



Effects of the addition of microencapsulated aromatic herb extracts on fatty acid profile of different meat products

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Abstract

The objective of this study was to evaluate the influence of addition of microencapsulated natural extracts of aromatic herbs in comparison with synthetic antioxidants on fatty acids profile of different meat products (restructured product of Tilapia mechanically separated fish meat (RP) and Fresh pork sausage (FS) stored under freezing and cold storage. Synthetic antioxidant sodium erythorbate was used in the RP and butylated hydroxytoluene (BHT) was used in the FS. Natural oregano extract (*Origanum vulgare*) was used in the RP, while the rosemary extract (*Rosmarinus officinalis*) was used in the FS. The fatty acid profiles of each formulation were obtained by gas chromatography (GC-MS). The oleic acid was the most abundant compound in both evaluated products, also, during the observed storage period there was an increase in SFA and MUFA composition ($p < 0.05$) in all the treatments. However, the treatments with addition of synthetic antioxidant and microcapsules loaded with different content of aromatic herb extracts showed similar behavior. Moreover, the addition of microencapsulated aromatic herb extracts increased the content of PUFA, improving the nutritional quality indexes in both RP and FS. Our results infer that the addition of oregano and rosemary extracts have antioxidant potential equivalent to synthetic antioxidants.

Keywords: antioxidant; fish meat; oxidation; bioactive compounds; gas chromatography.

Practical application: This work reveals the influence of the addition of microencapsulated natural extracts of herbs in comparison with synthetic antioxidants on the fatty acid profile of different meat products.

1 Introduction

The lipids are compounds of vital importance for human nutrition, because although they provide energy for the biological processes in the human body, the lipids contain essential fatty acids or liposoluble vitamins that are only obtained in diet. Moreover, the lipids are responsible for many desirable characteristics of meat and meat products (Adilah & Hanani, 2016; Lima et al., 2022), because they influence the flavor and contribute to the improvement of tenderness and succulence of meats (Ahmed et al., 2017; Domínguez et al., 2018; Rodrigues et al., 2022). The types of fatty acids (FA) in food products can influence both stability during storage and nutritional characteristics, being classified as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (Lucarini et al., 2018).

The consumption of polyunsaturated fatty acids (PUFAs), mainly the essential ones (Ω -3 e Ω -6) provide benefits to consumer's health such as decreased risk of cardiovascular diseases, prevention of cancer and neurological diseases. However, despite these benefits, food rich in PUFAs are very susceptible to nutritional and sensory changes during storage, due to the higher susceptibility to oxidative reactions (Fernandes et al., 2018; Qi et al., 2015; Alkuraieef et al., 2022), which may decrease the shelf-life and affect commercialization of fish and meat products. Meat products with fish and pork are usually rich in

(PUFAs), which makes them more susceptible to peroxidation, resulting in restrictions on processing and storage possibilities (Huang et al., 2015; McGlone, 2013; Xiong et al., 2020).

The addition of antioxidants in food rich in PUFAs is essential to increase their stability during storage, retaining nutritional and sensory quality of food of animal origin (Araújo et al., 2021; Bellucci et al., 2021; Rachtan-Janicka et al., 2021; Gelbe List Pharmindex, 2013). In this context, the interest related to natural antioxidants and natural compounds with antioxidant activity have increased notably in recent years, since consumers are increasingly concerned with their health and with the consumption of processed food containing synthetic ingredients (Romola et al., 2021; Priol et al., 2021; Lorenzo et al., 2014; Zou et al., 2021). Therefore, many research projects have been carried out to find natural alternatives to replace the synthetic antioxidants (e.g. sodium erythorbate, butylated hydroxytoluene), and then, contribute to the development of healthier products, but maintaining the food stability during storage (Barteková et al., 2021; Fernandes et al., 2018; Munekata et al., 2017; Rodrigues et al., 2020b).

The potential of aromatic herbs as source of antioxidant compounds, its health benefits and industrial applications are well documented in literature, representing an attractive innovation

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for the food sector, aiming the increased shelf-life and nutritional value of food (Abeysinghe et al., 2021; Celano et al., 2017; Chiappero et al., 2021; Gonçalves et al., 2020; Mahajan et al., 2020; Byun et al., 2021). However, the biologically active compounds of aromatic herbs are relatively unstable, which may induce the release of strong aroma, present low solubility in water and degrade quickly during processing and storage (Homayonpour et al., 2021; Pabast et al., 2018; Thakur & Kumar, 2021). Therefore, the technology of encapsulation may be used to avoid these technological problems, providing protection and a controlled and directed release of the encapsulated bioactive compounds (Santos et al., 2021; Mendes et al., 2021; Ribeiro et al., 2020). Furthermore, such technology is constantly evolving, providing new methods of preparation and application of delivery systems of encapsulated bioactive compounds (Dumitraşcu et al., 2021; Smaoui et al., 2021; Vinceković et al., 2020).

The objective of this study was to evaluate the influence of addition of microencapsulated natural extracts of aromatic herbs in comparison with synthetic antioxidants on fatty acids profile of different meat products (restructured product of Tilapia mechanically separated fish meat (RP) and Fresh pork sausage (FS)).

2 Material and methods

2.1 Aromatic herbs preparation

The aerial parts of oregano (*Origanum vulgare*) and rosemary (*Rosmarinus officinalis*) cultivated in the horticulture sector of Instituto Federal Goiano - Campus Morrinhos, were collected always in the morning (between 8 and 10 h). The plants were washed, sanitized with a solution of sodium hypochlorite at 100 µL.L⁻¹ for 15 min, rinsed with ultrapure water, and dried in stove (40 °C with forced air circulation). Then, they were grinded in a knife mill (Fortnox FT 50) and sifted using a 30-mesh stainless steel sieve to obtain a homogeneous powder.

2.2 Extracts production

For the obtainment of the extracts, the methodology described by (Vieitez et al., 2018) was used with some modifications. It was used the conventional technique at an initial proportion of 1:20 (5 g of aromatic herbs powder and 100 mL of an aqueous solvent). For the oregano extract, ultrapure water was used as a solvent, while a hydroethanolic solvent (50% water and 50% absolute ethanol) was used for rosemary, then, the solutions were shaken at ambient temperature for 1 h using the shaker Q261-22 (Quimis, São Paulo, Brasil). Then, the solutions were filtered in *Whatman* n° 4 paper and the final volumes were adjusted to 100 mL with their respective solvent. Finally, the extracts were evaporated in rotary evaporator (Fisatom 802, São Paulo, Brasil) (vacuum pressure of 600 mm Hg at 40 °C), bottled in amber glass flasks, sealed and stored in freezer (-18 °C) for further analysis.

2.3 Microcapsules preparation

The microspheres were obtained according to Dallabona et al. (2020) with modifications. The sodium alginate (2 g) was mixed with 100 mL of the respective extract under magnetic agitation.

Once homogenized, the solution (alginate + extract) was kept still for 2 h to remove the air bubbles. Then, the alginate solution with the active compounds was poured using a burette into 80 mL of 1.5% (w/v) calcium chloride solution. The extrusion speed was 30 mL/h, and the distance between the burette tip and the surface of the collection solution was adjusted to 7 cm. The capsules formed in this process were kept in the CaCl₂ solution for 15 min with agitation. Then, they were filtered through *Whatman* paper filter and washed three times with ultrapure water. Finally, the microspheres were dried spontaneously in ambient air (25 °C) for 24 h and kept in desiccator at 25 °C for further use.

2.4 Manufacturing of the Restructured Product (RP) based on tilapia mechanically separated fish meat (MSM) with microencapsulated oregano extract

The MSM were obtained right after the tilapia filleting, which were transported in isothermal container to the meat laboratory from Instituto Federal Goiano - Campus Morrinhos, where the products were manufactured. Five formulations for the restructured product were elaborated with tilapia MSM.

The fresh MSM was homogenized for 5 min with sodium chloride (1.5%), garlic and onion powder (1%) and microbial transglutaminase (0.5%) * (MTGM) ACTIVA WM by Ajinomoto Co. Inc. (Barentz, Poland). This basic formula was divided into five batches. The first was called RP0 and no additional ingredient was included. The second (RP1) was added synthetic antioxidant sodium erythorbate (0.5%). The third (RP2) received the oregano aqueous extract (0.5%). The fourth (RP3) received the microencapsulated oregano aqueous extract (0.5%) and the Fifth (RP4) received the microencapsulated oregano aqueous extract (1%).

After the complete homogenization of the ingredients, the obtained mass was molded in a cylindrical shape (Ø 6 cm) using a polyvinyl chloride film (PVC) according to the procedure described by Monteiro et al. (2015). Some holes were made with assistance of a syringe throughout the product to allow the release of retained air and keep uniform the product surface. Then, the samples were stored under refrigeration (4 ± 2 °C) for 24 h to obtain firmness following the transglutaminase manufacturer recommendations. After the storage period, the PVC was removed and the samples cut into medallion shape of 1 cm thickness. Finally, the treatments were packed separately in low-density Polyethylene (LDPE) packaging and kept under frozen storage (-20 ± 2 °C). All the analysis were carried out at days 0, 30, 60, 90 and 120.

2.5 Manufacturing of the fresh pork sausage with microencapsulated rosemary extract

The sausages preparation was carried out to the Brazilian standard for this product (Brasil, 1999). The fresh pork meat trimmings and the dorsal subcutaneous fat were ground into plates with holes of 0.8 and 0.6 cm, respectively. Then, the blend was homogenized for 5 min with sodium chloride (1.5%), garlic and onion powder (1%).

This basic formula was divided into five batches. The first was called FS0 and no additional ingredient was included. The second (FS1) was added synthetic antioxidant BHT (0.01%). The third (FS2) received the rosemary aqueous extract (0.5%). The fourth (FS3) received the microencapsulated rosemary aqueous extract (0.5%) and the Fifth (FS4) received the microencapsulated rosemary aqueous extract (1%).

The meat blends of each treatment were stuffed in natural pork intestine (caliber 32/34), packed in plastic bags and stored at 4 ± 2 °C. All the analysis were carried out at days 0, 5, 10, 15 and 20.

2.6 Fatty acids profile

Lipid extraction and analysis by Gas Chromatography Mass Spectroscopy (GC-MS)

The total lipids content was cold extracted according to Bligh & Dyer (1959), with some modifications. In short, 5 g of sample were mixed in a falcon tube with 4 mL of ultrapure water, 16 mL of methanol and 8 mL of chloroform. The mechanical agitation was carried out in a shaker during 30 min. Then, 8 mL of chloroform and Na₂SO₄ at 1.5% were added to promote a biphasic system. This blend was shaken for 2 min and centrifuged for 5 min at 3000 rpm.

The lower phase (chloroform) was evaporated, then, 15 mg of the lipid phase were extracted for the performance of the methylation process under acid conditions, in which 6 mL of HCL were added to methanol 10%, followed by heating bath (60 °C for 20 min) and ice bath for 5 min. Thereafter, 1 mL of ultrapure water and 1 mL of hexane were added and shaken in vortex for 1 min. The upper phase containing fatty acid methyl ester (FAME) (Chin et al., 1992; Conte-Junior & Soncin, 2007; Kishino et al., 2002) was injected (split ratio of 1:20) in a gas chromatograph (model Clarus 680-Perkin Elmer) attached to mass spectrometer (SQ8S), split injector and capillary column Elite 5MS (30 m x 0,25 mm x 0,5 mm).

The injector temperature was adjusted to 250 °C, using helium as carrier gas. After the injection of 2 µL, the initial temperature of the oven was kept in 60 °C, followed by an increase to 180 °C for 15 min, 245 °C for 2.5 min, then 280 °C, in which the sample is maintained for 1 min. The peaks identification was carried out by comparison of the retention times and the samples peak areas with a commercial standard containing 37 fatty acid methyl esters (Supelco CRM 47885, Sigma-Aldrich, St. Louis, Mo., USA). The temperatures of EI+ ion sources and transference lines of the MS were 220 °C. The m/z scanning range was 50 to 450 in MS Scan mode. Mass spectra were compared with reference compounds from the NIST library. The identified fatty acid peaks were expressed as mg of individual fatty acids/g of total fatty acid.

2.7 Statistical analysis

The experiments were carried out following an entirely casual experimental design, performed in triplicate. The data were submitted to variance analysis (ANOVA) and the means evaluated by Tukey test at a level of 5% of significance ($p < 0.05$) using the Action Stat software.

3 Results and discussion

3.1 Fatty acid profile of the Restructured Product (RP) based on tilapia mechanically separated fish meat (MSM) with microencapsulated oregano extract

The fatty acids (FAs) composition (mg of individual fatty acid/g of total fatty acid) of RP after 0, 30, 60, 90 and 120 days of frozen storage is shown in Table 1. The results show 19 FAs, being 5 saturated fatty acids (SFA), 6 monounsaturated fatty acids (MUFA) and 8 polyunsaturated fatty acids (PUFA).

The oleic acid (C18:1n9) was the major FA found in the restructured products, followed by linoleic acid (C18:2) and palmitic acid (C16:0), which is in accordance with other studies that evaluated the fatty acid composition of fresh water fish species (Baldissera et al., 2020; Memon et al., 2011; Rebolé et al., 2015). It is important to emphasize that the lipid content of fish is variable, depending on the specie, the food availability, the sexual maturation and the nutritional handling (Correia et al., 2020).

In relation to the storage period, the main significant changes in FA composition were observed in RP0 (most of detected FAs). Treatments RP1, RP3 and RP4 showed similar behavior ($p > 0.05$), indicating that the addition of oregano extracts microcapsules, as well as the synthetic antioxidant, could preserve the fatty acids composition of fish products.

Taking into consideration the storage period, at the end of 120 days was observed that the myristic acid (C14:0) showed lower proportions in treatments RP1, RP3 and RP4 compared to RP0 and RP2, which may indicate a positive result, since this FA is associated with induction of hypocholesterolemia in humans (Fernandes et al., 2014; Rodrigues et al., 2020a).

At a nutritional point of view, the oleic acid (C18:1n9), found in higher quantity in this product, have a lipid-lowering effect due to its capacity to reduce the LDL cholesterol (Bowen et al., 2019; Tarté et al., 2020), indicating a benefit of the restructured product. The oleic acid in every treatment increased during storage ($p < 0.05$), therefore, the antioxidant additives used in this study did not affect the stability of these fatty acids, since all the treatments showed similar values at the end of the storage period ($p > 0.05$).

In relation to the linoleic acid (C18:2), its composition maintained stable during the whole storage period in treatment RP4 ($p > 0.05$). Meanwhile, the FA composition of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) increased in all the treatments ($p < 0.05$). However, at the end of storage period, this increase was more pronounced in treatments RP3 and RP4, which may be beneficial, since EPA and DHA are widely known as essential for human health and development, including the neural function and reduction of cardiovascular and inflammatory diseases (Setty et al., 2019; So et al., 2021; Sprague et al., 2020).

There are no reports in literature about the effect of natural antioxidant on FA profile of restructured products of tilapia MSM, and studies evaluating the effect of addition of vegetable extracts on FA composition of fish products are scarce.

The sum of total fatty acids (TFA), the SFA, the MUFA and PUFA, of restructured tilapia MSM products stored for 0, 30,

Table 1. FA composition (mg of individual fatty acid/g of total fatty acid) of restructured product based on tilapia mechanically separated fish meat (MSM) formulations, stored at -20 ± 2 °C for up to 120 days.

Fatty acids	Days	Treatments				
		RP0	RP1	RP2	RP3	RP4
C14:0	0	0.05 ± 0.00 ^{Ac}	0.05 ± 0.00 ^{Abc}	0.04 ± 0.00 ^{Ab}	0.04 ± 0.00 ^{Ab}	0.04 ± 0.01 ^{Aab}
	30	0.07 ± 0.01 ^{Abc}	0.05 ± 0.00 ^{Bc}	0.05 ± 0.00 ^{Bb}	0.04 ± 0.00 ^{Bb}	0.04 ± 0.00 ^{Bb}
	60	0.09 ± 0.00 ^{Aab}	0.07 ± 0.00 ^{Bab}	0.09 ± 0.00 ^{Aa}	0.07 ± 0.00 ^{Ba}	0.06 ± 0.00 ^{Ba}
	90	0.10 ± 0.00 ^{Aa}	0.08 ± 0.00 ^{Ba}	0.09 ± 0.01 ^{Ba}	0.08 ± 0.00 ^{Ba}	0.06 ± 0.00 ^{Cab}
	120	0.11 ± 0.00 ^{Aa}	0.07 ± 0.00 ^{Ba}	0.10 ± 0.00 ^{Aa}	0.07 ± 0.03 ^{Ba}	0.06 ± 0.01 ^{Bab}
C16:0	0	3.31 ± 0.44 ^{Bd}	5.27 ± 0.14 ^{Ab}	5.69 ± 0.53 ^{Ac}	5.15 ± 0.35 ^{Aab}	5.43 ± 0.46 ^{Aab}
	30	6.07 ± 0.01 ^{Ac}	5.69 ± 0.19 ^{Ab}	5.20 ± 0.91 ^{Ad}	5.99 ± 0.96 ^{Aa}	5.70 ± 0.29 ^{Aab}
	60	7.52 ± 0.19 ^{Ab}	6.36 ± 0.27 ^{Ba}	6.71 ± 0.29 ^{Bbc}	6.38 ± 0.11 ^{Ba}	6.16 ± 0.40 ^{Ba}
	90	8.42 ± 0.34 ^{Aab}	5.24 ± 0.32 ^{Cb}	7.16 ± 0.06 ^{Bb}	5.20 ± 0.08 ^{Cab}	5.16 ± 0.03 ^{Cb}
	120	9.11 ± 0.84 ^{Aa}	5.30 ± 0.04 ^{Bb}	8.78 ± 0.20 ^{Aa}	4.31 ± 0.13 ^{BCb}	4.14 ± 0.06 ^{Cc}
C18:0	0	0.69 ± 0.18 ^{Ac}	0.64 ± 0.04 ^{Ac}	0.57 ± 0.01 ^{Ac}	0.67 ± 0.03 ^{Ab}	0.63 ± 0.03 ^{Ac}
	30	1.44 ± 0.03 ^{Ac}	0.66 ± 0.17 ^{Cc}	0.65 ± 0.17 ^{Cc}	0.85 ± 0.03 ^{BCb}	1.08 ± 0.08 ^{Bc}
	60	4.76 ± 0.08 ^{Ab}	2.46 ± 0.47 ^{Bb}	1.56 ± 0.32 ^{BCb}	1.52 ± 0.08 ^{Cb}	1.23 ± 0.11 ^{Cc}
	90	5.44 ± 0.28 ^{Ab}	3.49 ± 0.12 ^{Bab}	3.29 ± 0.15 ^{Ba}	3.65 ± 0.55 ^{Ba}	3.62 ± 0.19 ^{Ba}
	120	7.56 ± 0.98 ^{Aa}	4.52 ± 0.69 ^{Ba}	3.58 ± 0.43 ^{BCa}	3.46 ± 0.53 ^{BCa}	2.63 ± 0.44 ^{Cb}
C20:0	0	0.02 ± 0.00 ^{Aab}	0.02 ± 0.00 ^{Aab}	0.02 ± 0.00 ^{Aab}	0.02 ± 0.00 ^{Abc}	0.02 ± 0.00 ^{Abc}
	30	0.01 ± 0.00 ^{Cbc}	0.01 ± 0.00 ^{Cab}	0.02 ± 0.00 ^{Bab}	0.03 ± 0.00 ^{Aa}	0.03 ± 0.00 ^{Aa}
	60	0.00 ± 0.00 ^{Cc}	0.00 ± 0.00 ^{Cb}	0.01 ± 0.00 ^{Bb}	0.02 ± 0.00 ^{Ab}	0.02 ± 0.00 ^{Ab}
	90	0.00 ± 0.00 ^{Bc}	0.01 ± 0.00 ^{Aa}	0.01 ± 0.00 ^{ABb}	0.01 ± 0.00 ^{ABd}	0.01 ± 0.00 ^{Abc}
	120	0.02 ± 0.00 ^{Aa}	0.01 ± 0.00 ^{ABab}	0.02 ± 0.01 ^{Aa}	0.01 ± 0.00 ^{Ac}	0.01 ± 0.00 ^{Abc}
C22:0	0	0.08 ± 0.00 ^{Ac}	0.08 ± 0.00 ^{Ab}	0.09 ± 0.00 ^{Aa}	0.09 ± 0.00 ^{Ab}	0.10 ± 0.02 ^{Aa}
	30	0.10 ± 0.00 ^{Abc}	0.08 ± 0.00 ^{Ab}	0.09 ± 0.00 ^{Aa}	0.10 ± 0.00 ^{Aab}	0.10 ± 0.02 ^{Aa}
	60	0.11 ± 0.02 ^{Abc}	0.12 ± 0.02 ^{ABab}	0.10 ± 0.04 ^{Aa}	0.13 ± 0.01 ^{Aab}	0.15 ± 0.01 ^{Aa}
	90	0.12 ± 0.00 ^{ABab}	0.15 ± 0.01 ^{Aa}	0.12 ± 0.01 ^{Aa}	0.14 ± 0.01 ^{Aa}	0.15 ± 0.03 ^{Aa}
	120	0.15 ± 0.00 ^{Aa}	0.16 ± 0.03 ^{Aa}	0.14 ± 0.02 ^{Aa}	0.11 ± 0.01 ^{ABab}	0.11 ± 0.00 ^{Aa}
C16:1	0	3.13 ± 0.12 ^{Bd}	3.26 ± 0.07 ^{Bd}	3.70 ± 0.29 ^{ABd}	4.02 ± 0.86 ^{ABc}	4.54 ± 0.35 ^{Ad}
	30	5.35 ± 0.26 ^{Ac}	4.78 ± 0.16 ^{ABc}	4.18 ± 0.10 ^{Bd}	5.35 ± 0.37 ^{Ab}	4.73 ± 0.16 ^{ABd}
	60	7.57 ± 0.28 ^{Ac}	6.39 ± 0.50 ^{Bb}	6.54 ± 0.15 ^{Bc}	7.56 ± 0.34 ^{Aa}	6.59 ± 0.08 ^{Bc}
	90	11.48 ± 0.15 ^{Ab}	7.42 ± 0.34 ^{Ba}	10.89 ± 0.67 ^{Ab}	7.72 ± 0.25 ^{Ba}	7.43 ± 0.22 ^{Bb}
	120	20.91 ± 1.87 ^{Aa}	7.45 ± 0.40 ^{Ca}	16.10 ± 0.09 ^{Ba}	8.31 ± 0.33 ^{Ca}	8.79 ± 0.20 ^{Ca}
C17:1	0	0.06 ± 0.00 ^{Ae}	0.06 ± 0.00 ^{Ab}	0.06 ± 0.02 ^{Ac}	0.05 ± 0.00 ^{Aa}	0.05 ± 0.00 ^{Ae}
	30	0.12 ± 0.00 ^{Ad}	0.09 ± 0.01 ^{Ab}	0.12 ± 0.01 ^{Ab}	0.15 ± 0.01 ^{Aa}	0.09 ± 0.00 ^{Ad}
	60	0.16 ± 0.00 ^{Bc}	0.14 ± 0.00 ^{Ba}	0.14 ± 0.00 ^{Bb}	0.18 ± 0.00 ^{Aa}	0.15 ± 0.00 ^{Bc}
	90	0.26 ± 0.00 ^{Ab}	0.18 ± 0.00 ^{Ca}	0.15 ± 0.00 ^{Db}	0.21 ± 0.01 ^{Ba}	0.20 ± 0.01 ^{BCb}
	120	0.35 ± 0.05 ^{Aa}	0.19 ± 0.00 ^{Ca}	0.26 ± 0.02 ^{Ba}	0.22 ± 0.01 ^{BCa}	0.24 ± 0.01 ^{Ba}
C18:1n9	0	34.32 ± 1.24 ^{Ad}	34.15 ± 1.54 ^{Ac}	33.51 ± 0.64 ^{Ad}	33.59 ± 0.79 ^{Ac}	34.32 ± 1.33 ^{Ac}
	30	45.17 ± 0.68 ^{Ac}	47.08 ± 1.56 ^{Ab}	45.77 ± 0.43 ^{Ac}	43.84 ± 2.18 ^{Ab}	49.46 ± 4.17 ^{Ab}
	60	49.48 ± 0.36 ^{Bb}	52.49 ± 1.86 ^{Aa}	47.31 ± 1.06 ^{Cbc}	53.43 ± 0.08 ^{Aa}	52.66 ± 0.47 ^{Aa}
	90	52.42 ± 2.08 ^{Ab}	54.72 ± 1.15 ^{Aa}	50.95 ± 2.88 ^{Ab}	54.01 ± 1.52 ^{Aa}	54.96 ± 0.00 ^{Aa}
	120	56.10 ± 0.63 ^{Aa}	55.48 ± 0.05 ^{Aa}	57.02 ± 2.51 ^{Aa}	55.65 ± 0.25 ^{Aa}	57.00 ± 0.00 ^{Aa}
C20:1	0	0.07 ± 0.01 ^{Ae}	0.07 ± 0.00 ^{Ad}	0.09 ± 0.02 ^{Ad}	0.10 ± 0.01 ^{Ac}	0.11 ± 0.00 ^{Ac}
	30	0.15 ± 0.00 ^{Ad}	0.13 ± 0.01 ^{Ac}	0.05 ± 0.00 ^{Bd}	0.13 ± 0.01 ^{Ac}	0.12 ± 0.02 ^{Ac}
	60	0.32 ± 0.01 ^{Ac}	0.15 ± 0.00 ^{Bc}	0.16 ± 0.01 ^{Bc}	0.31 ± 0.16 ^{Ab}	0.15 ± 0.00 ^{Bc}
	90	0.61 ± 0.13 ^{Ab}	0.25 ± 0.03 ^{Cb}	0.46 ± 0.04 ^{Bb}	0.47 ± 0.02 ^{Ba}	0.28 ± 0.01 ^{Cb}
	120	0.70 ± 0.01 ^{Aa}	0.36 ± 0.05 ^{Ba}	0.62 ± 0.01 ^{Aa}	0.34 ± 0.03 ^{Bb}	0.35 ± 0.03 ^{Ba}
C22:1	0	0.27 ± 0.01 ^{Ab}	0.22 ± 0.02 ^{Aa}	0.25 ± 0.02 ^{Ab}	0.23 ± 0.03 ^{Ab}	0.25 ± 0.01 ^{Aa}
	30	0.13 ± 0.01 ^{Cc}	0.15 ± 0.00 ^{Cb}	0.09 ± 0.00 ^{Cd}	0.34 ± 0.04 ^{Aa}	0.25 ± 0.02 ^{Ba}
	60	0.16 ± 0.01 ^{Ac}	0.14 ± 0.00 ^{Ab}	0.17 ± 0.01 ^{Ac}	0.16 ± 0.00 ^{Abc}	0.15 ± 0.01 ^{Ab}
	90	0.26 ± 0.00 ^{Ab}	0.16 ± 0.02 ^{Bb}	0.25 ± 0.00 ^{Ab}	0.15 ± 0.04 ^{Bbc}	0.15 ± 0.02 ^{Bb}
	120	0.32 ± 0.00 ^{Aa}	0.15 ± 0.00 ^{Bb}	0.34 ± 0.01 ^{Aa}	0.14 ± 0.00 ^{Bc}	0.14 ± 0.00 ^{Bb}

A, B, C, D: different uppercase letters in the same row indicate significant difference (p < 0.05) between treatments of same storage period; a, b, c, d: different lower case letters in the same column indicate significant difference (p < 0.05) between storage days of a treatment. Values are expressed by mean ± standard deviation.

Table 1. Continued...

Fatty acids	Days	Treatments				
		RP0	RP1	RP2	RP3	RP4
C24:1	0	0.06 ± 0.01 ^{Ad}	0.06 ± 0.00 ^{Ab}	0.04 ± 0.02 ^{Ad}	0.06 ± 0.00 ^{Ac}	0.07 ± 0.00 ^{Ab}
	30	0.14 ± 0.03 ^{Bc}	0.16 ± 0.01 ^{Ba}	0.13 ± 0.00 ^{Bc}	0.26 ± 0.01 ^{Aa}	0.16 ± 0.01 ^{ABa}
	60	0.17 ± 0.01 ^{Bc}	0.18 ± 0.01 ^{ABa}	0.19 ± 0.00 ^{ABb}	0.20 ± 0.00 ^{Ab}	0.20 ± 0.01 ^{Aa}
	90	0.24 ± 0.00 ^{Ab}	0.18 ± 0.00 ^{Ca}	0.21 ± 0.00 ^{Bb}	0.18 ± 0.00 ^{Cb}	0.19 ± 0.00 ^{Ca}
	120	0.31 ± 0.00 ^{Aa}	0.17 ± 0.00 ^{Ba}	0.32 ± 0.01 ^{Aa}	0.18 ± 0.01 ^{Bb}	0.18 ± 0.00 ^{Ba}
C18:2	0	12.65 ± 0.23 ^{ABa}	12.50 ± 0.05 ^{Bab}	12.74 ± 0.12 ^{ABa}	12.78 ± 0.10 ^{ABa}	12.95 ± 0.04 ^{Aa}
	30	8.81 ± 0.17 ^{Bb}	12.46 ± 0.03 ^{Aab}	11.84 ± 0.71 ^{Aa}	12.28 ± 0.12 ^{Aa}	12.43 ± 0.43 ^{Aa}
	60	6.51 ± 0.13 ^{Cc}	11.31 ± 1.16 ^{Abc}	9.68 ± 0.20 ^{Bab}	11.52 ± 0.42 ^{Ab}	12.73 ± 0.32 ^{Aa}
	90	5.82 ± 0.68 ^{Dc}	10.49 ± 0.34 ^{Ba}	8.55 ± 0.37 ^{Cb}	12.33 ± 0.26 ^{Aa}	12.51 ± 0.33 ^{Aa}
	120	2.29 ± 0.09 ^{Dd}	10.35 ± 0.37 ^{Bc}	5.19 ± 0.29 ^{Cb}	11.63 ± 0.06 ^{Ab}	12.42 ± 0.38 ^{Aa}
C18:3	0	0.29 ± 0.00 ^{Aa}	0.28 ± 0.00 ^{ABa}	0.26 ± 0.00 ^{Ba}	0.28 ± 0.01 ^{ABa}	0.28 ± 0.01 ^{Ab}
	30	0.23 ± 0.05 ^{Ba}	0.25 ± 0.02 ^{ABa}	0.27 ± 0.00 ^{ABa}	0.27 ± 0.02 ^{ABa}	0.32 ± 0.00 ^{Aa}
	60	0.16 ± 0.00 ^{Cb}	0.26 ± 0.04 ^{ABa}	0.24 ± 0.01 ^{Ba}	0.27 ± 0.01 ^{ABa}	0.31 ± 0.00 ^{Aa}
	90	0.12 ± 0.00 ^{Dbc}	0.24 ± 0.00 ^{Ba}	0.18 ± 0.01 ^{Cb}	0.25 ± 0.02 ^{ABa}	0.29 ± 0.00 ^{Ab}
	120	0.08 ± 0.00 ^{Bc}	0.24 ± 0.02 ^{Ba}	0.12 ± 0.02 ^{Bc}	0.23 ± 0.01 ^{Aa}	0.23 ± 0.00 ^{Ac}
C20:2	0	0.13 ± 0.00 ^{Ca}	0.14 ± 0.00 ^{BCa}	0.13 ± 0.00 ^{BCa}	0.14 ± 0.00 ^{ABb}	0.15 ± 0.00 ^{Ab}
	30	0.12 ± 0.00 ^{Ca}	0.13 ± 0.01 ^{BCa}	0.12 ± 0.01 ^{BCa}	0.18 ± 0.00 ^{Aa}	0.15 ± 0.01 ^{ABb}
	60	0.10 ± 0.00 ^{Bb}	0.13 ± 0.02 ^{Ba}	0.13 ± 0.01 ^{Ba}	0.18 ± 0.03 ^{Aa}	0.18 ± 0.01 ^{Aa}
	90	0.07 ± 0.00 ^{Dc}	0.14 ± 0.00 ^{Ba}	0.11 ± 0.01 ^{Ca}	0.17 ± 0.00 ^{Aa}	0.18 ± 0.00 ^{Aa}
	120	0.04 ± 0.00 ^{Dd}	0.13 ± 0.00 ^{Ba}	0.08 ± 0.00 ^{Cb}	0.15 ± 0.00 ^{Bb}	0.15 ± 0.01 ^{Ab}
C20:3	0	0.08 ± 0.00 ^{Ba}	0.09 ± 0.01 ^{Ba}	0.09 ± 0.00 ^{Bab}	0.12 ± 0.00 ^{Aa}	0.12 ± 0.00 ^{Ac}
	30	0.05 ± 0.00 ^{Cab}	0.10 ± 0.00 ^{Ba}	0.11 ± 0.00 ^{Ba}	0.13 ± 0.00 ^{Aa}	0.13 ± 0.00 ^{Abc}
	60	0.04 ± 0.01 ^{Cb}	0.09 ± 0.00 ^{Ba}	0.08 ± 0.00 ^{Bab}	0.12 ± 0.00 ^{Aa}	0.13 ± 0.00 ^{ABb}
	90	0.02 ± 0.00 ^{Cb}	0.09 ± 0.00 ^{Ba}	0.07 ± 0.01 ^{Bbc}	0.13 ± 0.00 ^{Aa}	0.13 ± 0.00 ^{Aa}
	120	0.04 ± 0.00 ^{Cb}	0.09 ± 0.01 ^{Ba}	0.06 ± 0.00 ^{Cc}	0.13 ± 0.00 ^{Aa}	0.11 ± 0.00 ^{Ad}
C20:4	0	0.54 ± 0.00 ^{Aa}	0.53 ± 0.01 ^{Aa}	0.59 ± 0.07 ^{Aa}	0.59 ± 0.10 ^{Aa}	0.65 ± 0.03 ^{Aa}
	30	0.56 ± 0.04 ^{ABa}	0.54 ± 0.03 ^{Ba}	0.54 ± 0.02 ^{Bab}	0.63 ± 0.01 ^{ABa}	0.66 ± 0.06 ^{Aa}
	60	0.46 ± 0.00 ^{Cb}	0.54 ± 0.00 ^{Ba}	0.49 ± 0.02 ^{BCab}	0.63 ± 0.03 ^{Aa}	0.64 ± 0.04 ^{Aa}
	90	0.37 ± 0.02 ^{Cc}	0.53 ± 0.05 ^{Ba}	0.43 ± 0.03 ^{Cbc}	0.64 ± 0.03 ^{Aa}	0.64 ± 0.02 ^{Aa}
	120	0.32 ± 0.01 ^{Cc}	0.47 ± 0.08 ^{Ba}	0.30 ± 0.07 ^{Cc}	0.62 ± 0.02 ^{Aa}	0.62 ± 0.01 ^{Aa}
C20:5	0	0.08 ± 0.00 ^{Cc}	0.09 ± 0.00 ^{Cc}	0.11 ± 0.00 ^{Bc}	0.12 ± 0.00 ^{ABb}	0.13 ± 0.00 ^{Ac}
	30	0.06 ± 0.02 ^{Bc}	0.04 ± 0.00 ^{Bc}	0.08 ± 0.00 ^{ABc}	0.08 ± 0.00 ^{ABb}	0.12 ± 0.03 ^{Ac}
	60	0.22 ± 0.04 ^{Cb}	0.47 ± 0.03 ^{Bb}	0.42 ± 0.01 ^{Bb}	0.67 ± 0.08 ^{Aa}	0.64 ± 0.03 ^{Ab}
	90	0.48 ± 0.09 ^{Ca}	0.51 ± 0.02 ^{BCab}	0.63 ± 0.00 ^{ABa}	0.71 ± 0.03 ^{Aa}	0.74 ± 0.02 ^{Aa}
	120	0.56 ± 0.03 ^{BCa}	0.53 ± 0.03 ^{Ca}	0.65 ± 0.04 ^{Ba}	0.75 ± 0.03 ^{Aa}	0.75 ± 0.01 ^{Aa}
C22:2	0	0.02 ± 0.00 ^{Aa}	0.02 ± 0.00 ^{Ab}	0.02 ± 0.00 ^{Ab}	0.02 ± 0.00 ^{Ac}	0.02 ± 0.00 ^{Ab}
	30	0.02 ± 0.00 ^{ABa}	0.02 ± 0.00 ^{BCb}	0.01 ± 0.00 ^{Cb}	0.03 ± 0.00 ^{Abc}	0.03 ± 0.00 ^{ABb}
	60	0.01 ± 0.00 ^{Cab}	0.01 ± 0.00 ^{Cb}	0.02 ± 0.00 ^{BCab}	0.03 ± 0.00 ^{Bbc}	0.04 ± 0.00 ^{Aa}
	90	0.02 ± 0.00 ^{Ca}	0.04 ± 0.00 ^{Aa}	0.03 ± 0.00 ^{ABa}	0.04 ± 0.00 ^{Aab}	0.03 ± 0.00 ^{BCb}
	120	0.00 ± 0.00 ^{Cb}	0.04 ± 0.00 ^{Aa}	0.02 ± 0.00 ^{Bab}	0.04 ± 0.00 ^{Aa}	0.04 ± 0.00 ^{Aa}
C22:6	0	0.12 ± 0.00 ^{Ad}	0.12 ± 0.01 ^{Ad}	0.12 ± 0.00 ^{Ac}	0.13 ± 0.00 ^{Ad}	0.13 ± 0.01 ^{Ae}
	30	0.16 ± 0.00 ^{Bcd}	0.14 ± 0.00 ^{Cd}	0.13 ± 0.00 ^{Dc}	0.16 ± 0.00 ^{Bc}	0.17 ± 0.00 ^{Ad}
	60	0.21 ± 0.02 ^{ABbc}	0.18 ± 0.00 ^{Bc}	0.12 ± 0.00 ^{Cc}	0.18 ± 0.00 ^{Bc}	0.22 ± 0.01 ^{Ac}
	90	0.23 ± 0.00 ^{Bb}	0.26 ± 0.00 ^{ABb}	0.24 ± 0.04 ^{ABb}	0.27 ± 0.01 ^{ABb}	0.29 ± 0.00 ^{Ab}
	120	0.33 ± 0.04 ^{Ca}	0.34 ± 0.03 ^{BCa}	0.30 ± 0.01 ^{Ca}	0.41 ± 0.01 ^{Aa}	0.40 ± 0.02 ^{Aa}

A, B, C, D: different uppercase letters in the same row indicate significant difference ($p < 0.05$) between treatments of same storage period; a, b, c, d: different lower case letters in the same column indicate significant difference ($p < 0.05$) between storage days of a treatment. Values are expressed by mean ± standard deviation.

60, 90 and 120 days are presented in Figure 1. In Figure 1A can be seen that the level of TFA increased significantly ($p < 0.05$) during storage in all treatments, however, treatments RP0 (from 56.05 to 99.40 mg/g of fat) and RP2 (from 58.21 to 94.08 mg/g

of fat) presented higher values at the end of the storage period. It can also be observed that that RP1 was similar to RP3 and RP4 ($p > 0.05$), being in accordance with the results obtained by Aguirrezábal et al. (2000) and Fernandes et al. (2018). These

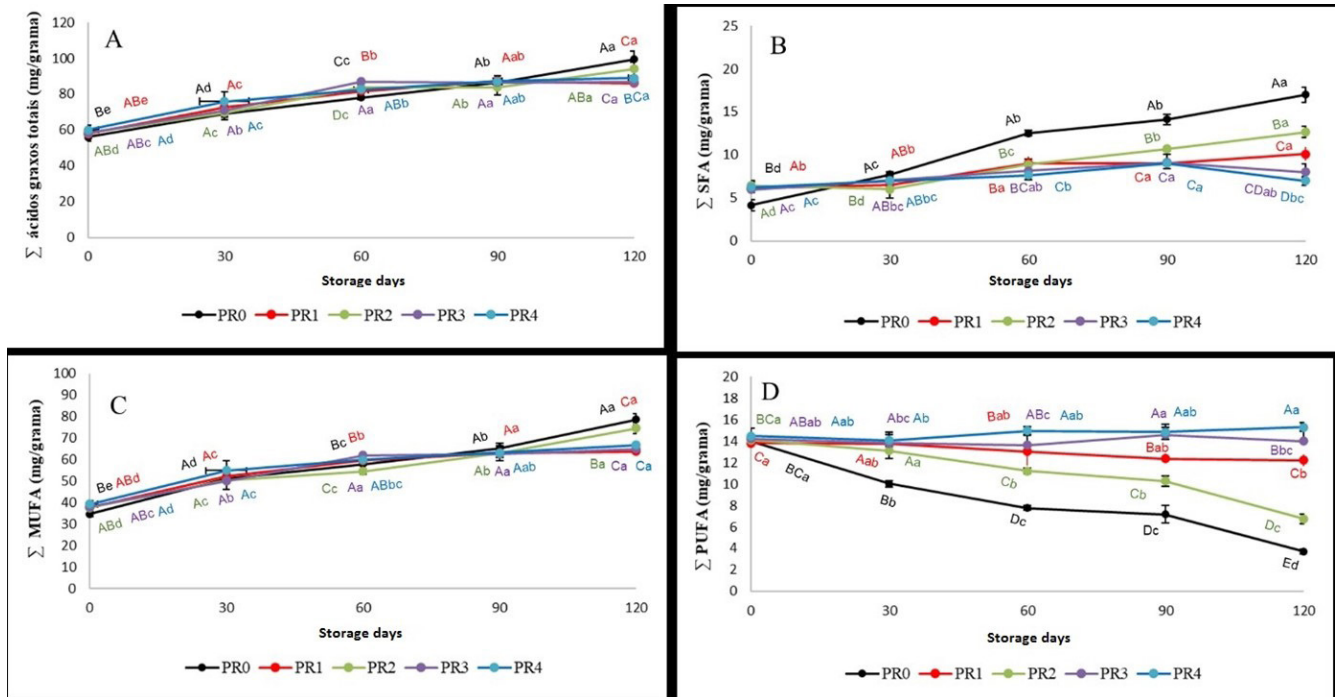


Figure 1. Fatty Acid (FA) composition (mg of individual fatty acids/g of total fatty acid) of restructured product without ingredients addition (RP0), with 0.5% sodium erythorbate addition (RP1), with 0.5% oregano (*Origanum vulgare*) extract addition (RP2), with 0.5% oregano (*Origanum vulgare*) extract microcapsules addition (RP3) and with 1% oregano (*Origanum vulgare*) extract microcapsules addition. (A) Σ TFA: sum of the composition of all fatty acids; (B) Σ SFA: sum of saturated fatty acids; (C) Σ MUFA: sum of monounsaturated fatty acids; (D) Σ PUFA: sum of polyunsaturated fatty acids. A-D: different uppercase letters indicate significant difference ($p < 0.05$) between treatments of the same storage period; a-d: different lowercase letters indicate significant difference ($p < 0.05$) between storage periods of the same treatment.

authors evaluated the effect addition of paprika and garlic to chorizo and the stability of sheep sausages with addition of different content of *Origanum vulgare* extract, respectively. Both studies described that the level of TFA increased in all treatments for 96 and 135 days, also that the spices showed antioxidant effect as effective as synthetic additives.

As can be seen in the Figure 1B-1C, there was an increase over time for the fractions SFA and MUFA ($p < 0.05$) in all treatments, however, at the end of storage, the lowest values in fatty acids composition were obtained in RP1, RP3 and RP4 ($p < 0.05$). A similar behavior was observed by Fernandes et al. (2018) while evaluating the influence of addition of different concentrations of natural extract of oregano in comparison with sodium erythorbate in lamb sausage stored for 135 days at -20 ± 2 °C. The findings of Serdaroğlu & Felekoğlu (2005), also showed similar results while studying the effect of frozen storage on oxidative quality of *Sardina pilchardus* meat added with rosemary extract and onion juice.

The highest content of SFA was also observed by Bitalebi et al. (2019) in samples of cold stored minced rainbow trout (*Oncorhynchus mykiss*) without treatment, compared to samples added with synthetic antioxidant or apple peel extract.

In general, the oxidative reactions occur mainly in PUFA (Álvarez et al., 2009), which act as excellent substrates for beginning of oxidation (Gobert et al., 2010), reacting with oxygen reactive species, leading to a series of secondary reactions

that, in turn, drive to lipid degradation and oxidative rancidity (Amaral et al., 2018).

In Figure 1D, there was a decrease in PUFA during storage for RP0 (13.94 to 3.70 mg/g of fat) and RP2 (14.10 to 6.75 mg/g of fat) ($p < 0.05$). Despite the trend to decrease, the reduction was not significant in RP1 (13.80 to 12.21 mg/g of fat), RP3 (14.21 to 13.99 mg/g of fat) and RP4 (14.45 to 14.44 mg/g of fat) ($p > 0.05$). This behavior confirm the higher susceptibility to oxidation of PUFA (Alfaia et al., 2010), generating less compounds in comparison with SFA and MUFA, similarly observed by Martín et al. (2000) during the manufacture of ripened ham under different processing conditions. Therefore, at the end of storage period, it was possible to observe the efficacy of the addition of oregano extract microcapsules as natural antioxidants (RP3 and RP4) and their equivalence with the synthetic antioxidant (RP1). This result indicating the lower PUFA oxidation is important because this fatty acid is associated with several health benefits (Boroski et al., 2012; Cheng et al., 2021; Pérez et al., 2021). Furthermore, our results are similar to the fatty acid profile found by Fernandes et al. (2016) while studying ovine hamburgers added with oregano extract and packed in modified atmosphere.

This effect in FA composition during storage of restructured products added of natural and synthetic antioxidants is associated with lipid oxidation that occurs naturally by incrementing a radical to a double bond or by abstracting hydrogen from unsaturated fatty acids, thus increasing the

degree of lipid saturation (Frankel et al., 1996; Monteiro et al., 2017) leading to a decrease in the content of unsaturated fatty acids. Moreover, the raw oregano extract added to the RO2 may have induced a pro oxidant effect due to the characteristic of its components (Seck et al., 2021). In this situation, many reactions may occur with the chemical structure of the phenolic compounds or their dispersion in the system, which change its efficacy to prevent oxidation (Wong & Kitts, 2002).

Jasour & Rahimabadi (2011) and Šimat et al. (2015) observed similar tendency in fatty acid profile during cold and frozen storage of *Oncorhynchus Mykiss* and *Boops boops* fillets, respectively.

3.2 Fatty acids profile of fresh pork sausage with microencapsulated rosemary extract

The results of FA composition (mg of individual fatty acids/g of total fatty acid) of the pork sausage formulations stored at 4 ± 2 °C for up to 20 days are presented in Table 2. It was detected 16 FA, being 4 SFA, 6 MUFA and 6 PUFA.

The oleic acid (C18:1 n9) was the most representative FA in the fresh pork sausage, which agrees with evaluations of fatty acid composition in swine of previous studies Kim et al. (2020), Vehovský et al. (2018) and Wood et al. (2008). These authors found similar profile, highlighting that the FA composition can be influenced by genetic and environmental factors, including race, diet, maturity stage, among others (Kim et al., 2020).

During the fresh sausage storage was possible to observe changes related to the content of different FA between treatments. The main significant changes were observed in sample FS0 in most FA detected. During the entire conservation period, the fatty acid composition of treatments FS1, FS3 and FS4 changed similarly, indicating a similar behavior of the rosemary extract microcapsules in comparison with the synthetic additive. Fernandes et al. (2018) also observed significant changes during storage in control sample and similarity between natural and synthetic antioxidants, when evaluating the stability of sheep sausage with addition of different concentrations of oregano (*Origanum vulgare*) extract.

Taking into consideration the storage period, at the end of 20 days, the composition of palmitic acid (C16:0), margaric acid (C17:0), stearic acid (C18:0) and heneicosanoic acid (C21:0) showed higher values ($p < 0.05$), in treatments FS0 and FS 2, while the treatments FS1, FS3 and FS4 decreased significantly ($p < 0.05$), considering that these FA are associated with negative changes in lipid profile of blood (Ye et al., 2020), this decrease can be evaluated as positive.

The linoleic (C18:2) and linolenic (C18:3) acids showed reduction ($p < 0.05$) during storage period in treatments FS0 and FS2, while in treatments FS1, FS3 and FS4 showed increase ($p < 0.05$). Gawlik-Dziki (2012) evaluating the antioxidant activity of spices confirmed that the oregano (*Origanum vulgare* L.) and rosemary (*Rosemarinus officinalis* L.) extracts were more efficient to prevent linoleic acid peroxidation in comparison with thyme (*Thymus vulgaris* L.), white and black pepper (*Piper nigrum* L.),

tarragon (*Artemisia dracuncululus* L.), basil (*Ocimum basilicum* L.) and cinnamon (*Cinamomum* sp.) extracts, which consequently improve the enzymatic and non-enzymatic antioxidant system, due to its anti-inflammatory properties.

The composition of EPA and DHA acids presented decreased values ($p < 0.05$) during the storage period in treatments FS0 and FS2, while the treatments FS1, FS3 and FS4 remained stable at the end of 20 days of cold storage. Higher concentrations of EPA and DHA are desired due to their several health benefits (Khan et al., 2021; Lorente-Cebrián et al., 2013; Rodrigues et al., 2020b).

In relation to the total FA showed in Figure 2A, it was observed that treatments FS3 and FS4 showed satisfactory results compared to BHT synthetic antioxidant (FS1). Our results are in accordance with the findings of Berasategi et al. (2011), whom evaluated the efficacy of *Melissa officinalis* natural antioxidant extract in formulations of Bologna enriched with omega-3, which was also similar to the formulation with BHT. Meanwhile, the treatments FS0 and FS2 showed higher susceptibility to oxidation during storage ($p < 0.05$).

The SFA composition (Figure 2B) increased during the cold storage period for all treatments. However, the treatments remained unaltered ($p > 0.05$) from the 10th day except in treatment FS0, which presented a constant increase during the whole storage. For the composition of MUFA, also occurred an increase over time ($p < 0.05$) in all treatments, but at the end of the storage, was not observed significant difference between treatments FS1, FS3 and FS4 ($p > 0.05$).

A significant reduction ($p < 0.05$) in levels of PUFAs was observed at the end of storage for treatments FS0 (16.26 to 5.22 mg/g of fat) and FS2 (14.91 to 8.49 mg/g of fat), and these changes were not observed in the treatments with synthetic antioxidant (FS1) and rosemary extract microcapsules (FS3 and FS4). Despite the presumption that these PUFAs would decrease, it showed a significant increase ($p < 0.05$) in treatments with synthetic antioxidant and the herb extract microcapsules. The reduction in PUFAs may have been caused by oxidative and hydrolytic reactions that occurred during storage (Amaral et al., 2018). Long chain hydrocarbons and the high unsaturation of the PUFA turned them more susceptible to oxidation and hydrolysis than SFA (Wójciak et al., 2015). The similar behavior between treatments FS0 and FS2 may be related to the increase of lipolysis resulted from the enzymatic action (Fernandes et al., 2018; Wang et al., 2014).

The higher levels of MUFA and SFA compared to PUFA, are probably derived from the lipid composition of the meat product. In swine meat, the triglycerides are mainly constituted of high levels of saturated fatty acids instead of polyunsaturated (Ma & Sun, 2020; Navarro et al., 2021).

The higher susceptibility to oxidation in treatments FS0 and FS2 may be justified by the low antioxidant efficiency influenced by the increased release of heme iron, present in high concentration in swine meat. The heme or non-heme iron can act as a catalyzer accelerating the lipid oxidation, which reduce the quality of meat and meat products (Ma et al., 2016; Zhou et al., 2019).

Table 2. FA composition (mg of individual fatty acid/g of total fatty acid) of fresh pork sausage formulations, stored at 4 ± 2 °C for up to 20 days.

Fatty acids	Days	Treatments				
		FS0	FS1	FS2	FS3	FS4
C16:0	0	21.13 ± 0.02 ^{Ac}	20.34 ± 0.33 ^{Bb}	20.87 ± 0.21 ^{Bc}	20.74 ± 0.09 ^{Bc}	20.90 ± 0.22 ^{Bb}
	05	23.43 ± 0.13 ^{Ad}	21.68 ± 0.42 ^{Bb}	22.69 ± 0.32 ^{Ad}	22.21 ± 0.56 ^{Bb}	21.46 ± 0.36 ^{Cb}
	10	25.30 ± 0.26 ^{Ac}	23.03 ± 0.77 ^{Bb}	24.65 ± 0.28 ^{Ac}	21.71 ± 0.42 ^{Cbc}	21.66 ± 0.41 ^{Cb}
	15	37.62 ± 0.14 ^{Ab}	32.44 ± 0.18 ^{Ba}	36.50 ± 0.44 ^{Ab}	32.58 ± 0.65 ^{Ba}	32.47 ± 0.62 ^{Ba}
	20	39.54 ± 0.16 ^{Aa}	33.36 ± 1.81 ^{Ba}	37.43 ± 0.36 ^{Aa}	32.08 ± 0.30 ^{Ba}	33.56 ± 1.92 ^{Ba}
C17:0	0	0.01 ± 0.00 ^{Ad}	0.01 ± 0.00 ^{Ab}	0.01 ± 0.00 ^{Ad}	0.01 ± 0.00 ^{Ab}	0.01 ± 0.00 ^{Aa}
	05	0.02 ± 0.01 ^{Ab}	0.01 ± 0.00 ^{Ab}	0.01 ± 0.00 ^{Ad}	0.02 ± 0.00 ^{Aab}	0.01 ± 0.00 ^{Aa}
	10	0.07 ± 0.01 ^{Aab}	0.04 ± 0.02 ^{ABab}	0.05 ± 0.00 ^{Abc}	0.02 ± 0.02 ^{Bb}	0.02 ± 0.02 ^{Ba}
	15	0.10 ± 0.01 ^{Aab}	0.05 ± 0.00 ^{Bab}	0.08 ± 0.01 ^{ABb}	0.03 ± 0.02 ^{Bab}	0.04 ± 0.02 ^{Ba}
	20	0.13 ± 0.02 ^{Aa}	0.07 ± 0.03 ^{Ba}	0.11 ± 0.00 ^{Ba}	0.06 ± 0.01 ^{Ba}	0.06 ± 0.03 ^{Ba}
C18:0	0	0.09 ± 0.01 ^{Ac}	0.11 ± 0.03 ^{Ab}	0.07 ± 0.04 ^{Ab}	0.06 ± 0.04 ^{Ac}	0.06 ± 0.04 ^{Ab}
	05	0.15 ± 0.03 ^{Ac}	0.13 ± 0.03 ^{Ab}	0.08 ± 0.04 ^{Ab}	0.13 ± 0.01 ^{Abc}	0.12 ± 0.03 ^{Ab}
	10	0.26 ± 0.02 ^{Ab}	0.15 ± 0.05 ^{Bab}	0.17 ± 0.01 ^{ABb}	0.14 ± 0.03 ^{Babc}	0.13 ± 0.03 ^{Bab}
	15	0.33 ± 0.02 ^{Ab}	0.17 ± 0.02 ^{Bab}	0.30 ± 0.01 ^{Aa}	0.22 ± 0.04 ^{Bab}	0.18 ± 0.03 ^{Ba}
	20	0.39 ± 0.02 ^{Aa}	0.23 ± 0.01 ^{BCa}	0.32 ± 0.06 ^{Ba}	0.23 ± 0.03 ^{BCa}	0.20 ± 0.01 ^{Ca}
C21:0	0	0.68 ± 0.20 ^{Ad}	0.71 ± 0.24 ^{Ac}	0.55 ± 0.16 ^{Ac}	0.49 ± 0.15 ^{Ab}	0.56 ± 0.14 ^{Ab}
	05	1.14 ± 0.12 ^{Abd}	0.81 ± 0.21 ^{Bbc}	0.61 ± 0.15 ^{Bc}	1.43 ± 0.19 ^{Aa}	1.42 ± 0.28 ^{Ab}
	10	6.03 ± 0.42 ^{Ab}	1.66 ± 0.58 ^{BCab}	2.22 ± 0.17 ^{Bb}	1.31 ± 0.06 ^{Ca}	1.24 ± 0.06 ^{Cab}
	15	4.46 ± 0.32 ^{Ac}	1.88 ± 0.12 ^{Ca}	3.37 ± 0.01 ^{Ba}	1.44 ± 0.28 ^{Ca}	1.38 ± 0.03 ^{Ca}
	20	8.24 ± 0.09 ^{Aa}	1.94 ± 0.24 ^{Ca}	3.45 ± 0.32 ^{Ba}	1.36 ± 0.43 ^{Ca}	1.30 ± 0.57 ^{Cab}
C16:1	0	1.21 ± 0.25 ^{Ab}	1.34 ± 0.28 ^{Aa}	0.97 ± 0.20 ^{Ac}	0.89 ± 0.19 ^{Ab}	1.00 ± 0.21 ^{Ac}
	05	1.86 ± 0.39 ^{ABb}	1.54 ± 0.36 ^{Aba}	1.09 ± 0.23 ^{Bc}	2.34 ± 0.50 ^{Ab}	1.56 ± 0.33 ^{ABbc}
	10	8.98 ± 1.94 ^{Aa}	2.91 ± 0.62 ^{Ba}	6.15 ± 1.32 ^{Aa}	1.67 ± 0.35 ^{Bab}	1.62 ± 0.34 ^{Babc}
	15	6.40 ± 1.38 ^{Aa}	3.19 ± 0.11 ^{Ba}	3.36 ± 0.72 ^{Bb}	2.38 ± 0.51 ^{Bab}	2.33 ± 0.50 ^{Bab}
	20	6.61 ± 0.00 ^{Aa}	3.36 ± 1.83 ^{ABa}	3.77 ± 0.31 ^{ABb}	4.38 ± 2.39 ^{ABa}	2.57 ± 0.32 ^{Ba}
C17:1	0	2.35 ± 0.08 ^{Ab}	2.57 ± 0.08 ^{Aa}	2.49 ± 0.06 ^{Ab}	2.65 ± 0.06 ^{Aa}	2.60 ± 0.11 ^{Aa}
	05	2.83 ± 0.05 ^{Aa}	2.26 ± 0.02 ^{Ab}	2.65 ± 0.08 ^{Ba}	2.32 ± 0.04 ^{Ca}	2.36 ± 0.04 ^{Cb}
	10	2.18 ± 0.06 ^{Abc}	2.01 ± 0.01 ^{Bc}	2.06 ± 0.05 ^{Bb}	2.19 ± 0.02 ^{Aa}	2.18 ± 0.00 ^{Ac}
	15	1.10 ± 0.01 ^{Bd}	2.06 ± 0.02 ^{Ac}	1.21 ± 0.34 ^{Bc}	2.28 ± 0.48 ^{Aa}	2.19 ± 0.00 ^{Ac}
	20	1.61 ± 0.09 ^{Ccd}	2.03 ± 0.02 ^{Bc}	1.55 ± 0.03 ^{Cc}	2.17 ± 0.02 ^{Aa}	2.22 ± 0.02 ^{Abc}
C18:1n9	0	36.19 ± 1.65 ^{Bc}	43.97 ± 2.33 ^{Ab}	29.00 ± 2.19 ^{Cc}	27.68 ± 0.50 ^{Cc}	32.05 ± 0.06 ^{BCb}
	05	43.70 ± 2.97 ^{Ad}	46.83 ± 2.55 ^{Ab}	36.71 ± 0.72 ^{Bd}	34.67 ± 1.09 ^{Bb}	36.63 ± 2.76 ^{Bb}
	10	50.71 ± 1.48 ^{Ac}	45.83 ± 0.86 ^{Bab}	47.04 ± 0.97 ^{Ac}	46.60 ± 1.54 ^{ABa}	45.81 ± 2.63 ^{Ba}
	15	58.39 ± 1.40 ^{Ab}	48.75 ± 0.52 ^{BCa}	53.04 ± 1.01 ^{Ab}	47.66 ± 2.44 ^{BCa}	46.05 ± 4.68 ^{Ca}
	20	68.38 ± 1.12 ^{Aa}	43.10 ± 0.00 ^{EB}	63.76 ± 0.00 ^{Ba}	44.88 ± 8.76 ^{Da}	46.44 ± 0.00 ^{Ca}
C20:1	0	0.02 ± 0.00 ^{Ac}	0.02 ± 0.01 ^{Abc}	0.01 ± 0.01 ^{Ac}	0.02 ± 0.00 ^{Aa}	0.02 ± 0.00 ^{Aa}
	05	0.02 ± 0.00 ^{Ac}	0.02 ± 0.00 ^{Ac}	0.01 ± 0.00 ^{Ac}	0.02 ± 0.00 ^{Aa}	0.02 ± 0.00 ^{Aa}
	10	0.11 ± 0.00 ^{Ab}	0.05 ± 0.00 ^{Bab}	0.10 ± 0.00 ^{Aa}	0.02 ± 0.01 ^{Ca}	0.02 ± 0.00 ^{Ca}
	15	0.13 ± 0.02 ^{Ab}	0.07 ± 0.00 ^{Ba}	0.09 ± 0.00 ^{ABa}	0.02 ± 0.00 ^{Ca}	0.02 ± 0.01 ^{Ca}
	20	0.16 ± 0.00 ^{Aa}	0.07 ± 0.00 ^{Ba}	0.06 ± 0.01 ^{Bb}	0.03 ± 0.01 ^{BCa}	0.02 ± 0.01 ^{Ca}
C22:1	0	0.14 ± 0.03 ^{Ac}	0.14 ± 0.08 ^{Aa}	0.13 ± 0.00 ^{Ab}	0.12 ± 0.03 ^{Aa}	0.12 ± 0.00 ^{Aa}
	05	0.17 ± 0.03 ^{Ac}	0.13 ± 0.01 ^{Aa}	0.13 ± 0.01 ^{Ab}	0.12 ± 0.01 ^{Aa}	0.12 ± 0.00 ^{Aa}
	10	0.26 ± 0.02 ^{Ab}	0.15 ± 0.03 ^{Ba}	0.15 ± 0.00 ^{Bb}	0.13 ± 0.04 ^{Ba}	0.12 ± 0.00 ^{Ba}
	15	0.31 ± 0.04 ^{Ab}	0.16 ± 0.03 ^{Ba}	0.26 ± 0.03 ^{Aa}	0.17 ± 0.02 ^{Ba}	0.14 ± 0.03 ^{Ba}
	20	0.34 ± 0.02 ^{Aa}	0.15 ± 0.02 ^{Ca}	0.30 ± 0.01 ^{Ba}	0.15 ± 0.01 ^{Ca}	0.14 ± 0.06 ^{Ca}
C24:1	0	0.16 ± 0.03 ^{Aa}	0.16 ± 0.04 ^{Aa}	0.15 ± 0.02 ^{Ab}	0.16 ± 0.03 ^{Aa}	0.15 ± 0.03 ^{Aa}
	05	0.11 ± 0.01 ^{Ab}	0.15 ± 0.02 ^{Aa}	0.15 ± 0.04 ^{Aa}	0.16 ± 0.04 ^{Aa}	0.15 ± 0.00 ^{Aa}
	10	0.09 ± 0.02 ^{BCb}	0.14 ± 0.02 ^{ABa}	0.07 ± 0.02 ^{Cc}	0.12 ± 0.03 ^{ABCa}	0.16 ± 0.01 ^{Aa}
	15	0.07 ± 0.02 ^{ABb}	0.11 ± 0.04 ^{ABa}	0.04 ± 0.00 ^{Bc}	0.14 ± 0.03 ^{Aa}	0.15 ± 0.03 ^{Aa}
	20	0.09 ± 0.00 ^{Bb}	0.08 ± 0.01 ^{Ba}	0.09 ± 0.00 ^{Bbc}	0.13 ± 0.05 ^{ABa}	0.16 ± 0.01 ^{Aa}
C18:2	0	14.12 ± 1.49 ^{Aa}	13.99 ± 0.99 ^{Ab}	13.09 ± 1.71 ^{Aa}	12.34 ± 1.52 ^{Ab}	13.17 ± 1.69 ^{Ab}
	05	13.59 ± 1.65 ^{Aba}	13.04 ± 1.31 ^{ABb}	11.51 ± 0.68 ^{Bab}	13.99 ± 1.71 ^{ABab}	16.30 ± 2.37 ^{Ab}
	10	11.84 ± 0.86 ^{Ca}	13.42 ± 0.36 ^{Bab}	10.68 ± 0.31 ^{Cb}	15.28 ± 0.40 ^{Ab}	15.94 ± 0.01 ^{Aab}
	15	7.70 ± 0.94 ^{Cb}	14.06 ± 0.64 ^{Bab}	7.57 ± 0.39 ^{Cc}	16.09 ± 0.69 ^{Aba}	16.41 ± 1.22 ^{Aab}
	20	3.75 ± 0.06 ^{Dc}	15.32 ± 0.34 ^{Ba}	5.81 ± 0.53 ^{Cc}	16.64 ± 1.10 ^{ABa}	17.50 ± 0.41 ^{Aa}

A, B, C, D: different uppercase letters in the same row indicate significant difference (p < 0.05) between treatments of same storage period; a, b, c, d: different lower case letters in the same column indicate significant difference (p < 0.05) between storage days of a treatment. Values are expressed by mean ± standard deviation.

Table 2. Continued...

Fatty acids	Days	Treatments				
		FS0	FS1	FS2	FS3	FS4
C18:3	0	1.83 ± 0.10 ^{Aa}	1.60 ± 0.36 ^{Ab}	1.53 ± 0.07 ^{Ab}	1.55 ± 0.07 ^{Aa}	1.62 ± 0.08 ^{Aa}
	05	1.40 ± 0.10 ^{Abc}	1.28 ± 0.16 ^{Ab}	1.62 ± 0.08 ^{Aa}	1.44 ± 0.19 ^{Aa}	1.56 ± 0.02 ^{Aa}
	10	1.31 ± 0.05 ^{Bc}	1.69 ± 0.24 ^{Aa}	1.38 ± 0.08 ^{ABc}	1.06 ± 0.02 ^{Cbc}	1.47 ± 0.03 ^{ABa}
	15	1.66 ± 0.15 ^{Ab}	1.51 ± 0.01 ^{ABab}	1.22 ± 0.01 ^{Cc}	1.03 ± 0.01 ^{Cc}	1.45 ± 0.03 ^{Ba}
	20	1.24 ± 0.06 ^{Ac}	1.08 ± 0.04 ^{Ab}	1.30 ± 0.06 ^{Ac}	1.37 ± 0.17 ^{Ab}	1.36 ± 0.48 ^{Aa}
C20:2	0	0.06 ± 0.02 ^{Aa}	0.07 ± 0.01 ^{Ab}	0.06 ± 0.01 ^{Aa}	0.06 ± 0.01 ^{Aa}	0.07 ± 0.00 ^{Aa}
	05	0.04 ± 0.00 ^{Ab}	0.07 ± 0.02 ^{Ab}	0.05 ± 0.00 ^{Aa}	0.08 ± 0.01 ^{Aa}	0.08 ± 0.01 ^{Aa}
	10	0.06 ± 0.02 ^{ABa}	0.12 ± 0.01 ^{Aa}	0.05 ± 0.01 ^{Ba}	0.07 ± 0.02 ^{ABa}	0.08 ± 0.02 ^{ABa}
	15	0.04 ± 0.00 ^{Ca}	0.13 ± 0.00 ^{Aa}	0.07 ± 0.00 ^{BCa}	0.07 ± 0.02 ^{BCa}	0.09 ± 0.01 ^{Ba}
	20	0.03 ± 0.01 ^{Ab}	0.12 ± 0.01 ^{Ac}	0.06 ± 0.01 ^{Ab}	0.11 ± 0.01 ^{Ab}	0.12 ± 0.01 ^{Ab}
C20:4	0	0.16 ± 0.05 ^{Ab}	0.16 ± 0.01 ^{Aa}	0.15 ± 0.03 ^{Ab}	0.16 ± 0.01 ^{Ab}	0.15 ± 0.01 ^{Ab}
	05	0.11 ± 0.01 ^{Ab}	0.15 ± 0.02 ^{Aa}	0.15 ± 0.00 ^{Ab}	0.16 ± 0.01 ^{Ab}	0.15 ± 0.03 ^{Ab}
	10	0.09 ± 0.02 ^{Aa}	0.14 ± 0.02 ^{Ba}	0.07 ± 0.02 ^{ABab}	0.12 ± 0.03 ^{Bb}	0.16 ± 0.01 ^{Bb}
	15	0.07 ± 0.02 ^{Ab}	0.11 ± 0.04 ^{Aa}	0.05 ± 0.02 ^{Ab}	0.14 ± 0.03 ^{Ab}	0.15 ± 0.03 ^{Ab}
	20	0.06 ± 0.00 ^{Ab}	0.08 ± 0.01 ^{Aa}	0.08 ± 0.02 ^{Aa}	0.13 ± 0.05 ^{Aa}	0.16 ± 0.01 ^{Aa}
C20:5	0	0.03 ± 0.01 ^{Aa}	0.01 ± 0.00 ^{Aa}	0.02 ± 0.00 ^{Aa}	0.02 ± 0.01 ^{Ab}	0.02 ± 0.01 ^{Ab}
	05	0.03 ± 0.00 ^{Aa}	0.04 ± 0.00 ^{Aa}	0.02 ± 0.00 ^{Aa}	0.04 ± 0.00 ^{Ab}	0.04 ± 0.01 ^{Ab}
	10	0.01 ± 0.00 ^{Cb}	0.03 ± 0.01 ^{Ca}	0.02 ± 0.00 ^{Ca}	0.08 ± 0.00 ^{Ba}	0.13 ± 0.00 ^{Aa}
	15	0.00 ± 0.00 ^{Cb}	0.03 ± 0.02 ^{Ca}	0.01 ± 0.00 ^{Cb}	0.07 ± 0.01 ^{Ba}	0.12 ± 0.01 ^{Aa}
	20	0.00 ± 0.00 ^{Bb}	0.01 ± 0.00 ^{Ba}	0.00 ± 0.00 ^{Bb}	0.07 ± 0.00 ^{ABa}	0.11 ± 0.06 ^{Aa}
C22:6	0	0.04 ± 0.00 ^{Aa}	0.05 ± 0.00 ^{Ac}	0.04 ± 0.00 ^{Ab}	0.04 ± 0.00 ^{Ab}	0.04 ± 0.00 ^{Ac}
	05	0.04 ± 0.01 ^{ABa}	0.05 ± 0.00 ^{ABC}	0.04 ± 0.00 ^{Ba}	0.09 ± 0.02 ^{Ab}	0.07 ± 0.01 ^{ABbc}
	10	0.03 ± 0.00 ^{Bab}	0.09 ± 0.01 ^{ABb}	0.03 ± 0.01 ^{Bab}	0.11 ± 0.02 ^{Ab}	0.12 ± 0.04 ^{Ab}
	15	0.01 ± 0.00 ^{Bbc}	0.11 ± 0.01 ^{Aa}	0.02 ± 0.00 ^{Bb}	0.12 ± 0.05 ^{Aa}	0.13 ± 0.01 ^{Aa}
	20	0.00 ± 0.00 ^{Cc}	0.13 ± 0.00 ^{Ba}	0.03 ± 0.01 ^{Cb}	0.14 ± 0.02 ^{ABa}	0.17 ± 0.00 ^{Aa}

A, B, C, D: different uppercase letters in the same row indicate significant difference ($p < 0.05$) between treatments of same storage period; a, b, c, d: different lower case letters in the same column indicate significant difference ($p < 0.05$) between storage days of a treatment. Values are expressed by mean \pm standard deviation.

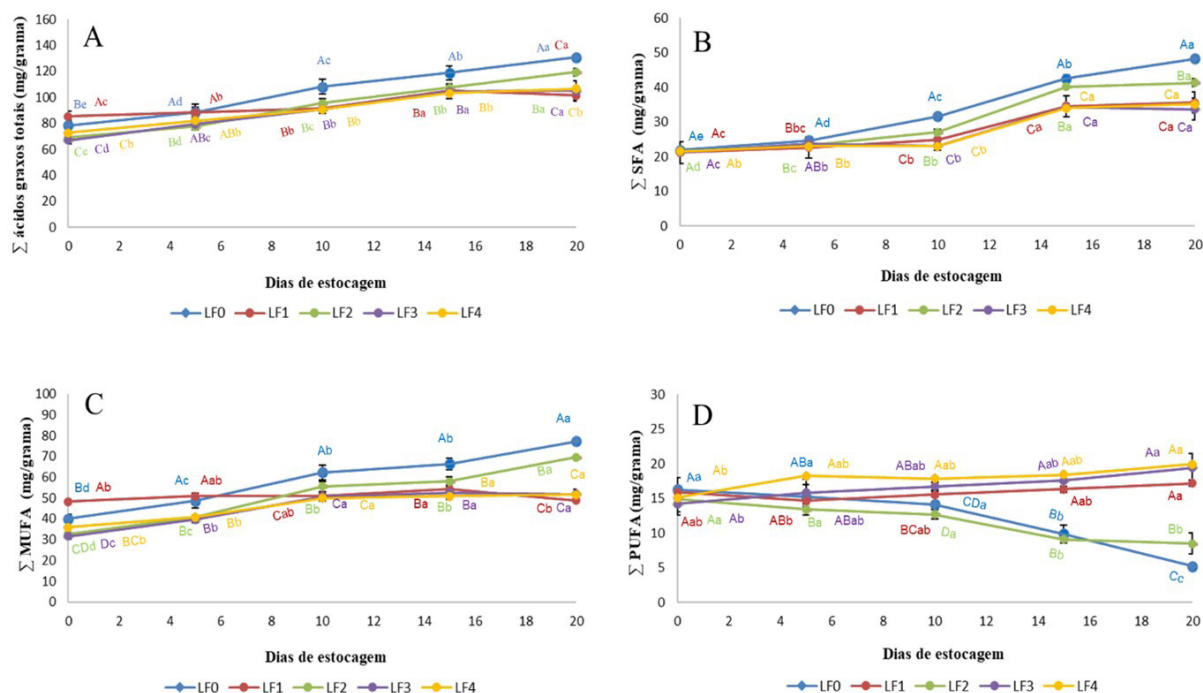


Figure 2. Fatty Acid (FA) composition (mg of individual fatty acids/g of total fatty acid) of fresh pork sausage without ingredients addition (RP0), with 0.5% BHT addition (RP1), with 0.5% rosemary (*Rosmarinus officinalis*) extract addition (RP2), with 0.5% rosemary (*Rosmarinus officinalis*) extract microcapsules addition (RP3) and with 1% rosemary (*Rosmarinus officinalis*) extract microcapsules addition. (A) Σ TFA: sum of the composition of all fatty acids; (B) Σ SFA: sum of saturated fatty acids; (C) Σ MUFA: sum of monounsaturated fatty acids; (D) Σ PUFA: sum of polyunsaturated fatty acids. A-D: different uppercase letters indicate significant difference ($p < 0.05$) between treatments of the same storage period; a-d: different lowercase letters indicate significant difference ($p < 0.05$) between storage periods of the same treatment.

4 Conclusion

Among the fatty acids concentrations evaluated in this study, in general, the composition of SFA and MUFA increased at the end of the storage period in samples without any treatment with antioxidant or with addition of only the herb extract. Furthermore, the addition of microencapsulated aromatic herbs extracts prevented the oxidation of PUFA and consequently its reduction, improving the nutritional quality indicators in both the restructured product based on tilapia MSM and the fresh pork sausage.

Our results suggest that the application of aromatic herbs extracts, more specifically *Origanum vulgare* and *Rosmarinus officinalis* in combination with techniques of microencapsulation may be an interesting natural alternative to improve the stability of fish and pork meat products by preventing oxidative rancidity as well as improve the nutritional quality of these products.

These findings open new perspectives for the application of microencapsulated herb extracts in substitution to synthetic antioxidants, aiming the manufacturing of products with a natural and health claim in order to achieve the new trends in the consumer market.

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