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Surimi-like protein ingredient from porcine spleen as lean meat replacer in emulsion-type sausages

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ABSTRACT

The aim of this work was to assess the influence of a porcine spleen surimi-like protein ingredient as pork meat replacer in emulsified cooked meat products (frankfurter-type sausages). The effects of the addition of porcine spleen protein isolate (SPI) in substitution of lean meat at concentrations of 5%, 10% and 15% on the physicochemical characteristics, microstructure, textural, and sensorial properties of the sausages were investigated. The addition of SPI did not affect the emulsion stability of raw meat batters nor the proximate composition of the cooked sausages, provided that sausages are formulated considering the differences in protein and fat content between pork meat and spleen protein fraction. Results showed that SPI was successfully applied as a meat replacer up to 15% of substitution level without producing significant modification on the physicochemical and techno-functional properties (water holding capacity and instrumental texture) of sausages. Meat replacement with SPI resulted in the formation of a stable and homogeneous protein gel network. Moreover, there were no negative effects on the sensory attributes in the cooked sausages containing 15% SPI as compared to the control ones. Therefore, the results of this study confirm that SPI up to 15% can be successfully used as a lean meat substitute in meat products.

1. Introduction

The world population is projected to rise from 7.8 to 9.9 billion by 2050, demanding at least to double the global food production (Population Reference Bureau (PRB), 2020). At the same time, diets are changing and achieving better nutritional outcomes across the globe due to economic growth and poverty reduction. However, the increasing wealthier segments of the population consume excessively large amounts of meat and dairy products. Meat production draws on intensive resources and is often inefficient, causing greater environmental impact than other food types (Wellesley, Happer, Froggatt, & House, 2015).

Meeting the protein gap will require utilising several alternative protein sources including not only plant crops (pulses, cereals, or oilseeds), insects, fungi, algae but also protein recovered from meat or fish processing by-products. There is an increasing market for alternative protein extracts and ingredients such as concentrates and isolates for their use as meat protein replacers, protein extenders, or meat analogues (Anzani, Boukid, Drummond, Mullen, & Álvarez, 2020). The utilisation of these protein sources contributes to a more sustainable future of the food industry while meeting the UN Sustainable Development Goals. Furthermore, developing products from meat by-products that can be used as ingredients rather than as finished products so that they can be integrated into food products is also a key strategy to ensure a sustainable development of the meat industry (European Commission, 2020; European Commission, 2019).

The meat industry generates large volumes of co-products and by-products that are costly to treat and dispose ecologically (Toldrá, Mora, & Reig, 2016). The carcass of slaughtered pigs represents about 70% of the total live weight, while the yield of edible meat by-products including offal (organs and viscera) is around 7–17% of porcine weight. Meat industry by-products are sources of high-quality proteins with excellent techno-functional properties and nutritional value, based on their amino acid profile. These products are nutrient-rich but are often discarded at the expense of loosing valuable nutrients and business opportunities. The majority of these by-products have a high protein content, between 15 and 20% (*w*/w), and good nutritional properties as they contain essential nutrients such as amino acids, vitamins, minerals, and fatty acids comparable to those that conform the muscular tissue (Alao, Falowo, Chulayo, & Muchenje, 2017; Rahman, Sahar, & Khan,

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2014). The underutilization of by-products not only leads to loss of potential revenue, but also results into an increase in their amounts and disposal costs (Shen, Zhang, Bhandari, & Gao, 2019).

Consequently, recovery and conversion of the meat by-products to human food would result in an increase of their added value. Likewise, the development of techniques that allow the efficient recovery and utilisation of low commercial value animal co-products as a source of cheaper and nutrient dense food ingredients has gained considerable interest (Boles, Rathgeber, & Shand, 2000; Lynch, Mullen, Neill, & Álvarez, 2018; Maysonnave et al., 2020; Parés et al., 2020; Selmane, Vial, & Djelveh, 2011).

The application of surimi technology for producing a surimi-like based material, obtained from meats other than fish, could provides a new approach towards increasing its value and utilisation at a reasonable cost. Earlier studies address the use of animal meat protein sources such as beef, pork, sheep meat, and poultry to develop surimi-like materials (Cortez-Vega, Fonseca, & Prentice, 2015; Tina, Nurul, & Ruzita, 2010; Villalobos-Delgado, Núñez-González, Alarcon-Rojo, & Silva-Avila, 2020). Research showed that surimi-like extracts display functional properties appropriate for their utilisation as meat or fat replacers, and these can be further utilised in the formulation of comminuted meat ingredients (Choi, Choe, Cho, & Kim, 2012; Ramadhan, Huda, & Ahmad, 2014; Seo, Yum, Kim, Jeong, & Yang, 2016).

The spleen is one of the less valued and most unexploited viscera generated in slaughterhouses. The porcine spleen contains about 17% protein with high biological value (Toldrà, Parés, Saguer, & Carretero, 2019) and also is a good source of iron with a high bioavailability value (Jayathilakan, Sultana, Radhakrishna, & Bawa, 2012). The adequate conditions to obtain protein extracts from porcine spleen (pH and salt concentration of the buffer solution and processing conditions), their physicochemical characteristics and techno-functional properties were determined previously (Toldrà, Parés, et al., 2019). Likewise, studies to determine the best conditions for washing the insoluble protein fraction from porcine spleen were also conducted. In a previous work, spleen-based protein extracts were successfully applied as techno-functional ingredients in emulsified cooked meat products, acting as sodium caseinate and/or soy protein substitutes (Toldrà, Parés, Saguer, & Carretero, 2020).

Spleen-based protein ingredients could be a suitable source of non-allergenic functional proteins for meat products due to their water-holding properties and acceptably effectiveness as fat emulsifiers. These characteristics also made porcine spleen surimi-like product a potential good replacer for pork meat or protein extender in meat products to optimize the valorization of underutilized meat by-products and satisfy the increasing global protein demand (Henchion, McCarthy, & O'Callaghan, 2016; Toldrà, Lynch, Couture, & Álvarez, 2019).

Therefore, this work aimed to determine the applicability of a surimi-like protein ingredient from porcine spleen as a lean meat replacer in cooked emulsified frankfurter-type sausages. The influence of replacing different percentages of ground pork meat by spleen protein ingredient was evaluated and compared through the assessment of the emulsion stability of raw meat batters, and the proximate composition, pH, water activity, internal colour parameters, water holding capacity, textural properties, microstructure, and sensorial characteristics of the frankfurter-type sausages.

2. Material and methods

2.1. Preparation of the spleen-based protein ingredient (SPI)

A total of 4 different batches (replicates) of insoluble protein fraction from porcine spleen productions were performed on different days but under the same conditions ($n = 4$), to obtain the amount of spleen-based protein ingredient (SPI) required to carry out all the experiments of meat substitution.

Fresh porcine spleens were supplied by a local industrial slaughterhouse (NORFRISA, Riudellots de la Selva, Girona, Spain) on the day of slaughter and transported refrigerated to the laboratory under hygienic conditions. Spleens came from male and female commercial crossbred pigs (Large White x Landrace x Pietran x Duroc), 6 months old and 100 kg of weight, fed with a diet based on cereals, soy, and vitamin supplements. Spleens were polished through removing fat and the splenic artery and vein with a scalpel and then cut into small cubical portions (1.5–2 cm side). Afterwards, a mixture of pieces from 4 to 5 different organs were packed in sterile polyethylene bags and frozen at −21 °C. Samples were thawed in the refrigerator at 4 °C overnight before the SPI preparation process.

According to previous studies (Toldrà et al., 2020), spleen pieces were first minced in a cutter SAMMIC CKE-5 (Sammic S.L., Barcelona, Spain) at 2000 rpm; subsequently, soluble proteins were extracted by mixing the ground spleens with sodium citrate buffer 0.1 M at pH 5 (1:0.5 *w/v*), at 400 rpm for 30 min. Insoluble and soluble fractions were separated by centrifuging at 20,000 x *g* for 15 min at 20 °C (Sorvall RC-SC plus, Dupont Co, Newton, CT, USA). The soluble fraction was decanted and discarded, and the insoluble fraction was submitted to three washing steps with tap water (1:5 w/v) at 400 rpm for 25 min in the cutter vessel. After each washing step, the insoluble protein fraction was filtered through a 1 mm mesh metal strainer and the coarse protein fraction retained by the sieve was reserved. The washing liquid was submitted to continuous centrifugation to further recover the insoluble fraction. A zonal TZ-28 rotor (Sorvall, Dupont Co, Newton, CT, USA), at an angular velocity of 23,000 x *g*, at 20 °C, and at 10 $L{\cdot}h^{-1}$ inlet flow, regulated by a Watson Marlow 313S Peristaltic Pump (Watson-Marlow Fluid Technology Group Global Office, Falmouth, UK) were used. The pellet recovered after continuous centrifugation was merged with the previously recovered insoluble fraction. The protein, moisture, and ash content, and the colour parameters of surimi-like spleen fraction were determined as described later in paragraph 2.3. Finally, SPI was vacuum packed in polyethylene bags and kept frozen at −21 °C until use.

2.2. Sausages production

In order to evaluate the applicability of the SPI in emulsion-type sausages, three trials (replications) consisting of laboratory scale emulsion-type sausage (frankfurter) productions were performed on different days but under the same conditions ($n = 3$). Each trial consisted of the production of four different substitution treatments containing 0% (control), 5%, 10% and 15% of SPI replacing lean meat. Formulations of sausages are presented in Table 1.

In each production, 1 kg of emulsified meat batter was made to obtain about 10–12 sausages. Raw materials, pork lean meat and back fat, from the same production lot were used in all the trials to minimize the variability factors between batches. Fresh pork ham (including main whole ham muscles *biceps femoris, semimebranosus,* and *semitendinosus*) and back fat were purchased in a local market. Connective tissue and visible fat were removed from the ham muscles. Lean and back fat were also previously cut into small cubes (1.5–2 cm side) and kept frozen at −21 °C until sausages manufacture. Aliquots of SPI from 4 batches were mixed and homogenized to be used in sausages' productions. The proportion of ingredients (lean meat and ice) used to produce 1 kg of sausage batter was adjusted based on the percentage of protein substitution, as well as considering the protein and moisture content of both meat and spleen protein extract.

Emulsion batter's preparation and stuffing, as well as frankfurter pasteurization of each treatment replication, were conducted according to the procedure reported by Toldrà et al. (2020). Sausages' productions were performed using a Stephan UMC-5 universal machine (Stephan Machinery GmbH, Hameln, Germany) and a RCWF-3L-H sausage stuffer (Royal Catering, Madrid, Spain). Frankfurters were stuffed into 20 mm diameter cellulose casings (ViskoTeepak N.V., Lommel, Belgium) and cooked in a water bath at 80 °C until reaching a minimum core temperature of 70 ± 2 °C and achieving a pasteurization value equivalent to

Table 1
Formulation (expressed in g·kg^{-1}) of frankfurter-type sausage as function of different substitution treatments.

Ingredient	Control (g/kg)	5% replacement (g/kg)	10% replacement (g/kg)	15% replacement (g/kg)
Pork lean meat	440	418	396	374
Pork back fat	180	180	180	180
Ice	315	296	278	259
Spleen protein fraction	0	41	81	122
Sodium chloride [a]	17	17	17	17
Sodium nitrite [a]	0.2	0.2	0.2	0.2
Sucrose	15	15	15	15
Sodium caseinate [b]	15	15	15	15
Maize starch	10	10	10	10
Sodium polyphosphate [a]	5	5	5	5
Sodium ascorbate [b]	0.5	0.5	0.5	0.5
Dehydrated smoke aroma[c]	0.5	0.5	0.5	0.5
Black pepper powder	1.5	1.5	1.5	1.5

[a] Panreac Química S.A. (Barcelona, Spain).
[b] Induxtra de Suministros (Girona, Spain).
[c] BDF Natural Ingredients S.L. (Girona, Spain).

13 D_{70} for *Streptococcus faecalis* (PV10/70 = approx. 40 min). After cooking, the sausages were washed and immediately cooled by immersion in cold water at 4 °C. Frankfurters were dried in a laboratory oven Conterm (JP Selecta, Abrera, Barcelona, Spain) at 55 °C for 15 min and at 80 °C for 5 min, before and after cooking, respectively. Finally, all sausages were vacuum-packed (6 units per bag) and stored at 4 ± 2 °C in a domestic refrigerator until further analyses within 2–3 d.

2.3. Physicochemical analysis

Standard methods were used to analyse the proximate composition of the raw material (SPI), as well as the frankfurter-type sausages. The proximate composition was carried out on homogenates obtained by mincing samples in a blades grinder (Moulinex Moulinette MR, Écully, France). Moisture, ash, protein, and fat contents were determined by following the procedures of the Association of Official Analytical Chemists methods (AOAC (Association of Official Analytical Chemists), 2000). Moisture and ash contents were determined gravimetrically using a hot air oven at 110 °C overnight and a muffle furnace at 550 °C for 24 h, respectively (AOAC 950.46 and 920.153, respectively). Protein content was determined through the Kjeldahl method by using a semi-automatic digestion system Gerhardt KB20 (Gerhardt GmbH & Co. KG, Königswinter, Germany) and a distillation unit Buchi K314 (Büchi Labortechnik AG, Flawil, Switzerland). Protein content was calculated by multiplying by 6.25 the total Kjeldahl nitrogen (AOAC 928.08). Total fat content was estimated by Soxhlet extraction with diethyl ether (AOAC 991.36). A colorimetric method for the determination of hydroxyproline content was used (Kolar, 1990). Total collagen content was calculated by multiplying the percentage of hydroxyproline by 8.

The pH measurements were carried out using a pH meter with a penetration electrode (LPG Crison 22, Barcelona, Spain) directly placed into minced samples. The water activity (a_w) of minced samples was analysed at 25 °C in a Labmaster electrolytic hygrometer (Novasina AG, Lachen, Switzerland).

All measurements were determined at least in duplicate for each type of sausage.

2.4. Emulsion stability determination

Emulsion stability of raw meat batters was measured as described in Toldrà et al. (2020). Approximately 10 g (exact weight recorded) of each meat batter formulation were placed in a 50 mL polycarbonate centrifuge tube (six samples per each treatment per replication) and centrifuged at 2000 x*g* for 1 min at 15–20 °C (Sorvall RC-SC plus, Dupont Co, Newton, CT, USA). After that, the tubes were placed in a water bath at 70 °C and heated for 30 min, and subsequently centrifuged again at 2500 x*g* for 3 min. The pelleted samples were removed and weighed; the percentages of TEF (Total Expressible Fluid) were calculated from the weight loss caused by the combined treatment of heating and centrifuging. The supernatants were poured into crucibles and weighed, dried overnight in an oven at 100 ± 2 °C, and reweighed. The residues remaining in the crucibles after drying were used to calculate the percentage of fat in the TEF.

2.5. Water holding capacity (WHC)

Water holding capacity of cooked sausages was determined following the method reported by Parés et al. (2018). Cores (10 ± 1 g) of the sausages from each treatment were cut and placed in glass jars, closed, and heated in a water bath at 90 °C for 10 min. After heating, samples were cooled to room temperature, carefully removed from the jar using forceps, wrapped in cotton cheesecloth, and placed into 50 mL polycarbonate centrifuge tubes (containing absorbent cotton wool at the bottom). Samples were centrifuged at 9000 x*g* for 10 min at 4 °C (Sorvall RC-SC plus, Dupont Co, Newton, CT, USA). After centrifuging, the cheesecloth was removed, and sample weights were recorded. The results were reported as percentage (*w*/w) of water retained after centrifugation relative to the total water content of the sample. Three samples were measured for each treatment per replication.

2.6. Texture profile analysis (TPA)

The textural properties of cooked sausages were analysed one day after production, using a Texture Analyser TA.XT2 (Texture Technologies Corp., Scarsdale, NY, USA). Three cores (diameter = 2 cm; height = 1.5 cm) were cut from different sausages per each treatment per replication and were axially compressed by a two-cycle compression test to 50% of their original height using an aluminium 50 mm cylindrical probe. Determinations were performed at room temperature (22–23 °C). Force-time deformation curves were recorded with a 25 kg load cell at a crosshead speed of 1 mm/s. Hardness, adhesiveness, springiness, cohesiveness, and chewiness were calculated. Hardness (expressed in N) is the peak force required for the first compression; adhesiveness (in Ns) is the negative force area after the first compression; springiness (%), considered as the distance the sample recovers after the first compression as related to the original height; cohesiveness (dimensionless) is the ratio of positive force peak area of the second and the first compression; and chewiness (N·mm) expressed as the product of hardness by cohesiveness and springiness.

2.7. Colour properties

The colour properties of spleen protein fraction and the internal colour of cooked sausages were measured by determining the CIE L*, a*

and b* colour parameters using a Minolta Chroma Meter CR-400 with a CR-A33f glass light projection tube (Minolta Co, Ltd., Japan). Results were expressed as L* representing the lightness on a scale of 0 (dark) to 100 (white); a* the redness-greenness value; and b* the yellowness-blueness value. Measurements were made using diffuse illumination, a D_{65} light source and 2° standard observer. The colorimeter had been calibrated using a standard white ceramic plate (L* = 97.15, a* = −5.28 and b* = +7.82). The colour parameters were measured in triplicate per each treatment per replication on the inner face of sausages slices (1 cm height). The angular coordinates of Chroma (C*) and Hue angle (h°) were calculated according to the following equations: $C^* = \sqrt{(a^{*2} + b^{*2})}$ and $h^\circ = \tan^{-1}(b^*/a^*)$.

Also, the total colour differences (ΔE*) of each spleen-based sample respect to control sausage were calculated as: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$, where ΔL*, Δa* and Δb* are the differences between the L*, a*, and b* values of the treatment and control samples.

2.8. Microstructure

The microstructure of cooked sausages was examined using Scanning Electron Microscopy (SEM) according to the procedure described by Parés et al., 2018. Small cubes of sausages were obtained by cutting from the interior of the frankfurter samples and fixed with a mixture (*w/v*) of glutaraldehyde 2.5% and sodium cacodylate 0.1 M buffer (pH 7.4), at 4 °C for 2–4 h. After first washing with cacodylate buffer and after that twice with distilled water, samples were successively dehydrated in ethanol by subjecting them to a serial of increasing concentrations from 50% to 100%, dried at the critical point with CO_2 as transition fluid in an Emitech K850 CPD instrument (EMIntegrated Technology, Kent, UK), and carbon-coated in an Emitech K950 Turbo Evaporator. Samples were examined under a Hitachi S-4100 FE-SEM microscope (Hitachi High-Technologies Corporation, Tokyo, Japan). At least two representative pictures at each magnification (×100, ×500, and x5000) were taken for each sample. Quartz PCI software (Quartz Imaging Corporation, Vancouver, Canada) was used to acquire and process digital images.

2.9. Sensorial analysis

The sensorial analyses were performed on control and test sausages containing 15% of SPI replacing lean meat. The sensory evaluation of frankfurters was assessed by a six-member expert panel of IRTA (Institute of Agrifood Research and Technology, Monells, Girona, Spain). Panellists had been trained and chosen according to international norms ASTM (1981); ISO 8586-1 (1993); and ISO 8586-2 (1994), with a minimum of 3 years' experience in making quantitative descriptive profiles of meat products. The appropriate descriptive attributes that best described the sausages samples and characterize their sensory properties was agreed on by the assessors in a previous attribute generation session of open discussion. A total of 18 descriptors (2 for odour, 5 for texture in mouth and 3 for tactile texture, 5 for flavour and 3 for visual appearance) were selected for the final descriptive profile of frankfurters (Table 2).

A complete block design over a three-session period was used to provide three replicate assessments ($n = 3$) for each treatment (control and 15% SPI) and balancing the possible effect of the order of samples' presentation (Macfie, Bratchell, Greenhoff, and Vallis (1989). Samples were presented fresh at room temperature for the evaluation of visual appearance and tactile texture; after that, vacuum packaged sausages (CRYOVACK Cooking HT 3000, Sealed Air, Italy) were immersed in boiling water and kept at 100 °C for 2 min, and afterward served for taste and odour/flavour evaluation. In each session, randomized samples of one whole sausage per each treatment were presented to each assessor; samples were coded with three-random numbers and were presented in a white plastic plate of 17 cm diameter to the assessors balancing the first order and the carry-over effects according to Macfie et al. (1989). Each assessor cut each sausage into two parts; one-half was used to assess the visual appearance and tactile texture and the other to assess the smell, taste and texture in mouth. The attributes included in the descriptive profile were evaluated using a non-structured quantitative scale (Amerine, Pangborn, & Roessler, 1965) from 0 (absence) to 10 (maximum intensity). The sensory evaluation sessions were carried out in a standardized sensory room (UNE, 1979) under white light (8 W daylight fluorescent) at 20 ± 2 °C. Each assessor had bread sticks and room temperature mineral water to rinse their palates between samples.

Table 2
Sensory attributes included in the descriptive profile of frankfurter-type sausages with their description and evaluation procedure.

Attribute	Description	Evaluation method
Odour		
Smoked	Intensity of odour associated with burning wood	Assessment of the attribute by performing 3 or 4 short inhalations repeatedly
Animal	Intensity of odour associated with live animals (includes barn and feather)	
Texture in mouth		
Crunch	Auditory sensation related to resistance to bite	To compress the sample transversely between the molars to break its outer surface
Cohesiveness	Binding degree of the meat product	To evaluate the attributes during chewing of a 2 cm cross section of the product
Crumbliness	Degree of product desintegration during chewing	
Graininess	Sensation of granules or small particles during chewing	
Juiciness	Amount of liquid released when chewing the sample	
Flavour/Taste		
Smoked	Flavour associated with smoke given off by burning wood	To evaluate the attributes during chewing of a 1 cm cross section of the product (to rinse the mouth with water and bread sticks between samples)
Sweet	Basic taste associated with sugars such as sucrose or lactose	
Salty	Basic taste associated with common salt (NaCl)	
Lactic notes	Flavour associated with milk	
Animal	Flavour associated with live animals (includes barn and feather)	
Visual appearance		
External colour (from ivory-pink to greyish)	Greyish colour intensity of the external surface of the sample	Assesment of the attribute under normalized light
Cylindrical appearance	Appearance associated with an elongated geometric body of circular section	
Internal colour (from ivory-pink to greyish)	Greyish colour intensity of the internal area of the sample	Assessment of the attribute in a cros-section of the sample
Tactile texture		
Sponginess	Elastic and porous structure	Assessment of the attribute in in a cros-section of the sample
Turgidity	Quality of being firm and tight	To hold the sample transversely by the center with the index finger and thumb making a slight movement from top to bottom and from bottom to top
Exudate	Amount of liquid released when a force is applied to the sample	To press transversely half of a sausage with the index finger and thumb and to observe the section

2.10. Statistical analysis

The statistical analyses were carried out using the SPSS software package version 25 for Windows (IBM SPSS Statistical software Inc., Chicago, IL, USA). In all cases, data were submitted to ANOVA using the general linear model procedure (Proc GLM) and considering spleen protein ingredient concentration as a fixed effect, and replication (experimental batch) as a random effect. When significant effects were obtained, the Tukey's test was used for the post hoc analysis to compare means. For the sensory evaluation data, ANOVA variance analysis was applied including product and assessor as fix effects and session-in-assessor as random effect. The significance level for all tests was established at $P < 0.05$.

3. Results ANS discussion

3.1. Physicochemical analysis

The mean values of SPI composition are shown in Table 3. Apart from its moisture (86.82%), protein is the main component of SPI (11.14%). Due to the additional washing process stage, the protein content of dry matter was lower than the insoluble spleen protein extract previously produced by Toldrà et al. (2020). Likewise, the CIE L*a*b* colorimetric parameters showed that the spleen protein fraction had a more neutral colour (light pink), because of the more intense drag of heam and myoglobin chromatic proteins during the washing process, coinciding with previous studies (Toldrà et al., 2020).

On the other hand, regarding the composition of the frankfurter-type sausages (Table 4), values were at the normal ranges for these types of products (Novakovic et al., 2019). There were no significant differences ($P > 0.05$) in the moisture, fat and collagen contents between control and test formulations up to 15% meat substitution. Nevertheless, significant differences ($P < 0.05$) in protein and total ash contents between control and 15% protein substitution sausages were found, even though sausages formulations have been calculated so that all frankfurters (control and treatments) would have a similar composition. Sausages with 15% of SPI had slightly higher protein content (12.7%) compared to control ones (11.4%), whereas protein values in sausages with 5% and 10% of SPI were not significantly different from the other treatments. Similarly, it can be observed that the percentages of total ash did not indicate much variation between all types of sausages, even though sausages with 5% of SPI had a little higher but significant ($P < 0.05$) ash content (1.9%) than control ones (1.8%). The small differences in protein and ash contents between treatments can be due to uncontrollable sources of experimental error when formulating meat products. The composition of the sausages were similar to other emulsion-type sausages with pork meat (Álvarez, Drummond, & Mullen, 2018; Choe, Kim, Lee, Kim, & Kim, 2013; González-Viñas, Caballero, Gallego, & García Ruiz, A., 2004; Seo et al., 2016).

Neither the water activity (a_w) nor pH values of the sausages were affected by the replacing treatment ($P > 0.05$), being similar values (a_w = 0.95–0.96; pH = 6.9–7.0) to those observed in frankfurter samples by Pereira et al. (2011) and Massingue et al. (2018) in sausages elaborated with mechanically deboned poultry meat.

Table 3
Proximate composition (%, wet basis) and CIE L*a*b* colour parameters of spleen protein fraction (means ± standard deviation, $n = 4$).

Parameters	
Moisture (%)	86.82 ± 1.08
Protein (%)	11.14 ± 1.99
Total ash (%)	0.52 ± 0.14
L* (lightness)	67.21 ± 1.31
a* (redness)	4.67 ± 0.87
b* (yellowness)	13.91 ± 0.83
C* (Chroma)	14.68 ± 1.05
H° (Hue)	71.57 ± 2.90

Table 4
Emulsion stability of raw meat emulsion batters, expressed as total expressible fluid (TEF) and fat content (FAT) in TEF, and proximate composition (%, wet basis), water activity and pH of frankfurter-type sausages prepared with different levels of spleen protein fraction. Values are mean ± standard deviation ($n = 3$).

Parameters	Substitution level			
	Control (0%)	5%	10%	15%
Raw batter				
TEF	5.19 ± 0.13 [a]	5.03 ± 0.63 [a]	5.47 ± 1.14 [a]	5.81 ± 0.51 [a]
FAT (%)	9.74 ± 0.26 [a]	10.08 ± 0.29 [a]	10.25 ± 0.18 [a]	10.06 ± 0.44 [a]
Proximate composition				
Moisture (%)	66.25 ± 1.5 [a]	66.25 ± 0.49 [a]	65.75 ± 1.67 [a]	66.19 ± 1.76 [a]
Protein (%)	11.42 ± 0.57 [a]	12.06 ± 0.50 [ab]	12.39 ± 0.15 [ab]	12.69 ± 0.57 [b]
Fat (%)	19.10 ± 1.40 [a]	18.23 ± 0.90 [a]	18.96 ± 1.00 [a]	18.34 ± 0.55 [a]
Total ash (%)	1.78 ± 0.10 [a]	1.90 ± 0.07 [b]	1.86 ± 0.06 [ab]	1.82 ± 0.10 [ab]
Collagen (%)	2.21 ± 0.27 [a]	2.56 ± 0.3 [a]	2.83 ± 0.22 [a]	2.24 ± 0.26 [a]
Physicochemical				
a_w (25 °C)	0.955 ± 0.02 [a]	0.945 ± 0.03 [a]	0.957 ± 0.02 [a]	0.960 ± 0.01 [a]
pH	6.91 ± 0.14 [a]	6.86 ± 0.13 [a]	7.01 ± 0.07 [a]	7.00 ± 0.09 [a]

Different letters across columns indicate significant differences between treatments ($P < 0.05$).

3.2. Emulsion stability of meat batters

The emulsion stability of the raw batter and the overall emulsifying quality of the meat emulsion can be estimated throughout TEF and FAT parameters of the raw batter formulation and the WHC of cooked sausages. In our study, no significant differences ($P > 0.05$) could be appreciated on frankfurter-type sausages in terms of TEF (Table 4). Thus, protein from the spleen extract incorporated in sausages performs as well as lean meat protein in terms of emulsifying capacity. Moreover, there were no significant differences ($P > 0.05$) with the fat content of the expressible liquid fluid (FAT), indicating that the spleen-based ingredient also resulted as effective as meat protein as a fat binder.

3.3. Water holding capacity (WHC)

The WHC of the cooked sausages was also the same for control sausages and sausages containing SPI (Table 5). In the same way as TEF values, WHC of cooked frankfurters was not significantly affected ($P > 0.05$) by the meat replacement with spleen protein fraction. Good water retention capacity is important not only for a reasonable yield but also to prevent exudation inside the sausages' package during the storage and commercialization periods as well as to guarantee a juicy meat product after the culinary treatment before consumption (Toldrà et al., 2020).

3.4. Textural properties

According to the results of the texture profile analysis (Table 5), no significant differences between control and test frankfurters were found ($P > 0.05$), which is consistent with the proximate composition and techno-functional results exposed before. The texture profile of meat products is related to the water holding capacity of proteins, emulsification properties, and interactions between protein molecules (Choe et al., 2013). In general, the textural parameters analysed were comparable to those obtained by other authors in similar studies (Massingue et al., 2018; Toldrà et al., 2020). Although not significantly different, a

Table 5
Water-holding capacity (WHC), TPA parameters, and internal colour parameters of frankfurter-type sausages prepared with different levels of spleen protein fraction.

Parameters	Substitution level			
	Control (0%)	5%	10%	15%
WHC (%)	78.49 ± 1.45 [a]	80.04 ± 1.45 [a]	81.43 ± 3.45 [a]	80.33 ± 6.01 [a]
Texture Profile Analysis (TPA)				
Hardness (N)	17.63 ± 0.67 [a]	18.91 ± 1.12 [a]	20.27 ± 1.53 [a]	19.04 ± 2.24 [a]
Adhesiveness (Ns)	−0.54 ± 0.08 [a]	−0.48 ± 0.03 [a]	−0.58 ± 0.02 [a]	−0.55 ± 0.04 [a]
Springiness (%)	82.27 ± 1.86 [a]	82.91 ± 1.35 [a]	81.60 ± 0.60 [a]	81.8 ± 0.29 [a]
Cohesiveness	0.66 ± 0.02 [a]	0.66 ± 0.01 [a]	0.66 ± 0.00 [a]	0.67 ± 0.01 [a]
Chewiness (N·mm)	138.44 ± 8.95 [a]	149.84 ± 11.16 [a]	160.92 ± 10.16 [a]	156.23 ± 32.60 [a]
Internal CIE colour parameters				
L* (lightness)	72.15 ± 0.43 [a]	71.93 ± 1.18 [a]	72.58 ± 2.01 [a]	71.51 ± 1.14 [a]
a* (redness)	6.38 ± 0.19 [a]	6.68 ± 0.24 [a]	6.13 ± 0.67 [a]	6.15 ± 0.59 [a]
b* (yellowness)	9.25 ± 1.13 [a]	9.24 ± 0.46 [a]	9.67 ± 0.48 [a]	10.02 ± 0.66 [a]
C* (Chroma)	11.25 ± 1.04 [a]	11.41 ± 0.25 [a]	11.47 ± 0.35 [a]	11.78 ± 0.36 [a]
H° (Hue angle)	55.27 ± 2.41 [a]	54.07 ± 2.28 [a]	57.62 ± 3.72 [a]	58.41 ± 3.87 [a]
ΔE*	–	1.45 ± 0.61	2.14 ± 0.78	1.62 ± 0.46

Different letters across columns indicate significant differences between treatments ($P < 0.05$).
Values are means ± standard deviation ($n = 3$).

slight increase in hardness was observed as a function of SPI addition, which could be related to the slightly higher total protein content. This tendency for increased hardness was also reported by Seo et al. (2016) when pork meat protein was replaced by cattle heat surimi on Frankfurter sausages formulation.

3.5. Colour parameters

Due to the washing process stages of the insoluble spleen fraction, residual haem and myoglobin pigments present in the splenic protein ingredient had been removed and SPI showed higher L* values (lightness) and lower values of a* (redness) coordinates (Table 3) compared with pork meat colour (Fernández-López, Sayas-Barberá, Pérez-Alvarez, & Aranda-Catalá, 2004). Usually, a* values have been related to meat red components (haem pigments). Nevertheless, no significant differences ($P > 0.05$) regarding the CIE L*a*b* internal colour parameters of control and test cooked sausages samples were observed (Table 5). The CIE L*a*b* absolute values obtained were also close to those measured in other studies conducted with Irish breakfast sausages (Álvarez et al., 2018) and frankfurters manufactured with lean pork and beef (Seo et al., 2016). Since no colouring additives were added to sausages, the colour of both control and spleen ingredient containing samples was clearer and paler as well as less yellow than other frankfurters described in the literature (Hayes, Desmond, Troy, Buckley, & Mehra, 2005). Fig. 1 shows one sample of each treatment, in which no colour differences could be noticed visually. Likewise, the total colour differences values (ΔE*) between frankfurters with added spleen protein with respect to control samples were between 1.45 and 2.14 units. The variable ΔE* measures the total colour change by accounting for combined changes in L*, a*, and b* parameters. According to Fernández-López et al. (2019), only values of total colour differences for ΔE* > 3 units were visually detectible and would be distinguished by an observer. Therefore, the addition of spleen protein fraction did not have any negative effect on the instrumental colour attributes of the sausages.

Fig. 1. Frankfurter-type sausages prepared with different percentage of porcine spleen protein fraction (control, 5%, 10%, and 15%).

3.6. Microstructure

Scanning electron microscopy (SEM) was conducted to investigate the protein matrix structure of the sausages and to evaluate the effects of lean pork substitution with SPI on the sausages microstructure. Fig. 2 shows the microphotographs of frankfurters samples prepared with different percentages of spleen protein fraction replacing lean meat.

Overall, common features are observed in all microstructures, such as a spongy appearance caused by dense protein aggregates with irregular cavities, as well as the presence of fat globules and some salt crystals dispersed in the continuous protein matrix. Similar structures had been previously described for this kind of meat product (Ayo, Carballo, Solas, & Jiménez-Colmenero, 2008; Hurtado, Saguer, Toldrà, Parés, & Carretero, 2012; Parés, Saguer, Pap, Toldrà, & Carretero, 2012; Tahmasebi, Labbafi, Emam-Djomeh, & Yarmand, 2016). Lots of similarities could be appreciated with the microstructure described in analogous emulsified meat products by Horita, Messias, Morgano, Hayakawa, and Pollonio (2014) and Hurtado et al. (2012). Microstructure from frankfurters were slightly porous and consisted of a coarse structure with few cavities, mixed with meat aggregates and with the fat globules finely suspended in the continuous protein matrix. These irregular conformations were very similar to the spongy structures reported by Jiménez-Colmenero, Herrero, Pintado, Solas, and Ruiz-Capillas (2010) and Tahmasebi et al. (2016) in pork frankfurters.

When the effect of meat replacement on the morphology of the meat matrix is analysed, the most noticeable tendency is a small reduction in the size of pores and the sponginess of the matrix when the percentage of replacement increases, with this trend being most noticeable in SEM images of 100 and 500 magnification (Fig. 2). This lower fluffiness could be related to the slightly higher protein content and, likewise, with the tendency of higher hardness of the treatment sausages with respect to control ones, though no significant differences were observed in textural properties. In any case, these small differences in the microstructure of sausages with different SPI contents did not led to differences in other technofunctional parameters, such as WHC and texture parameters.

3.7. Sensory evaluation

Table 6 presents the results of the ANOVA for the attributes included in the profile of the sausages samples. It was observed that both types of samples have a slight intensity of animal smell and taste, mild smoky smell, intermediate salty taste and low milky notes, and a medium intensity of sweetness and smoky flavour. In terms of texture, both types of samples show a knack, cohesiveness, moderate crumbling, granularity, juiciness, sponginess and turgidity and a slight exudate after cooking. It is noticeable that no significant differences ($P > 0.05$) were found between control and test sausages in terms of odour, flavour/taste, texture in mouth, cylindrical visual aspect, and tactile texture attributes.

Regarding the appearance attributes, significant differences ($P <$ 0.05) were observed only for internal and external colour, being from

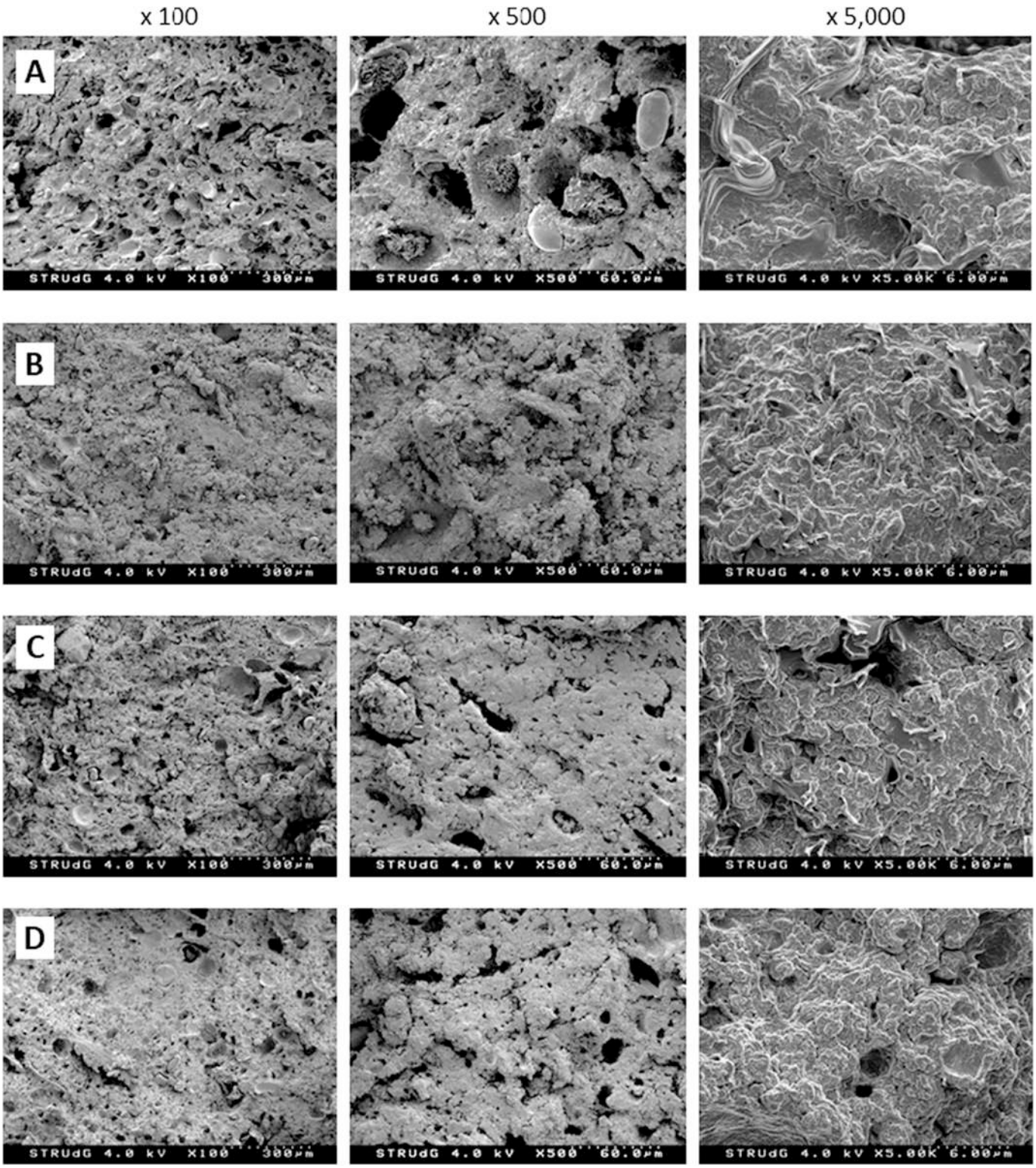

Fig. 2. Scanning electronic microscopy (SEM) images of frankfurter-type sausages formulated with different percentage of porcine spleen protein fraction: control (A), 5% (B), 10% (C), and 15% (D). Images magnified x 100, x 500, and x 5000 (from left to right).

ivory-pink to greyish. Specifically, the treatment samples (with 15% SPI) present a greyer colour, and less pink, than the control ones, despite the values of the instrumental colour parameters not being significantly different. In fact, only experienced observers would be able to discriminate colour differences ($1 < \Delta E^* < 2$) (Álvarez et al., 2018). Notwithstanding, the small differences between the two kinds of samples could be minimized by the application of food colouring.

4. Conclusions

The results of this study showed that the insoluble porcine spleen fraction can be successfully applied to emulsified meat sausages at 15% or less as a pork lean meat replacer without producing significant modification on the physicochemical and techno-functional parameters, as long as they are formulated considering the difference in composition

Table 6
Results of sensory analysis: attributes intensity for both control and 15% spleen protein fraction (test) cooked frankfurter-type sausages.

Attributes	*P*-value	Substitution level	
		Control (0%)	Test (15%)
Odour			
Smoked	0.189	4.1 ± 1.57	3.7 ± 1.26
Animal	0.492	2.0 ± 1.82	1.8 ± 1.31
Texture in mouth			
Crunch	1.000	3.6 ± 1.01	3.6 ± 1.15
Cohesivity	0.540	4.3 ± 0.96	4.1 ± 0.87
Crumbliness	0.175	5.0 ± 1.51	5.4 ± 1.35
Graininess	0.961	3.9 ± 1.59	3.9 ± 1.91
Juiciness	0.882	5.1 ± 1.64	5.0 ± 1.60
Flavour/Taste			
Smoked	0.914	3.3 ± 1.85	3.3 ± 1.57
Sweet	0.236	4.3 ± 1.13	3.9 ± 1.36
Salty	0.211	2.1 ± 1.16	2.2 ± 0.99
Lactic notes	0.320	1.8 ± 0.60	2.0 ± 1.57
Animal	0.580	1.2 ± 1.43	1.1 ± 1.04
Visual appearance			
External colour (from ivory-pink to greyish)	**<0.0001**	**2.8^{b}** ± 1.15	**5.0^{a}** ± 1.03
Cylindrical aspect	0.518	5.5 ± 1.85	5.9 ± 1.90
Internal colour (from ivory-pink to greyish)	**<0.0001**	**2.6^{b}** ± 1.45	**4.9^{a}** ± 1.55
Tactile texture			
Sponginess	0.198	3.9 ± 1.28	4.4 ± 1.18
Turgidity	0.389	4.6 ± 1.25	4.3 ± 0.94
Exudate	0.734	3.2 ± 1.41	3.0 ± 1.47

Different superscript letters indicate significant differences ($P < 0.05$).
Values are means ± standard deviation ($n = 3$).

between the pork meat and the insoluble protein fraction. Texture and WHC were not affected by the replacement, according to instrumental analyses. Regarding the sensory characteristics, no significant changes in the flavour, taste, odour, and texture attributes were found in sausages with 15% SPI added. Although panellists only reported significant differences in terms of colour between control and 15% SPI sausages, the instrumental colour attributes were not significantly modified.

The results highlight the potential use of porcine spleen extract as a protein ingredient in the food industry. The incorporation of spleen-based protein extract into meat products as meat replacer or meat extender is a good strategy to upgrade the meat by-product and contribute to the sustainability of the meat industry. Therefore, spleen protein can be used in sausage formulations in order to increase the access of the world population to proteins, increasing the competitiveness of meat industries and diminishing environmental pollution.

Author statement

Each author has made substantial contributions to the present work and has approved the submitted version. Their individual contributions are specified below.

Declaration of Competing Interest

None.

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