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Research Article

Effect of functional ingredients and vegetable oils on the technological properties and microstructure of emulsified cooked sausages

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Abstract

The aim of this study was to investigate the effect of the incorporation of vegetable oils (chia, milk thistle and pumpkin seed oils) and non-meat functional ingredients, such as quinoa flour or potato starch, into the manufacture of emulsified cooked sausages with reduced animal fat content. The samples were evaluated with respect to the texture properties, emulsion stability, colour and microstructural characteristics of the reformulated meat products. The sausages formulated with quinoa flour were found to have better emulsion stability and lower hardness, gumminess and adhesiveness values according to the results of the TPA analysis. Furthermore, higher L^* and b^* and lower a^* values were measured in these samples compared to the analogical samples prepared with potato starch. The milk thistle oil led to the highest a^* and the lowest b^* and *h (C)* values, whereas the samples containing pumpkin seed oil were characterised by increased values in the yellow spectrum and the highest lightness of all samples studied. The SEM images of the samples formulated with the addition of vegetable oils and quinoa flour exhibited a more uniform distribution of the individual components restructured into a more compact protein network, unlike the samples made with potato starch, which had rougher and less organised morphological structure.

Keywords
animal fat, colour, emulsion stability, potato starch, quinoa flour, substitution, texture

Abbreviations
ES – emulsion stability, PUFA – polyunsaturated fatty acids, SEM – scanning electron microscopy, TPA – texture profile analysis

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Introduction

Over the last decade, the meat industry has gradually adopted a so-called "functional" trend towards the development of new meat foods that possess a number of physiologically functional properties that ensure protection against certain diseases. On the other hand, the changes in people's living conditions and eating habits have led to a constant increase in the consumption of ready-made meat foods high in fats composed of predominantly saturated fatty acids (Ansorena et al. 2004; Grasso et al. 2014; Pintado et al. 2015). Specialists assume that this dietary change is largely due to the increased occurrence of diseases such as diabetes, cardiovascular diseases, etc. (Skeaff et al. 2009). The PUFA of the ω -6 and ω -3 groups, which are a natural and efficient regulator of the lipid metabolism balance in the human organism, exert considerable influence on people's health (Carvalho et al. 2020). These discoveries call for prompt and radical adaptation of the manufacture of emulsified meat products to healthier alternatives through their enrichment with various functional ingredients and optimisation of their fatty acid composition (Bis-Souza et al. 2019; Silva et. al. 2019; Guiné et al. 2020; Badar et al. 2021). Vegetable oils and proteins, soluble fibres, inulin, polydextrose and different types of oleogels efficiently enhance sausage quality owing to their nutritional and functional properties and health-promoting activity (Felisberto et al. 2015; Paglarini et al. 2019; Bis-Souza et al. 2020; Akterian et al. 2022; Suryawanshi et al. 2022). Chia and pumpkin seed oils are typical examples of sources rich in bioactive compounds, such as polyunsaturated fatty acids, minerals, vitamins, pyrazine derivatives, phytosterols, pigments and phenolic compounds (Nederal et al. 2014; Rodrigues et al. 2018). Other attractive bioactive compounds are those contained in milk thistle oil, such as silymarin, mixtures of different flavonolignans and fat-soluble vitamins (Scott, 1998). The direct incorporation of vegetable oils as animal fat substitutes is related to the occurrence of problems of technological nature in the formation of the meat batter texture and the sensory profile of the reformulated product (Kim et al. 2020).

Quinoa is a suitable additive to the formulation of meat batter for sausages since it contributes to a number of technological benefits, such as improved water-retention, gelation and emulsifying capacity (Pellegrini et al. 2018; Zambrano et al. 2019; Fernández-López et al. 2020a). In addition, it is a valuable source of essential amino acids and PUFA, plant anti-oxidants, vitamins, minerals and dietary fibre (Srujana et al. 2019).

The aim of the present study was to investigate the possibility of a rational design and reformulation of meat emulsions by using non-meat functional ingredients, such as chia, milk thistle and pumpkin seed oils, in the manufacture of cooked sausages, and to evaluate the technological role of quinoa flour and potato starch in the restructured meat matrices with reduced animal fat content.

Materials and Methods

Materials. Six types of emulsified sausage samples were prepared for the purposes of the experiment, with partial replacement of the animal fat with a pre-made emulsion of the respective vegetable oil, sodium caseinate and water, as shown in Table 1. In a previous experiment, which aimed to replace the pork back fat with chia oil emulsion, the optimum quantitative fat to chia oil emulsion ratio in emulsified cooked sausage was determined (20% fat :80% chia oil emulsion) by using simplex-centroid mixture design. The data indicated that the characteristics of the resultant emulsified sausage having optimised composition were the closest to those of the control sausage. The present experiment was conducted on this basis for the purpose of comparing the effect of the different vegetable oils and functional ingredients studied on the technological properties and microstructure of emulsified sausages. The ingredients for the formulation of the emulsions were the vegetable oils, sodium caseinate and water in a 5:1:5 ratio. Quinoa flour was also added to the meat batter in three of the samples, and potato starch was added to the other three. The technological steps in the manufacture of the experimental samples have been presented in Fig. 1. The finished cooked sausages were kept at 4 ± 2°C until the time of the analyses.

Table 1. Formulations of cooked meat sausages with vegetable oil emulsions and quinoa flour or potato starch

Ingredients, g.kg^{-1}	**Samples**					
	1	**2**	**3**	**4**	**5**	**6**
Pork meat	790	790	790	790	790	790
Pork back fat	42	42	42	42	42	42
Emulsion (chia oil + water + sodium caseinate)	168	168	-	-	-	-
Emulsion (milk thistle oil + water + sodium caseinate)	-	-	168	168	-	-
Emulsion (pumpkin seed oils + water + sodium caseinate)	-	-	-	-	168	168
Sodium chloride	20	20	20	20	20	20
Sodium nitrite	0.05	0.05	0.05	0.05	0.05	0.05
Black pepper	4	4	4	4	4	4
Nutmeg	1	1	1	1	1	1
Sugar	2	2	2	2	2	2
Phosphates	2	2	2	2	2	2
Potato starch	30	-	30	-	30	-
Quinoa flour	-	30	-	30	-	30
Sodium caseinate	10	10	10	10	10	10
Water / ice	290	290	290	290	290	290

Methods. For the analysis of the texture profile of the finished 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 for the test from the sausage samples. 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).

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

The colour parameters were determined spectrophotometrically using a Minolta Chroma meter (model CR 410, Osaka, Japan) in the CIELab system.

Scanning electron microscopy (SEM) analysis was carried out with an SEM Philips 515, digitised.

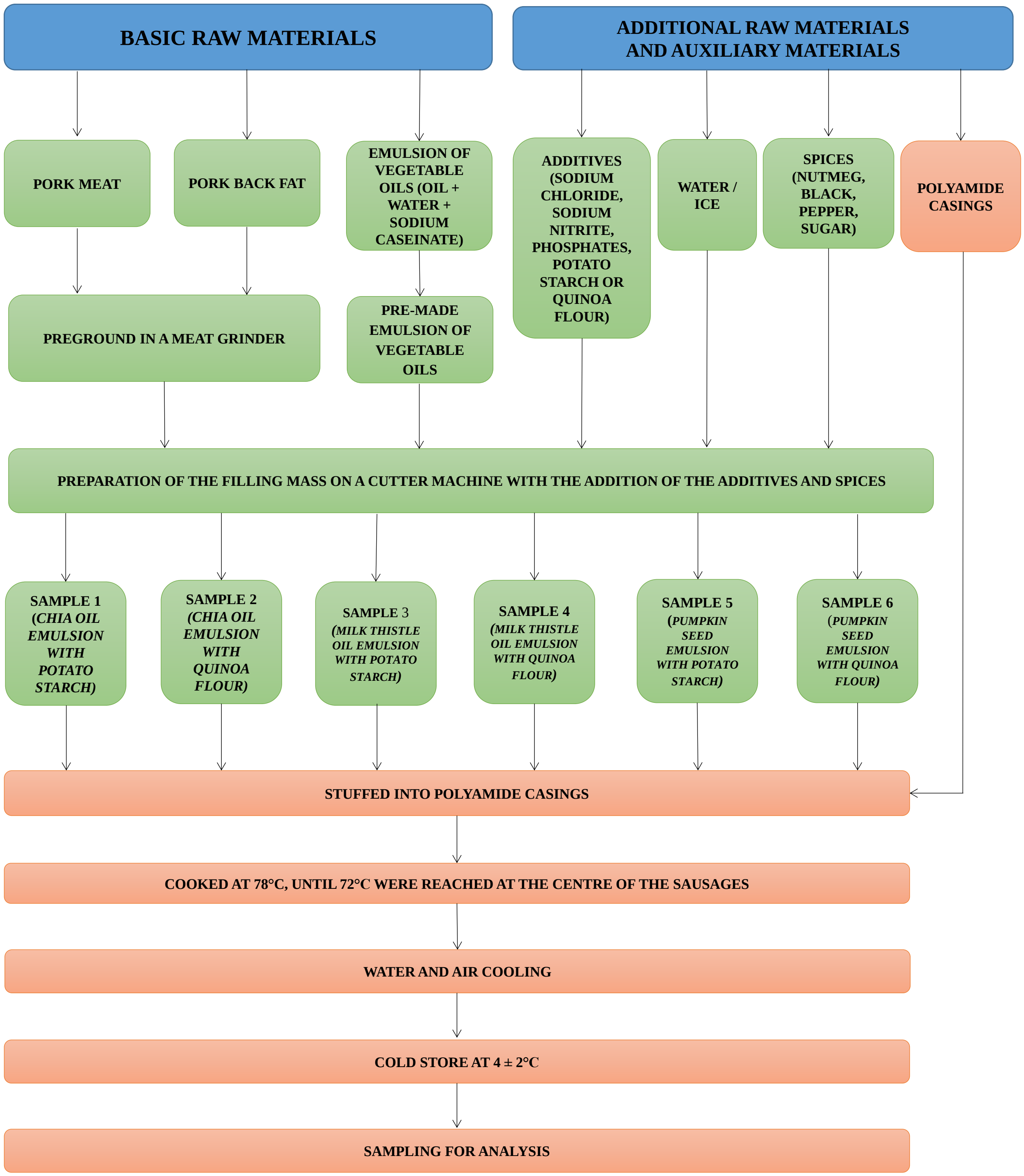


Figure 1. Processing diagram of reformulated cooked sausages

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 Electron Imaging mode. The accelerating voltage applied during the analysis was 8 eV. Photographs were taken at 500x and 1000x magnifications for each sample.

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

Results and Discussion

Texture properties and emulsion stability. Texture properties are a commonly accepted and indisputable criterion of the quality of reformulated meat products, cooked sausages in particular, where the integrity of the meat tissue is broken during the technological process and animal fat and/or vegetable oil is added. The comparison of the effect of the vegetable additives used showed significant differences in the hardness, resilience and adhesiveness parameters of the samples (Table 2). The sausage samples prepared with the addition of potato starch exhibited a clear trend towards an increase in hardness and decrease in resilience compared to the analogical samples made with quinoa flour. This was due to their characteristic technical and functional properties (surface activity, solubility, water retention and emulsifying capacity, etc.) resulting from the specific chemical composition and structure of quinoa flour and potato starch. The data obtained on the adhesiveness and gumminess of sausages indicated that quinoa flour contributed greatly to their reduction.

The experimental results obtained on the emulsion stability of the meat batter and the heat-treated sausages have been presented in Table 3. The meat batter emulsion stability values ranged from 93.58% to 96.2%. A well-outlined trend to an increase in the emulsion stability was observed in the samples formulated with the addition of quinoa flour, the highest and statistically discernible difference having been reported for sample 6 ($p < 0.05$). One of the main components of quinoa is cellulose (Repo-Carrasco-Valencia and Serna 2011). The structure of its macromolecule differs from that of starch in the number, type, spatial arrangement and linking of the components, each one of which corresponds to one β-glucose molecule. Unlike starch, the β-glucose residues are linked into straight, unbranched chains to form the polysaccharide chains in the cellulose molecule.

Table 2. TPA of cooked meat sausages with vegetable oil emulsions and quinoa flour or potato starch

Indicator	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Hardness, N	29.14 ± 3.30^{b}	27.48 ± 2.42^{ab}	29.12 ± 1.28^{b}	26.84 ± 4.07^{a}	32.97 ± 2.38^{c}	27.78 ± 1.54^{ab}
Springiness	0.92 ± 0.01^{a}	0.94 ± 0.02^{a}	0.94 ± 0.03^{a}	0.92 ± 0.05^{a}	0.93 ± 0.03^{a}	0.91 ± 0.04^{a}
Homogeneity	0.57 ± 0.03^{b}	0.58 ± 0.04^{b}	0.58 ± 0.05^{b}	0.53 ± 0.03^{a}	0.55 ± 0.03^{ab}	0.56 ± 0.03^{b}
Resilience	0.23 ± 0.04^{a}	0.34 ± 0.04^{b}	0.36 ± 0.01^{bc}	0.38 ± 0.01^{c}	0.34 ± 0.02^{b}	0.38 ± 0.01^{c}
Gumminess	20.09 ± 0.76^{a}	19.32 ± 1.19^{a}	20.69 ± 1.16^{a}	19.41 ± 3.04^{a}	23.04 ± 1.82^{b}	19.96 ± 1.57^{a}
Chewiness, N	22.25 ± 1.37^{b}	20.58 ± 1.1^{a}	22.09 ± 0.97^{ab}	21.13 ± 2.82^{ab}	24.87 ± 1.5^{c}	21.86 ± 1.13^{ab}
Adhesiveness, Nmm	-0.6 ± 0.23^{bc}	-0.5 ± 0.08^{cd}	-0.91 ± 0.12^{a}	-0.43 ± 0.06^{de}	-0.7 ± 0.10^{b}	-0.38 ± 0.05^{e}

Sample description: **sample 1**: chia oil emulsion with potato starch; **sample 2**: chia oil emulsion with quinoa flour; **sample 3**: milk thistle oil emulsion with potato starch; **sample 4**: milk thistle oil emulsion with quinoa flour; **sample 5**: pumpkin seed emulsion with potato starch; **sample 6**: pumpkin seed emulsion with quinoa flour

The values for the respective sample are the means of the measurements for the given indicator.

$^{a\text{-}e}$-values within the same row bearing a common superscript did not show statistically significant differences ($p > 0.05$)

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

Indicator	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
ES of meat batter, %	93.58±1.02^{a}	95.37±0.9^{bc}	93.76±1.15^{ab}	95.88±0.8^{c}	92.89±1.11^{a}	96.2±0.9^{c}
ES of sausages, %	89.62±0.5^{a}	91.11±0.3^{b}	89.08±0.8^{a}	91.52±0.9^{b}	89.18±0.7^{a}	91.74±0.8^{b}

Sample description: sample 1: chia oil emulsion with potato starch; sample 2: chia oil emulsion with quinoa flour; sample 3: milk thistle oil emulsion with potato starch; sample 4: milk thistle oil emulsion with quinoa flour; sample 5: pumpkin seed emulsion with potato starch; sample 6: pumpkin seed emulsion with quinoa flour
The values for the respective sample are the means of the measurements for the given indicator.
$^{a-c}$ -values within the same row bearing a common superscript did not show statistically significant differences ($p > 0.05$)

This typical linear structure of cellulose microfibrils perhaps underlies their better water-binding capacity (Medronho et al. 2018) and the improved meat emulsion stability of the three samples (2, 4 and 6) compared to potato starch. Another reason for the water and fat separation during the emulsion stability analysis was the difference in the functional properties, density in particular, of the vegetable oils and the protein-protein interaction network in the restructured meat batter. The release of a liquid was also facilitated by the meat matrix contraction as a result of the heat treatment. This transfer depended mainly on the technical and functional properties, the physical state and the swelling capacity of the individual components that make up the meat batter: proteins, fats, fibres, water, etc. These data are in conformity with the results obtained by other authors, who used quinoa paste, rice flour and mango fibres to improve the stability of meat emulsions (Winuprasith et al. 2013; Pereira et al. 2016; Pellegrini et al. 2018).

Colour characteristics. Colour is a very important parameter in the development of new, reformulated meat products since it is one of the primary, most essential factors considered by consumers in their evaluation of these products (Pintado et al. 2015a).

Table 4. Colour characteristics of cooked sausages made with different vegetable oils and potato starch or quinoa flour

Samples	Colour characteristics on the 7th day of storage				
	L	a*	b	C	h
Sample 1	72.29±1.65^{a}	5.97±0.49^{b}	7.55±0.36^{bc}	9.47±0.32^{b}	58.46±0.4^{bc}
Sample 2	74.87±0.51^{bc}	5.26±0.22^{a}	8.05±0.31^{c}	9.62±0.36^{b}	56.83±0.79^{b}
Sample 3	73.48±0.77^{ab}	6.05±0.19^{bc}	6.94±0.7^{a}	9.37±0.32^{ab}	49.72±1.24^{a}
Sample 4	74.16±0.45^{bc}	5.58±0.45^{ab}	7.15±0.32^{ab}	8.92±0.39^{a}	51.1±4.76^{a}
Sample 5	75.3±0.22^{c}	5.24±0.19^{a}	9.41±0.18^{d}	10.65±0.26^{c}	61.23±1.05^{cd}
Sample 6	75.49±0.29^{c}	5.17±0.23^{a}	9.96±0.14^{d}	11.26±0.22^{d}	62.25±0.48^{d}

Sample description: **sample 1**: chia oil emulsion with potato starch; **sample 2**: chia oil emulsion with quinoa flour; **sample 3**: milk thistle oil emulsion with potato starch; **sample 4**: milk thistle oil emulsion with quinoa flour; **sample 5**: pumpkin seed emulsion with potato starch; **sample 6**: pumpkin seed emulsion with quinoa flour
The values for the respective sample are the means of the measurements for the given indicator.
$^{a-d}$ -values within the same column bearing a common superscript did not show statistically significant differences ($p > 0.05$)

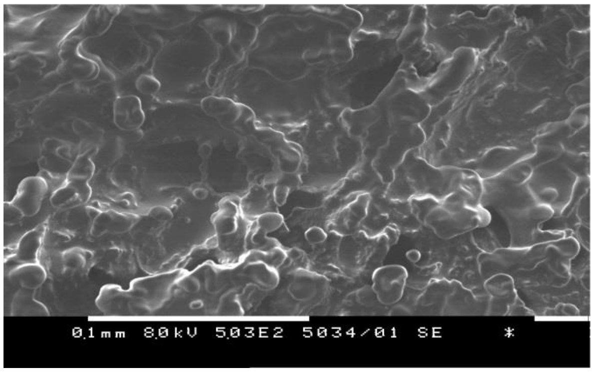
The results on the effect of the vegetable additives used on the indicators characterising the colour of the reformulated sausages have been presented in Table 4. The data obtained on the changes in L*, a*, b*, C and h showed a statistically significant difference in the values measured for the individual samples ($p < 0.05$), which was largely dependent on the type and condition of the non-meat ingredients used (Kulkarni et al. 2011; Youssef et al. 2011; Choe et al. 2013). On the whole, the samples prepared with quinoa flour had higher *L** values than the potato starch samples, sample 1, where chia oil emulsion and potato starch had been used, being the darkest. The higher *L** values in samples 2, 4 and 6 were probably due to the functional properties of quinoa flour leading to the formation of a meat emulsion, in which the oil droplets were considerably smaller in diameter that those in the emulsions prepared with potato starch (Fig. 2). That was the reason for the higher degree of reflection of light in these samples. A similar effect of the use of vegetable additives on the lightness of emulsion gels and veal burgers was reported by other authors as well (Poyato et al. 2014; Shokry, 2016). As regards the red component, there was a decrease in the *a** values in all sausage samples made with quinoa flour, but a statistically significant difference between the values was only observed in the chia oil samples ($p < 0.05$), (Table 4). Upon comparison of the effect of the type of vegetable oils used on *a**, greater intensity was registered within the red colour spectrum in the sausages formulated with chia and milk thistle oil emulsions. The *b** values of the samples and their colour hues (*h*) could be explained by the specific colour characteristics of the individual vegetable oils, as has also been reported in other research where olive oil, linseed oil and chia oil were used in reformulated meat products (Heck et al. 2019). For instance, pumpkin seed oil is yellow-green in colour unlike chia oil and milk thistle oils that have a more yellow colour. On the other hand, the *b** values demonstrated a trend to a more yellow colour of the sausages made with chia oil and pumpkin seed oil. This fact could be attributed to the translucent effect of milk thistle oil leading to lesser retention of the *b** values of those samples within the yellow colour spectrum (Lima et al. 2021). Fernández-López et al (2020b), who studied the effect of adding black quinoa wet-milling co-products to bologna-type sausages on their colour, reported a decrease in the *a** values and increase in *b**, *C* and *h* in the quinoa-containing samples. A similar trend was observed in our experimental samples, too.

SEM analysis. The investigation of the effect of different vegetable additives on the microstructure of cooked sausages is of particular interest for the evaluation of the technological characteristics of reformulated meat matrices that determine the quality indicators of the finished products. The comparison of the micrographs of the individual samples showed the formation of empty spaces, zones of different optical density and different degrees of coherence of the morphological elements in the newly formed meat matrices (Fig. 2).

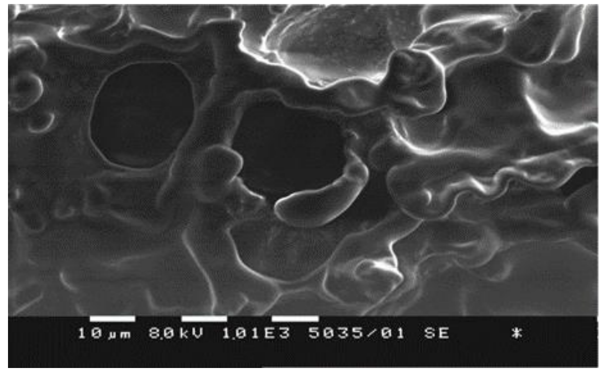
Within these structures, there were cavities and spongy structure in the sausages that played an essential role in the meat emulsion stabilisation. This fact could be due to some aspects of the technical and functional behaviour of meat and vegetable components, such as proteins, fats, carbohydrates, water and/or air, which form the meat batter for cooked sausages.

Similar results related to the cavities in the structural space have also been reported by other authors, who studied the change in the microstructure of emulsified meat products using olive oil and other vegetable oils (Jiménez et al. 2010; Delgado-Pando et al. 2011; Salcedo-Sandoval et al. 2013). The functional properties (gelation, emulsifying and water-binding capacity) of the vegetable additives used, integrated in an extremely complicated way with the occurring physical and biochemical transformations, affect the formation of the new multi-component structure of cooked sausages (Pintado et al. 2015a; Serdaroğlu et al. 2016).

The meat batters prepared with the addition of vegetable oils and quinoa flour (samples 2, 4 and 6) showed a more uniform distribution of the individual components, restructured into a more compact protein network with considerably fewer suspended grains in the morphological structure (Fig. 2).



Sample 1- 500x



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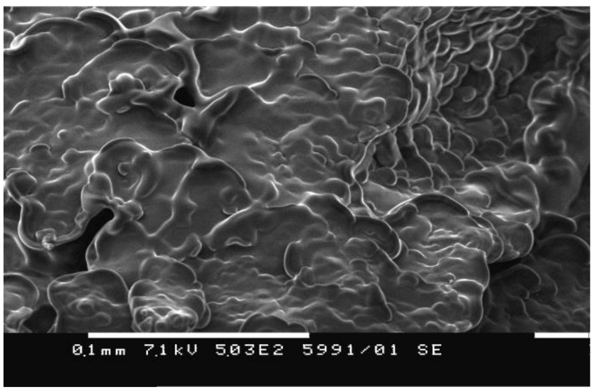

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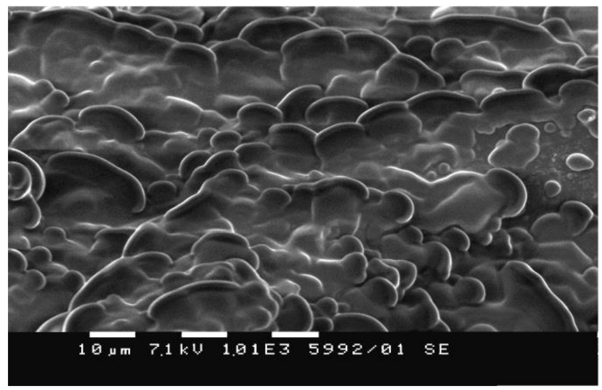

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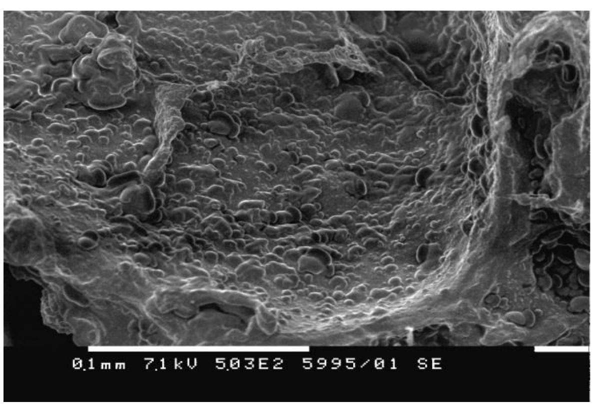
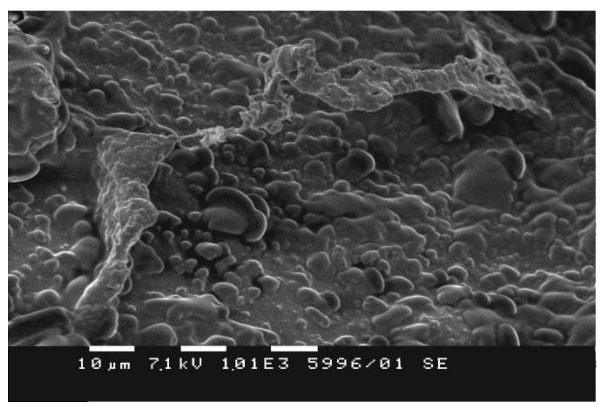


Sample 3- 500x



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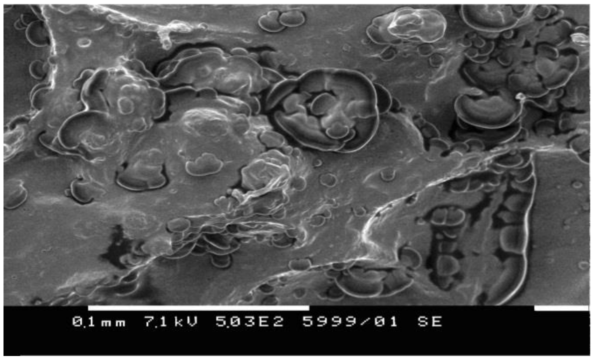
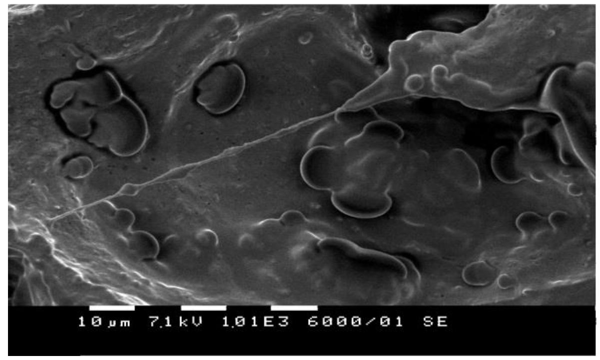
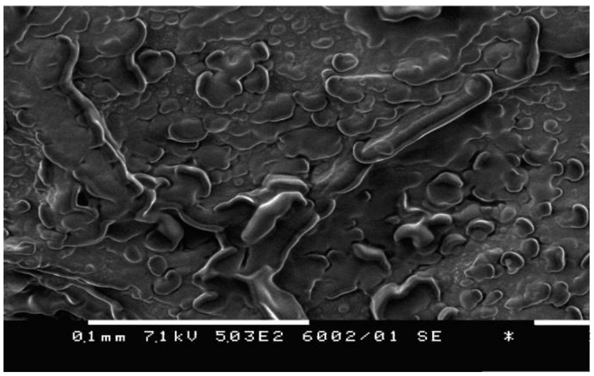


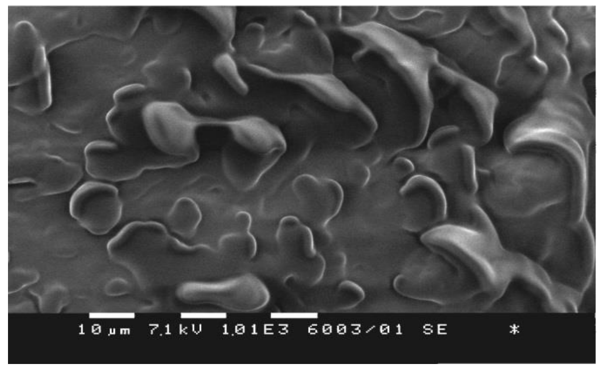
Sample 4- 500x



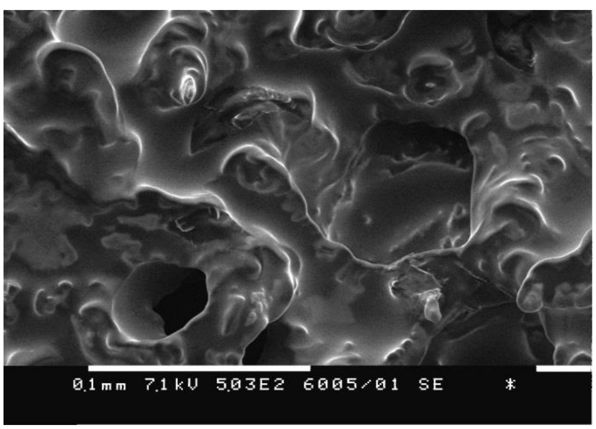
Sample 4- 1000x



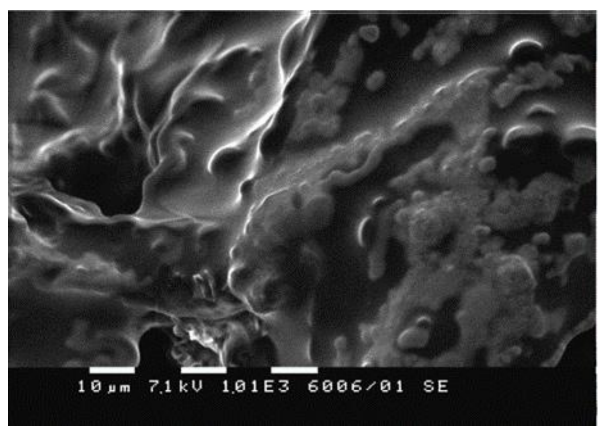
Sample 5- 500x



Sample 5- 1000x



Sample 6- 500x



Sample 6- 1000x

Figure 2. SEM micrographs of cooked sausages with different vegetable oils and potato starch or quinoa flour; magnification 100x and 500x. Sample description: sample 1: chia oil emulsion with potato starch; sample 2: chia oil emulsion with quinoa flour; sample 3: milk thistle oil emulsion with potato starch; sample 4: milk thistle oil emulsion with quinoa flour; sample 5: pumpkin seed emulsion with potato starch; sample 6: pumpkin seed emulsion with quinoa flour

This could be attributed to the occurrence of better protein-lipid and protein-water interactions (Dickinson, 2012; 2013) in the complex matrix of the meat batter that create the technological conditions required for the formation of a more stable emulsion with a weaker spongy structure in the sausages. In the samples made with potato starch, rougher and less organised morphological structure was observed (Fig. 2). The instability and greater disorganisation of the structure formed in samples 1, 3 and 5, perhaps due to the worse interaction of the emulsified fat with the other morphological elements, could be displayed in the release of water and fat during cooking. Furthermore, in the SEM images of the samples formulated with quinoa flour, an off-white film was clearly visible, which is related to the formation of a more specific kind of protein cover that has a positive effect on the capacity for immobilisation of the dispersed fat and free water in cooked sausages (Cáceres et al. 2008; Lima et al. 2021). This fact corresponded well to the data obtained on the emulsion stability of the samples (Table 3).

Conclusions

The research conducted showed that emulsions formulated with vegetable oils from chia, milk thistle and pumpkin seeds in combination with quinoa flour are promising ingredients for the adequate design and production of emulsified cooked sausages with regard to their quality and nutritional aspects in terms of animal fat substitution. Quinoa flour, which is rich in proteins and fibres, contributed to the formation of a more compact protein microstructure and improved the water and fat binding capacity, as confirmed by the increased emulsion stability and improved texture parameters of the reformulated meat products.

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