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Original article

Crude Fat Analysis from Some Meat Products by Differential Scanning Calorimetry[1]

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Abstract

This paper presents the thermal analysis of crude fat from salami samples which were reformulated by their lipid profile differentiation using the differential scanning calorimetry (DSC) method. Salami samples were manufactured by partial substitution of the back fat with vegetable oils and walnuts. Thermal curves profile of the crude fat was correlated with the lipids profile determined by gas-chromatography/mass spectrometry (GC/MS) method. The addition of lipids from vegetable sources determined a decrease of the ratio of saturated/unsaturated fatty acids from 0.639 (control) to 0.283 (salami with oil) and 0.218 (salami with oil and walnuts). The thermal curves obtained were different between the samples. For each sample, the crystallization profile showed an exothermic event for the reformulated samples and two events for the control sample, for different onset temperatures: 15.41^{0}C (control sample), 1.73^{0}C (salami with oil) and -5.12^{0}C (salami with oil and walnuts). The same profile was observed for two different heat flow rates: 10^{0}C/min and 20^{0}C/min, respectively. The melting profile showed three endothermic events for the reformulated samples and two events for the control sample. The samples were different regarding the onset temperature of the last endothermic event which was 11.82^{0}C for the control sample, 18.73^{0}C for salami with oil and 15.15^{0}C for salami with oil and walnuts, respectively, for both heat flow rates. DSC showed the physical properties and thermal behaviour for each chemical composition of the fat. DSC is a promising and rapid method for assessing the thermal fingerprint of a meat product by analyzing the crude fat.

Keywords: DSC, Lipids, Meat products, Thermal profile.

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INTRODUCTION

Lipids for human consumption are mostly saturated lipids, coming from the animal kingdom and particularly unsaturated lipids, from the plant area. In terrestrial animals, the composition of triacylglycerols (TAGs) has the following predominant fatty acids (FAs): palmitic acid, stearic acid and oleic acid. Lipids from the vegetable area may contain a wide range of FAs, but oleic acid and linoleic acid occur in most commercial oils. The DSC analysis protocols have highlighted the potential of the method for characterizing lipids physical properties regarding temperature and time. Tan and Che Man (2000), have shown that the thermal events of vegetable oils are specific for each type of oil. Similar results have been obtained in other studies that have focused on the sustainability of the DSC method regarding the analysis of the physical characteristics, determined by the chemical composition of oils and fats. Thus, in the evaluation of the thermal characteristics of oils, Nassu, (1999) and Fasina, (2008) obtained specific thermal curves for each type of commercial oils. The olive oil was the most studied one for geographical origin (Chatziantoniou et al., 2014) and authenticity assessment (Chiavaro et al., 2008), as well as cocoa butter (Chiavaro, 2015), sesame oil (Fahimdanesh, 2014), chia seed oil (Timilsena et al., 2017). Different researches in the field have studied the thermal characteristics of mixtures of oils with animal fats in order to detect the falsification. (Márquez et al., 2003; Marikkar et al., 2012; Dahimi et al., 2014) Sasaki (2006) showed that major peaks in DSC curves were similar among types of adipose tissue but the temperatures of the melting peak and conclusion point differed among them, because the physical properties of the mixtures of different types of TAGs had specific thermal properties. The melting/crystallization temperatures of TAGs are influenced by the FAs composition through the polymorphic form, according to Belitz (2009), FAs chain lengths, the number of double bonds and their position, isomerism. (Hidalgo and Zamora, 2005) The most studied physical properties of lipids by DSC were the melting and crystallization profiles. The aim of this preliminary study was to identify the thermal characteristics specific to the lipid component from the composition of a meat product which has a substantially improved nutritional value by addition of functional ingredients. The crude fat was extracted from pasteurized salami samples, manufactured simultaneously by the same technology but with lipids from different sources. The lipid profile differentiation was achieved using meat from different species (pig and beef), with different percentages of back fat, mixture of vegetable oils and walnuts. The obtained DSC curves were correlated with the FAs composition of the crude fat, determined by gas-chromatography/mass spectrometry (GC/MS). The thermal curve profiles were found to be specific for each sample through the number of thermal events and the temperatures of the exo (endo)thermic events. The complex composition of TAGs was dependent of the lipid sources. A good reproducibility of the results was obtained, maintaining the same solvent type, the same extraction method and the same DSC method. These conditions were also specified by Angiuli (2009) regarding the results reproducibility.

Materials and Methods

Experimental sample salami

Materials used for this study consisted in 3 salami samples which were differentiated from compositional point of view (the quantity of the ingredients is presented in descending order) as follows:

1. Control sample (labelled as P6T) - country salami, made from pork, water, back fat, vegetable and animal protein, spices, common technological additives, preservatives, antioxidants.

2. Salami sample (labelled as P5M) made from pork, vegetable oil mixture (sunflower, grape seeds, corn), water, cranberry, back fat, sodium caseinate, spices, common technological additives, antioxidants.

3. Salami sample (labelled as P4N) made from pork, beef, water, walnuts, vegetable oil mixture (sunflower, grape seeds, corn), sodium caseinate, back fat, spices, common technological additives, antioxidants.

The salami samples were manufactured according to the technology of cooked-smoked salami, matured for 48 hours at temperatures of 4 – 6 °C, vacuum packed, pressurized at 600 MPa for 3 min, using HPP technology (Spain), sliced and packed using the skin pack technology. The manufacturing and packaging technology ensures samples innocuity and removes the mass losses during storage. The samples were taken in the analysis after 6 days from the date of manufacture.

Crude fats extraction

The crude fats was extracted according to AOAC Method 965.33/2006 with some modifications. 100 ml of chloroform (Chemical, RO) in the presence of 40 g of anhydrous Na_2SO_4, p.a. (Chemical, RO) were added to 70 g of finely chopped sample. The mixture was vigorous stirred for 10 min, rested in the dark for 25 min and filtrated. The solvent was evaporated using a rotovap (Hei-Vap ML, type G6), followed by removal of the solvent traces by drying under liquid nitrogen to a constant mass. Crude fat extraction was performed in duplicate for each salami sample.

Lipid profile of crude fats

The lipid profile of crude fats (CF) was determined using two reference standards: SRM 2377 – a mixture of 26 FAMEi (fatty acid methyl esters) with rated mass fraction (mg/g) certified by NIST and F.A.M.E. Mix. C4-C24 – a mixture of 37 FAMEi, with mass percentages (%) for the 37 FAMEi. All solvents and reagents used in the experiments were of chromatographic purity / ACS / residual analysis (petroleum ether, 5.4 M sodium methoxide solution, 14% boron trifluoride methanol solution, sodium chloride, methanol, isooctane, etc.). FAME was prepared by transesterification of the extracted fat from salami samples with sodium methoxide solution and BF_3 methanol solution in accordance with SR EN ISO 12966-2: 2011. FAME analysis was performed in accordance with SR EN ISO 12966-4: 2015, with

modifications from the reference by using a gas chromatograph coupled with the mass spectrometer (Trace GC Ultra/TSQ Quantum XLS, Thermo Fisher Scientific, USA). A capillary column with high polarity was used (TR-FAME, 60 m x 0.25 mm x 0.25 μm). The analysis of the derivatized sample extracts was carried out in the positive electron impact (EI^+) ionization mode, SIM mode (Selected Ion Monitoring) by using 24 segments. The ion source temperature was 250°C, the furnace temperature was programmed at 100 °C for 0.2 min, rising to 240°C, with 2°C/min and holding for 15 min at this temperature. Helium 5.0 (99.9995% purity) was used as mobile phase at a constant flow rate of 1 ml/min. 0.5 μL of extract was injected at 240 °C in split mode with a split ratio of 1:50 and a splitting flow rate of 50 ml/min. Injections were performed in duplicate. Instrument control, data acquisition and processing were performed using the Xcalibur software. Peak identification from the food matrices was performed by comparing with the retention times of the FAMEi components from the reference standards used and by the mass/charge (m/z) ratio which is characteristic for each component. The composition in fatty acids from the studied matrices was determined as relative concentration (mass percentages, %) based on correction factors (F_{cor}). F_{cor} were determined from the both reference standards, SRM 2377 and F.A.M.E. Mix. C4-C24 (23 FAMEi common to both standards, 3 FAMEi specific to SRM 2377 and 14 FAMEi specific to F.A.M.E. Mix. C4-C24).

The composition in FAs, individual and total, SFAs (saturated FAs), MUFAs (monounsaturated FAs) and PUFAs (polyunsaturated FAs) from CFs extracted from salami samples was determined based on correction factors (F_{cor}) and evaluated according to the repeatability limit (r) and the absolute difference between 2 results (Δ). Thus, it was followed that Δ of two independent analysis results to have $r < 5\%$.

Determination of the thermal characteristics of crude fats

Determination of the thermal characteristics of the CFs extracted from the salami samples was performed using a differential scanning calorimeter (DSC 8000, Perkin Elmer, USA) with power compensation (the power was independently compensated between the pans with the sample and the pans without sample (reference)). Calibration of the temperature and heat of the fusion was carried out in the temperature range of -40°C to + 300°C using indium (ΔH_f = 28.5 J/g and Tm = 156.5°C). From each CF sample, 5 mg were approximately weighed in hermetically pans (Perkin Elmer) with help of a microbalance (Mettler Toledo, d = 0.1 μg). The pans were conditioned at 20°C for 20 hours. The samples were encapsulated in duplicated for each cooling/heating heat flow rate and analyzed according to a similar method described by Peyronel and Marangoni (AOCS, Lipid Library) and modified according to the method of Chiavaro (2015). Cooling/melting heat flow rate: r (dq/dt) = $10°Cmin^{-1}$ and r (dq/dt) = 20°C/min, in the temperature range -40°C - +80°C, with an isothermal time of 15 min at -40°C and +80°C. Nitrogen 5.0 (99.999% purity) was used as purge gas at a rate of 20 ml/min. Curves were processed by the Pyris software and To, Tpeak (maxim) and Tend (°C) were calculated from

crystallization and melting profiles. The following method steps were employed to study the melting and crystallization of crude fat from salami samples:

Step 1: Initial temperature 20°C.

Step 2: Melting sample at a heat flow rate of 10°C/min up to 80°C to complete the melting of crystals.

Step 3: Isothermal for 15 min in order to equilibrate the sample temperature, remove polymorphic transformations and erase all memory of the crystal structure.

Step 4: Cooling sample at a heat flow rate of 10°C/min from 80°C to -40°C to format the crystalline networks in the mass of the sample.

Step 5: Isothermal for 15 min at -40°C to balance the mass of crystals.

Step 6: Melting sample at a heat flow rate of 10°C/min, up to 80°C to complete melting crystals of sample.

The method assures the deletion of the thermal memory of the mixtures of TAGs by subjecting to changes from the liquid phase into the solid phase and then in the liquid phase.

Statistical analysis

All the determinations were done in duplicate. The data were expressed as mean ± SD.

Results and Discussion

Lipid profile of crude fats

The lipid profile of CFs from the three salami formulations changed significantly for each composition. Analyzing the chemical composition of TAGs in each sample (Table 1), a quantitative differentiation based on fatty acid groups was observed, but also a qualitative differentiation due to the presence of some FAs in all samples. Some FAs were present only in the compositions of salami with lipids addition from vegetable sources and the presence of FAs specific to each salami composition were also observed.

Table 1. Fatty acids composition in crude fat[a]

Samples	P4N	P5M	P6T
Butanoic (C4:0)	0.04±0.00	0.03±0.00	-
Caproic (C6:0)	0.03±0.00	0.01±0.00	-
Caprylic (C8:0)	0.02±0.00	0.02±0.00	-
Capric (C10:0)	0.07±0.01	0.07±0.01	-
Lauric (C12:0)	0.06±0.00	0.08±0.01	-
Myristic (C14:0)	1.11±0.02	1.28±0.20	-
Pentadecanoic (C15:0)	0.08±0.00	0.04±0.01	-
Palmitic (C16:0)	9.72±0.73	12.58±0.18	24.48±0.55
Margaric (C17:0)	0.49±0.04	0.36±0.04	-
Stearic (C18:0)	3.93±0.16	6.09±0.09	14.52±0.45
Arachidic (C20:0)	0.46±0.05	0.37±0.03	-
Heneicosanoic (C21:0)	-	0.03±0.00	-
Behenic (C22:0)	0.73±0.07	0.52±0.03	-
Tricosanoic (C23:0)	0.03±0.00	0.02±0.00	-
Lignoceric (C24:0)	0.25±0.03	0.18±0.01	-
Σ SFA (Saturated fatty acids)	**17.02**	**21.68**	**39.00**
Myristoleic (C14:1n5) c*is*-5-	0.07±0.00	-	-
Palmitoleic (C16:1n7) c*is*-7-	2.83±0.07	3.29±0.49	2.11±0.06
Heptadecenoic (C17:1n7) *cis*-7-	-	0.21±0.03	-
Oleic (C18:1n9) c*is*-9-	24.13±0.31	33.7±0.09	42.84±0.03
Vaccenic (C18:1n11) c*is*-11-	1.16±007	1.93±0.08	2.82±0.11
Gondoic (C20:1n9) c*is*-9-	1.14±0.12	1.51±0.12	-
Erucic (C22:1n9) *Cis*-9-	-	0.02±0.00	-
Σ MUFA (Monounsaturated fatty acids)	**29.33**	**40.66**	**47.77**
Linoleic (C18:2n6) *cis*-11,14-	48.28±0.26	35.33±0.45	13.23±0.22
Eicosadienoic (C20:2n6) *cis*-11,14-	0.34±0.04	0.54±0.04	-
Docosadienoic (C22:2n6) *cis*-13,16-	0.07±0.01	-	-
Σ PUFA 2n6 (Polyunsaturated fatty acids)	**48.69**	**35.87**	**13.23**
γ-linolenic (C18:3n6)	-	0.04±0.00	-
Linolenic (C18:3n3)	4.32±0.08	0.93±0.10	-
Dihomo-γ-linolenic (C20:3n6)	0.13±0.02	0.13±0.01	-
Arachidonic (C20:4n6)	0.39±0.04	0.43±0.03	-
Eicosatrienoic (C20:3n3) *cis*-11,14,17-	0.06±0.01	0.13±0.01	-
Docosapentaenoic (C22:5n3) *cis*-7,10,13,16,19-	0.06±0.01	0.09±0.00	-
Docosahexaenoic (C22:6n3) *cis*-4,7,10,13,16,19-	-	0.03±0.00	-
Σ PUFA 3n3, 3n6	**4.96**	**1.78**	**0**

a Fatty acids composition expressed in g/100g fat. The data were expressed as mean ± SD.

SFAs decreased quantitatively by reducing the back fat content from the salami compositions at P6T to P4N. Palmitic acid and stearic acid were the major SFAs constituents of CF and they were present in all samples; the highest amount was in P6T sample. There were many other SFAs in the samples with vegetable oils addition, and myristic and behenic acids were in more significant quantities.

Oleic acid was the main MUFA present in all samples, which was quantitatively reduced: from 42.84% CF in P6T to 24.13% CF in P4N, as the amount of back fat in the composition was lower. Other MUFAs common to the three samples were: *cis*-vaccenic acid that was reduced, similarly to oleic acid, the smallest quantity was in P4N sample. Palmitoleic acid was in the highest amount in P5M sample, because the vegetable oils were the main source of palmitoleic acid. Gondoic acid was additionally identified in the samples with lipids from plant sources: 1.14% CF in P4N sample and 1.51% CF in P5M sample. *cis*-10-Heptadecenoic acid (0.21% CF) and erucic acid (0.02% CF) were found only in the P5M sample. P5M sample had the highest vegetable oil content, which was the main source of these fatty acids.

PUFAs fatty acids were grouped into PUFAs with two double bonds, PUFAs with three or more double bonds, correlating the impact of increasing the number of acyl groups and double bonds with the thermal characteristics of CFs. Linoleic acid is the main FA component of the group found in all samples, which increased in quantity in the following order: 13.23% CF in P6T < 35.33% CF in P5M < 48.28% CF in P4N, as the percentage of back fat replaced by the lipids from vegetable sources decreased in the sample composition. *Cis*-11,14-Eicosadienoic acid was found in the samples with lipids from vegetable sources, namely, sample P4N (0.34% CF) and sample P5M sample (0.54% CF), while *cis*-13,16-docosadienoic acid was only identified in the P4N sample (0.07% CF) due to the presence of the walnuts in this salami composition. Walnuts are the main source of *cis*-13,16-docosadienoic acid.

PUFAs with three double bonds were found only in the samples with lipids from vegetable sources. Linolenic acid was in the highest amount in P4N (4.32% CF) as compared with P5M sample (0.93% CF), the walnuts being an important source of linolenic acid. In a small amount *cis*-4,7,10,13,16,19-docosahexaenoic acid (0.03% CF) was found only in the P5M sample. The data obtained showed that each salami composition had a unique lipid profile.

Thermal characteristics of crude fats

Figure 1 and figure 2 shows the comparison of the thermal curves with scanning rate of 10^0C/min and 20^0C/min. It was observed that the beginning of crystallization took place at different temperatures for each crude fat. The number of exothermic event is different for the samples with lipids addition from vegetable sources (P4N and P5M) as compared to the sample with animal fat (P6T). The increase in the cooling rate from r = 10°C/min to r = 20°Cmin^{-1} showed a different crystallization process of CFs. For P4N sample, a single exothermic event was produced for both cooling rates; for P5M sample,

a second exothermic event occurred at r = 10°C/min, while for P6T sample, 3 exothermic events took place at both cooling rates. Samples P4N and P5M presented wider peaks, TAGs mixture was more heterogeneous because of the higher content of unsaturated fatty acids. P6T sample presented 3 different sharp peaks at r = 10^0C/min si 2 evenimente exoterme la r = 20^0C/min, in domeniu de temperatura ales. Each thermal event was defined by the onset crystallization (Toc) temperatures; Tpeak (maximum) crystallization (Tc) and the end of the thermal phenomenon temperature (Tend) for crystallization. Tpeak – To (in which the maximum energy yield/ acquisition occurred) and Tend – To (where full crystal formation took place).

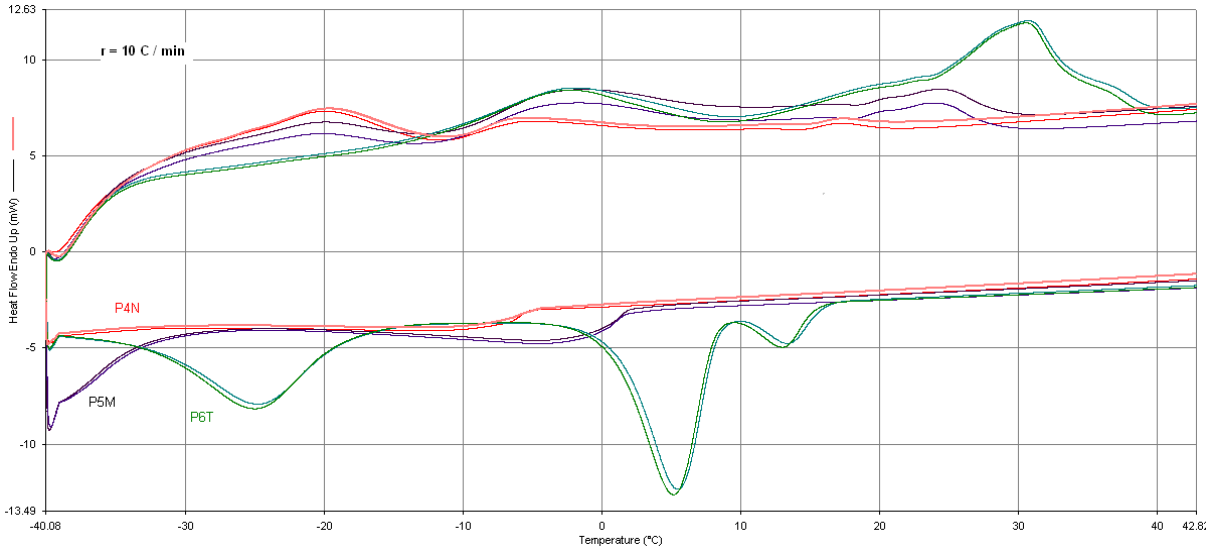

Figure 1. Thermal curves: r (dq/dt) = 10°C/min; Heat Flow Endo Up (mW). P4N - salami with addition of vegetable oils and walnuts, P5M - salami with addition of vegetable oils, P6T - salami with back fat. Every sample was scanned in duplicate.

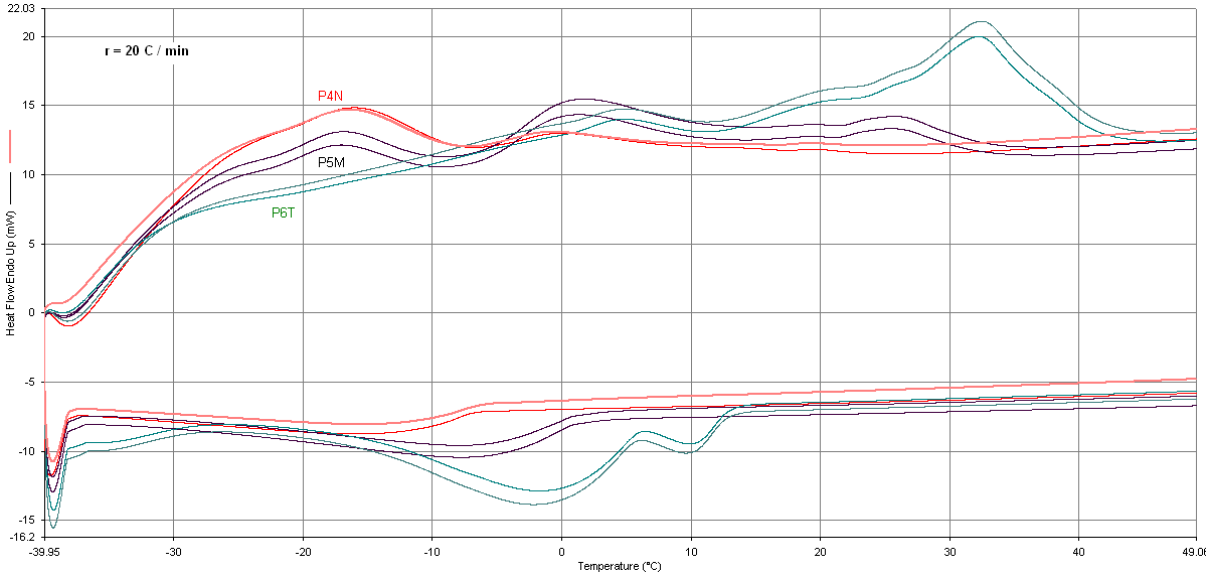

Figure 2. Thermal curves: r (dq/dt) = 20°C/min. Heat Flow Endo Up (mW). P4N - salami with addition of vegetable oils and walnuts, P5M - salami with addition of vegetable oils, P6T - salami with back fat. Every sample was scanned in duplicate.

The values for temperatures are presented in Table 2.

Table 2. Crystallization profiles for crude fats[a]

Heat flow rate	Samples	Exothermic events	Toc (^{0}C)	Tc (^{0}C)	Tend (^{0}C)	Tc - Toc	Tend - Toc
r (dq/dt) 10°C/min	**P4N**	1	-5.12±0.25	-10.63±0.06	-24.60±0.27	5.51	19.48
	P5M	1	1.73±0.13	-3.78±0.03	-17.92±0.14	5.51	19.65
	P6T	1	15.41±0.27	13.29±0.21	10.53±0.14	2.12	4.88
		2	8.15±0.11	5.29±0.16	0.83±0.22	2.86	7.32
		3	-18.52±0.02	-24.83±0.11	-32.76±0.18	6.31	14.24
r (dq/dt) 20°C/min	**P4N**	1	-6.52±0.31	-15.01±0.2	-33.52±0.97	8.49	27.00
	P5M	1	0.87±0.03	-7.85±0.00	-25.28±1.14	8.72	26.15
	P6T	1	12.57±0.08	10.18±0.13	6.74±0.15	2.39	5.83
		2	5.74±0.15	-2.00±0.20	-15.99±0.06	7.74	21.69

[a] Values are expressed as mean ± SD. SD < 1 °C

It was observed that for both cooling rate, Toc for P4N sample had negative value, while for P5M sample, the value was positive. As for P6T sample, the values were positive for first two exothermic events and negative for the third one. For sample P4N, the maximum energy yield, Tc – Toc, was produced at 5.5°C at r = 10°C/min and 8.5°C at r = 20°C/min, and for sample P5M was produced at 5.5°C at r = 10°C/min and 8.7°C at r = 20°C/min. In the nucleation process, TAGs are arranged to optimize intra and intermolecular interactions with the aim to form a wrapping structure as stable as possible. Although the crystallization network formed in the polymorph mixture of TAGs is strongly influenced by fatty acids composition: P4N sample with oils and walnuts addition had a PUFAs content of 48.28% CF linoleic acid and 4.32% CF linolenic acid compared to P5M sample with oils addition had a PUFAs content of 35.33% CF linoleic acid and 0.93% CF acid linolenic (Table 1). Da Silva, (2013), observed a similar behaviour to the dilution of lard with soybean oil. The explanation was attributed to the formation of different crystals from number and size point of view, depending on the ratio of saturated/unsaturated fatty acids. Increasing of cooling rate displace Tc along x axis to negative values with 4.38^{0}C (P4N) and 4.07^{0}C (P5M). The marginal position of unsaturated fatty acids in the glycerol esterification forms strong bonds in the intermolecular rearrangements of TAGs during the heating or cooling process. (Chiavaro, 2015). The greater the number of *cis* double bonds, the more negative the temperature required for crystal formation is. Thus, a high content of PUFAs with two or more *cis* double bonds in P4N sample resulted in crystals fraction formation at more negative temperatures than in P5M sample. This aspect was observed at both cooling rates. Dahimi, (2014), observed movement peaks can be a very strong indicator on the contamination levels associated to the capability of small doses (0.1% – 1.0%) of lard to change the crystallisation regime of mixed chicken

fat and beef tallow edible lipid materials. The FAs composition in P6T sample had the lowest linoleic acid content (13.23% CF) and the highest MUFAs (47.77% CF) and SFAs (39.8% CF) content (Table 1) and the crystals fraction formation occurred at positive temperatures with a higher energy release. The onset temperature for the first two exothermic events was positive, and for the third exothermic event it was negative at r = 10°C/min (Table 2). At r = 20°C/min, exothermic events had positive onset temperatures at 12.57°C and 5.74°C. For P6T sample, the temperature range in which exothermic events occurred was 26.44°C at r = 10°C/min and 27.52°C at r = 20°C/min. These values were approximately equal to the values of the samples with vegetable oils addition at r = 20°C/min. It was noted that each lipid sample had a specific crystallization profile, significantly different between the sample with back fat and the samples with lipids addition from vegetable sources. Tan (2000) noted that although the DSC method did not provide detailed information on the chemical composition of TAGs, the thermal characteristics were strongly influenced by this. (Tan et al., 2000). The amounts of PUFAs, MUFAs and SFAs were strongly correlated with the number of exothermic events and the temperature range in which each event occurred.

The analysis of thermal melting curves highlighted a number of three different endothermic events for the samples with vegetable oils addition and two events for the sample with back fat, for both heating rates. The melting profiles are unique for each sample. Similar results were obtained by Fasina (2008), who analyzed 14 commercial oils by DSC.

The onset temperature for the first endothermic event took place at negative values close to those for the samples with TAGs from vegetable oils for both cooling rates (Table 3).

Table 3. Melting profiles for crude fats[a]

Heat flow rate	Samples	Endothermic events	Tom (^{0}C)	Tm (^{0}C)	Tend (^{0}C)	Tm - Tom	Tend - Tom
r (dq/dt) 10^0C/min	**P4N**	1	-26.95±0.78	-19.88±0.14	-12.39±0.95	7.07	14.56
		2	-10.02±0.20	-5.61±0.20	2.98±0.55	4.41	13.00
		3	15.15±0.15	17.22±0.05	19.95±0	2.07	4.80
	P5M	1	-27.61±0.45	-20.54±0.15	-14.91±0.13	7.07	12.70
		2	-10.05±0.4	-2.64±0.26	6.42±0.93	7.41	16.47
		3	18.73±0.15	24.48±0.16	27.87±0.19	5.75	9.14
	P6T	1	-12.22±0.10	-2.66±0.15	6.64±0.53	9.56	18.86
		2	11.82±0.8	30.60±0.9	40.41±0.01	18.78	28.59
r (dq/dt) 20^0C/min	**P4N**	1	-23.05±1.77	-15.96±0.36	-9.33±0.08	7.09	13.72
		2	-5.80±0.38	-0.45±0.4	6.70±0.63	5.35	12.50
		3	17.59±0.26	19.48±0.16	22.00±0.42	1.89	4.41
	P5M	1	-22.44±0.08	-17.02±0.14	-11.16±0.12	5.42	11.28
		2	-5.53±0.25	1.00±0.26	10.80±0.22	6.53	16.33
		3	21.82±0.08	26.28±0.13	30.52±0.02	4.46	8.70
	P6T	1	0.76±0.02	4.80±0.07	9.86±0.07	4.04	9.10
		2	13.05±0.1	32.35±0.16	45.86±0.13	19.3	32.81

[a]Values are expressed as mean ± SD. SD < 1 °C

Although Tm – Tom had similar value for both samples, the melting of the crystal network (Tend – Tom) was finalized on a higher temperature interval for P4N sample as compared to P5M, probably due to the higher unsaturated fatty acid content in P4N sample.

The second endothermic event took place at the same Tom = –10.02°C for both samples (P4N and P5M). It was found that the maximum energy requirement for melting crystals (Tm - Tom) was lower in case of P4N sample, while the complete crystals melting (Tend – Tom) had a higher value for P5M sample, for both cooling rate. This endothermic event is generated by TAGs with a higher content of SFAs and MUFAs from P5M sample as compared to P4N sample (Table 1).

The third endothermic event occurred at Tom = 15.15°C for sample P4N and Tom = 18.73°C for sample P5M. Tm and Tend had more positive value in case of P5M sample, while Tm-Tom and Tend-Tom were almost twofold higher in P5M sample as compared to P4N sample. In case of a complex mixture of TAGs, the melting of the crystal network in different temperature intervals is influenced by the saturated/unsaturated fatty acids ratio. The ratio for P5M was 0.283, which is higher than the ratio for P4N (0.218). The influence of TAGs composition in the thermic behaviour was similar for both

heating rates. Thus, in the case of P4N sample with MUFAs content of 29.33% CF and PUFAs content of 53.65% CF, the first endothermic event had the highest energy requirement of 7.07°C, followed by 2 events that had a decreasing energy requirement (4.41°C and 2.07°C) at r = 10°C/min, which was maintained at r = 20°C/min. P5M sample, with MUFAs content of 40.66% CF and PUFAs content of 37.65% CF, had the first two endothermic events close to the energy requirement of 7.07°C and 7.41°C and a lower energy requirement at the third endothermic event of 5.75°C, which was maintained at r = 20°C/min. It was observed that in the case of P4N sample, the energy requirement for melting crystals is directly dependent on the unsaturated FAs and the maximum melting temperature (Tm) migrated on the axis to negative values. Bezerr (2017), showed that the shifting of the endothermic events towards negative values took place when the percentage of oil in animal fat was increased. Similar results were also obtained by Marikkar (2012) in the case of sunflower oil contamination with lard, beef tallow and chicken fat (minimum amount of 2%) through different positions of Tm on the melting curves.

The endothermic curve for P6T sample differed significantly from samples with vegetable oils but also according to the heating rate. The onset temperatures of the first endothermic event were different depending on the heating rate. Thus, at r = 10°C/min, Tom was negative (–12.22°C) and at r = 20°C/min, Tom had a positive value (0.76°C). It is noted that depending on the heating rate, both the Tm – Tom and Tend – Tom differences had a double value at r = 10°C/min compared to the value at r = 20°C/min, showing the influence of heating rate over TAGs with saturated FAs. In the case of sample P6T with the highest SFAs (39.0%CF) and MUFAs (47.77%CF), the second endothermic event is characterized by positive value of To, Tm and Tend, with similar value for both heating rates. Tm had the highest value among the samples, being characteristic to TAGs with TAGs with SFAs and MUFAs.

Conclusions

Differentiation of the thermal profiles of the crude fat extracted from salami samples with lipids addition from vegetable sources was correlated with the fatty acid composition of the crude fat. Differentiation of the thermal behaviour presented two major aspects: the onset temperatures of the thermal events may be close as values, but the temperature range in which the thermal events occurred are different and both the onset temperature and the temperature range at which the thermal events occurred were different. The results obtained support the conclusion of different studies according to which the thermal characteristics of a mixture of TAGs were strongly influenced by the chemical composition of TAGs, using the same working conditions and DSC method. Differential scanning calorimetry was proved to be an easy and relevant method for analyzing the physical properties of lipids that have a defined history in a food system. The thermal profile represents a thermal fingerprint of the fat from some meat products.

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