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PII: S0309-1740(17)30190-0
DOI: doi: 10.1016/j.meatsci.2017.04.260
Reference: MESC 7267

To appear in: *Meat Science*

Received date: 14 February 2017
Revised date: 24 April 2017
Accepted date: 28 April 2017

Please cite this article as: K. Shikha Ojha, Camila A. Perussello, Carlos A. Garcia, Joesph P. Kerry, Daniel Pando, Brijesh K. Tiwari , Ultrasonic-assisted incorporation of nano-encapsulated omega-3 fatty acids to enhance the fatty acid profile of pork meat, *Meat Science* (2017), doi: 10.1016/j.meatsci.2017.04.260

Ultrasonic-assisted incorporation of nano-encapsulated omega-3 fatty acids to enhance the fatty acid profile of pork meat

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Abstract

In this study, ultrasound was employed to enhance the diffusion of microencapsulated fatty acids into pork meat. Nanovesicles of fish oil composed of 42% EPA (eicosapentanoic acid) and 16% DHA (docosahexanoic acid) were prepared using two different commercial Pronanosome preparations (Lipo-N and Lipo-CAT; which yield cationic and non-cationic nanovesicles, respectively). The thin film hydration methodology was employed for encapsulation. Pork meat (*Musculus semitendinosus*) was submerged in the nanovesicles suspension and subjected to ultrasound (US) treatment at 25 kHz for either 30 or 60 min. Samples were analysed for fatty acid composition using gas chromatography-flame ionisation (GC-FID). The content of long-chain PUFAs, especially omega-3, was found to increase following the US treatment which was higher for Lipo-CAT compared to Lipo-N nanovesicles. Samples subjected to Lipo-N had higher atherogenic and thrombogenic indices, indicating higher levels of saturated fatty acids compared to the Lipo-CAT. The omega-6/omega-3 ratio in pork meat was significantly reduced following the US treatment, thus indicating an improved fatty acid profile of pork.

1. Introduction

Pork is a rich source of proteins, possessing high biological value and a number of bioactive molecules, including taurine, B vitamins and minerals. However, pork also contains high levels of lipids, which have been a topic of discussion for meat consumers due to their associated health implications. Relationships between dietary fat intake and incidence of various lifestyle disorders, including cardiovascular diseases, is well established and several health agencies have specific guidelines in this regard (Dugan, Vahmani, Turner, Mapiye, Juárez, Prieto, et al., 2015; Troy, Tiwari, & Joo, 2016). For instance, the World Health

Organisation, recommends that various dietary fat fractions should contribute <15–30%, <10%, <5–8%, <1–2% and <1% of the total energy intake from total fat, saturated fatty acids, n(6)-polyunsaturated fatty acids, n(3)-polyunsaturated fatty acids and trans fatty acids, respectively (WHO, 2003). Lipid content of pork generally varies from 4-15% on a fresh basis, depending on several factors, including; geographical origin, genotype, feeding regime and meat cut (Park, Kim, Lee, Jang, Kim, Lee, et al., 2012; Wood, Enser, Fisher, Nute, Sheard, Richardson, et al., 2008).

According to Jiménez-Colmenero, Cofrades, Herrero, Fernández-Martín, Rodríguez-Salas, and Ruiz-Capillas (2012) and Olmedilla-Alonso, Jiménez-Colmenero, and Sánchez-Muniz (2013), the nutritional quality of meat products can be improved towards the reduction of caloric value and fat content, enhancement of fatty acid profile (e.g. reduction of saturated fatty acids and increase in MUFAs and PUFAs), reduction of cholesterol content, increase of amino acid quality, enrichment with minerals, vitamins and antioxidants, and reduction of sodium, nitrite and phosphate contents.

Genetic and environmental factors largely affect meat quality. Animal tissues vary in composition according to species, breed, age, sex, feeding, climate, rearing and slaughtering conditions (Bonneau & Lebret, 2010). According to Żak and Pieszka (2009), genomic manipulation may result in improved carcass and meat quality (lean and fat content). Carcass and meat quality are influenced simultaneously by genes known as quantitative trait loci (QTLs) and environment. Additionally, animal feeding composition and portion affects growth rate, hormone metabolism and quantity and quality of fat within porcine muscles. The fatty acid profile of pork meat can also be modified by changing the dietary composition of fatty acids, either by adding vegetable oils or supplementing conjugated linoleic acid (CLA) to the pigs' diet, which improves feed conversion (Bonneau & Lebret, 2010; Rosenvold &

Andersen, 2003). Studies have shown that the alteration of feeding regime e.g. grass fed vs grain fed can improve meat fatty acid profile (Daley, Abbott, Doyle, Nader, & Larson, 2010).

In the last decade, specific strategies for increasing the level of fatty acids with beneficial health effects, while reducing the content of saturated fatty acids, have been a subject of active research (Horcada, Polvillo, Juárez, Avilés, Martínez, & Peña, 2016; Mapiye, Vahmani, Mlambo, Muchenje, Dzama, Hoffman, et al., 2015; Pouzo, Fanego, Santini, Descalzo, & Pavan, 2015; Xu, He, Liang, McAllister, & Yang, 2014). Different strategies have also been adopted to particularly focus on intramuscular fat (Scollan, Hocquette, Nuernberg, Dannenberger, Richardson, & Moloney, 2006). One such approach is the incorporation of encapsulated polyunsaturated omega-3 fatty acids into meat to enhance its fatty acid profile, while oils are preserved from oxidation and further degradation. Studies have shown that microencapsulated omega-3 fatty acids from a range of sources, such as fish, can be achieved successfully. Several techniques are available in order to encapsulate targeted fatty acids; the most popularly employed approaches being; coacervation, spray drying, spray chilling, extrusion coating and liposome entrapment by thin film hydration (TFH) (Kaushik, Dowling, Barrow, & Adhikari, 2015; Martín, Varona, Navarrete, & Cocero, 2010). Incorporation of microencapsulated oils have been carried out in a range of food matrices for various potential health benefits (Bilia, Guccione, Isacchi, Righeschi, Firenzuoli, & Bergonzi, 2014; Gallardo, Guida, Martinez, López, Bernhardt, Blasco, et al., 2013; Jiménez-Colmenero, 2013). However, incorporation of encapsulated ingredients in solid foods such as meat is challenging compared to liquid foods, since diffusion rates are low and the oil is not effectively dispersed into a solidfood matrix. Among the techniques investigated to date, ultrasound has shown its potential for use in the assisted diffusion of a number of ingredients within food matrices. For example, the application of ultrasound can enhance NaCl diffusion rates, thereby allowing for faster and more uniform diffusion of NaCl into

meat tissues (Carcel, Benedito, Bon, & Mulet, 2007; Jayasooriya, Torley, D'Arcy, & Bhandari, 2007; Ojha, Keenan, Bright, Kerry, & Tiwari, 2016; Ozuna, Puig, García-Pérez, Mulet, & Cárcel, 2013). However, the application of ultrasound for enhancing diffusion of microencapsulated ingredients has not been investigated to date. The objectives of this study were (a) to assess the potential for ultrasound application to improve the diffusion of encapsulated oils into a pork meat matrix, in terms of fatty acid profile of cooked and uncooked pork meat using a chemometric approach and (b) characterise both cationic (Lipo-CAT) and non-cationic (Lipo-N) nanoencapsulated fatty acids.

2. Material and methods

2.1 Preparation of nanovesicles

Fish oil for encapsulation was purchased from Neoalgae (Oviedo, Spain). EPA (eicosapentanoic acid) and DHA (docosahexanoic acid) contents were 42% and 16%, respectively. Two different nanovesicles were prepared using the Pronanosome Lipo-N and Pronanosome Lipo-cat blend techniques. The first one was employed to generate non-cationic nanovesicles, while the second one yielded cationic nanovesicles. Although both formulations are confidential, all ingredients employed were of food grade. The nanovesicles were generated by means of the TFH method (Martín, Varona, Navarrete, & Cocero, 2010). Briefly, specific amounts of fish oil and the selected Pronanosome (Lipo-N or Lipo-cat) formulation were solubilized in chloroform; then the solvent was evaporated firstly using a rotatory evaporator and finally under nitrogen stream. The dried films obtained were rehydrated for 20 min using ultrapure water at 55ºC. Subsequently, the samples were homogenised at 15000 rpm for 15 min using a SilentCrusher M homogenizer (Heidolph). Finally, in order to obtain a homogenous size distribution, a further extrusion step (Avesting, LiposoFast LF50) was employed using a 500 nm pore size cellulose filter (Millipore). Non-

encapsulated fish oil was removed using two purification steps. Firstly, samples were centrifuged at 5000 rpm for 1h and the supernatant, which contained the non-encapsulated oil, was carefully decanted. Following this step, the pellets were dialysed as follows: samples were filled into 10k MWCO SnakeSkin dialysis tubing (Thermo Fischer) and submerged in ultrapure water for 24h. The final composition of the nanovesicles was 100 mg/mL of Pronanosome formulation and 10 mg/mL of fish oil, respectively, for both cationic and non-cationic nanovesicles.

2.2. Nanovesicles characterisation

Vesicle characterisation was carried out according to the protocol described by Pando, Beltran, Gerone, Matos, and Pazos (2015). Measurement of size and determination of Polidispersity Index (PDI) of the samples were carried out using Dynamic Light Scattering (DLS) (Zetasizer NanoZS90, Malvern Instruments). Ten microliters of the sample was diluted into 990 μL of ultrapure water and the measurements were carried out in triplicate. Zeta potential was determined using the Mixed Measurement Mode-Phase Analysis Light Scattering technology (M3-PALS) (ZetaSizer NanoZS90, Malvern Instruments). The particle concentration of the sample was determined using Nanoparticle Track Analysis (NTA) in a Nanosight LM10 device (Malvern Instruments). The samples were diluted at 1:200 using ultrapure water and three video captures were recorded to calculate the particle concentration of samples.

2.3 Sample preparation and ultrasound treatment

Meat used for all treatments was porcine *M. semitendinosus* which was obtained from a local supplier (Dublin Meat Company, Blanchardstown, Co. Dublin, Ireland) at 6 days post-

mortem. The muscles were derived from Large White crosses, slaughtered at approx. 96 kg live weight. All visible fat was manually removed from each muscle, prior to slicing and subsequent treatments. Samples were cut into slices of approximately similar weight and size (4 x 4 x4 mm, length x width x height). Meat cubes were subjected to treatments immediately after slicing.

Pork meat pieces were submerged in the corresponding nanovesicles suspension in a glass beaker and were placed in an ultrasonic bath operating at a frequency of 25 kHz (Elma Schmidbauer GmbH, Germany) for either 30 or 60 min. Control (no US treatment) samples were placed in a beaker containing nanovesicles suspension. During the experiments, temperature was kept constant at 4.0±1.0°C by circulation of cold water using a temperature-controlled refrigerated water bath. After treatment, samples were removed, blot-dried and vacuum packed in high gas barrier laminated pouches. Control, ultrasound treated and raw pork samples were cooked in hot water bath (90 ^{o}C) to a core temperature of 70ºC for 10 min. The cooked and uncooked samples were cooled, vacuum packed and stored at -80 ^{o}C for fatty acid analysis.

2.5 Microwave- assisted preparation of fatty acid methyl esters (FAMEs)

Microwave-assisted FAME preparation was conducted using a MARS 6 Express 40 position Microwave Reaction System (CEM Corporation, Matthews, NC, USA). Reactions took place in PFA 55 mL reaction vessels. For FAME preparation, 1.0 g of pork samples was added to the reaction vessel containing a 10 mm stir bar. To this, 10 mL of potassium hydroxide (2.5%, w/v) in methanol was added along with 100 µL of internal standard (ISTD) (C23:0 methyl ester; final concentration following extraction is 0.1 mg/mL in pentane), and the reaction vessel was heated in the MARS 6 Express system to 130°C within 4 min and

held at this temperature for 4 min. The reaction vessels were then removed from the carousel and cooled on ice for 5 min, or until they had reached room temperature before being opened. Derivatisation was then carried out by adding 15 mL of 5% (v/v) acetyl chloride in MeOH solution and heated to 120°C within 4 min and holding at this temperature for 2 min. The reaction tubes were removed again and cooled on ice to room temperature. To the cooled tubes, 10 mL of pentane was added, and the reaction tubes were shaken to extract the FAMEs into the upper pentane layer. Subsequently, 15 mL of a saturated salt solution was added, and the solution was mixed again. Following separation of the layers, the top pentane layer was removed and aliquoted into amber GC vials (1.5 mL).

2.6 Gas chromatography-flame ionisation detector (GC-FID) analysis

Gas chromatography was carried out using a Clarus 580 Gas Chromatograph fitted with a flame ionisation detector. A CP-Sil 88 capillary column (Agilent, Santa Clara, California, USA) with a length of 100 m x 0.25 mm ID and 0.2 µm film was used for the separation. The injection volume was 0.5 µL, and the injection port was set to 250°C. The oven was set to 80°C with an initial temperature ramp of 6.2°C/min to 220°C which was held for 3.2 min. A second temperature ramp of 6.3°C/min to 240°C followed and was held for 6.5 min (total runtime was 35 min for each sample). The carrier gas was hydrogen at a flow of 1.25 mL/min, and the split ratio was set at 10:1. The FID was set at 270°C. Compounds were identified by comparing their retention times with those obtained from the Supelco 37 FAME standard (Sigma Aldrich, Wicklow, Ireland). The content of each fatty acid was calculated using the following equation (Eq. 1).

$$FA\ content = \frac{Peak\ Area\ (FAME)}{Peak\ Area\ (ISTD)}\ x\ \frac{ISTD\ weight}{Sample\ weight}\ x\ ISTD\ purity\ x\ 10\ x\ 0.96 \qquad \text{(Eq. 1)},$$

where, FA content is the content of fatty acid in the sample (mg/g), 10 is the dilution factor and 0.96 is the conversion factor for the internal standard.

2.7 Lipid quality factors

Atherogenic index (AI) and thrombogenic index (TI) were calculated as per Ghaeni, Ghahfarokhi, and Zaheri (2013):

$$AI = [(4 \times C14{:}0) + C16{:}0 + C18{:}0]/ [\Sigma MUFA + \Sigma PUFA\ n6 + \Sigma PUFAn3] \quad \text{(Eq. 2)}$$

$$TI = (C14{:}0+C16{:}0+C18{:}0)/(0.5MUFA+0.5PUFAn6+3PUFAn3+ PUFAn3/PUFAn6) \quad \text{(Eq. 3)}$$

2.8 Statistical analysis

Experiments were carried out as per experimental design shown in Table 1. Four parameters (cooking, ultrasound treatment, nanovesicles' formulation and sonication time) were investigated at two different levels. Principal Component Analysis (PCA) and Analysis of Variance (ANOVA) were performed using the software Statistica (StatSoft, ver 7.0). Means were separated using Tukey's test. Samples were considered significant at 95% confidence level ($p<0.05$). Factor loadings analysis was performed and graphs containing the experimental treatments were constructed using the first three principal components (PC1, PC2 and PC3).

3. Results and discussion

3.1 Nanovesicles characterisation

The properties of the nanovesicles suspensions are shown in Table 2. The appearance of the suspension was white homogeneous liquid. There was no statistical difference ($p>0.05$) regarding the average size of the vesicles. Similarly, the PDI values were not statistically different as a result of the formulation employed.

On the other hand, zeta potential was significantly affected by nanovesicle formulation ($p<0.05$). This value is an indication of suspension stability: absolute values closer to 0 are found in low-stable suspensions and consequently, prone to aggregation and precipitation, while higher absolute values are found in highly-stable suspensions. In this case, Lipo-N can be considered as having incipient stability, while Lipo-Cat suspension possessed a good stability (American Society for Testing and Materials, 1985). Finally, particle concentration is placed in the same order of magnitude, supporting the fact that minor differences can be found based on formulation pertaining to fatty acid content.Thus, it can be assumed that size and size distribution did not impact upon the results obtained in this study. On the contrary, zeta potential is the one factor that might have an impact on the performance of the vesicles in terms of lipid contents in treated meats.

3.2. Principal component analysis

Similarities and differences between samples were analysed by Principal Component Analysis, as illustrated in Figures 1 and 2. According to the scatterplot of the loading factors, while most of the fatty acids remained unchanged after treatment, PUFAn3, PUFAn6 and AI, TI and N6/N3 were affected by the process variables (Figure 1a). In fact, several omega-3 and omega-6 fatty acids (e.g. C18:3n3, C20:5n3, C22:6n3 and C20:4n6) are located in the

same quarter as total PUFAn3 and PUFAn6, confirming the consistency of this PCA analysis. Since most of the samples were located in the second and third quarters of the PC1 X PC2 plot, the samples could be further differentiated based on spectral details described by the second (PC2) and third (PC3) principal components. The PC1 × PC2 scatterplot of the principal component scores (Figure 1b) allowed identification of a slight separation between cooked and uncooked samples by P2, and between Lipo-N and Lipo-CAT nanovesicles also by P2.

Although PC1 and PC2 represented around 85% of the data variance, the similarities and differences between samples were better evidenced by PC2 and PC3 (Figure 2), indicating that the data are explained only in the details. The scatterplot of the loading factors (Figure 2a) showed four clear distinct groups, as indicated in green. Samples subjected to CAT emulsion are clustered in the region correspondent to higher levels of PUFAn3 and PUFAn6 (1^{st} and 4^{th} quarters), PUFAn3 prevailing over PUFAn6. These samples showed, in particular, high levels of C18:3n3/α-linolenic acid, C22:6n3 (DHA), C22:2 (cis-13,16-docosadienoic acid methyl ester) and C20:5n3 (EPA). On the other hand, samples subjected to N emulsion have higher atherogenic and thrombogenic indices (AI and TI, respectively), meaning that they have greater amounts of saturated fatty acids. These samples also had higher ratios between omega-6 and omega-3 fatty acids.

PC3 clearly separated cooked from non-cooked samples (Figure2b), as previously represented by the PC1 × PC2 scatterplot (Figure 1b). The slight differentiation between samples which were and were not subjected to ultrasound by PC2 in Figure 2b indicates that ultrasound enhanced the content of unsaturated over saturated fatty acids. Differences between samples could be clearly observed after the introduction of a third PC (PC3), which accounted for 7.33% of the data variance. This means that the differences ascribed to the

process variables are only subtle, which is in agreement with the results found by the Pareto/ANOVA analysis presented in the following section.

3.3 Effects of process variables on the fatty acid profile of pork meat

The effect of process variables was evaluated in 33 responses related to the fatty acid composition (Table 3), including individual fatty acids, AI and TI indices, and the ratio between n6/n3, following a Pareto analysis at a 95% confidence interval. The detailed results presented in Figure 3 are summarized in Table 3.

It was found that the total fatty acids (TFA) were not affected by any of the treatments at the confidence interval aforementioned. This means that the overall fat content of the meat samples remained constant following the ultrasound treatment. However, it was found that the level of PUFA3 was positively affected by the treatment, regardless of the nanovesicle formulation. This effect is mainly ascribed to the increased amount of EPA (C20:5n3), which was the main component of the fish oil employed for encapsulation, and DHA (C22:6n3), which was the second fatty acid of importance in the fish oil studied. These two fatty acids were primarily affected by ultrasound treatment and time. Longer treatments significantly increased ($p<0.05$) the amount of n3 fatty acids present in the final product. In general terms, all long chain PUFAs (which are present in the encapsulated fish oil), experienced a positive effect following ultrasound treatment. These results indicate that ultrasound has a positive effect on the fatty acid profile of pork meat, enhancing the mass transfer of encapsulated oil into the meat samples. However, it has been reported that ultrasound had a negative effect on the fatty acid composition of edible oils (Chemat, Grondin, Sing, & Smadja, 2004). In addition, cooking resulted in higher contents of certain fatty acids (Figure 3), probably owing to the water loss incurred during cooking.

No significant differences ($p>0.05$) were found regarding the type of formulation employed for oil encapsulation. It means that both, cationic and non-cationic nanovesicles, can be successfully employed to increase the levels of MUFAs and PUFAs in pork meat.

3.4 Lipid quality indices

To further characterise the fatty acid profile, two lipid quality factors were studied. The atherogenic index (AI) indicates the relationship between the sum of the main saturated fatty acids and that of the main classes of unsaturated, the former being considered pro-atherogenic (favouring the adhesion of lipids to cells of the immunological and circulatory system), and the latter anti-atherogenic (inhibiting the aggregation of plaque and diminishing the levels of esterified fatty acid, cholesterol, and phospholipids, thereby preventing the appearance of micro- and macro-coronary diseases). The second factor analysed was the thrombogenic index (TI), which represents the tendency to form clots in the blood vessels. This is defined as the relationship between the pro-thrombogenetic (saturated) and the anti-thrombogenetic fatty acids (MUFAs, PUFAs-n6 and PUFAs-n3).

No statistical differences were determined when the AI was analysed (Table 3). The values of AI ranged from 0.41±0.01 to 0.49±0.00 (Table 4). These values are similar to those found in rabbit meat (Dal Bosco, Castellini, Bianchi, & Mugnai, 2004) and fermented pork sausages (Stajić, Živković, Perunović, Šobajić, & Vranić, 2011). On the other hand, the reduction of TI ascribed to the ultrasound treatment and the effect of time (Table 4) were statistically significant (Table 3), which means that the pork obtained showed a better relationship between pro-thrombogenic and anti-thrombogenetic fatty acids. The values for this index ranged from 1.07±0.05 (when ultrasound was not applied) to 0.81±0.16 (after 60 minutes of ultrasound treatment using cationic nanocapsules). These results suggest that

healthier pork meat results from the novel technique applied here. In the same way, the ratio of n6/n3was found to decrease with ultrasound treatment, as shown in Table 3 and Figure 3. This ratio is of key importance for well-balanced and healthy diets. In western diets this ratio is around 15-20/1, when it should be closer to 1/1 (Simopoulos, 2008). As the ratio n6/n3 in pork meat was reduced from 9.1±1.4 to 8.5±1.6 after the US treatment (Table 4), it resulted in a more balanced and healthier product. Compared to other studies (Kasprzyk, Tyra, & Babicz, 2015; Peiretti, Gai, Brugiapaglia, Mussa, & Meineri, 2015), the lipid quality indices of treated samples were found to have similar values of atherogenic index (0.42-0.47), higher (thus unwanted) values of thrombogenic index (1.01-1.15) and considerably higher (thus undesirable) n6/n3 ratios (12.61-35.32).

Conclusions

A combination of novel technologies e.g. nanoencapsulation and ultrasound, has been applied in order to improve the lipid profile of pork meat. This study has demonstrated the positive effect that ultrasound application had in increasing the amount of healthy fatty acids in pork meat. It was found that, regardless of the type of formulation employed, there was an increase in the content of DHA and EPA fatty acids. This increase was more noticeable as the length of ultrasound treatment was extended. The higher content of these fatty acids improved the lipid quality indices. Although the AI remained constant after treatment, TI and n3/n6 ratio values were beneficially modified.

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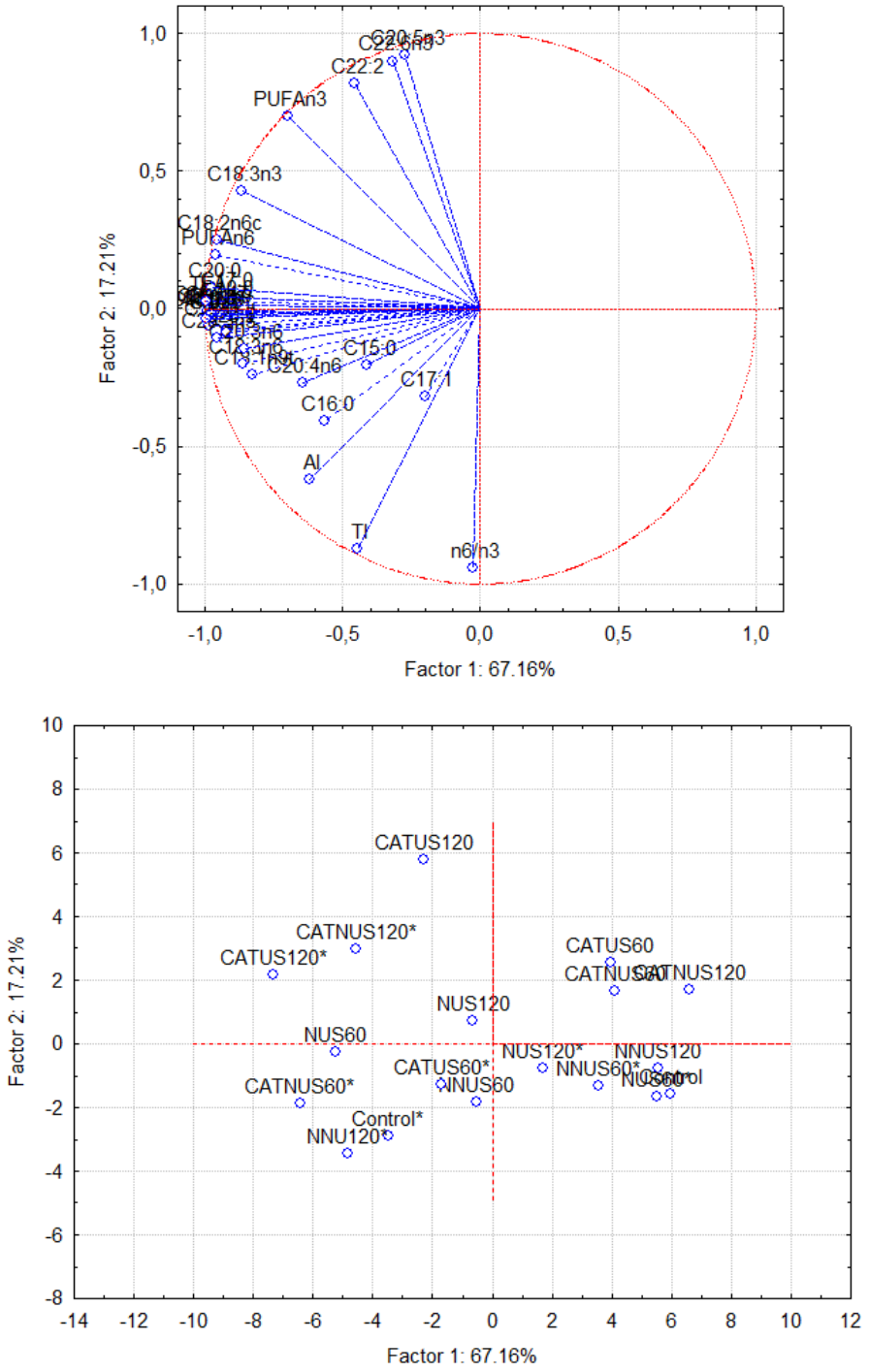


Figure 1 – PCA (PC1 X PC2) showing the differences between samples of pork meat treated according to the experimental design outlined in Table 1: (a) Loadings and (b) Scores.

(Cooked samples are marked with*)

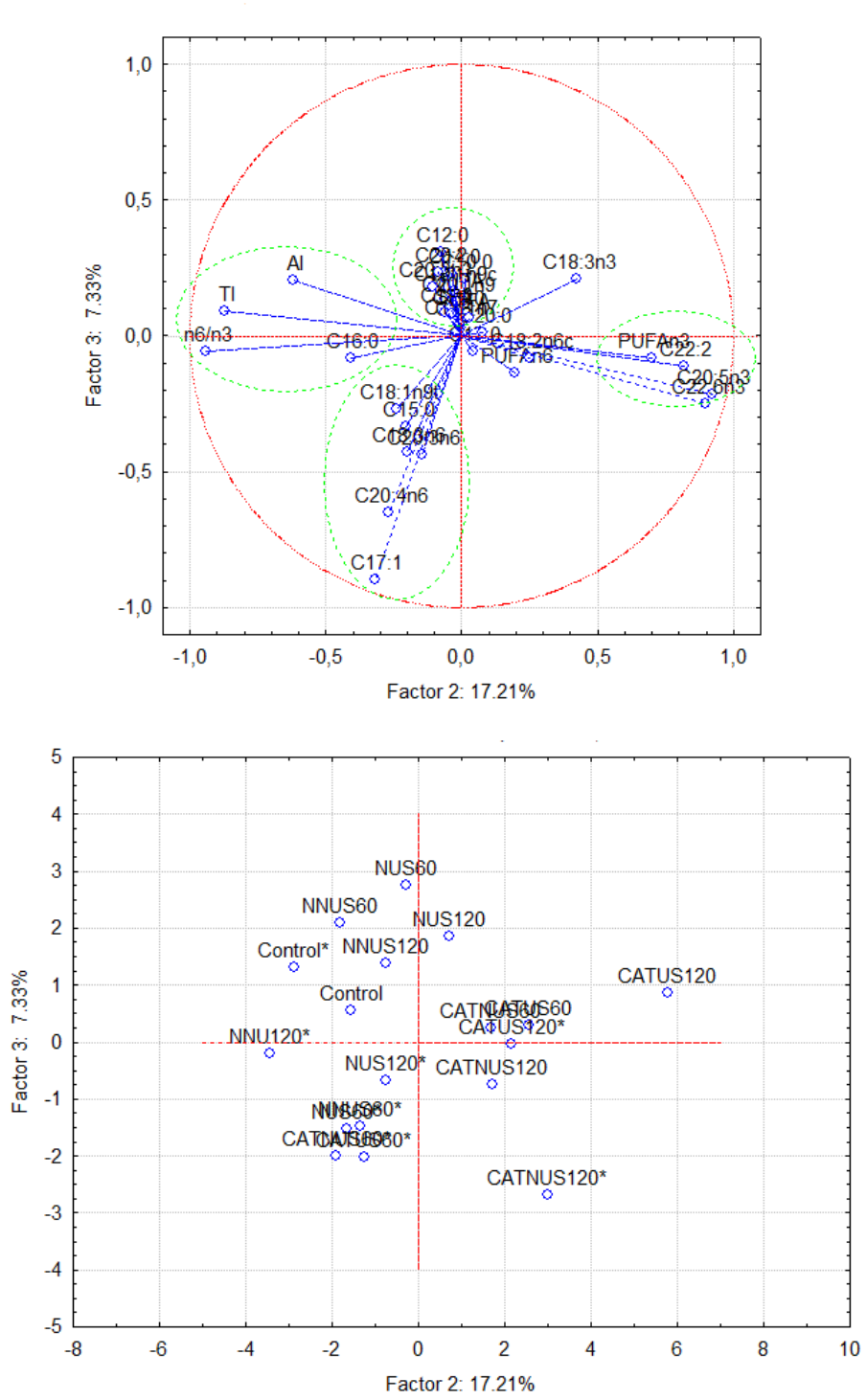
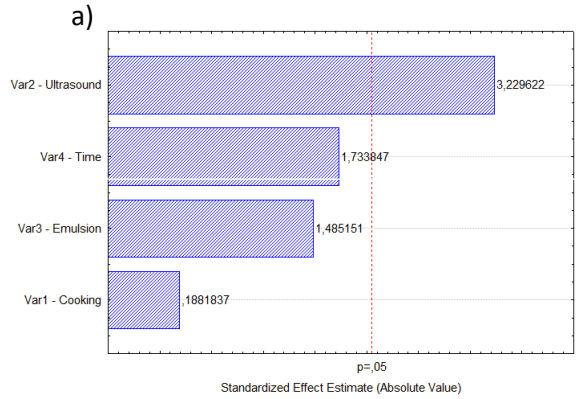


Figure 2 – PCA (PC2 X PC3) showing the differences between samples of pork meat treated according to the experimental design outlined in Table 1: (a) Loadings and (b) Scores.

(Cooked samples are marked with*)

a)



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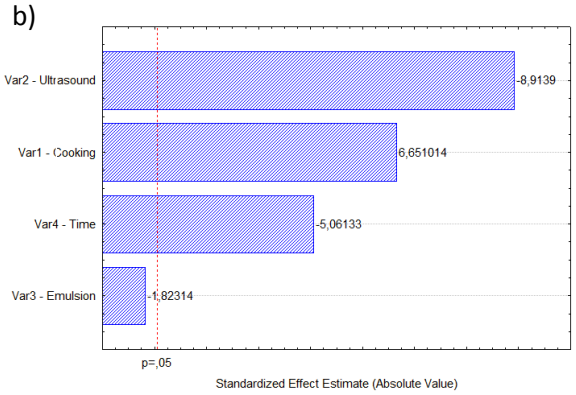
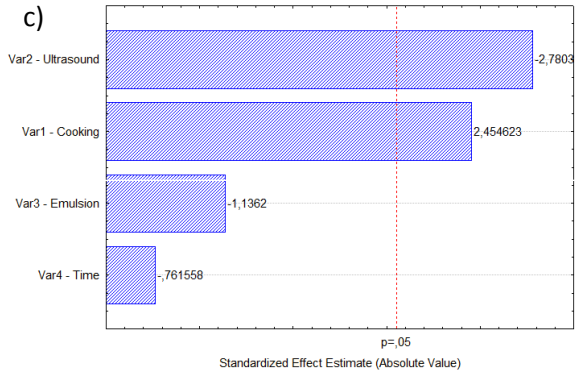
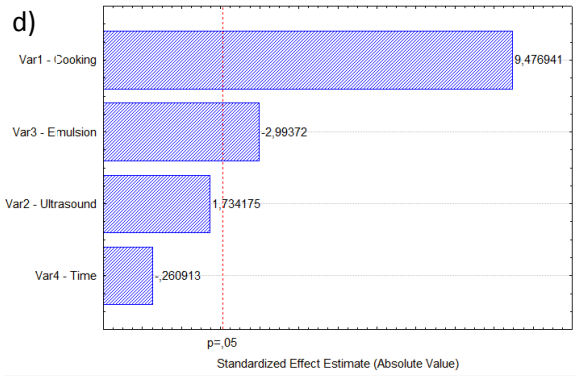


c)



d)



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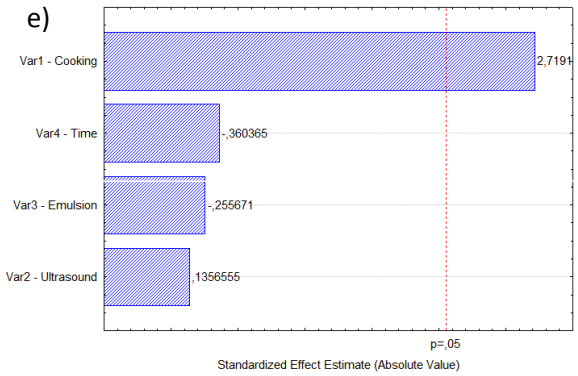

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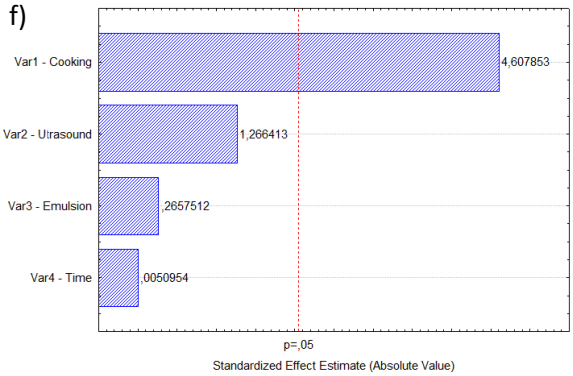

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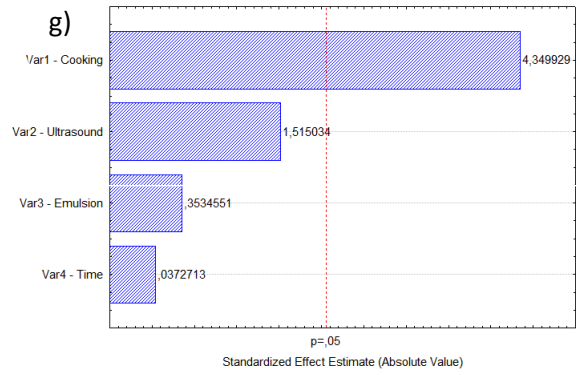

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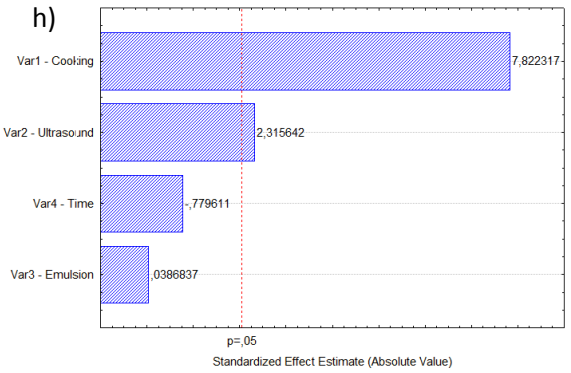

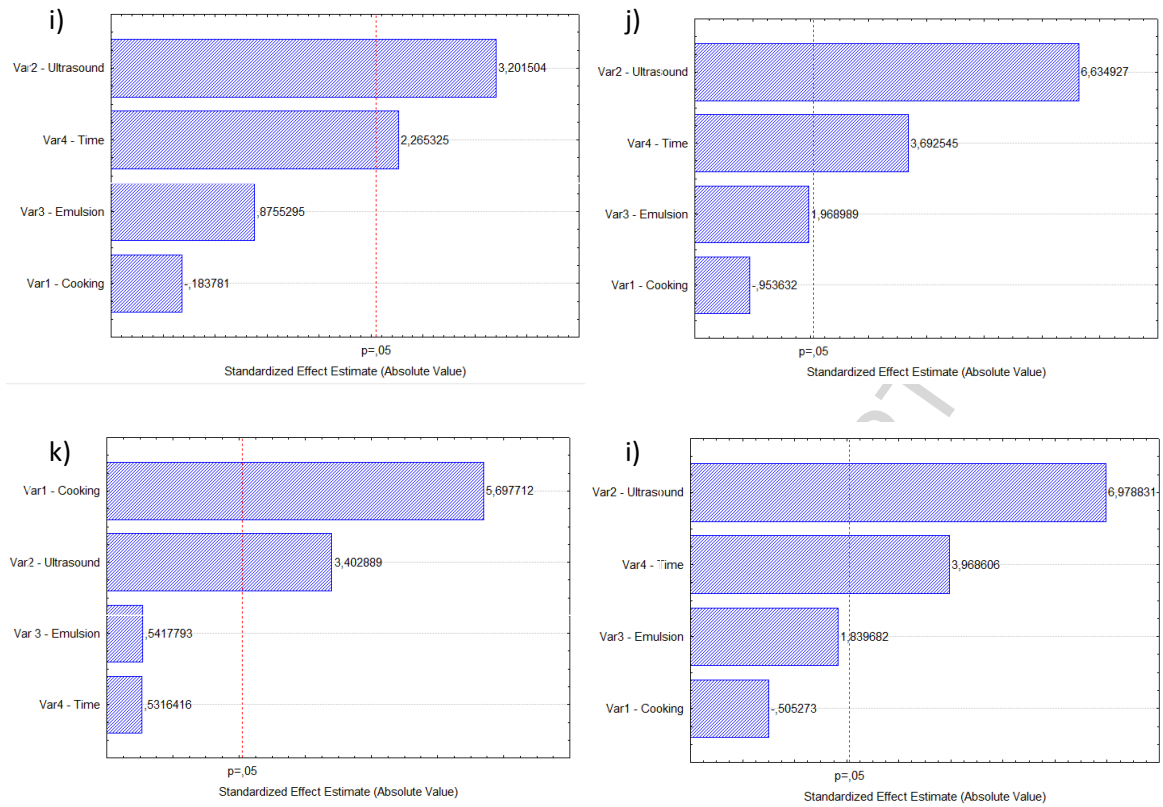

Figure 3 – Pareto charts of the main effects of treatments. All fatty acids and indices analysed that were significantly affected (p> 0.05) by one or more of the four parameters studied are represented. Only 12 out of 33 responses analysed were affected: (a) PUFAn3; (b) n6/n3; (c) TI; (d) C17:1; (e) C18:1n9; (f) C18:3n6; (g) C20:3n6; (h) C20:4n6; (i) C22:2; (j) C20:5n3; (k) C22:5n3; (l) C22:6n3. Those remained unaffected are not represented.

Table 1 – Experimental design (factorial 2^4).

Variable	Code	Level	
		-1	**+1**
Cooking	X1	No	Yes
Ultrasound	X2	No	Yes
Emulsion	X3	N	CAT
Immersion time (min)	X4	60	120

Table 2 – Characteristics of nanovesicles employed for fish oil encapsulation based on their formulation.

Emulsion	Average size (nm)	Polidispersity index	Zeta potential (mV)	Particle concentration (vesicles/mL)
Lipo-N	352.8±8.5[a]	0.266±0.037[a]	14.4±0.1[b]	5.8 x 10^{12a}
Lipo-Cat	345.9±5.1[a]	0.319±0.010[a]	51.3±1.0[a]	7.2 x 10^{12a}

[a,b] Values followed by same superscript are not significantly different (p<0.05).

Table 3 – Effects of process variables on the fatty acid profile of pork meat.

	Response	Effect(s)
	MUFA	Not affected by any variables
	PUFAn3	Positively affected by US treatment
	PUFAn6	Not affected by any variables
	SFA	Not affected by any variables
	TFA	Not affected by any variables
	AI	Not affected by any variables
	n6/n3	Affected by US treatment and time(-), and cooking (+)
	TI	Affected by US treatment (-) and cooking (+)
SFA	C10:0	Not affected by any variables
	C12:0	Not affected by any variables
	C14:0	Not affected by any variables
	C15:0	Not affected by any variables
	C16:0	Not affected by any variables
	C16:1	Not affected by any variables
	C17:0	Not affected by any variables
	C17:1	Affected by cooking (+) and emulsion (-)
	C18:0	Not affected by any variables
	C20:0	Not affected by any variables
PUFAs and MUFAs	C18:1n9t	Positively affected by cooking
	C18:1n9c	Not affected by any variables
	C18:1n7	Not affected by any variables
	C18:2n6c	Not affected by any variables
	C18:3n6	Positively affected by cooking
	C20:1n9	Not affected by any variables
	C18:3n3	Not affected by any variables
	C20:2	Not affected by any variables
	C20:3n6	Positively affected by cooking
	C20:3n3	Not affected by any variables
	C20:4n6	Positively affected by cooking and US treatment
	C22:2	Positively affected by US treatment and time
	C20:5n3	Positively affected by US treatment and time
	C22:5n3	Positively affected by US treatment and cooking
	C22:6n3	Positively affected by US treatment and time

Table 4 – Lipid quality factors in pork meat submerged in nanovesicles after different treatments.

Treat	Emulsion	US	Time (min)	n6/n3	AI	TI
Cooked	Control	Control	Control	10.75±0.00	0.49±0.00	1.06±0.00
	N	NOUS	60	10.59±0.02	0.43±0.01	0.95±0.02
	N	NOUS	120	10.72±0.11	0.49±0.5	1.07±0.11
	cat	NOUS	60	9.41±0.04	0.47±0.02	1.01±0.04
	cat	NOUS	120	7.24±0.10	0.45±0.03	0.90±0.10
	N	US	60	10.89±0.02	0.43±0.01	0.93±0.02
	N	US	120	9.42±0.01	0.45±0.01	0.96±0.01
	cat	US	60	9.53±0.05	0.45±0.01	0.95±0.05
	cat	US	120	7.74±0.03	0.46±0.01	0.93±0.03
Uncooked	Control	Control	Control	9.41±0.04	0.45±0.01	0.95±0.04
	N	NOUS	60	9.69±0.01	0.47±0.01	1.00±0.01
	N	NOUS	120	8.93±0.05	0.45±0.02	0.93±0.05
	cat	NOUS	60	7.39±0.02	0.44±0.01	0.87±0.02
	cat	NOUS	120	7.21±0.05	0.41±0.01	0.82±0.05
	N	US	60	9.55±0.16	0.45±0.07	0.97±0.16
	N	US	120	7.80±0.07	0.46±0.02	0.92±0.07
	cat	US	60	7.05±0.10	0.42±0.03	0.83±0.10
	cat	US	120	5.87±0.16	0.42±0.06	0.81±0.16

Highlights

- Ultrasound can enhance diffusion of encapsulated fatty acids in pork meat
- Nanovesicles can be employed for improved nutritional profile of meat
- Lipid quality indices were significantly influenced by cooking