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FULL PAPER

Microstructure and technological properties of cooked meat sausages prepared with emulsions of vegetable oils as substitutes for animal fat

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Abstract

This paper investigated the functional possibilities of using two types of vegetable oil emulsions of chia and grape seeds, as substitutes for animal fat in the cooked sausage formulation. Scanning electron microscopy (SEM) and texture profile analysis (TPA) were used to assess the effect of different levels of fat substitution with the chia and grape seed oils emulsions on the structural changes and the emulsion stability of sausages. A direct correlation was observed between the hardness index in the TPA analysis and the addition of the chia oil emulsion. A decrease in the adhesiveness of the test samples in relation to the control sample ($P>0.05$) was observed when the fat was fully or partially replaced by a grape seed oil emulsion and, contrariwise, adhesiveness increased in the samples to which a chia oil emulsion had been added, the increase being directly proportional to its quantity. SEM images of sausages showed that the samples in which the porcine adipose tissue was partially substituted by pre-emulsified chia and/or grape seed oils exhibited a more compact network with smaller fat droplets compared to the control sample and the emulsified grape seed oil sample. To sum up, the use of chia and grape seed oil emulsions was found to be a possible alternative for the substitution of animal fat in emulsified meat sausages; pre-emulsified chia oil addition resulted in products that were close to the standard one in terms of texture, microstructural indices and emulsion stability, along with improved nutritional image and wholesomeness of the end product.

1. Introduction

The emulsion-type cooked sausages are among the most popular and most widely-eaten meat products worldwide. They are made from meat, fat, salt, sugar, nitrites and various spices and additives (Ayo *et al.,* 2008). These sausages contain between 20% and 30% of animal fats, which are technologically important for obtaining a stable emulsion and for the formation of the rheological, structural and sensory properties of the products (Hughes *et al.,* 1997; Pietrasik and Duda, 2000). Due to their high saturated fatty acid and cholesterol content, however, these meat products are often considered unhealthy by consumers because of their association with metabolic disorders such as obesity, hypertension and cardiovascular diseases (Vural and Javidipour, 2002; Özvural and Vural, 2008).

The dietary shift towards foods of lower fat content has placed the modern focus of the meat processing industry on the modification of traditional into re-formulated innovative products that contain less animal fat and have an improved health profile (Bloukas and Paneras, 1993; Mittal and Barbut, 1994; Garcia *et al.,* 2002; Choi *et al.,* 2009). A promising strategy in that respect is the substitution of saturated animal fat with unsaturated vegetable oils, which underlies the growing interest in the latter and the number of studies on their complete inclusion in the composition of new food products. Each vegetable oil has its own fatty acid profile that largely determines its physical properties such as colour, aroma, taste, emulsifying and textural properties (Pappa *et al.,* 2000). The health potential of chia and grape seed vegetable oils is widely recognised in relation both to cholesterol levels and to their cardioprotective, antidiabetic and anticancer effects (Choi *et al.,* 2010; Antonini *et al.,* 2020). It makes them suitable for

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inclusion in sausages characterised by a high undesirable fat content. However, their direct inclusion as a substitute for animal fat is not advisable from a technological point of view and could result in the deterioration of the overall quality of sausages and their sensory perception (Bolger *et al.*, 2018; Chen *et al.,* 2020). Therefore, it is advisable to include vegetable oils in emulsion before adding them to the meat batter, as this would lead to their better incorporation and improvement of the texture of the sausages (Cáceres *et al.,* 2008; Asuming-Bediako *et al.,* 2014; Qi *et al.,* 2020). One of the most important factors for obtaining cooked sausages with optimum technological and quality parameters is the meat 'emulsion' stability (Bertram *et al.,* 2002; Di Luca *et al.,* 2011). The dispersed phase in the meat emulsion consists of lipid particles dispersed in the viscous meat batter due to the formation of a protein film around them. The protein hydrophilic groups are oriented towards the water phase, whereas the hydrophobic groups are oriented towards the lipid phase, and the 'emulsion' is thus stabilised (Choi *et al.,* 2016). In this respect, the preliminary emulsification of the vegetable oil by using a protein, such as sodium caseinate, as an emulsifier, would result in the improvement of the emulsifying capacity of the meat batter for the production of these emulsion-type cooked sausages.

All of this outlines the aim of our research, i.e. investigation of the possibility of animal fat substitution by pre-emulsified chia and grape seed vegetable oils and evaluation of their effect on the meat emulsion stability and the textural and microstructural characteristics of cooked sausages.

2. Materials and methods

For the experiment, we made seven test formulations with different amounts of pork back fat and the type and amount of vegetable oil emulsions according to Table 1. The pork and other non-meat raw materials were obtained from local retailers in Plovdiv (Bulgaria). The vegetable oil emulsions, sodium caseinate and hot water (60°C) (in a 5:1:5 ratio) were prepared on a cutter (model CL/5, FIMAR, Italy) in advance, one day before they were used. The sodium caseinate and the hot water were stirred for 2 min, then the oils were added and the mixture was chopped for another 3 min. The emulsion was cooled to 6-8°C. The experimental sausages were made by grinding the pork meat and back fat in a meat grinder through a 4 mm grinder plate, then the meat was placed in the cutter working at a slow speed and the sodium chloride, nitrite, polyphosphate and half of the ice were added. The emulsion was chopped at a high speed and after the water was absorbed, the sodium caseinate, spices and the rest of the ice were added. The chopping continued until a temperature of 6-8°C was reached, and then the fat and/or emulsions were added. The chopping continued until 12°C and finally, the starch was added. The chopping went on until 14°C. The finished filling mass was transferred to a stuffer and stuffed into 50mm polyamide casings, the individual pieces weighing 0.250 kg each. Heat processing was performed at 65°C until 45°C was reached at the centre of the sausage; then the cooking continued at 78°C until 72°C was reached at the centre of the sausage, and held for 5 mins. After cooling, the sausages were kept in a cold store at 4±2°C until the time of the analyses.

2.1 Texture profile analysis

For the analysis of the texture profile of the finished

Table 1. Sample formulations of emulsified meat sausages with vegetable oil emulsions.

Ingredients, $g.kg^{-1}$	Formulations						
	Sample 1 (control)	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Pork lean meat	790	790	790	790	790	790	790
Pork Back fat	210	-	-	105	105	-	70
Emulsion (chia oil + water + sodium caseinate)	-	210	-	105	-	105	70
Emulsion (grape seed oil + water + sodium caseinate)	-	-	210	-	105	105	70
Sodium chloride	20	20	20	20	20	20	20
Sodium nitrite	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Black pepper	4	4	4	4	4	4	4
Nutmeg	1	1	1	1	1	1	1
Sugar	2	2	2	2	2	2	2
Phosphates	2	2	2	2	2	2	2
Potato starch	30	30	30	30	30	30	30
Sodium caseinate	10	10	10	10	10	10	10
Water/ice	290	290	290	290	290	290	290

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sausages (Bourne, 1978), a TA-XT Plus (Stable Micro Systems, Surrey, GB) texture analyser was used. The diameter of the cylinder was 50 mm. Discs 30 mm in diameter and 19±2 mm in height were made from the sausage samples for the test. The samples were compressed at a rate of 2 mm s^{-1} to 5 mm deformation. The relaxation time between two compressions was set at 5 s. The hardness, springiness, chewiness, adhesiveness, homogeneity, resilience, and gumminess were calculated (Bourne, 1978; Bourne, 2002; Kim *et al.,* 2009).

2.2 Emulsion stability

For the determination of the emulsion stability, the method described by Zorba and Kurt (2006) was used. Approximately 30 g of each sample before and after heat treatment were weighed into a centrifuge tube and heated in a water bath at 70°C for 30 mins. Immediately after heating, the tubes are centrifuged at 2000 min^{-1} for 10 mins and the separated water and oil are weighed and used to calculate the emulsion stability (ES).

2.3 Scanning electron microscopy analysis

Scanning electron microscopy (SEM) analysis was carried out with an SEM Philips 515 digitised. The sausage samples were cut into 10 mm × 10 mm × 5 mm pieces and frozen in a freezer for 24 hrs at -20°C, then lyophilised at a temperature of -50°C and pressure of 10 Pa for 72 hrs. The pre-lyophilised samples were coated with gold-palladium using cathode sputtering of a gold-palladium target. The morphology of the samples was revealed by Scanning Electron Microscopy (SEM) in Secondary Electrons Imaging mode. The accelerating voltage applied during the analysis was 8 eV. Photographs were taken at 100× and 500× magnifications for each sample.

The experiments were performed with two replicates, as all test measurements were performed five times, and the data in the tables are the average of the measured values. The statistical processing of the data was performed using the software product STATGRAPHICS 16 (2010). One-way analysis of variance (ANOVA) was performed and the significant difference between the groups was analyzed with Duncan's multiple-range tests. The significance was set at the level of $p < 0.05$.

3. Results and discussion

The experimental data obtained from the texture analysis (Table 2) demonstrated a significant difference in the hardness of the individual samples. Consistency in meat products is related to their fat content (Ventanas *et al.,* 2010) and fat type (Youssef and Barbut, 2010; Zhang *et al.,* 2013). In the samples, there was a trend to higher hardness values obtained for the chia oil samples (sample 2 and sample 4) compared to the control sample, which contains only back fat. In contrast, the addition of the grapeseed oil emulsion did not demonstrate this trend, and the hardness values were statistically indistinguishable ($P<0.05$) compared to the control sample. The rest of the samples did not show any considerable differences for this parameter.

Regarding chewiness, which is a product of the hardness, homogeneity and springiness and provides information on the force or work needed for the chewing of the sample studied (Bourne, 2002), sample 4 was found to be related to the greatest masticatory force required during consumption, followed by sample 2. Both samples contained chia vegetable oil emulsion. These data are consistent with the results on the chewiness of the samples studied, in view of the fact that this is a secondary parameter dependent on hardness (Selgas *et al.,* 2005; Ktari *et al.,* 2014). Similar results were obtained on the other parameters which reflect the structural and mechanical properties of the samples

Table 2. Texture profile analysis (TPA) of cooked meat sausages.

Sample	Texture profile analysis (TPA)						
	Hardness (N)	Chewiness (N)	Resilience	Gumminess	Adhesiveness (Nmm)	Homogeneity	Springiness
1	21.54±3.05^{ab}	12.79±2.56^{a}	0.51±0.04^{b}	13.4±2.14^{a}	-0.15±0.03^{b}	0.62±0.04^{b}	0.95±007^{a}
2	28.93±4.21^{c}	16.2±2.78^{b}	0.47±0.04^{a}	16.73±2.46^{b}	-0.24±0.05^{a}	0.58±0.05^{a}	0.97±0.04^{ab}
3	22.03±3.3^{b}	13.3±2.43^{a}	0.51±0.05^{b}	13.71±2.28^{a}	-0.07±0.07^{c}	0.62±0.05^{b}	0.97±0.03^{ab}
4	29.45±5.3^{c}	17.76±3.54^{c}	0.51±0.04^{b}	18.43±3.39^{c}	-0.18±0.03^{b}	0.63±0.04^{b}	0.96±0.04^{ab}
5	19.43±3.49^{a}	11.98±2.37^{c}	0.52±0.04^{b}	12.31±2.24^{a}	-0.08±0.01^{c}	0.63±0.04^{bc}	0.97±0.03^{ab}
6	19.74±3.49^{ab}	12.55±2.43^{a}	0.55±0.04^{c}	12.84±2.3^{a}	-0.17±0.02^{ab}	0.65±0.06^{c}	0.97±0.03^{b}
7	19.75±3.3^{ab}	12.11±2.3^{a}	0.51±0.04^{b}	12.38±2.15^{a}	-0.16±0.03^{ab}	0.63±0.04^{b}	0.97±0.03^{b}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($P<0.05$). Sample description: Sample 1: only pork back fat, Sample 2: only chia oil emulsion, Sample 3: only grape seed oil emulsion, Sample 4: equal proportions of back fat and chia oil emulsion, Sample 5: equal proportion of back fat and grape seed oil emulsion, Sample 6: equal proportion of grape seed oil and chia oil emulsions, Sample 7: equal proportion of pork back fat, chia oil and grape seed oil emulsions.

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tested that are associated with their behaviour during consumption, i.e. resilience and gumminess. The higher values of the gumminess parameter in samples 2 and 4 where chia emulsion was used were closely related to the hardness data of the same samples. Our results were in conformity with the results obtained by (Baek *et al.,* 2016; Afshari *et al.,* 2017), who reported an increase in the texture parameters when vegetable oils were substituted for fats. The situation with the grapeseed oil was different since it led to products that did not differ significantly from the control.

In view of the fact that adhesiveness expresses the degree of the product's adhesion to the cylinder of the texture analyser, and the teeth, respectively, the considerable differences in the adhesiveness of the different samples also indicate that a different taste perception will be caused by the substitution of fats by the emulsions in the sausages. A decrease in the adhesiveness of the experimental samples compared to the control sample (P>0.05) was established when grape oil emulsion was fully or partially substituted for fat. In contrast, it increased compared to the control sample in the samples made with the addition of chia oil, this increase is directly proportional to the emulsion quantity.

The substitution of the back fat in the sausages made with emulsions had no significant effect on homogeneity, except for sample 2 prepared with 100% chia oil substitution. In terms of the springiness parameter, the inclusion of vegetable oil emulsions slightly increased its values compared to the control sample, although a statistically significant difference was only established in samples 6 and 7. A possible reason for the better recovery of the height of these samples after the first compression could be found in the addition of sodium caseinate which is part of the composition of the emulsions used and improves the three-dimensional structure of the product, as indicated by the results of the microscope pictures for samples with pre-emulsified oils.

Table 3 shows the results obtained on the emulsion stability of the meat batter before and after heat processing. The highest and statistically distinguishable emulsion stability value in relation to the other samples was established in the meat batter of the control sample 1 (P>0.05), where the animal fat was in accordance with the basic sausage formulation, while its substitution with vegetable emulsions led to its marked decrease. In the samples where the back fat was completely substituted by emulsions, the meat batters made with chia oil only (Sample 2) demonstrated higher stability than the one made with grape seed oil only (sample 3), but this trend was not observed in the other samples where the saturated fats had only been partially substituted. Thus, the fat and chia oil emulsion sample showed considerably lower stability of the meat batter compared to the sample made with the same proportional substitution with grape seed oil. Furthermore, sample 4 was seen as the least stable sample with regard to the emulsion obtained prior to cooking.

After heat processing, control sample 1 again exhibited the best emulsion stability, whereas the stability decreased significantly in the other samples. Nevertheless, its values in the chia oil samples were considerably higher than those in the grapeseed oil samples. According to Pires *et al.* (2017) who studied the effect of the reduced addition of sodium chloride on the Bologna sausage quality, problems in the texture and structure of the finished sausages occurred when the emulsion stability was 85-88% or lower. In our studies, however, all emulsion stability values were above that percentage, the lowest value being 88.02% in sample 5, and the highest 92.95% in sample 2.

Figure 1 depicts the changes in the structure of the freeze-dried sausages that occurred when pre-emulsified vegetable oils were added. As shown in the SEM images, the sausages to which vegetable oils had been added had smaller fat globules and a less porous, close and firm network. In contrast, the control sausage sample (sample 1) showed larger fat globules, a less pronounced three-dimensional structure and relatively small cavities compared to the other samples. The structure of sample 7 was most similar to the microstructural characteristics of the control sample in comparison to the other test samples. These results correspond well with the observation of Miklosa *et al.* (2013) that the physicochemical properties of the fats added affected the fat distribution pattern and size as vegetable oils resulted

Table 3. Emulsion stability (ES, %) of meat batters and cooked sausages.

Indicator	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
ES of meat batter, %	99.95±0.01^{g}	99.69±0.01^{e}	97.19±0.01^{b}	93.15±0.01^{a}	99.91±0.01^{f}	99.32±0.01^{d}	99.21±0.01^{c}
ES of sausages, %	95.73±0.01^{f}	92.95±0.01^{e}	89.43±0.01^{c}	89.41±0.01^{b}	88.02±0.01^{a}	90.27±0.01^{d}	90.28±0.01^{d}

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different (P<0.05). Sample description: Sample 1: only pork back fat, Sample 2: only chia oil emulsion, Sample 3: only grape seed oil emulsion, Sample 4: equal proportions of back fat and chia oil emulsion, Sample 5: equal proportion of back fat and grape seed oil emulsion, Sample 6: equal proportion of grape seed oil and chia oil emulsions, Sample 7: equal proportion of pork back fat, chia oil and grape seed oil emulsions.

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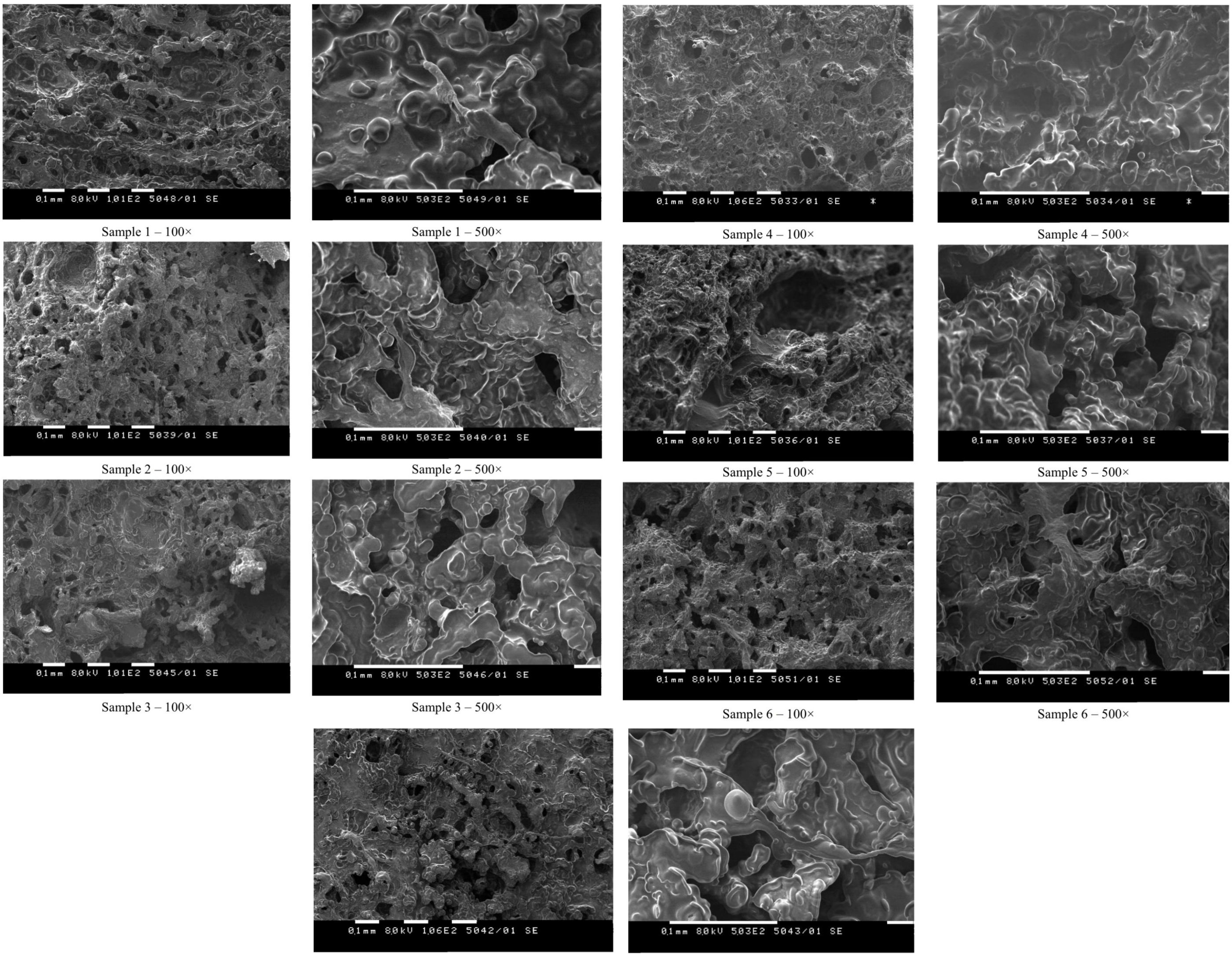

Figure 1. SEM micrographs of cooked sausages with emulsified vegetable oils. Sample 1: only pork back fat, Sample 2: only chia oil emulsion, Sample 3: only grape seed oil emulsion, Sample 4: equal proportions of back fat and chia oil emulsion, Sample 5: equal proportion of back fat and grape seed oil emulsion, Sample 6: equal proportion of grape seed oil and chia oil emulsions, Sample 7: equal proportion of pork back fat, chia oil and grape seed oil emulsions.

in emulsions of smaller size compared to animal fat. The smaller-diameter fat globules are those that contribute to the more uniform distribution of fat in the meat emulsion (Hammer, 1992; Youssef *et al.,* 2011). In addition, Youssef and Barbut (2009) note that apart from the fat/oil type, and its hardness, respectively, another important determinant affecting the emulsion homogeneity was the protein content, since more aggregation and a less homogeneous meat matrix structure were observed in the higher protein meat emulsion. In the samples obtained with the addition of pre-emulsified oils, a higher structural density of the protein network was established, which could be the result of the sodium caseinate added.

Although the microstructural images of sausages are considered difficult to compare/correlate with the texture parameters (Parés *et al.,* 2018), the higher hardness values of samples 2 and 4 are established through the TPA analysis in our study correlated well with the densest microstructure observed in these samples.

The formation of a more intense protein network leads to enhanced protein matrix strength of the sausages and thereby to higher values of the textural parameters: hardness and chewiness (Intarasirisawat, 2014). Despite the tight protein network, the presence of large cavities observed in sample 4 might have been related to its higher emulsion destabilisation resulting in more fat/water lost during sausage cooking. Cheetangdee (2017) suggested that an emulsified meat matrix with a more uniform cavity formation could retain water more effectively. Homogeneous gel-like compact structures were more stable compared to highly aggregated gels (Milkosa *et al.,* 2013) and kept the water from moving out of the structure (Youssef and Barbut, 2009). When vegetable oils were used, more homogeneous matrices containing small fat droplets were formed. When back fat was used, a heterogeneous structure containing fat globules of different sizes was formed, and they were dispersed rather than truly emulsified (Labudde and Lanier, 1995; Feiner, 2006).

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4. Conclusion

On the basis of the analysis, a conclusion can be drawn that with the exception of sample 4, the pre-formulated sausages with partial substitution of emulsified chia and grape seed oils for the animal fat did not differ considerably from the sample made according to traditional fat formulation in terms of its texture parameters and exhibited satisfactory emulsion stability. In the sample that only contained chia oil emulsion, there was an increase in the values of the texture parameters compared to the control sample and the grapeseed oil samples, except for the homogeneity and resilience indicators. The inclusion of vegetable oil emulsions resulted in products of good emulsion stability, although its values were lower than those of the control sample. The micrography of the sausages showed that the samples with partial substitution of pre-emulsified chia and/or grapeseed oils for porcine adipose tissue had a more compact network with smaller fat droplets compared to the control sample and the grape oil emulsion sample. Thus, the inclusion of this pre-emulsified chia oil could be an efficient approach to fat substitution and the manufacture of products with improved nutritional profiles via animal fat reduction and enrichment with wholesome vegetable oil.

Conflict of interest

The authors declare no conflict of interest.

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