FOOD
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Frankfurter sausage texture is affected by using isolate, concentrate and flour of *Lupinus albus* and pork skin proteins

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Abstract

Frankfurters are typically produced using a wide range of ingredients comprised of various animal or vegetable proteins, including legume and oleaginous seeds, mainly soybean. *Lupinus* and collagen from pork skin have several functional properties which contribute to stabilizing and forming emulsions. The aim of this study was to determine the effect of collagen obtained from pork skin and isolate, concentrate or flour of *Lupinus* on texture frankfurters measured using different probes attached to a texture analyzer. Frankfurters were prepared by adding flour, isolated and concentrate of *Lupinus* in proportion of 0.5, 1.0, 1.5 y 2.0; and collagen solutions obtained from pork skin (0, 1, 2 y 3%). The proximal analysis in isolate, concentrate, and flour of *Lupinus*, and collagen in pork skin solutions were measured. Textural properties were evaluated in frankfurters. The results showed no differences (P>0.05) between *Lupinus* flour, protein isolate and concentrate vs control in firmness and cohesiveness. However, higher values for resilience and springiness than sausages prepared with *Lupinus*. Warner Bratzler firmness values using 3% collagen were lower than other treatments. The treatment had less firmness was AIS-100, and the least cohesiveness was CON-150, control had the most resilience, and CON-100 treatment was the most adhesiveness. The sausages added with isolate, concentrate or flour of *Lupinus albus* in quantities of 50, 100 or 150 g, respectively produce an excellent texture meat product. When collagen is used in 3% the texture of sausages is firm and should be recommended to use for industrial production of meat products.

1. Introduction

Sausages composition is diverse; pork, turkey or poultry are used, also it is added with several additives, e.g. phosphates, gums and other ingredients. Back fat and meat extenders are usually used to produce sausages (Abdolghafour and Saghir, 2015). There are several non-meat proteins used in the preparation of frankfurters and other meat products which include starches, vegetable protein, dairy protein, antioxidant, extenders and so on (Tarté, 2009; Abdolghafour and Saghir, 2015). Soybean protein isolate is one of the most used in meat technology (Egbert and Payne, 2009). Another protein used is collagen which improves sensory properties, and enhances gelling, water holding capacity and increase protein content (Sousa *et al*., 2017).

Collagen is added to meat products as connective tissue, but it is physical form, composition, and stability to heat must be important for use in emulsified products (Prabhu *et al*., 2004). When this protein is used in meat products, it must be purified or hydrolysate (Tarté, 2009). Prabhu *et al*. (2004) suggest that collagen improved protein functionality through facilitating immobilized free water and increasing the stability of the product. Collagen used at low levels is effective in reducing the shortening and texture control of meat products. An alternative to using soybean is a leguminous named *Lupinus*, which has been used as food for humans since ancient times (Prusinski, 2017). *Lupinus* seed has 42% protein in some varieties (Schumacher *et al*., 2011). *Lupinus* proteins improve nutritional quality, texture, and color of food products (Prusinki, 2017). The functional properties of these proteins are to facilitate food emulsions (Porras-Saavedra *et al*., 2013) and contribute to sausage structure. Pork skin proteins increase the firmness and stability of meat

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products. Collagen, the main protein skin, becomes gelatin during heat treatment. Gelatin is commonly used in cooked sausages due improve firmness (Feiner, 2006). The aim of this study is to determine the effect of collagen, *Lupinus* flour, protein isolate and concentrate on frankfurters texture using different attachments to measure them.

2. Materials and methods

2.1 Collagen, Lupinus flour, and protein isolate preparation

Collagen was obtained from pork skin incubated at 80°C for 4 hours in a 1.5M NaCl solution at pH 9. *Lupinus albus* flour was detoxified by exposing the seeds to hot water (95°C) for five minutes and alkaloids were removed using continuous water for 15 hours (Carvajal-Larenas *et al.*, 2016). The seeds were the sun-dried. They were then ground (Krups model 203) and sieved (Standard Testing Sieve No. 25, Tyler equivalent 24 mesh, 0.710 mM). The flour was defatted using hexane (1:4 w/v) for 8 h under constant shaking at 8°C. The flour was then dried in a chamber at 50°C to remove any solvent residues. In order to obtain *Lupinus* protein isolate, the flour was washed three times for 30 minutes each time at room temperature using isopropyl alcohol 80% and 60%. The collagen was analyzed by hydroxyproline content. *Lupinus* flour and protein isolate was evaluated for soluble protein. The proximate analysis (dietary fiber, ash, nitrogen content as protein, fat) in all samples.

2.2 Frankfurter sausage preparation

Meat batters were formulated using a typical frankfurter sausage base, using pork meat without any seasoning ingredients (Table 1). The lean pork meat was purchased from local market. Ground meat, salt, sodium nitrate, commercial phosphates mixture and a third part

ice were mixed for one min in a Dito Sama K35 cutter (Dito Sama, Aubusson, France). Back fat was added and emulsified for one minute with another third of ice for two minutes. Collagen, *Lupinus* flour, protein isolate or concentrate and the remainder of the ice were added and homogenized for another three minutes, controlling the temperature to less than 10°C. Meat batters were manually stuffed into polyamide casings, cooked to an internal temperature of 68°C, maintained for 15 min, cooled in an ice bath, stored at 4°C for 24 h, vacuum packed and kept under refrigeration until analysis. A total of three batches (0.5 kg) of each treatment were manufactured.

2.3 Texture profile analysis (TPA) of sausages prepared using collagen, flour, concentrate or protein isolate from Lupinus

Samples were kept in refrigeration 24 hours before analysis. The sausage samples were then cut 2 cm thick, 2.5 cm diameter and subjected to two cycles compression test and analyzed using a a texture analyzer (TA.HDi Texture Analyzer, TA Instruments, Surrey, UK). Five samples of each treatment were compressed to 75% of their height according to Bourne (1978). A Perspex probe of 2.5 cm diameter attached to a load cell of 50 kg was compressed twice at a speed of 1 mm/sec. TPA parameters were obtained as follows: hardness, cohesiveness, resilience, and springiness.

2.4 Warner-Bratzler analysis of sausages prepared using collagen, flour, concentrate or protein isolate from Lupinus

A V-shaped blade with a thickness of 3.20 mm, the height of 125 mm and width of 70 mm was assembled to the TA.HDi Texture Analyzer with an attached a load cell of 50 kg. A tested sample was placed on the plate, under the v-blade, cutting down at a pre-test speed of 2 mm/s, and 1.0 mm/s test speed. The analyzer had

Table 1. Frankfurter sausages formulation used in collagen and *Lupinus* derivates treatments.

Ingredients	Control	Collagen treatment (Trial 1)			*Lupinus* treatment (Trial 2)			
		T1	T2	T3	T1	T2	T3	T4
Pork meat (%)	60	60	60	60	60	60	60	60
Pork Back fat (%)	15	15	15	15	15	15	15	15
Water (%)	25	25	25	25	25	25	25	25
Salt (g)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Phosphates mix (g)	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
Nitrites (g)	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Flour (g)	4	4	4	4	4	4	4	4
Carrageenan (g)	1.5	1.5	1.5	1.5	2.5	2	1.5	1
Soybean isolate (g)	5	0	0	0	0	0	0	0
Collagen, *Lupinus* flour, protein isolate or concentrate (g)	0	5	10	15	0.5	1	1.5	2.0

attached a 50-kg cell load and was configuring to run a test speed 1mm/s and 2 mm/s before and after the test. The samples used for this analysis were cut 100 mm of large and 25 mm diameter and each sample was assessed five times.

2.5 Kramer analysis of sausages prepared using collagen, flour, concentrate or protein isolate from Lupinus

Kramer device involves a combination of shearing and compression forces; the determinations were performed as described by Szczesniak (1963). Samples were sheared using a TA texture analyzer (TA Instruments, Surrey, UK) with a 10-blade Kramer shear compression attached, 3 mm thick and 100 cm wide blades that down through a box having a corresponding number of slots using a 50 kg load cell. The maximum peak force (kg) was recorded as the shear force required shearing through the sample.

2.6 Statistical Analysis

To evaluate collagen effect on texture properties of frankfurter sausages, a one-way analysis was used (0, 1, 2 y 3%). *Lupinus* flour, concentrate, and protein isolate was analyzed by two-way analysis using percentage used (0.5, 1, 1.5 y 2%) and *Lupinus* isolate, concentrate and flour as factors. All results were evaluated using a PROC GLM procedure and a Tukey test comparison by means of SAS software (SAS, 1999).

3. Results and discussion

3.1 Composition of Lupinus flour, protein isolate, concentrate and collagen extract

Table 2. Proximal analysis of *Lupinus* seed, flour, concentrate and collagen content of pork skin.

Analysis	*Lupinus* derivates				Pork skin extract
	Seed	HD	HDD	C	
Moisture (%)	9.0	9.0	9.9	9.6	ND
Ash (%)	3.8	2.5	2.7	2.5	ND
Protein (%)	34.6	41.5	43.8	83.1	57.92
Dietary fiber (%)	ND	7.1	8	ND	ND
Fat (%)	14.53	16	0	0	ND
Collagen (g/100g)	ND	ND	ND	ND	53.1

HD = Detoxified flour, HDD = Detoxified and defatted flour, C = Protein concentrate, ND= Non-determined

Proximate analysis of *Lupinus* flour, protein isolate and collagen content of extract of pork skin are shown in Table 2. Moisture values were between 9 and 9.95%, ash content was higher in seed than flour or concentrate of *Lupinus*. Ruiz and Sotelo (2001) reported similar results, but Güemes-Vera (2012) reported lower values. The protein percentage of concentrate of *Lupinus* was

83.12%, Ruiz and Sotelo (2001); Fraser *et al.* (2005) reported 40.73 and 36% for wild *Lupinus* and *Lupinus angustifolius*, respectively. Güemes-Vera (2012) analyzed *Lupinus mutabilis* and found 49.4, 70 and 93.5% of protein for flour, concentrate and isolate. The average fat was 14.53 and 16%, respectively. Ruiz and Sotelo (2001) reported 7.09% for wild *Lupinus*.

3.2 Effects of utilize Lupinus flour, protein isolate and concentrate in frankfurter texture

Results from TPA analysis are shown in Table 3. All variable analyzed showed differences (P<0.05) between treatments. Frankfurters elaborated using 2% of *Lupinus* protein concentrate had higher firmness than control. This effect is due to higher protein content and better water holding capacity; whereby both of these factors contribute to be a stable emulsion.

The adhesiveness was similar in all the treatments although the CON-150 showed greater value (P< 0.05). With respect to the resilience were significant differences (P< 0.05) between the witness and the other treatments. Springiness showed differences (P< 0.05) but only between CON-200 and the rest of the treatments. With regard to other studies in which diverse vegetable products were added to sausages with the aim of modifying their functional properties, the serum protein and the tapioca starch used in sausage manufacturing increased firmness, adhesiveness, gumminess, and chewiness. However, neither the effect on resilience nor on the cohesiveness of the product were observed (Hughes *et al.*, 1998). Osburn and Keeton (2004) used different flour levels of Konjac in the elaboration of lamb inlays with the purpose of reducing the amount to be used. When the TPA was performed, differences in hardness, chewiness, and gumminess, indicating that the fat diminution resulted in a dense product but with a greater proportion of myofibrillar protein for the intermolecular union, and for this reason a greater force for compression was required.

3.3 Effect of the protein inclusion extracted from pig skin on the sausage texture using the knife of Warner Bratzler.

Table 3 shows the results obtained for texture using Warner Bratzler blade where significant differences were observed (P<0.05). The sausages which were incorporated with 3% pig skin proteins showed the lowest firmness. These results were similar to those reported by Schilling *et al.* (2005), whose reported that the addition of 1.5% of collagen of chicken, in chicken products did not find any differences in the protein-protein union and concluded that collagen can improve the functional nature of proteins. Prabhu *et al.* (2004)

Table 3. Texture profile analysis of frankfurters added with *Lupinus* derivates.

Treatment	Hardness (g)	Cohesiveness	Resilience	Springiness (negative)
Control	1196.15 ± 90.38[b]	0.34 ± 0.01 [ab]	0.95 ± 0.09[a]	39.39 ± 4.29[a]
AIS-50	1016.31 ± 257.50[b]	0.29 ± 0.03[ab]	0.23 ± 0.01[b]	52.06 ± 36.70[ab]
AIS-100	776.70 ± 63.03[c]	0.24 ± 0.02[ab]	0.20 ± 0.01[b]	51.82 ± 19.47[ab]
AIS-150	1416.10 ± 182.79[ab]	0.20 ± 0.24[b]	0.21 ± 0.01[b]	83.13 ± 30.56[ab]
AIS-200	1469.83 ± 81.02[ab]	0.36 ± 0.12[a]	0.21 ± 0.01[b]	88.51 ± 13.57[ab]
CON-50	994.39 ± 3.71[b]	0.26 ± 0.05[ab]	0.20 ± 0.03[b]	67.06 ± 37.97[ab]
CON-100	1300.93 ± 56.94[ab]	0.25 ± 0.03[ab]	0.21 ± 0.01[b]	135.14 ± 87.23[ab]
CON-150	1141.44 ± 11.26[bc]	0.13 ± 0.04[b]	0.18 ± 0.02[b]	179.51 ± 147.01[b]
CON-200	1521.94 ± 49.99[a]	0.43 ± 0.13[a]	0.19 ± 0.02[b]	82.34 ± 51.93[ab]
HAR-50	1184.33 ± 36.13[b]	0.30 ± 0.02[ab]	0.24 ± 0.00[b]	86.27 ± 53.19[ab]
HAR-100	897.51 ± 203.77[c]	0.21 ± 0.03[b]	0.18 ± 0.00[b]	40.35 ± 8.44[a]
HAR-150	1165.82 ± 5.48[bc]	0.20 ± 0.03[b]	0.16 ± 0.03[b]	82.76 ± 1.56[ab]
HAR-200	1039.8 ± 0.78[bc]	0.20 ± 0.01[b]	0.30 ± 0.15[b]	113.21 ± 1.26[ab]

Different letters in same column indicate differences significantly (P<0.05).

also reported that no difference exists between treatments used in ham and sausages. Nevertheless, it was observed that as the levels of collagen in the product are increased, the values of maximum cut force increased. Choe *et al.* (2013) found that the addition of gelatin into frankfurter sausages increases hardness. However, Schilling *et al.* (2003) found that the use of collagen increases protein union using meat PSE. Several vegetal products modify functional properties of sausages. The tapioca starch is used in sausage manufacture for increase firmness, adhesiveness, gumminess, and chewiness (Hughes *et al.*, 1998). Osburn and Keeton (2004) reported that Konjac flour in sausage lamb decreases fat quantity, firmness, chewiness, and gumminess. When fat is low, the product is firm, and higher myofibrillar protein content is needed for intermolecular union.

3.4 Effects of protein extracted from pork skin on sausage texture using Warner bratzler blade

Sausage texture using collagen from pork skin is shown in Table 4. The firmness of the sausage was different between treatments (P<0.05), but there was no stress (P>0.05). Sausages added with 3% of pork skin protein showed less firmness. Chilling *et al.* (2005) observed that collagen from poultry improved the protein functionality and there was no interaction between proteins. Prabhu *et al.* (2004) showed no differences between treatments used in ham and sausages preparation. However, when collagen percentage was increased firmness values were higher. Kenney *et al.* (1992) reported restructured beef products using washed muscle mixed with 0.2% NaCl and observed that washing enhance instrumental texture measures of product.

Table 4. Effect of protein skin on texture of frankfurter.

Treatment	Kramer cell		Warner Bratzler	
	Maximum Force (g)	Firmness (g/s)	Maximum Force (g)	Firmness (g/s)
0	10377	121354	759.41[a]	9374
1	12129	133281	776.49[a]	9604
2	9671	115689	717.81[a]	9533
3	29809	83794	522.37[b]	7589

Different letters in same column indicate differences significantly (P<0.05).

Conclusion

The use of 3% of collagen produces less firmness of sausages using Warner Bratzler blade. There were no differences between the isolate, the concentrate or the flour of *Lupinus* in terms of control in firmness, cohesiveness, and springiness. The treatment which showed the least firmness was AIS-100, and the least cohesiveness was CON-150, control had the most resilience while CON-100 was the most adhesive treatment. The sausages added with isolate, concentrate or flour of *Lupinus albus* in quantities of 50, 100 or 150 g, respectively produce an excellent texture meat product. When 3% collagen was used, the texture of sausages is firm and should be recommended to be used for industrial production of meat products.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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