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Healthy vegetable oils and ingredients for reduced pork back fat content and evaluation of their impact on the nutritional aspects of comminuted cooked sausages

Здравословни растителни масла и съставки за намалено съдържание на свинска гръбна мазнина и оценка на въздействието им върху хранителните аспекти на смлените варени колбаси

Maria MOMCHILOVA[1] (✉), Dilyana GRADINARSKA-IVANOVA[2], Albena PARZHANOVA[1], Dinko YORDANOV[2]

[1] Food Technology Division, Institute of Food Preservation and Quality, Plovdiv, 154 Vasil Aprilov Blvd., Plovdiv, Agricultural Academy, Bulgaria

[2] Department of Meat and Fish Technology, Technological Faculty, University of Food Technologies, 26 Maritsa Blvd., Plovdiv 4002, Bulgaria

✉ Corresponding author: masha821982@abv.bg

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ABSTRACT

This study compares three healthy vegetable oils, from chia, milk thistle and pumpkin seeds, added as emulsions for partial substitution of animal fat and evaluation of the effect of quinoa flour as a potato starch substitute in the meat matrix of cooked sausages. For the purpose of evaluating the nutritional properties of the samples, the fatty acid composition, the cholesterol content and the oxidative changes in the lipid fraction were determined, and testing was performed for examination of sensory perception of the reformulated cooked sausages. Lower malondialdehyde (MDA) quantities were found in the sausages made with the addition of quinoa flour compared to the respective potato starch samples. In the reformulated meat products, the highest polyunsaturated fatty acid/saturated fatty acid (PUFA/SFA) ratio of 0.67 was recorded in the sample made with chia oil and quinoa flour. The same sample was given the highest overall sensory score, whereas the respective sample made with potato starch received a lower overall score, statistically indiscernible in relation to the milk thistle samples (P>0.05). The lowest sensory acceptance was reported for the samples made with pumpkin seed oil. The milk thistle oil, characterised by negligible cholesterol content and high phytosterol content, proved most interesting and capable of enrichment with unsaturated fatty acids.

Keywords: sausages, chia oil, milk thistle oil, pumpkin seed oil, quinoa, fatty acids, cholesterol content

РЕЗЮМЕ

В настоящото изследване е направено сравнение между три здравословни растителни масла от чия, бял трън и тиквени семки, вложени под формата на емулсии за частична замяна на животинската мазнина и оценка на влиянието на брашното от киноа като заместител на картофеното нишесте в месната матрица на варени колбаси. За оценка на хранителните свойства на пробите са установени мастнокиселинния състав, холестероловото съдържание и окислителните изменения в липидната фракция и беше проведено тестване за изследване на сетивното възприятие на преформулираните варени колбаси. По-ниски количества на малоновдиалдехид (MDA) са установени в колбасите с добавката на брашно от киноа, спрямо аналогичните проби с картофено нишесте. С най-високо съотношение на полиненаситени мастни киселини/наситени мастни киселини (PUFA/SFA) от преформулираните месни продукти се характеризира пробата с използването на масло от чия и брашно киноа, съответно 0,67. Отново при нея е получен и най-висок общ сензорен бал, докато аналогичната ѝ проба, с картофено нишесте, е получила по-ниска обща оценка, която е и статистически неразличима спрямо оценките за пробите с маслото от бял трън (*P*>0.05). С най-ниска сензорна приемливост са отличени пробите с маслото от тиквени семки. Особено интересно и обещаващо за обогатяване с ненаситени мастни киселини е маслото от бял трън, което има и незначително съдържание на холестерол и високо съдържание на фитостероли.

Ключови думи: колбаси, масло от чия, масло от бял трън, масло от тиквени семки, киноа, мастни киселини, съдържание на холестерол

INTRODUCTION

The initiatives for food reformulation aimed at the improvement of people's health and quality of life are regarded as an important element of the strategies for limiting the distribution of the main non-infectious diseases (cardiovascular diseases, new malignant formations, chronic respiratory diseases and diabetes) resulting from unhealthy eating habits. For example, the reduction in the intake of SFA, which are related to the risk of cardiovascular diseases, is recommended by World Health Organization (FAO/WHO, 2008); moreover, their partial replacement with PUFA is advocated to reduce the latter events (Lenighan et al., 2019).

To meat food manufacturers, however, the technological strategies for lipid reformulation do not consist solely in animal fat removal or its replacement with vegetable oils in the product formulation. Consistency is a basic problem of these strategies (Sisik et al., 2012; Nasonova and Tunieva, 2019), along with the stronger trend towards oxidation of unsaturated fatty acids. Therefore, it is of utmost importance to adapt the processing technologies in order to guarantee that animal fat substitution would indeed improve the nutritional properties of products without compromising its sensory quality and safety. Authoritative scientific research demonstrates the huge potential of emulsion gels for application in emulsion type sausages, contributing to the manufacture of meat products with acceptable quality attributes (Paglarini et al., 2018; Palagrini et al., 2019). On the other hand, the addition of technological additives, including binders or health promoting ingredients such as dietary fibre, can produce the desired effect on the meat emulsion stabilisation and the texture of the finished sausages (Bis-Souza et al., 2020; Younis et al., 2022).

Regardless of the constantly increasing number of studies focused on the potential of various vegetable oils for animal fat substitution in meat products and on the adaptation of the technologies for their addition, there have been no, or very few, studies that combine approaches aimed at the modification of the fatty acid profile of the finished products and improvement of the overall nutrient density of products by optimising their desirable components, such as antioxidants, dietary fibre, and technical and functional characteristics. The high amounts of unsaturated fatty acids (UFA), vitamins, minerals, dietary fibre, proteins and polyphenol compounds in quinoa flour determine the considerable interest in its use for enrichment of different foods, including meat products (Vega-Gálvez et al., 2010; Pellegrini et al., 2018; Zambrano et al., 2019). Chia oil, pumpkin seed oil and milk thistle oil were used for comparison of the effect of different emulsions of cold-pressed vegetable oils. Unlike chia and pumpkin seed oil, no scientific results are available on the incorporation of milk thistle oil in food regardless of the reports on the potential benefit of this oil for human health (Meddeb et al., 2017; 2018) and the low cost of its production.

This study was conducted to evaluate the nutritional properties of comminuted, emulsion-type pork sausages when pork fat was partially replaced with an emulsion of three different vegetable oils and addition of quinoa flour as a binder and extender additive instead of potato starch. The nutritional properties of the samples were evaluated by investigation of the fatty acid composition, cholesterol content and oxidative changes in the lipid fraction and performance of testing for determining sensory perception of the reformulated cooked sausages.

MATERIAL AND METHODS

The experiment was repeated two times and six types of comminuted cooked sausage samples were prepared for the purposes of the research, as shown in Table 1 and Figure 1.

Emulsions of the respective vegetable oils, sodium caseinate and water were pre-made in a 5:1:5 ratio. The meat raw materials were purchased from the local market, and the potato starch, quinoa flour and vegetable oils from specialised health food stores.

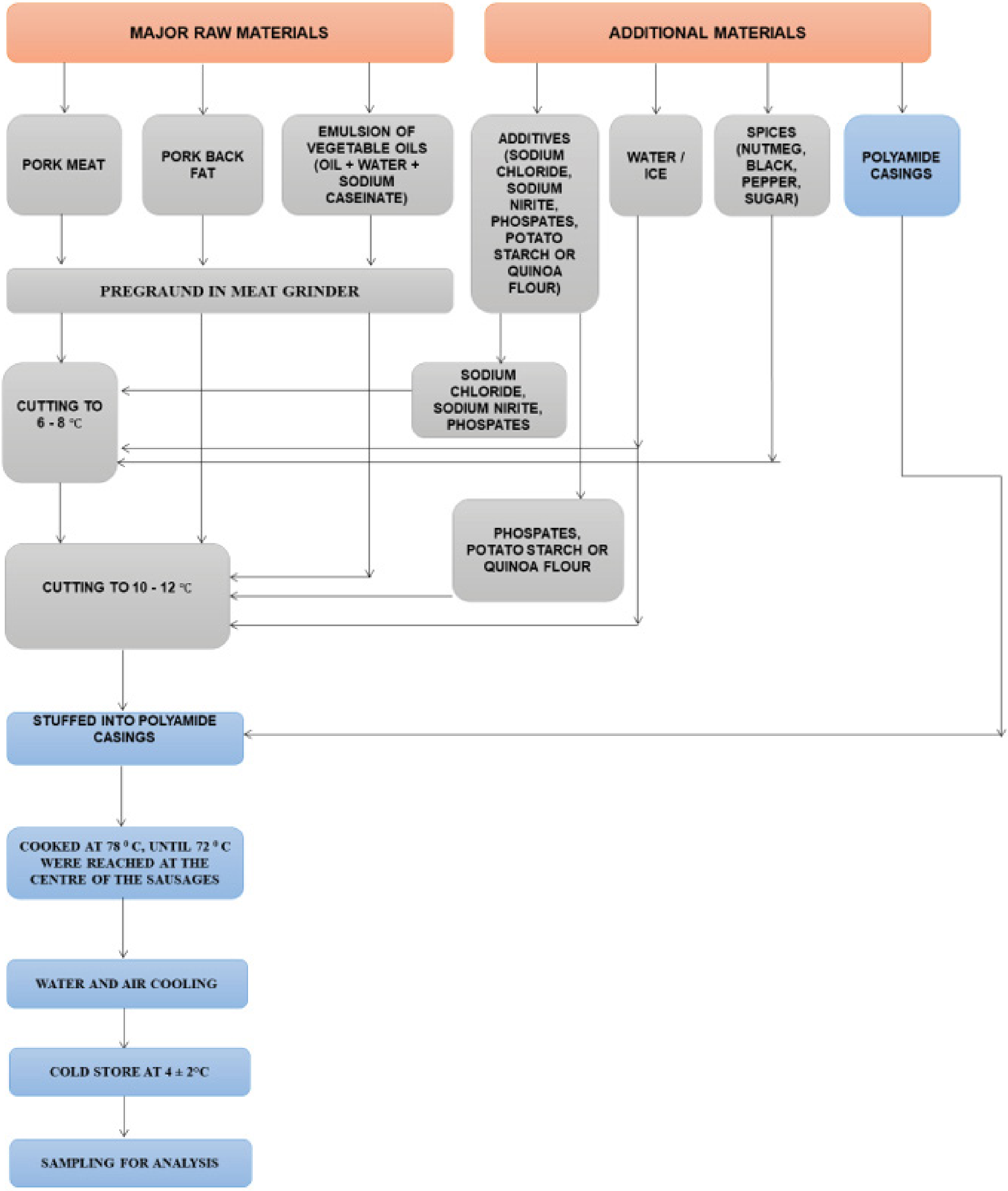

Figure 1. Processing diagram of reformulated comminuted cooked sausages

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

Ingredients, g/kg	Sample formulations					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 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

In our previous experiment the optimal replacement of pork back fat with chia oil emulsion in the emulsified cooked sausages was determined (20% fat : 80 % chia oil emulsion), by using simplex centroid mixture design.

The emulsified sausages with this optimized composition have the most similar characteristics compared with those of the control sausages. Based on that, the present experiment was conducted with this ratio of fat : vegetable oil emulsion.

Fatty acid composition

The fatty acid composition of fats was determined by gas chromatography (GC) (ISO 12966-4:2015). The determination of the fatty acid methyl esters (ISO 12966-2:2017) was performed on a gas chromatograph (HP 5890 series II) equipped with a 30 m x 0.25 mm x 25 μm capillary SUPELCO column and a flame ionisation detector. The column temperature programme ranged from 70 °C (hold for 1 min) at 6.0 °C /min to 190 °C at 10 °C /min, to 250 °C; the injector and detector temperature was 250 °C. The individual composition was determined by the relative retention time as well as using a standard mixture of fatty acid methyl esters, and finally adjusted/normalized to 100%.

The results on the individual identified fatty acids have been presented as a relative percentage of the area of the respective peaks recorded in the chromatogram in relation to the quantity of extracted lipids.

Analysis of sterols

The quantification of sterols was carried out spectrophotometrically after isolation of sterols from other unsaponifiable mix (Petkova et al., 2015; Ivanov et al., 1972). The sterol content was determined on an HP 5890 series II gas chromatograph (Hewlett Packard GmbH, Vienna, Austria) equipped with a 25 m x 0.25 mm

(I.D.) x 25 µm (film thickness) DB–5 capillary columns (Agilent Technologies, J&W Scientific Products, Santa Clara CA, USA) and a flame ionisation detector. The temperature gradient ranged from 90 °C (hold for 2 min) up to 290 °C at a rate of change of 15 °C/min; then up to 310 °C at a rate of 4 °C/min (hold for 10 min); detector temperature: 320 °C; injector temperature: 300 °C, and carrier gas: hydrogen (H2), split, 1:50. An analytical mixture containing cholesterol (stabilised, 95% purity, New Jersey, USA), stigmasterol (Sigma-Aldrich, 95% purity, St. Louis, MO, USA) and β-sitosterol was used to confirm the retention times of the individual sterols in the analysed samples (ISO 12228-1:2014).

Analysis of lipid oxidation

In order to evaluate the lipid oxidation level in the samples, an assay was performed of the secondary products of the oxidation reactions in the sausages in term of thiobarbituric acid reactive substances (TBARS) in them during storage (on the 7^{th}, 21^{st}, and 45^{th} days). The TBARS were measured according to the method of Li et al. (2020).

Sensory evaluation

The consumers' perception of the reformulated sausages was evaluated by sensory testing of the samples on the 7^{th} day of their refrigerated storage. The sensory panel included a total of 10 panellists which were acquainted with the sensory protocol and trained in sensory analysis and they were familiar with the problems of emulsified cooked sausage assessment. The products were submitted to the panellists with ambient temperature. A five-digit scale was used during sensory evaluation, where 5 corresponded to the completely satisfactory value and 1 to the entirely unsatisfactory state for the given parameter. The sausages were evaluated according to the following sensory parameters: appearance, colour, consistency, flavour, juiciness, aroma and overall assessment of the sensory perception of the products. An emphasis was given on the acceptability of the sensory perceptions for the descriptors flavour and aroma.

Statistical analysis

All the data obtained were statistically analysed by one-way analysis of variance (ANOVA) using the STATGRAPHICS 16 software product. Significant (P<0.05) differences between the treatments were determined using Duncan's post hoc test. The analyses were carried out with two samples of a batch of sausages (experiment), and the measurements were performed in triplicate for the determination of TBARS, fatty acid composition and cholesterol content and once for stigmasterol and β-sitosterol content of the vegetable oils. The data presented in the tables and figures were expressed as means ± standard deviation.

RESULTS AND DISCUSSION

In view of the fact that oxidative stability is an important index affecting the shelf life of processed meat products, the change in the TBARS values was monitored on the 7^{th}, 21^{st} and 45^{th} day of the refrigerated storage of the sausages reformulated with the three vegetable oils (Figure 2).

Initially, on the seventh day of the study, oxidative changes were established in the samples and expressed in mg MDA/kg of sausage, ranging from 0.17 to 0.47 mg MDA/kg. These values increased later, this increase being more pronounced and statistically significant on the 45^{th} day of the refrigerated storage of the sausages (P<0.05); however, the quantities measured remained lower than the value of 1 mg MDA/kg of sausage indicated for rancidity (Ockerman, 1985). An exception of the general trend was only registered with sample 6 prepared with a pumpkin seed oil emulsion and quinoa flour addition, where the TBARS value measured on the 45^{th} day was lower than the one measured on the 7^{th} day but higher than that measured on the 21^{st} day.

Another trend in the lipid oxidation changes observed in the sausages was related to the lower MDA quantities in the samples made with the addition of quinoa flour compared to the respective potato starch samples (Figure 2). This higher oxidative stability in the quinoa flour samples was consistent with the antioxidant properties reported for quinoa seeds (Tang et al., 2015).

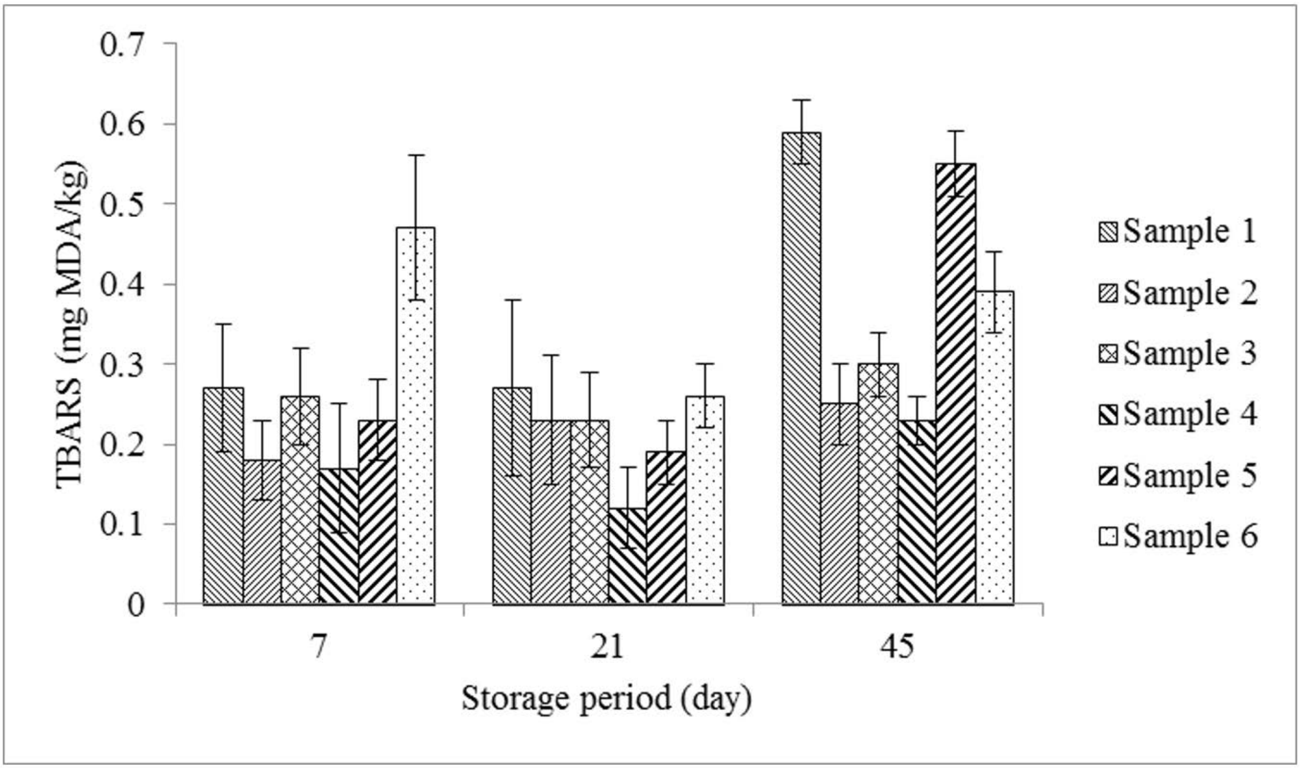

Figure 2. Lipid oxidation, expressed by changes in the TBARS values of reformulated cooked sausages prepared with emulsions of different vegetable oils and the addition of potato starch or quinoa flour during refrigerated storage

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 oil emulsion with potato starch; sample 6: pumpkin seed oil emulsion with quinoa flour

Table 2 and Table 3 show the results on the fatty acid composition and cholesterol content of the vegetable oils and pork back fat used and the lipid fraction of the sausage samples. Significant differences ($P<0.05$) were registered not only in the fatty acid profile of the sausages but also in their cholesterol content (Table 3). The differences resulted directly from the specific fatty acid composition of the vegetable oils used and their sterol content (Table 2).

Thus, for instance, the oleic acid quantity was the greatest in the chia oil, followed by palmitic acid, whereas the total PUFA content were in a statistically significant lower quantity in relation to the quantities in the chia and pumpkin seed oils. In this respect, the chia oil was closest to the fatty acid composition of the lipids extracted from pork back fat (Table 2). The pumpkin seed and milk thistle oils were characterised by a considerably higher UFA, especially PUFA, and the pumpkin seed oil exhibited the highest PUFA/SFA ratio of 1.66, followed by the milk thistle oil with a ratio of 1.55. The milk thistle oil also contained the highest stigmasterol and β-sitosterol quantities, these two phytosterols being among the main plant-derived sterols, associated with health-promoting effects in humans (Moreau et al., 2002).

Although animal fat is regarded as the main source of dietary cholesterol intake by people, a number of edible vegetable oils also contain cholesterol, the quantity of which can reach about 200 mg/kg, as is the case with camelina oil (Okpuzor et al., 2009; Shukla et al., 2002). Out of the vegetable oils studied, the highest cholesterol quantity was measured in the chia oil, followed by the pumpkin seed oil, whereas the cholesterol quantity in the milk thistle oil was very low (Table 2). These results suggested that the milk thistle oil had the best characteristics of all three oils with regard to enrichment with UFA and beneficial phytosterols in reformulated cooked sausages.

Table 2. Fatty acid composition and cholesterol, stigmasterol and β-sitosterol content in the chia, milk thistle and pumpkin seed vegetable oils and the extracted lipids from the pork back fat used for the manufacture of the reformulated comminuted cooked sausages

Fatty acids	Type of oil/fat			
	Chia oil	Milk thistle oil	Pumpkin seed oil	Pork back fat
C6:0	0±0[a]	0.2±0.1[b]	0.2±0.1[b]	0±0[a]
C 8:0	0.4±0.1[b]	0.1±0.1[a]	0±0[a]	0±0[a]
C10:0	0.5±0.2[b]	0±0[a]	0±0[a]	0±0[a]
C12:0	0.1±0.05[a]	0±0[a]	0±0[a]	0.1±0.1[a]
C13:0	0.2±0.1[b]	0±0[a]	0±0[a]	0.1±0.1[ab]
C14:0	0.2±0.1[a]	0.1±0.05[a]	0.1±0.07[a]	3±1[b]
C15:0	0±0[a]	0±0[a]	0±0[a]	0.2±0.1[b]
C15:1	0±0[a]	0.1±0.06[b]	0±0[a]	0±0[a]
C16:0	39.8±1.2[d]	10.5±1.8[a]	14.9±2.1[b]	34.4±0.1[c]
C16:1	0.1±0.05[a]	0.1±0.08[a]	0.1±0.08[a]	1.2±0.1[b]
C17:0	0.1±0.1[a]	0.1±0.1[a]	0.1±0.1[a]	0.7±0.1[b]
C17:1	0.5±0.1[b]	0.2±0.1[a]	0.1±0.05[a]	0.4±0.1[b]
C18:0	6.4±1.2[a]	7.1±1.3[a]	6.9±1.2[a]	9.5±0.1[b]
C18:1	44.1±0.1[c]	31.4±1.6[a]	38.3±2.3[b]	49.9±0.1[d]
C18:1 Trans	0.2±0.1[b]	0±0[a]	0±0[a]	0±0[a]
C18:2	2.3±0.1[a]	40.5±1.5[b]	38±2.6[b]	0±0[a]
C18:2 Trans	0±0[a]	0±0[a]	0±0[a]	0±0[a]
C18:3	3.7±0.1[b]	0.2±0.1[a]	0.2±0.1[a]	0.1±0.05[a]
C20:0	0.9±0.1[a]	4.2±1.1[b]	0.5±0.1[a]	0±0[a]
C20:1	0.4±0.1[b]	1±0.3[c]	0.2±0.05[ab]	0±0[a]
C20:2	0.2±0.1[b]	0±0[a]	0±0[a]	0±0[a]
C20:4	0±0[a]	0±0[a]	0±0[a]	0±0[a]
C22:0	0±0[a]	3±0.2c	0.2±0.05[b]	0±0[a]
C22:1	0±0[a]	0±0[a]	0±0[a]	0±0[a]
C23:0	0±0[a]	0.1±0.05[b]	0±0[a]	0±0[a]
C24:0	0±0[a]	0.1±0.05[b]	0±0[a]	0±0[a]
C24:1	0±0[a]	0.8±0.1[b]	0.1±0.05[a]	0±0[a]
SFA	48.8	26.2	23	48.1
UFA	51.2	73.8	77	51.9

Continued. Table 2.

Fatty acids	Type of oil/fat			
	Chia oil	Milk thistle oil	Pumpkin seed oil	Pork back fat
MUFA	45.2	33.1	38.8	51.8
PUFA	6	40.7	38.2	0.1
PUFA / SFA	0.122	1.553	1.661	0.002
MUFA/ PUFA	7.533	0.813	1.016	0.927
UFA / SFA	0.953	0.355	0.299	0.927
Stigmasterol, mg/100g oil	-	1.2 mg	0.9 mg	0.1
β-sitosterol, mg/100g oil	171.6	171.8	98.2	77.9
Cholesterol, mg/100g oil	12±2.0[b]	0.1±0.02[a]	2.2±1.2[a]	43.6±3.4[c]

The values given are the arithmetic means of three measurements for the given fatty acid in the relevant sample oil/fat
[a-d] values within the same row bearing a common superscript did not show statistically significant differences (*P*>0.05)

However, these expectations were not met after the analysis of the lipid fraction of the experimental sausage samples. The lipid fraction of the individual experimental samples showed significant differences with regard not only to the UFA composition but also to the saturated fatty acids. Nevertheless, the comparison of the effect of quinoa flour addition to the three pairs of samples demonstrated that its use led to a reduced MUFA/PUFA ratio at the expense of the increased PUFA quantity in those sausages. These results were in certain disagreement with the data reported by Pereira et al. (2019), who found that MUFA dominated the fatty acid composition of quinoa (approximately 40%), followed by PUFA (31–33%) and SFA (27–29%).

In all samples, the percentage of UFA was higher than that of SFA, and MUFA were present in it in a larger quantity. Oleic acid (C18:1) was the dominant fatty acid in this group, its content being higher in the chia oil and pumpkin seed oil samples. In view of the fact that a diet rich in MUFA is related to advantages for the human health (López-Miranda et al., 2006), the reformulation of comminuted cooked sausages with these oils could enhance their health-related properties. As regards the PUFA fraction of the sausage samples, it was affected not only by the phospholipid composition of the meat raw materials and the fatty acid composition of the vegetable oil used, but also by the addition, or no addition, of quinoa flour. PUFA have also demonstrated their health-promoting potential with regard to blood plasma cholesterol levels, blood pressure and cardiac arrhythmia Lorente-Cebrián et al. (2013). The main PUFA in the experimental sausage samples was linoleic acid (C18:2), the highest values having been recorded in the samples made with the addition of chia oil (Table 3), although it was present in larger quantities in the milk thistle and pumpkin seed oils (Table 3).

Perhaps, the heat treatment of the cooked sausages had led to a reduced quantity of the PUFA in these samples as a result of the more intensive oxidation reactions occurring in them since they are known to depend on temperature and on the UFA composition of the fat (Choe and Min, 2006). Other researchers, who also studied the effect of chia addition to meat products for animal fat substitution, reported a similar increase in PUFA in ready-made low-fat burgers (Heck et al., 2017). As regards linolenic acid (C18:3), it was reported to range between 0.1 and 0.2 %, this variation being dependent not only on its content in the oils used but also on the addition of quinoa flour.

Table 3. Fatty acid composition and cholesterol determined in the lipid fraction of reformulated comminuted cooked sausages

Fatty acids	Sample formulations					
	1	2	3	4	5	6
C6:0	0±0^{a}	0.1±0.05^{a}	0.6±0.08^{c}	0.5±0.1^{bc}	0.1±0.06^{a}	0.4±0.1^{b}
C 8:0	0.4±0.1^{c}	0±0^{a}	0.2±0.1^{b}	0.2±0.1^{b}	0±0^{a}	0.2±0.08^{b}
C10:0	1.1±0.8^{b}	0.1±0.08^{a}	0.1±0.1^{a}	0±0^{a}	0±0^{a}	0±0^{a}
C12:0	0.7±0.2^{b}	0.1±0.05^{a}	0±0^{a}	0.1±0.06^{a}	0.1±0.1^{a}	0.1±0.1^{a}
C13:0	0.6±0.05^{b}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
C14:0	1.9±0.12^{c}	0.9±0.09^{a}	1.4±0.2^{b}	1.4±0.1^{b}	1.2±0.2^{ab}	1.2±0.3^{ab}
C15:0	0±0^{a}	0.1±0.05^{b}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
C15:1	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
C16:0	26.2±2.8^{b}	21.4±1.2^{a}	24.1±1.8^{ab}	23.5±2.2^{ab}	25.1±2.8^{ab}	25.2±1.9^{ab}
C16:1	1.3±0.9^{ab}	1.4±1.1^{ab}	1.9±1.2^{b}	1.7±0.9^{ab}	1.7±1.1^{ab}	0±0^{a}
C17:0	0.1±0.05^{a}	0.2±0.1^{ab}	0.4±0.1^{c}	0.3±0.1^{bc}	0.3±0.1^{bc}	0.4±0.1^{c}
C17:1	0.3±0.1^{a}	0.2±0.1^{a}	0.5±0.1^{b}	0.2±0.1^{a}	0.2±0.1^{a}	0.3±0.09^{a}
C18:0	3.8±1.8^{a}	8.5±1.6^{b}	13.7±1.8^{c}	13.3±1.8^{c}	15.1±1.5^{c}	13.9±1.8^{c}
C18:1	41.1±2.9^{a}	38.6±2.2^{a}	38.8±2.9^{a}	37.5±2.1^{a}	41.3±2.8^{a}	40.9±2.5^{a}
C18:1 Trans	0.3±0.1^{b}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0.2±0.1^{b}
C18:2	18.0±2^{c}	23.3±2.4^{d}	13.2±1.8^{ab}	16.6±2.1^{bc}	12.6±2.3^{a}	13.8±2.6^{ab}
C18:2 Trans	0±0^{a}	0.1±0.05^{b}	0±0^{a}	0.1±0.1^{b}	0±0^{a}	0±0^{a}
C18:3	0.2±0.1^{a}	0.2±0.1^{a}	0.1±0.05^{a}	0.2±0.1^{a}	0.1±0.05^{a}	0.2±0.1^{a}
C20:0	0±0^{a}	2.3±1.2^{c}	1.7±0.9^{bc}	0.7±0.05^{ab}	0.6±0.1^{a}	0.4±0.1^{a}
C20:1	0.3±0.1^{a}	0.9±0.1^{c}	0.8±0.1^{c}	0.3±0.1^{a}	0.6±0.1^{b}	0.4±0.1^{a}
C20:2	0±0^{a}	0.2±0.05^{b}	0.2±0.1b^{b}	0±0^{a}	0.2±0.1^{b}	0.2±0.1^{b}
C20:4	0.1±0.02^{b}	0.1±0.05^{b}	0±0^{a}	0±0^{a}	0.1±0.05^{b}	0±0^{a}
C22:0	2.0±0.05^{c}	1.5±0.9^{bc}	1.2±0.3^{bc}	1.1±0.6^{b}	0.3±0.2^{a}	0.1±0.05^{a}
C22:1	0.1±0.05^{ab}	0.2±0.1^{b}	0.2±0.1^{b}	0±0^{a}	0±0^{a}	0±0^{a}
C23:0	0±0^{a}	0.3±0.1^{b}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
C24:0	0±0^{a}	0.4±0.1^{b}	0.3±0.1^{b}	0.3±0.1^{b}	0.1±0.05^{a}	0±0^{a}
C24:1	0±0^{a}	0±0.1^{a}	0.3±0.1^{b}	0.3±0.1^{b}	0.1±0.05^{a}	0±0^{a}
SFA	38.8	35.9	44	42.6	42.9	41.9
UFA	61.8	65.2	56	57.4	57.1	58.1
MUFA	43.5	41.3	42.5	40.6	44	43.9
PUFA	18.3	23.9	13.5	16.8	13.1	14.2

Continued. Table 3.

Fatty acids	Sample formulations					
	1	2	3	4	5	6
PUFA / SFA	0.472	0.666	0.307	0.394	0.305	0.339
MUFA/ PUFA	2.377	1.728	3.148	2.417	3.359	3.092
Cholesterol mg/100g	38.36±3.6[c]	37.3±2.2[c]	12.62±1.8[a]	13.93±2.1[a]	27.6±2.9[b]	28.67±1.2[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 oil emulsion with potato starch; sample 6: pumpkin seed oil emulsion with quinoa flour

The values given are the arithmetic means of three measurements for the given fatty acid in the relevant sample

[a-d] values within the same row bearing a common superscript did not show statistically significant differences (*P*>0.05)

Higher values were recorded in the sausages containing quinoa flour compared to those measured in the analogical samples made with potato starch.

A parameter widely used for assessment of the health-promoting profile of the lipid fraction of food is the PUFA/SFA ratio, the desirable values being over 0.4 (Decker and Park, 2010; Hathwar et al., 2012). It was only the lipid fraction of the samples made with chia oil emulsion that met this requirement (PUFA/SFA = 0.47 and 0.67 for sample 1 and 2, respectively) In the other samples, the ratio was below 0.4, although in sample 4, made with milk thistle emulsion and quinoa flour, it was 0.39, i.e. slightly less than the target value. Nevertheless, in view of the fact that the replacement of SFA with UFA (cis-MUFA and cis-PUFA) plays an essential role in the health benefits of food, especially with regard to the prevention of cardiovascular diseases (Domínguez et al., 2016; EFSA, 2011) as long as UFA make up at least 55 – 70% of the total fatty acids (EU Reg. 432/2012), all sausage samples could meet this health target.

A statistically significant difference (*P*<0.05) was registered in the cholesterol content of the different samples, the lowest level measured in the samples made with a milk thistle oil emulsion, and the highest one in those formulated with a chia oil emulsion. These differences were directly related to the cholesterol content in the source oil. A daily intake of less than 300 mg of cholesterol has been indicated for prevention of cardiovascular and coronary diseases (American Heart Association, 2004), which suggests that the consumption of 100 g of the reformulated sausages in samples 6, 4 and 3 will have a nutritional advantage over samples 5, 2 and 1, although the consumption of all samples in 100 g quantities would not exceed the limit specified.

The results of the sensory analysis made on the 7th day of the sample preparation have been presented in Table 4. All sausage samples received statistically indiscernible scores above 4 for the following sensory descriptors: appearance, consistency and aroma. The comparison of the scores given to the colour parameter showed scores below 4 for samples 5 and 6, lower than the scores for the other sausage samples. The panellists described the colour of these samples as lighter, having untypical hues, perhaps due to the natural greenish colour of the pumpkin seed oil. In the quantity used, the quinoa flour was barely perceptible to the panellists and was not reported to result in any sensory defects regarding the taste and aroma.

The highest overall sensory score was given to the sample containing chia oil and quinoa flour, whereas the analogical sample formulated with potato starch received a lower score, statistically indiscernible from samples 3 and 4, where milk thistle oil had been used (*P*>0.05). The other two samples, 5 and 6, prepared with the pumpkin seed oil emulsion and either potato starch or quinoa flour, were marked as the reformulated sausages having the lowest sensory acceptance.

Table 4. Sensory evaluation of cooked sausages made with different vegetable oils and potato starch or quinoa flour during storage

Samples	Sensory evaluation						
	Appearance	Color	Consistency	Flavour	Juiciness	Aroma	Overall liking
Sample 1	4.2±0.71[a]	4.15±0.88[ab]	4.4±0.66[a]	4.35±0.75[ab]	4.3±0.35[ab]	4.05±0.55[a]	4.2±0.54[ab]
Sample 2	4.15±0.85[a]	4.55±0.64[b]	4.65±0.41[a]	4.6±0.32[b]	4.4±0.66[b]	4.3±0.59[a]	4.6±0.39[b]
Sample 3	4.25±0.59[a]	4.25±0.82[ab]	4.5±0.47[a]	4.35±0.63[ab]	4.3±0.42[ab]	4.2±0.54[a]	4.3±0.63[ab]
Sample 4	4.3±0.48[a]	4.15±0.78[ab]	4.35±0.75[a]	4.05±0.76[ab]	4±0.58[ab]	4.05±0.64[a]	4.05±0.55[ab]
Sample 5	4.2±0.71[a]	3.75±1.11[ab]	4.3±0.75[a]	3.95±0.86[a]	4.1±0.52[ab]	4.1±0.57[a]	3.95±0.8[a]
Sample 6	4.05±0.83[a]	3.55±1.26[a]	4.35±0.53[a]	4.05±0.64[ab]	3.85±0.63[a]	4.05±0.55[a]	3.95±0.8[a]

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 oil emulsion with potato starch; sample 6: pumpkin seed oil emulsion with quinoa flour

[a-b] values within the same column bearing a common superscript did not show statistically significant differences (*P*>0.05)

CONCLUSIONS

The vegetable oils from chia, milk thistle and pumpkin seeds used in the form of emulsions for partial substitution of pork back fat in comminuted cooked sausages contributed to a general improvement of the lipid profile of the finished products. The reformulated meat products where quinoa flour and either chia oil or milk thistle oil emulsion had been used showed the highest PUFA/SFA ratio. The combination of the vegetable oils, which are susceptible to oxidation, with quinoa flour in the formulation of cooked sausages could contribute to a delay in the oxidation reactions during the subsequent storage of the sausages without any effect on the sensory properties of the finished products. Although the fatty acid composition of chia oil was closest to that of pork back fat compared to the other oils studied, and its use resulted in the highest sensory scores for the respective reformulated sausage samples, the milk thistle oil, characterised by a negligible cholesterol content and high phytosterol content, is particularly interesting with regard to the enrichment of meat products with MUFA and PUFA.

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