, but, sensory quality as well as product stability could be affected. Animal fat can be replaced or reduced by adding more water in the product

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or by substituting with vegetable fats and oils, or by adding hydrocolloids like dextrin, starches and gums in the product (Halwai, 2012).

Consumer demand healthier meat products that are low in fat, salt, cholesterol, nitrates and calories in general and contain in addition health-promoting bioactive components such as carotenoids, unsaturated fatty acids, sterols and fibers on the other hand; furthermore, consumer accept these level meat products with altered formulations to taste, look and smell the same way as they are traditionally formulated and processed counterparts. At the same time competition is forcing the meat processing industry to use the increasingly expensive raw material i.e. meat more efficiently and produce products at lower costs (Jochen *et al.,* 2010).

Lin *et al.* (2004) theorized that an increase in the risk of colorectal cancer is associated with dietary saturated fat. People are reluctant to increase sausage consumption because of SFA content in it and these fatty acids are associated with increased risk of coronary heart diseases (Gines *et al.,* 2005). Therefore, dietary guidelines for Americans suggested reducing sodium, fat and cholesterol in diets. Moreover, the American Health Association suggested limiting fat consumption to < 30 % of calories, cholesterol to < 300 mg and sodium to < 3g daily (Matulis *et al.,* 1995).

The fact that rats receiving the high fat-cholesterol diet supplemented with wheat germ presented a reduced liver steatosis could be the result of various mechanisms such as lower intestinal absorption of dietary lipids, impaired hepatic uptake of intestinal lipoproteins, reduced biosynthesis or increased catabolism of cholesterol and fatty acids in the liver. Recent advances made in the understanding of lipoprotein metabolism have established that unlike the apo B, E receptors specific for LDL, the apo Ereceptors responsible for hepatic uptake of chylomicron remnants are not subjected to regulation Mahleyr and Innerarityt (1983). The various observed modifications of lipid metabolism induced by the addition of wheat germ to the diet could probably be attributed to a lesser uptake and/or intestinal secretion of neutral lipids and cholesterol in the rat fed the high fat-cholesterol diet. Taking into account the low dose of wheat germ added to the diet (7%) and the low fiber content of wheat germ Fisher (1985), it seems very unlikely that such low amounts of ingested fibers could be responsible for the marked metabolic effects observed.

The aim of this investigation was carried out to reduce the sheep fat in the sausage using wheat germ at level 25, 50 and 75% replacement the sheep fat. Physical and chemical were determined during storage period (three month) in beef sausage and its formulae and also sensory characteristics and texture profile analysis was evaluated. Moreover the parameters in the serum hypercholesterolemia rats for the biological experimental were evaluated.

Materials and Methods

Materials

Fresh lean beef meat from boneless round and fat tissues (sheep tail) were purchased from the private sector shop in the local market at Egypt. Other ingredients used in sausage preparation such as rusk, salt and spices were purchased from local market at Egypt.

Wheat germ was obtained from North Cairo flour Mills Company El-Salam City, Egypt. Moreover, flavonoids and phenolic acids were purchased from standard from Sigma Co.

Methods

Extraction of natural antioxidant compounds from wheat germ

Air dried wheat germ were finely powdered as previously described and extracted with petroleum ether (40-60ºC) to remove fats and resinous materials. The residues were exhaustively separately extracted with 500 ml of ethanol (70%). The extract was filtrated through Whatman no., 1 filter papers and the filtrates were evaporated to dryness under reduced pressure on a rotary evaporator (RE 300/MS) at 40ºC.

Determination of phenolic compounds from wheat germ using HPLC

Phenolic compounds were determined by HPLC according to the method of Goupy *et al.* (1999) as follow: 5 g of sample were mixed with ethanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtered through a 0.2 µm Millipore membrane filter then 1-3 ml was collected in a vial for injection into HPLC Agilant (series 1200) equipped with auto-sampling injector, solvent degasser, ultraviolet (UV) detector set at 280 nm and quaternary HP pump (series 1100). The column temperature was maintained at 350C. Gradient separation was carried out with ethanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. phenolic acid standard from sigma Co, were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculate the phenolic compounds concentration by the data analysis of HEWLLET Packared software.

Determination of flavonoids compounds from wheat germ using HPLC

Flavonoids compounds were determined by HPLC according to the method of Mattila *et al.* (2000) as follows: 5 g of sample were mixed with ethanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtered through a 0.2 µm Millipore membrane filter then 1 - 3 ml was collected in a vial for injection into HPLC Hewllet Packared (series 1050) equipped with auto-sampling injector, solvent degasser, ultraviolet (UV) detector set at 330 nm and quarter HP pump (series 1050). The column temperature was maintained at 350C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at a flow rate of 1 ml/min. Flavonoids standard from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculation of phenolic compounds concentration by the data analysis of HEWLLT Packared software.

Preparation of beef sausage from wheat germ

Three formulae of sausage were manufactured in this study. The sausage control formula was prepared according to Rocco *et al.* (2003), as follows: lean beef meat (62.0%), sheep fat tail (20.0%), ice water (10.0%), rusk (5.0%), salt (2.0 %) and spices (1.00%). Other sausage treatments were prepared by reducing added fat level from 20% in sausage control to 25, 50 and 75% replacement by wheat germ flour.

The sausage control and other sausage formulae were prepared by mixing the minced lean beef meat with other ingredients for 8 – 10 min by using a laboratory emulsifier (Hobbart Kneading machine, Italy) specialized for sausage manufacturing. The obtained emulsion was stuffed into a nature casings which were hand linked at about 15 cm intervals. All treatments were aerobically packaged in a foam plate, wrapped with polyethylene film and stored at -18°C for 3 months. Samples were taken for analysis every month periodically.

Physical and chemical analysis of sausage formulae

Water Holding Capacity (WHC) and plasticity of different sausage formulae were measured immediately after processing and during frozen storage period according to the filter press method of Soloviev (1966). The cooking loss of sausage and their formula during storage period were determined as the method described by AMSA (1995). Thiobarbituric acid (TBA) value was determined as described by Egan *et al.* (1981) and total volatile nitrogen (TVN) and trimethylamine-nitrogen (TMA-N) were determined according to the method published by Winton and Winton (1958).

Texture profile analysis

The texture profile analysis (TPA) indices of different sausage formulae were determined using a texture analyzer (Cometech, B type, Taiwan). The conditions of texture analyzer were provided with software, 35 mm diameter compression disc was used. Two cycles were applied at a constant crosshead velocity of 1 mm/s, to 30% of sample depth and then returned. From the resulting force-time curve the values for texture attributes, i.e. Hardness, gumminess, chewiness, adhesiveness, cohesiveness, springiness and resilieness were calculated from TPA graphic according to Bourne (2003).

Organoleptic evaluation of different sausage formulae

Organoleptic evaluation of beef sausage and its formulae was carried out according to Watts *et al.* (1989) by aid of ten members. Judging scale for each factor was as follows: excellent (8-9), Very good (7-<8), Good (6-<7), Fair (5-< 6), Poor (4-<5) and Rejected (<4).

Nutritional experiments

Male adult rats (30 rats) weight ranging 140-155 g were purchased from National Organization for Drug and Control Research, Giza, Egypt. Animals were housed in individual cages with screen bottoms and fed on basal diet for eight days. The basal diet consisted of corn starch 70%, casein 10% corn oil 10%, salt mixture 4%, vitamin mixture 1% and cellulose 5% according AOAC (1995). After feeding on basal diet for eight days, rats were divided into two groups. The first group (6 rats) was fed on the basal diet for another four weeks (30 days) and considered as negative control. The second main group (24 rats) was fasted overnight and injected with aloha solution into the leg muscle (150 mg /1kg body weight) to induce hypercholesterolemic rats according to Buko *et al.* (1996). After 48 hr of injection the second main group was divided into four sub groups (6 rats for each). The first one (6 rats) was continued to be fed on basal diet and considered as positive control. From the second to three subgroup (6 rats for each) were fed on 20% from sausage formulae as a substitute of the basal diet. The hypercholesterolemia rats group 1 fed on basal diet substitute with sausage formula 1 had contained 25% wheat germ as alternative sheep fat. The hypercholesterolemia rats groups 2 and 3 fed on sausage formulae 2 and 3 had contained 50 and 75% wheat germ as alternative sheep fat. Each rat was weighted every two days and the food consumption was calculated. At the end of experimental period (four weeks), the blood samples were taken with drawn from the orbital plexus and centrifuged at 3000 rpm to obtain the sera. After that, the sera were kept on a deep freezer at -20ºC until their analyses. Total lipids, total cholesterol and triglycerides were determined according to Allain *et al.* (1974) Fossati and Prencipe (1982) and Tietz (1986), respectively. High and low density lipoprotein- cholesterol in serum was determined according to Burstein (1970) and Fruchart (1982).

Statistical analysis

Statistical analysis for each of the collected data was done of variance (ANOVA) test described the procedure outline by Armitage and Berry (1987). The treatment means were compared using the least significant difference test (LSD) at 5% level of probability as outline by Waller and Duncan (1969).

Results and Discussion

Polyphenolic fractions of wheat germ extract

Polyphenolic compounds in wheat germ extract were fractionated using HPLC apparatus and the results are reported in Table (1). From the results it can be noticed that the wheat germ extract contained a higher value of the polyphenolic compounds. Total phenolic compounds were fractionated using HPLC and the results illustrated that the salycilic, E-vanillic and pyrogallol and catechein acids were the highest amounts (177.99, 100.60 and 87.61 mg/100g in black rice extract, respectively). Meanwhile, the catechein, alpha-coumaric, catechol, chlorogenic, protocatchuric, vanillic, ferullic and 3-oH-tyrosol acids was a medium amounted in wheat germ (22.82, 13.96, 13.75, 13.57, 12.74, 11.66, 11.40 and 10.28 mg/100g, respectively). Moreover, the phenolic compounds remaining were less than 10 mg/100g in wheat germ extract.

The results observed that the wheat germ extract had the highest flavonoids content such as hispertin, apigenin, kampferol, rosmarinic, narengin, rutin and quercetrin (107.16, 104.78, 100.45, 99.88, 92.31, 87.35, and 76.83 mg/100g, respectively). Luteolin, and quercetin were the lowest amounted of flavonoids compound in wheat germ extract.

Flavonoids are a class of secondary plant phenolics having potential beneficial effects on human health with significant antioxidant amounts and chelating properties in the human diet. Over the years, they have been found to be an important part of the human diet and are considered to be active principles in some medicinal plants. The antioxidant activity of flavonoids is efficient in trapping superoxide anion (O2·-), hydroxyl (OH·), peroxyl (ROO·) and alcohoxyl (RO·) radicals (Wang *et al.,* 2003).

Table 1: Polyphenols content (mg/100g) in ethanol extract from wheat germ

Phenolic compounds	mg/100g	Flavonoids compound	mg/100g
Gallic	2.82	Luteolin	59.52
Pyrogallol	87.61	Narengin	92.31
4- Amino-benzoic	2.10	Rutin	87.35
3-OH- Tyrosol	10.28	Hisperidin	45.76
Protocatchuic	12.74	Rosmarinic	99.88
Chlorogenic	13.57	Quercetrin	76.83
Catechol	13.75	Quercetin	22.04
Catechein	22.82	Hispertin	107.16
Caffeine	5.51	Kampferol	100.45
P-OH-benzoic	1.85	Apegnin	104.78
Caffeic	5.00		
P-coumaric	5.24		
Ferulic	11.40		
Iso- ferulic	1.52		
Reversetrol	5.64		
Ellagic	8.21		
Vanillic	100.60		
Alpha-coumaric	13.96		
3,4,5- methoxy- cinnamic	8.31		
Coumarin	2.99		
Salycilic	177.99		
Cinnamic	3.83		

Physical properties of sausage formulae

Changes in physical properties of wheat germ sausage formulae containing different ratios of wheat germ at different ratios (25, 50 and 75 % as substitutes for fat sheep) during frozen storage at -18ºC for three months are presented in Table (2). From these results, it was observed that the water holding capacity (WHC) was increased with increasing the wheat germ amounts in sausage formulae at zero time compared with control. This increase in WHC of sausage formulae contained wheat germ may due to that the fibers leading to bind the water and increase of WHC. After 3 months frozen storage, the WHC were decreased for sausage formulae from 5.21 to 4.82 cm2/0.3g compared with control ranged from 6.54 to 2.98 cm^2/0.3g, respectively.

It was observed that the plasticity was increased with increasing the wheat germ amounts in sausage formulae at zero time compared with the control sample, ranged from 4.98 to 5.88 and 4.65 cm2/0.3g, respectively. During frozen storage, the plasticity decreased in all sausage formulae. During frozen storage, the water holding capacity (WHC) and plasticity were decreased (i.e., separated free water were increased) with advancement of storage time for all samples. The loss of WHC and plasticity during storage may be attributed to increase the protein denaturation and loss of protein solubility. The rate of decrease in WHC and plasticity was lower in sausage formulae prepared with different levels of wheat germ when compared with control sample. From results in Table (2) it could be noticed, also, that the replacement of sheep fat by wheat germ in sausage formulae led to decrease the cooking loss (frying loss) from the beginning of processing compared with the control sample, ranged from 17.08 to 14.94 and 19.54 %, respectively. During frozen storage, the cooking loss increased in all the tested samples. Therefore, the wheat germ formulae had the lowest values of cooking loss all over the stage period starting from processing point, during frozen storage and also at the end of the storage period compared with the control sample. The cooking yield increased with addition of wheat germ compared with control at zero time, ranged from 82.92 to 85.06 and 80.46%, respectively. It was noticed, also, that at the end of frozen storage the values were ranged from 67.44 to 72.85 and 62.04%. From the above results, it can be noticed that the cooking yield decreased with increasing frozen storage time.

Table 2: Changes in physical properties of sausage formulae

Formulae	Storage periods	WHC (cm^2/0.3g)	Plasticity (cm^2/0.3g)	Cooking loss (%)	Cooking yield (%)
Control	0	2.98	4.65	19.54	80.46
	1	4.10	4.14	22.40	77.60
	2	5.51	3.55	28.84	71.16
	3	6.54	2.98	37.96	62.04
Formula 1	0	2.52	4.98	17.08	82.92
	1	3.35	4.36	19.13	80.87
	2	4.23	3.83	25.27	74.73
	3	5.21	3.21	32.56	67.44
Formula 2	0	2.18	5.48	15.97	84.03
	1	3.02	4.89	17.72	82.28
	2	4.20	4.30	23.06	76.94
	3	5.08	3.65	30.10	69.90
Formula 3	0	1.85	5.88	14.94	85.06
	1	2.73	5.33	16.82	83.18
	2	3.69	4.61	22.05	77.95
	3	4.82	3.93	27.15	72.85

Chemical properties of sausage formulae

Changes in chemical properties of sausage formulae containing wheat germ and during frozen storage at-18ºC for up three months were presented in Table (3). Thiobarbituric acid (TBA) values, total volatile nitrogen (TVN) content and trimethylamine-nitrogen (TMA-N) content have long been assessed and accredited as a reliable indexes for quality of sausage, which is result from nature of sausage changes, storage temperature, treatment conditions and length of storage period. From results of Table (3), it was observed that the thiobarbituric acid (TBA) values decreased with addition of wheat germ compared with control at zero time ranged from 0.92 to 0.83 and 0.96 mg malonaldhyde/kg, respectively, and until the end of frozen storage compared with control ranged from 2.26 to 1.64 and 2.44 mg malonaldhyde/kg, respectively, may be due to antioxidant properties of phenolic and flavonoids compounds of wheat germ**;** this confirmed that replacement a part of sheep fat by wheat germ during sausage formulae led to decrease of (TBA**)** values from zero time to the end of storage period.

Duh and Yen (1997) cited that the addition of the antioxidants to food is effective in retarding fat oxidation. It is impressive that many substance have been identified which prevent lipid peroxidation. Some of these compounds are synthetic antioxidants and others occur as natural dietary constituents. This confirmed that replacement a part of fat sheep by wheat germ flour during sausage formulae led to decrease the TBA values from zero time to the end of storage period.

From the results in the same table it was noticed that replacement part of sheep fat by wheat germ led to decrease of TVN levels in fish fingers compared with control ranged from 10.72 to 8.37and 12.02 mg/100g respectively at zero time. While, ranged from 19.21 to 12.98 and 26.10 mg/100g, respectively until the end of frozen storage, may be due to that the wheat germ has antimicrobial activity. During frozen storage, it was

observed from results that the TVN contents increased for all samples. But the rate of increase in the control sample was higher than for samples contained wheat germ during storage until the end of frozen storage.
From the results in Table (3) it was found that the production of trimethylamine-nitrogen (TMA-N) followed a pattern similar to that of TVN. From the results it could be noticed that the TMA-N levels were decreased with addition of wheat germ compared with control at zero time ranged from 3.63 to 2.86 and 4.13 mg /100g, respectively at zero time. It may be due to that the wheat germ had contained polyphenol and flavonoid as antimicrobial activity**.** During frozen storage the TMA-N levels increased for all samples and the control sample had higher TMA-N level than these samples. During storage until the end of frozen storage ranged from 7.08 to 4.79, and 9.02 mg /100g, respectively until the end of frozen storage.

Table 3: Changes in chemical properties of sausage formulae

Formulae	Storage periods	TBA mg /kg	TVN mg /100g	TMA-N mg /100g
Control	0	0.96	12.02	4.13
	1	1.46	16.68	5.74
	2	1.97	21.37	7.36
	3	2.44	26.10	9.02
Formulae 1	0	0.92	10.72	3.63
	1	1.35	13.57	4.77
	2	1.70	16.35	5.90
	3	2.13	19.21	7.08
Formula 2	0	0.88	9.60	3.19
	1	1.16	11.81	4.04
	2	1.48	13.97	4.95
	3	1.75	16.19	5.87
Formula 3	0	0.83	8.37	2.86
	1	1.07	9.93	3.55
	2	1.30	11.40	4.17
	3	1.51	12.98	4.79

TBA : Thiobarbituric acid (mg/kg sample). TVN: Total volatile nitrogen (mg/100g). TMA-N: Trimethylamine-nitrogen (mg/100g).

Sensory properties of sausage formulae made with wheat germ

Data present in Table (4) showed that the sensory properties (appearance, aroma, texture, taste and overall acceptability) of sausage formulae containing wheat germ. From the results it was observed that the addition of wheat germ improved the sensory characteristics at replacement sheep fat 25, 50 and 75% wheat germ. From the results it could be observed that the best scores of sensory properties were in wheat germ formulae 1, 2 and 3 had recorded 8.38، 8.50 and 8.63 in overall acceptability and near then was the control sausage 8.28. This results showed that the wheat germ had contained high amounts from of total phenolics compounds and total flavonoids may be improve the acceptability of sausage formulae.

Dietary antioxidants are food compounds that impede the deleterious effects of reactive oxygen species, reactive nitrogen species, or both, on the normal physiological function in humans (Dimitrios, 2006). Dietary antioxidants include ascorbate, tocopherols, carotenoids and bioactive plant phenols. ROS; oxygen ions, free radicals, and peroxides and reactive nitrogen species (RNS); nitrous anhydride, peroxynitrite, and nitrogen dioxide radicals, causes oxidation, nitration, halogenation and deamination of biomolecules of all types, including lipids, proteins, carbohydrates, and nucleic acids, with the resultant formation of toxic and mutagenic products (Castrol and Freeman, 2001). Biological systems control these oxidative factors by a variety of antioxidative mechanisms that restrict the reactivity of ROS and RNS and oxidation catalysts (Baublis *et al.,* 2000).

Table 4: Sensory properties of sausage formulae made with wheat germ

Fish fingers formulae	Sensory properties				
	Appearance	Aroma	Texture	Taste	Overall acceptability
Control	8.60^{ab}	7.90^{bc}	8.40^{a}	8.20^{bc}	8.28^{ab}
Formula 1	8.50^{ab}	8.20^{b}	8.50^{a}	8.30^{bc}	8.38^{ab}
Formula 2	8.40^{ab}	8.50^{a}	8.60^{a}	8.50^{ab}	8.50^{ab}
Formula 3	8.30^{b}	8.70^{a}	8.80^{a}	8.66^{a}	8.63^{a}
LSD at 5%	0.296	0.293	0.305	0.277	0.367

Texture profile analyses of sausage and its formulae

Table (5) showed that the results of texture properties analysis after replacing fat with wheat germ in sausage and their formulae. Firmness was between 4.29 and 9.61g; cohesiveness between 0.51 and 1.73%, gumminess between 2.12 to 10.54g, chewiness between 1.51 and 2.34(g/mm), springiness between from 0.60 to 0.69(mm) and finely, resilience was between from 0.45 to 0.52g. The addition of wheat germ was influenced the texture of sausages and its formulae may be wheat germ was high contained total dietary fiber. Chambers and Bowers (1993) suggested that of the characteristics of texture, hardness is the most significant factor in influencing consumer preference towards meat products, so this study primarily examined the hardness of Chinese-style sausages. The addition of wheat fiber significantly increased the hardness of Chinese-style sausages. An experiment by García *et al.* (2002) involving the addition of dietary fiber to dry-fermented sausages also found that the addition of wheat and oat fibers significantly increased the hardness of meat products.

Viuda-Martos *et al.* (2010) found that adding 1% orange dietary fiber to the Spanish emulsified meat mortadella significantly increased the hardness of the product, primarily because the bonding capacity of fiber particles and the emulsified protein system were strengthened through the heating process (Viuda- Martos *et al.,* 2009). Gumminess and chewiness were determined from the following calculations: gumminess = hardness × cohesiveness; chewiness = hardness × cohesiveness × springiness. Consequently, the resulting gumminess and chewiness of the sausages after the addition of different types of dietary fiber followed the same general trend as the hardness of the sausage.

Table 5: Texture profile analyses of sausage and its formulae substituted with wheat germ

Formulae	Firmness (g)	Cohesiveness (%)	Gumminess (g)	Chewiness (g/mm)	Springiness (mm)	Resilience (g)
Control	4.29	0.51	2.12	1.51	0.60	0.45
Formula1	5.32	0.79	5.24	1.86	0.64	0.47
Formula2	7.24	1.25	8.36	2.11	0.67	0.49
Formula3	9.61	1.73	10.54	2.34	0.69	0.52

Biological investigation

Changes in body weight and daily feed intake

The data concerning the changes in body weight and daily feed intake for rats on sausage diet containing 25, 50 and 75% wheat germ flour as a replacement fat in sausage formulae and the results are reported in Table (6). At the end of experimental, the results showed that the changes in body weight in case hypercholesterolemia rats in control positive was decreased by -21.88 % followed by group 3 made from 75% wheat germ and 25% sheep fat was -17.63%. Whereas, the group no., 1 and 2 showed lower in body weight (-8.71 and -9.55%) made from 25 and 50% wheat germ as alternative sheep fat than healthy control negative (37.29) fed on basal diet. Moreover, the results from daily feed intake showed that the healthy rats in control positive was the highest in daily feed intake (12.54) followed by hypercholesterolemia rats in control positive was 11.34. The hypercholesterolemia rats in all groups were decreased gradually in daily feed intake when the wheat germ increased in sausage formulae, may be caused the wheat germ flour had contained rich amounts in dietary fiber.

Table 6: Means of initial and final body weight, changes in body weight and daily feed intake on normal and hypercholesterolemia rats fed on sausage formulae:

Groups	Initial body weight	Final body weight	Changes in body weight		Daily feed intake
			gram	%	
Control negative	144.50±0.88	198.39±2.98	+53.89	+37.29	12.54±1.07
Control positive	154.84±2.92	120.53±1.49	-34.31	-21.88	11.34±0.43
Group 1	150.10±1.62	137.02±3.49	-13.08	-8.71	10.76±0.43
Group 2	152.92±2.46	138.31±1.94	-14.61	-9.55	9.39±0.65
Group 3	141.23±1.89	123.60±3.13	-17.63	-13.43	8.40±0.71

Effect of wheat germ on serum lipid patterns

Total lipid, triglyceride, total cholesterol and cholesterol fractions as low density lipoprotein (LDL) and high density lipoprotein (HDL) were determined to evaluate the role of sausage formulae made from wheat germ on lipid metabolism and the results are reported in Table (7). From the resultant it could be noticed that the total lipids, triglycerides, total cholesterol and LDL were decreased when the hypercholesterolemia rats in the group (3) fed by increasing wheat germ (75%) in sausage formula and the results were 198.70, 125.60, 94.37 and 44.26 mg/dl. The results in group 3 were nearly to the results from the healthy rats in control negative, 291.75, 119.72, 85.42 and 31.43 mg/dl. The decreasing in lipid pattern could be referred to multi factors beside the role of dietary fiber and antioxidants may be playing a part of this action. By scavenging radicals, flavonoids can inhibit LDL oxidation in vitro. This action protects the LDL particles and theoretically, flavonoids may be having protective action against atherosclerosis (Nijveldt *et al.,* 2001).

Table 7: Means of serum total lipids, triglycerides and total cholesterol (mg/dl) in rats fed on sausage formulae

Groups	Total lipids	Triglycerides	Total cholesterol	LDL	HDL
Control negative	291.75±5.77	119.72±6.00	85.42±8.61	31.43±4.11	79.86±1.91
Control positive	387.36±7.72	171.49±6.22	185.65±11.60	121.58±10.97	42.36±4.09
Group 1	324.91±4.98	149.04±4.16	142.92±6.69	73.39±5.85	64.33±3.73
Group 2	311.52±6.60	133.45±6.48	129.73±5.65	66.82±5.83	71.98±2.29
Group 3	298.70±6.83	125.60±9.58	94.37±6.78	44.26±5.60	75.18±3.36

LDL Low density lipoprotein *HDL High density lipoprotein*

Hence, it can be recommended that the wheat germ can be used as alternative for fat sheep till 75% from sheep fat. This treatment resulted also improvement of physical, chemical and sensory characteristics of sausage formulae. The results showed the substituted sheep fat with wheat germ in sausage formulae decreased the total lipids, triglycerides and total cholesterol on the hypercholesterolemia rats.

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