Development and utilization of pork skin emulsion in mortadella as a soy protein substitute

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Abstract
The aim of this study was to develop an emulsion formulation of pork skin and evaluate the effect of partial substitution of soy protein for this emulsion in a formulation of mortadella. For the emulsion development was used a 23 factorial experimental design with central point in order to evaluate the best process condition in terms of amount of pork skin (60-80%), salt concentration (1.5 to 2.5%) and emulsifier disk size (2 to 3 mm). From the experimental design set up the best conditions for the preparation of the emulsion pork skin later to be applied in the mortadella formulation. Only the lipid emulsion content was influenced significantly (p <0.10) by the amount of pork skin, salt and by the discs emulsifier hole size. The pork skin emulsion was used in a mortadella formulation and compared to a standard formulation (similar to commercial). For the test formulation 6.4% of emulsion skin and 2.9% of soy protein (reduction of 27.5% in soybean protein added) were used. Mortadella developed met the standards established by the Brazilian legislation and analysis of texture showed higher hardness, cohesiveness, chewiness and adhesiveness (p <0.05) for the mortadella with pork skin emulsion against standard mortadella. In the sensory evaluation, the mortadella with pork skin emulsion was preferred by 71.4% of the panelists. Based on the results obtained, it was concluded that it is feasible to use pork skin emulsion in mortadella and this may partially replace soybean protein without harming the quality of the product and allowing to add value to this byproduct of the meat industry.

Keywords
Soy substitutes
Collagen
Optimization
By-products
Meat products
Texture

Introduction
In the meat industry, a major goal is to obtain the highest yield and commercial utilization of slaughter by-products in order to reduce environmental impact and improve economic performance, or at least, reduce the cost of managing these wastes (Brasil et al., 2014).

The pork skin is considered an edible byproduct of slaughter and represents about 3% to 8% of the live weight of the animal (Ockerman and Hansen, 1994). It is commonly used as feedstock for the production of collagen and gelatin (Nollet and Toldrá, 2011). In Europe about 80% of the edible gelatin is extracted from pork skin (FSA, 2006). Products derived from skin have been used in foods, drugs and cosmetics.

One of the ways to use the skin in meat products is through emulsions. Emulsions consist of two immiscible liquids (usually oil in water) in which one liquid is dispersed in another in the form of small droplets (McClements, 2005). Emulsifiers to stabilize the emulsions are used, among them are proteins. In the meat industry soy protein is most used mainly in emulsified products such as sausages and mortadellas. The mortadellas are among the meat products the most commonly consumed in Brazil and according to the latest survey data from the IBGE (2010) the consumption reached 0.827 kg/per capita/year, whereas the South Region showed higher domestic consumption (1.599 kg/capita/year).

The study of the emulsifying properties of collagen have been reported in recent studies (Santana et al., 2011; Santana et al., 2012; Moraes and Cunha, 2013; Choe et al., 2013). The skin application in the form of emulsion in mortadella may be an alternative to avoid the “off-flavor” caused by soy protein (Hsu and Sun, 2006; Youssef and Barbut, 2011) and reduce the texture problems and release of liquid, without modifying the organoleptic characteristics of the product (Prabhu and Doerscher, 2003), in addition to providing cost reductions in the formulation (Schilling et al., 2003). According Feiner (2006), the addition of...
skin emulsion is common in sausages formulations, because it contributes to the improvement of texture, because the collagen becomes gelatin as a result of heat treatment.

The aim of this study was to develop an emulsion formulation of pork skin and evaluate the effect of partial substitution of soy protein for this emulsion in a mortadella formulation.

**Material and Methods**

**Preparation of pork skin emulsion**

The development of the pork skin emulsion and sensory evaluation were conducted in a Food Industry at Chapecó, SC, Brazil. The physico-chemical analysis and the mortadela development were performed in URI Erechim (Erechim, RS, Brazil). The texture profile was developed at Federal University of Santa Maria (Santa Maria, RS, Brazil). The study was previously approved in the URI Erechim Ethics Committee under the number 23479013.3.0000.5351.

For the development of the different formulations of pork skin emulsion were used a 2³ factorial design with three replications in the center point as shown in Table 1. The dependent variables or responses analyzed were: moisture, water activity (Aw), proteins, lipids and collagen. For the development of the emulsions was followed the procedure suggested by Sadler and Young (1993) and Choe et al. (2013) with some modifications as follows: classification of the raw material (skin of loin, leg and palette), scalding for about 15 minutes (water at 60°C up to 58°C internal), cooling for about 20 minutes (water 0°C to skin reach 3°C internally) and subsequent grinding at disc of 5 mm (Cozzini, Chicago, USA).

The skin pre-heating aims to reduce the negative effects of the addition of raw skin in flavor and texture of the product as suggested by Sadler and Young (1993). After grinding, the skin (60%, 70% or 80%) was mixed with mechanically separated meat, salt (1.5%, 2.0% or 2.5%) and ice (6.4%) by a mixing machine (Cozzini, Chicago, USA) for 15 minutes to obtain a homogeneous mass. Then the mass passed through the emulsifier in the emulsification process (Cozzini, Chicago, USA) varying in 2.0, 2.5 or 3.0 mm disc as the experimental design (Table 1). The maximum temperature at the exit of the emulsifier was 4°C.

After the process of emulsification, the skin emulsion was characterized to evaluate the best process conditions and subsequently was used in a mortadella formulation called test (only the best experimental design condition).

**Preparation of mortadella added pork skin emulsion**

The mortadella preparation was held at the URI Erechim Meat Pilot Plant (Erechim, RS, Brazil). Two formulations were prepared. One was called standard (similar to that used industrially) and one called test (with partial substitution of soy protein for pork skin emulsion obtained using the best conditions of the experimental design) (Table 2). For the test formulation were used 6.4% of pork skin emulsion and 2.9% of soy protein (reduction of 27.5% in soybean protein added). For the development of the formulations was followed the Technical Regulation of Identity and Quality of Mortadella (MAPA, 2000) and the Ordinance n. 1004 (BRASIL, 1998).

For the mortadella preparation the raw material and mechanically separated meat were ground (disc 8 mm) in a grinder (Visa, Brusque, SC, Brazil), and then were taken to the cutter (Frigomaq, Chapecó, SC, Brazil) for emulsification and mixing with the other ingredients and additives in the following sequence: addition of the skin emulsion (only in the formulation test), ice, offal, salt, condiments and additives according to Table 3. The total time emulsification process was 8 minutes to a maximum final temperature of 12°C. Subsequently, the mass was embedded in plastic casings (nylon and polyethylene) with a caliber of 62 mm pre-hydrated in warm water (38°C for 30 minutes). Each piece was embedded with weight approaching 500 g. The mortadellas were subjected to boiling (80°C for 100 minutes) until they reach 72°C internally. The cooling of the pieces was performed in a conventional refrigerator at 4°C until 7°C internally and the pieces were kept in refrigeration (7°C) until further analysis.

**Physicochemical characterization of pork skin emulsion**

The emulsions were characterized crude, for moisture determination (indirect gravimetric method at 105°C), pH (electrometric determination with addition of distilled water), protein (Kjeldahl method), mineral residue (method of incineration in a muffle furnace at 550°C), lipids (Soxhlet method), collagen (colorimetric method for determination of

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**Table 1. 23 factorial design with center point used for the development of pork skin emulsions**

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Levels</th>
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<tbody>
<tr>
<td>Pork skin (%)</td>
<td>60 70  80</td>
</tr>
<tr>
<td>Salt (%)</td>
<td>1.5 2.0 3.0</td>
</tr>
<tr>
<td>Diameter of the holes in the disc (mm)</td>
<td>2.0 2.5 3.0</td>
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</table>

|&nbsp;*mulsifier disc of the equipment used*
the amino acid hydroxyproline multiplied by 8), all according to the methods of the AOAC (1990) and IAL (2005). All analyses were performed in triplicate.

**Physicochemical and chemical characterization of mortadella**

As for the mortadella characterization, analyzes of lipids and proteins as previously mentioned methodologies were performed. The mortadella texture profile (TPA) was held in an universal texturometer TA.XTplus (Stable Micro Systems, Godalming, UK) at 20°C, with five replicates for each treatment, with results provided by the software Texture Exponent Lite (Stable Micro Systems). The samples were previously cutted into cylinders of 1 cm of thick and 3 cm of diameter and subjected to a dual compression using 80% of compression plate of 75 mm of diameter at a constant speed of 1.0 mM.sec⁻¹ with an interval of 5 seconds between the first and second compression.

According to the TPA curve generated, the following parameters were evaluated: hardness (N), determined by the total peak area or peak force during the first compression; adhesiveness (N.mm), negative area of the curve after the first compression; cohesiveness, ratio between the areas of the second and first compression; elasticity (mm), the distance between the pointed end of the first compression and second compression peak; chewiness (N.mm), obtained by the product of hardness, cohesiveness and flexibility; resilience, calculated as the ratio between the area of the first decompression and compression of the first area (Ramos and Gomide, 2007).

**Sensory analysis of mortadella**

For sensory analysis the preference test was used (Queiroz and Treptow, 2006) with the aim of evaluate if there was significant difference (p < 0.05) in consumer preference among the mortadella formulations developed (standard and test). Forty two untrained and frequent consumers of meat products participated. The sensory evaluation was carried out in a Food Industry at Chapecó, SC, Brazil. The evaluation was performed individually in a climatized room where each panelist received a cube of each sample (approximately 2 cm edge) in containers properly coded randomly.

The physicochemical analyzes results (protein, fat, moisture, water activity, pH and collagen) for the pork skin emulsion and the mortadella (lipids, proteins, texture profile and sensory evaluation) were analyzed using the software Statistica version 8.0. Were processed three replicates for each test and the data were treated by analysis of variance ANOVA and the comparison of means by Tukey test at 90% level of confidence to the results of emulsions and 95% for the results of mortadella.

**Results and Discussion**

**Physicochemical characterization of pork skin emulsion**

Table 2 presents the matrix of 23 complete factorial design and responses (dependent variables) to the lipid content, pH, water activity, protein, moisture and collagen obtained for each treatment used in the process development of pork skin emulsions. It was observed that the lipid content of the pork skin emulsion samples obtained in the different treatments ranged from 17.44% to 26.62%. The lipid content was found to T2, where employed lesser amount of skin and smaller diameter disc and the greatest amount of salt. The highest levels of lipid were found to T3 where employed the less salt, the
The pH, aw, collagen, protein and moisture the results showed no statistically significant difference (p > 0.10) for these parameters. This can be explained by the small change in salt concentration (1.5 to 2.5%), a small variation in diameter of the disc (2 to 3 mm) and minor variation in the proportion of skin (60 to 80%), or the conditions tested were not enough to significantly change the characteristics. Furthermore, the actual variation in the composition of the raw material (pig skin) may have influenced the results. Only the lipid content was significantly affected by the independent variables (salt, disc diameter and proportion of skin) which can be justified by the extraction of fat.

Figure 1 presents the Pareto chart for the estimated effects (absolute value) in the 23 factorial design for lipid response to the treatments used for development of pork skin emulsions. It was observed that the variables, quantity of skin and the interaction salt concentration and disk diameter positively influenced (p < 0.10) in the lipid content. In this regard, lipid content found in the pork skin emulsion was influenced by the added amount of skin in the formulation. According to Silva (1995) about 70% fat pork is located in the layer located beneath the skin which can vary depending on the level of toilet or trimming performed in the feedstock or can vary due to the origin of the skin (loin, leg, belly, etc.). With respect to the interaction between salt concentration and disk diameter, there was a significant positive effect (p < 0.10), i.e. occurred higher levels of lipids when employed discs with smaller holes.

Salmon (2004) investigating the production of pork skin emulsion with different cuts obtained average lipid content of 18.70%, protein 15.8% and 61.01% of moisture. The pH values were below 7.49 ± 0.02 cited by Choe et al. (2013) for mixing pork skin and wheat fiber. When analyzing the results in Table 2, it was observed that the pH values (6.83 to 7.09), water activity (0.980 to 0.990), protein (15.06 to 18.78 g/100g), humidity (58.17 to 63.60%) and collagen (5.84 to 7.44 g/100g) showed little variation between treatments, and these were not affected significantly (p > 0.10) by the amount of skin and salt and by the hole size of the disks employed in the process.

Physicochemical characterization of mortadella

Analyzing Table 4, it can be seen that the
amount of protein found in both in mortadellas made with soy protein (standard) and the mortadellas produced using pork skin emulsion (test) showed no significant difference (p > 0.05) each other. The two formulations met the legislation by presenting minimum standard of 12% protein (MAPA, 2000).

There was a significant difference (p < 0.05) for lipid content for the elaborated mortadellas, where the test formulation showed higher lipid content. This value can be explained by the process of preparing the pork skin emulsion has allowed greater utilization of fat cut.

Even with increased lipid content in the two mortadella formulations no met the maximum of 30% fat established by the law (MAPA, 2000). Were tested as a 6.4% of emulsion skin, the maximum limit of 30% fat in the final product (mortadella) and maximum of 10% of skin added are two limiting the use of the emulsion in high proportions. In a study on the partial replacement of animal fat by vegetable oils in mortadella, Yunes (2010) obtained lipid levels between 16.19% and 12.51%. Cenci (2013) to investigate the influence of process variables such as the ratio of raw material frozen, chilled, temperature and speed emulsification for chicken mortadella, had values in the range from 23.25 to 25.45% of fat.

Choe et al. (2013) tested a mixture of pork skin and wheat fiber as fat substitutes in sausage. The use of the mixture increased moisture content and proteins because of the higher water retention capacity of the fiber and the proteins present in pork skin. The average results were 58.14% and 12.60% and 68.13% and 13.79% for moisture and protein respectively, for the standard and test mortadellas with 20% mixture. The standard (without skin and wheat fiber) had higher lipid content (26.17%) than the test (13.09%) with skin and wheat fiber. The lipid content can be explained by the removal of subcutaneous fat in the preparation of the skin, a procedure that was not performed in the present study.

Sadler and Young (1993) investigated tendons of the hind legs of cattle as a substitute lean meat in sausages. The tendons were preheated at different temperatures before being added. These authors observed changes in hardness compared to the standard when the tendons were heated to 50°C. For high temperatures not occured change in hardness of the sausages.

Puollanne and Ruusunen (1981) already mentioned the importance of the pork skin connective tissue in order to increase the hardness and water retention capacity in sausages. In the study by Choe et al. (2013) the fat replacement by a mix of pork skin and wheat fiber provided a more stable emulsion, increase in hardness, cohesiveness, gumminess and chewiness. Li (2006) evaluated the use of 6% collagen from discard chicken in cooked ham. The addition of this ingredient gave higher hardness, suggesting that the proteins of small size when added affected the texture of the hams, which was verified by increasing the hardness of 11.96 to 16.91 N. The addition of this extract caused no difference in properties adhesiveness, springiness, cohesiveness and chewiness. Prestes et al. (2012) also observed increased in the sheer force (7.61 to 11.64 N) in turkey ham added by hydrolyzed collagen, modified starch and guar gum.

In the study by Choe et al. (2013) hardness, cohesiveness, gumminess and chewiness for sausage with mixing of wheat fiber and pork skin were 82.25 N; 0.34; 26.69 N and 20.63 N, respectively. Also observed a significant increase in these parameters compared to standard sausage (no pork skin). The differences between the results obtained by these authors in comparison to the results obtained in this study can be explained by differences in the products formulations (sausage Vs. mortadella), differences

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Table 4. Levels of protein, lipids and texture profile obtained for the standard and test (with addition of emulsion pigskin) mortadellas

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Standard Mortadella</th>
<th>Test Mortadella</th>
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<tbody>
<tr>
<td>Protein (%)</td>
<td>12.49 ± 0.05*</td>
<td>12.09 ± 0.18*</td>
</tr>
<tr>
<td>Lipids (%)</td>
<td>16.60 ± 0.04*</td>
<td>17.19 ± 0.03*</td>
</tr>
<tr>
<td>Hardness (N)</td>
<td>84.74 ± 1.32</td>
<td>98.61 ± 3.99</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>0.69 ± 0.03</td>
<td>0.73 ± 0.01</td>
</tr>
<tr>
<td>Adhesiveness (N.mm)</td>
<td>46.83 ± 0.94</td>
<td>56.22 ± 1.12</td>
</tr>
<tr>
<td>Elasticity (mm)</td>
<td>1.27 ± 0.07</td>
<td>1.24 ± 0.04</td>
</tr>
<tr>
<td>Chewiness (N.mm)</td>
<td>69.36 ± 0.89</td>
<td>81.25 ± 1.45</td>
</tr>
</tbody>
</table>

* Means ± standard deviation followed by the same letter/line indicate no significant difference at level of 5% (Tukey test).

** With the addition of pork skin in the proportion of 6.4% and partial substitution of soy protein (27.5% reduction).
in sample size for evaluation of the texture and also
due to differences in raw materials and production
process.

Sensory analysis of mortadella
In the sensory evaluation was perceived difference
(p <0.05) between samples of mortadella developed. A sample of mortadella test (with addition of pork
skin emulsion and partial substitution of soy protein)
showed the greatest preference for tasters (71.4%).
In the work presented by Choe et al. (2013), no differences in the color, flavor, tenderness, juiciness
and overall acceptability of sausages with a mixture
of pork skin and wheat fiber as fat substitutes were noted.

In contrast, in the study of Sadler and Young
(1993) where cattle tendons were tested as substitutes
for lean meat in sausages, the sensory evaluation of
texture, flavor and overall acceptability decreased
these parameters due to the substitution of lean meat
per tendon. In the study by Choe et al. (2013), it was concluded that in general the quality characteristics
of reduced fat sausages were improved with the addition of a mixture of pork skin and wheat fiber.

Conclusion
In this study was developed an emulsion of pork
skin for use in mortadella using a 2³ factorial design
with central point. Only the lipid content of the
emulsion was influenced significantly (p <0.10) by
the amount of pork skin (60, 70 or 80%), salt (1.0,
2.0 or 2.5%) and by the diameter of the holes of disks
emulsifier (2.0, 2.5 or 3 mm).

The emulsion of pork skin was used in a
mortadella formulation and compared to a standard
formulation (similar to commercial). For the test
formulation were used 6.4% of skin emulsion (pork
skin 80%, 1.5% of salt, and disk of 3 mm of diameter)
and 2.9% soy protein (reduction of 27.5% of the
soybean protein added).

The mortadella developed met the standards
established by the Brazilian legislation and analysis
of texture showed higher hardness, cohesiveness,
chewiness and adhesiveness (p <0.05) for the
mortadella with pork skin emulsion against standard
mortadella. In sensory evaluation mortadella with
pork skin emulsion was preferred by 71.4% of the
panelists. Based on the results obtained, it was
concluded that it is feasible to use pork skin emulsion
in mortadella and this may partially replace soybean
protein without harming the quality of the product
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meat industry.

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