

RESEARCH ARTICLE

Effects of cooking over the stability of fatty acids as bioactive compounds in enriched pasta with a fish by-product

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Abstract

Background and objectives: Fusilli pasta enriched with sea bass concentrate (*Dicentrarchus labrax*) was developed with the aim of increasing its content in proteins and especially in polyunsaturated fatty acids (PUFA) like Ω -3. Pasta made from two types of cereals (wheat and spelt) and fish by-product with or without a natural antioxidant were cooking prior to consumption, and nutritional and physicochemical characteristics were evaluated.

Findings: Enriched developed pasta showed high levels of protein, fat, and fiber, and the fatty acid profiles confirmed a substantial enrichment in bioactive compounds (Ω -3 fatty acids). The cooking of pasta before consumption was able to reduce bacterial loads guaranteeing food safety and improving nutritional availability. Furthermore, an increase in the MUFA and PUFA content was revealed, which could represent an advantage to offer a better source of functional ingredients (EPA & DHA).

Conclusions: The combination of heat from cooking with formulations containing antioxidants seems to offer a remarkable synergistic effect to preserve unsaturated fatty acids with desirable nutritional properties.

Significance and novelty: Pasta enriched with bioactive compounds from fish by-product after cooking treatment before consumption appears to be an effectiveness option to improve healthy human nutrition.

KEYWORDS

cooking, EPA/DHA, fish by-product, food enrichment, fresh pasta, ω -3 fatty acids

1 | INTRODUCTION

Fish constitutes a basic food present in the diet of many households with an important content in polyunsaturated fatty acids (25%–45%) that are the main responsible for

energetic value in this kind of food. The ingest of Ω -3 fatty acids is related to a decrease in cardiovascular risk factors (Martínez et al., 2005). On the other hand, pasta supposes one of the most consumed foods due to its versatility, low cost, easy preparation, high shelf life, and nutritional

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quality (Foschia et al., 2015). For these reasons, pasta could be an excellent option to add some nutritive components thus improving its nutritive and organoleptic properties (Filipović et al., 2014; Kadam & Prabhasankar, 2010). Based on the above, the main purpose to use fish in pasta products is to offer an excellent source of quality nutrients as essential amino acids and polyunsaturated fatty acids (Oliveira et al., 2015; Stevanato et al., 2010).

The presence in foods of an appropriate amount of Ω -3 fatty acids is considered a need for reducing disease risks (Babuskin et al., 2014). Within this group, the α -linoleic acid—ALA—(C18:3 n-3), eicosapentaenoic acid—EPA—(C20:5 n-3), docosapentaenoic acid—DPA—(C22:5 n-3), and docosahexaenoic acid—DHA—(C22:6 n-3) are the most important (EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA), 2010). European regulation (EC) 1924/2006 “nutrition and health claims made on foods” and its modification Commission Regulation (EU) 116/2010 establishes that only those foods providing a significant amount of omega-3 fatty acids at their level of consumption can bear those claims. Furthermore, the conditions of use should establish a minimum quantity requested per 100 g and 100 kcal of product.

Despite their nutritional benefits, these fatty acids are susceptible to suffer oxidative reactions that can affect flavor, texture, color, odor, and nutritional value (Chaiyasit et al., 2007; Glodde et al., 2018). With the purpose of preventing or delaying oxidation, antioxidant substances are added in the food. In the last years, a growing interest in natural antioxidants has been evident. One of the most important natural antioxidants is rosemary extract (*Rosmarinus officinalis L.*) (Andrade et al., 2018; Sánchez-Camargo & Herrero, 2017).

Adequate heat treatment in foods is fundamental to guarantee its preservation by inactivating pathogens, decreasing a_w , improving its sensorial properties, and increasing nutrients availability. Nevertheless, cooking treatment may result in a loss of amino acids, synthesis of toxic compounds, or negative effects in flavor, texture, or color (Badui, 2006); Maillard reactions can proceed (Trevisan et al., 2016) giving rise to a number of

molecules that may affect organoleptic, functional, technological, and nutritional characteristics. Temperature and time control are thus crucial factors for assuring desirable food characteristics (Badui, 2006). As can be seen in other studies, the heat treatment can lead to a loss of Ω -3 fatty acids and thus a decrease in their biological availability for nutritional purposes (Lee et al., 2006; Türkkan et al., 2008). For that reason, the main objective of this study was to evaluate both fat stability and microbiological quality of pasta enriched with sea bass concentrate concerning the cooking method used and the addition of an antioxidant. The purpose is that the product that reaches the consumer maintains the amount of Ω -3 fatty acids in the raw pasta and also presents a microbiological and biochemical quality.

2 | MATERIAL AND METHODS

2.1 | Fish concentrates preparation

To obtain the concentrates from by-product seabass, fish cuts from a local fish factory (Scanfisk[®]) were dipped in saline solution 8% and dried in oven (Verinox, Mod. Junior 1100, Italy) (60°C for 24 hr) according to the procedure described in previous studies (Ainsa et al., 2021; Calanche et al., 2019). Two different concentrates from fish manual debone meat—MDM—(with antioxidant and without antioxidant) were made. The natural antioxidant used was rosemary extract powder (Marbys[®], Barcelona, Spain), which was added during the manufacture of concentrates.

2.2 | Preparation of pasta

In pasta making, two types, Durum (D) and Spelt (S), were developed. Their names correspond to the cereals were they from. Durum was a pasta made with semolina from durum wheat supplied by a local factory (Innova Obrador S.L), MDM, rehydrated fish soup (DMR[®]), and mushroom extract powder (Glucanfeed[®]), while Spelt was made with

TABLE 1 Formulations of enriched pasta with seabass concentrated

| Durum | | Durum with antioxidant | | Spelt | | Spelt with antioxidant | |
|---------------------------|------|---------------------------|--------|------------------|----|------------------------|--------|
| | % | | % | | % | | % |
| Durum wheat | 64.5 | Durum wheat | 64.5 | Spelt wheat | 55 | Spelt wheat | 55 |
| Fish concentrate | 10 | Fish concentrate | 10 | Spelt bran | 10 | Spelt bran | 10 |
| Aromatic herbs and spices | 0.5 | Aromatic herbs and spices | 0.5 | Fish concentrate | 10 | Fish concentrate | 10 |
| Water | 25 | Water | 25 | Water | 25 | Water | 25 |
| Rosemary extract | 0 | Rosemary extract | 15 ppm | Rosemary extract | 0 | Rosemary extract | 15 ppm |

commercial spelt wheat (Nurture[®]), MDM, and spelt bran flour (El granero integral[®]). Semolina from durum pasta showed 11.7% of protein and 0.9% of ash, while spelt wheat was 14% of protein and 0.7% of ash. As quality control in both kinds of pasta, moisture parameter was measured which showed values of 9.50% and 10.50%, respectively. Treatments that including natural antioxidants in each formulation (Durum +antioxidant—DA—& Spelt +antioxidants—SA) were developed to compare pasta with antioxidant from those without it. Regarding developed formulations, they are shown in Table 1, and pasta were produced using the machine “Bottene” (Bottene, Mod. Lillodue 14057CE, Italy) in fusilli shape. All ingredients were mixed for 10 min, and then, the dough was extruded. Batches of 0.5 kg of pasta were processed for each type following the procedure described in our previous study (Calanche et al., 2019).

For the study of cooking pasta, the determination of the optimal cooking time was made by measuring the hardness of the cooked pasta using a texturometer (ANAME Instrumentation Scientific, mod. TA-XT2i) with a flat Warner-Bratzler probe. In this way, the times obtained were 300 s for durum pasta and 270 s for spelt pasta. Pasta was cooked at these temperatures, then were cooled at room temperature, dried, and analyzed.

2.3 | Fatty acid profile

Fatty acid content was determined following method described by Bligh and Dyer (1959) and with the conditions explained in Ainsa et al. (2021). Each sample was homogenized with an Ultraturrax device (IKA-WERKE, T-25 basic) using chloroform, methanol, KCl, and water. Then, it was centrifuged for 10 min at 1,434 g and the fat was extracted. Finally, incorporating butylated hydroxytoluene, solvents were evaporated. Posteriorly, 0.03 g of this fat was mixed with an intern pattern (C23:0), 2 ml of hexane and 1 ml of KOH saturated. The analysis was made with a gas chromatograph (HP-6890 II Hewlett-Packard) with a column SP-2380 (100 m × 0.25 mm × 0.20 μm). Fatty acid content was quantified as total area (%) of identified fatty acids.

2.4 | TBARS index

Lipid oxidation was determined according to Pfalzgraf et al. (1995). The steps to follow are described in previous studies (Ainsa et al., 2021; Calanche et al., 2019). Using 1,1,3,3-tetrametoxipropane, TMP, a curve pattern was made. Two grams of sample was weighted and homogenized with trichloroacetic acid (TCA). Samples were

centrifuged at 4°C for 30 min at 4,000 rpm and then introduced in a thermostatic bath (J. P. Selecta, Unitronic 2000, Barcelona, Spain) at 97°C for 20 min. Measures were obtained in a spectrophotometer (UNICAM, 5625 UV/VIS) at 532 nm.

2.5 | Acidity index

Acidity index was determined according to standard NTE INEN 0038 (1969). Samples were homogenized with petroleum ether, which was evaporated gently heating. Afterward, 10 ml of neutralized ethanol was added, and samples were titrated with a solution of NaOH 0.1 N. The result was expressed as g oleic acid/100 g of pasta.

2.6 | Microbial counts

This microbial study was carried out to assess food safety in developed pasta (control & antioxidant) and to establish the effectiveness of the cooking applied to reduce initial microbial loads. A Standard on Microbiology was followed of food for human consumption to correctly carry out the analysis preparation and follow the recommendations described (UNE-EN ISO4833-1, 2013; UNE-EN ISO7218, 2017). The samples were prepared in accordance with the provisions of the UNE-EN ISO6887-2, 2017, which proposes to resuspend 10 g of sample in 100 ml of peptone water (Merck[®]) and subsequent homogenization. After that, it was necessary to make decimal dilutions in all cases except for cooked pasta, which was sown from the initial dilution.

A Standard on Microbiology was followed of food for human consumption, to correctly carry out the analysis preparation and follow the recommendations described (UNE-EN ISO 7218:2007/A1:2013). These microbial counts quality was studied to guarantee its microbial quality as well as the effect of the applied treatments over reduction initial microbial charge. The samples were prepared following the provisions of the UNE-EN ISO 6887-1:2017, which proposes to resuspend 10 g of sample in 100 ml of peptone water (Merck[®]) and subsequent homogenization. After that, it was necessary to make decimal dilutions in all cases except for cooked pasta, which was sown from the initial dilution.

2.6.1 | Total viable mesophilic count

Based on the procedure established by UNE-EN ISO4833-1, 2013, the Total viable mesophilic count (TVM) analysis was performed. For this, a planting was carried out by mass homogenization on PCA agar—Plate Count Agar—(Merck[®]). The reading was done after incubation for 24–48 hr at 37°C.

2.6.2 | Psychrotroph count

The count of psychrotrophic bacteria was carried out in the same way as the count of total viable mesophiles, using PCA medium (Merck®), but incubating for 7 days at 10°C.

2.6.3 | Enterobacteriaceae count

Bacteria of the *Enterobacteriaceae* family were counted according to the UNE-EN ISO, 21528-2, 2018 Standard. The samples, previously diluted, were inoculated in mass and after that covered with a second layer with VRBD medium (glucose, bile, and violet red agar) (Merck®) and incubated for 24–48 hr at 37°C.

2.6.4 | Mold and yeast count

The mold and yeast count was carried out in Agar Saboraud medium (Merck®) by the mass homogenization method, as described in the UNE-EN ISO, 16212, 2017. Once incubated for 48 hr at 25°C, the reading was performed.

2.7 | Statistical analyses

All the results obtained in this work were analyzed with Microsoft Excel and its statistical software XLSTAT (Version 16, Addinsoft®). First, a univariate analysis allowed checking the normality of the data and the detection of outliers. After checking the assumptions (normality of the data and homogeneity of the variances), two ways analysis of variance—ANOVA—(antioxidant-cooking, and their interaction) for each type of pasta was carried out using the Fisher's multiple comparison (LSD) as a posteriori test, with an interval of 95% confidence. On the other hand, a principal component analysis—PCA—was carried out as a method for a comprehensive understanding of the results and establishing relationships between variables and treatments studied.

3 | RESULTS

3.1 | Stability of fatty acids

Among all analyzed fatty acids, only the six most important due to their abundance and significant differences were selected for this study (C16, C18:1 n-9, C18:2 n-6, C18:3 n-3 -ALA-, C20:5 n-3 -EPA-, C22:6 n-3 -DHA-). In general, fatty acid profiles before and after cooking were similar in shape, although absolute values increased significantly by effect of cooking (Table 2). The interaction between cooking

TABLE 2 Fatty acid profile of both types of enriched pasta with seabass concentrated

| Treatments | Cooking | | |
|--------------------------------|-----------|---------|------|
| | Before | After | ΔC |
| Durum (D) | | | |
| <i>C16:0</i> | 15.01 a A | 16.34 b | 1.33 |
| <i>C18:1 n-9</i> | 24.75 a B | 27.26 b | 2.51 |
| <i>C18:2 n-6</i> | 32.35 a B | 36.18 b | 3.83 |
| <i>C18:3 n-3 (ALA)</i> | 3.80 B | 3.97 | 0.17 |
| <i>C20:5 n-3 (EPA)</i> | 2.12 a | 2.52 b | 0.40 |
| <i>C22:6 n-3 (DHA)</i> | 3.19 a | 3.87 b | 0.68 |
| Durum +antioxidant (DA) | | | |
| <i>C16:0</i> | 13.34 a B | 15.32 b | 1.98 |
| <i>C18:1 n-9</i> | 22.50 a A | 25.61 b | 3.11 |
| <i>C18:2 n-6</i> | 28.55 a A | 34.13 b | 5.58 |
| <i>C18:3 n-3 (ALA)</i> | 3.45 a A | 3.91 b | 0.46 |
| <i>C20:5 n-3 (EPA)</i> | 2.05 a | 2.25 b | 0.20 |
| <i>C22:6 n-3 (DHA)</i> | 3.12 a | 3.57 b | 0.45 |
| | Before | After | |
| Spelt (S) | | | |
| <i>C16:0</i> | 13.76 a | 14.81 b | 1.05 |
| <i>C18:1 n-9</i> | 26.92 a | 29.56 b | 2.64 |
| <i>C18:2 n-6</i> | 31.97 a B | 33.53 b | 1.56 |
| <i>C18:3 n-3 (ALA)</i> | 3.55 a B | 3.78 b | 0.23 |
| <i>C20:5 n-3 (EPA)</i> | 1.85 a A | 2.14 b | 0.29 |
| <i>C22:6 n-3 (DHA)</i> | 2.54 a | 2.94 b | 0.40 |
| Spelt +antioxidant (SA) | | | |
| <i>C16:0</i> | 14.00 | 14.87 | 0.87 |
| <i>C18:1 n-9</i> | 26.72 a | 28.93 b | 2.21 |
| <i>C18:2 n-6</i> | 30.31 a A | 32.96 b | 2.65 |
| <i>C18:3 n-3 (ALA)</i> | 3.41 a A | 3.68 b | 0.27 |
| <i>C20:5 n-3 (EPA)</i> | 1.99 a B | 2.14 b | 0.15 |
| <i>C22:6 n-3 (DHA)</i> | 2.68 a | 2.88 b | 0.20 |

Note: ΔC = Variation (%) in fatty acids composition. Lower case letters within each row for the same pasta show significant differences ($p < .05$) before and after cooking inside each treatment. Capital letters within columns show significant differences ($p < .05$) between treatments (with or without antioxidant) for each group of pasta (durum and spelt).

treatment and the addition of antioxidant did not show any significant differences. Particularly, in pasta Durum (D & DA), palmitic acid (C16) showed a higher increase. Similar behavior was detected for linoleic (C18:2 n-6) acid, the one with the greatest amount, and oleic acid (C18:1 n-9) with a significant rise after cooking. For its part, ALA showed a change (ΔC) higher in DA than in D while the variations of EPA and DHA were higher in pasta D.

Regarding S pasta, palmitic acid showed the same behavior as in D pasta. Oleic acid presented a similar change

(ΔC) in both kind of pasta (S & SA) while linoleic acid had a higher change in SA. Finally, after cooking, both S and SA showed higher concentrations of ALA than in raw pasta. The addition of antioxidant in all enriched pasta had no significant effect over fatty acid composition after cooking, which demonstrated that the bioavailability did not change despite the treatments applied (cooking and antioxidant). However, it is important to highlight that unsaturated fatty acids, especially ALA, EPA and DHA, showed a trend to increase their quantities in all cooked pasta.

According to the above, ratios of fatty acids for enriched pasta showed in Table 3 confirmed our findings. Concerning durum pasta, %MUFA and %PUFA had an increase after cooking whereas %SFA showed a reduction causing an P/S ratio increase while $\omega 6/\omega 3$ ratio had no significant differences in any pasta. The above suggests that an increase of more than 1 in P/S ratio of enriched pasta would reduce the risk of atherosclerosis and coronary heart disease (EFSA, 2010). Finally, for physicochemical parameters, TBARS showed low values in all pasta (Durum and Spelt) for both treatment (raw and cooked) which set around 1 mg MDA/kg indicating little or no unfavorable sensory perception of lipid oxidation; meanwhile, in the case of acidity index (%), a certain decrease was produced after cooking in all developed pasta, too.

TABLE 3 Nutritional Ratios for enriched pasta with seabass concentrated

| Treatments | Cooking | | Treatments | Cooking | |
|--------------------------------|-------------|-------------|--------------------------------|-------------|---------|
| | Before | After | | Before | After |
| <i>Durum (D)</i> | | | <i>Spelt (S)</i> | | |
| %SFA | 25.00 b B | 16.34 a | %SFA | 18.53 | 18.68 |
| %MUFA | 31.30 a B | 34.83 b | %MUFA | 32.87 a | 36.87 b |
| %PUFA | 43.71 a B | 48.95 b | %PUFA | 42.31 a B | 44.57 b |
| P/S ratio | 1.75 a A | 3.24 b | P/S ratio | 2.28 a B | 2.39 b |
| $\omega 6/\omega 3$ ratio | 3.48 B | 3.36 | $\omega 6/\omega 3$ ratio | 3.97 B | 3.68 |
| Acidity Index | 0.21 b B | 0.03 a A | Acidity Index | 0.18 b B | 0.04 a |
| TBARS (mg MDA/kg) | ≤ 1.00 | ≤ 1.00 | TBARS | ≤ 1.00 | 1.32 |
| <i>Durum +antioxidant (DA)</i> | | | <i>Spelt +antioxidant (SA)</i> | | |
| %SFA | 20.12 A | 20.94 | %SFA | 18.84 | 19.37 |
| %MUFA | 28.58 a A | 32.93 b | %MUFA | 33.44 a | 36.11 b |
| %PUFA | 39.73 a A | 46.33 b | %PUFA | 40.96 a A | 44.57 b |
| P/S ratio | 1.97 a B | 2.21 b | P/S ratio | 2.17 a A | 2.30 b |
| $\omega 6/\omega 3$ ratio | 3.29 A | 3.50 | $\omega 6/\omega 3$ ratio | 3.67 A | 3.69 |
| Acidity Index | 0.16 b A | 0.04 a B | Acidity Index | 0.13 b A | 0.04 a |
| TBARS (mg MDA/kg) | ≤ 1.00 | ≤ 1.00 | TBARS | ≤ 1.00 | 1.20 |

Note: Lower case letters within each row for the same pasta show significant differences ($p < .05$) before and after cooking inside each treatment. Capital letters within columns show significant differences ($p < .05$) between treatments (with or without antioxidant) for each group of pasta (durum and spelt).

3.2 | Microbial counts

Microbial counts for raw and cooked pasta are shown in Figure 1a for Durum pasta and Figure 1b for Spelt pasta. The effect of cooking clearly produced a significant decrease. Mesophiles—MTV—as well as mold and yeast count—M&Y—showed drastic reductions of up to three cycles log/cfu. However, the decrease became dramatic in the case of psychrotrophs—Psychrotroph count (PST)—(up to 4 log/cfu) and *Enterobacteriaceae*—*Enterobacteriaceae* count (ET)—(3 log/cfu). Besides this, it was possible to find some significant differences with the pasta with antioxidant which showed lower initial counts.

3.3 | Principal components analysis

A principal components analysis was carried out in order to establish interactions between the types of pasta and their respective fatty acid compositions. The resulting biplot is shown in Figure 2 as to Durum (A) and Spelt (B) pasta. Concerning durum pasta, the two first components (F1 and F2) described 90.18% of the variability of the analysis. Raw pasta was situated on the left, whereas cooked pasta was on the right, which evidenced the effect of heat treatment. Raw pasta

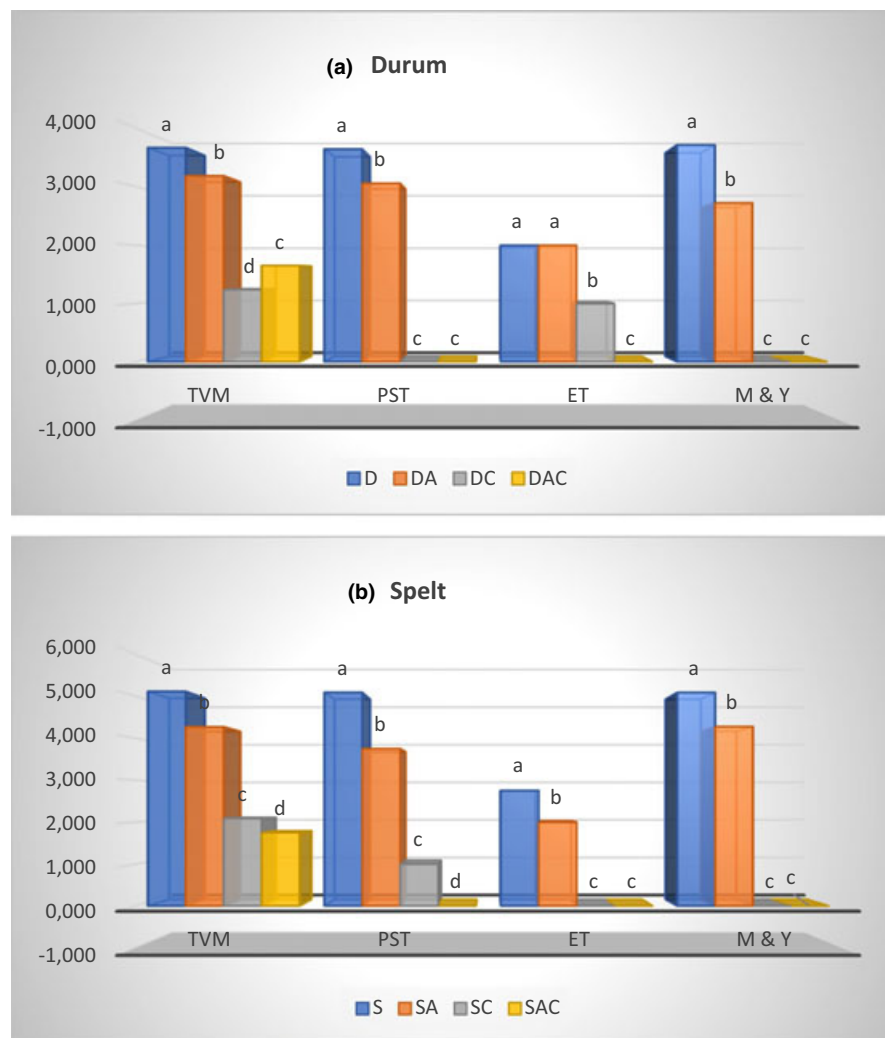


FIGURE 1 Microbiological counts for each type of pasta. (a) Durum pasta (b) Spelt pasta TVM, total viable mesophilic count; PST, psychrotrophic count; ET, enterobacteriaceae count; M&Y, mold and yeast count; D, durum; DA, durum with antioxidant; DC, durum cooked; DAC, durum with antioxidant cooked; S, spelt; SA, spelt with antioxidant; SC, spelt cooked; SAC, spelt with antioxidant cooked

was related to microbial counts, whereas fatty acids were found in abundance in cooked pasta. The same effect was produced in Spelt for both types of pasta (raw and cooked) showing a separation in the first axis with 90.90%. While in the second component, although only represents 8.31%, it was possible to see a relation between pasta with antioxidant and the concentrations of DHA and EPA after cooking.

4 | DISCUSSION

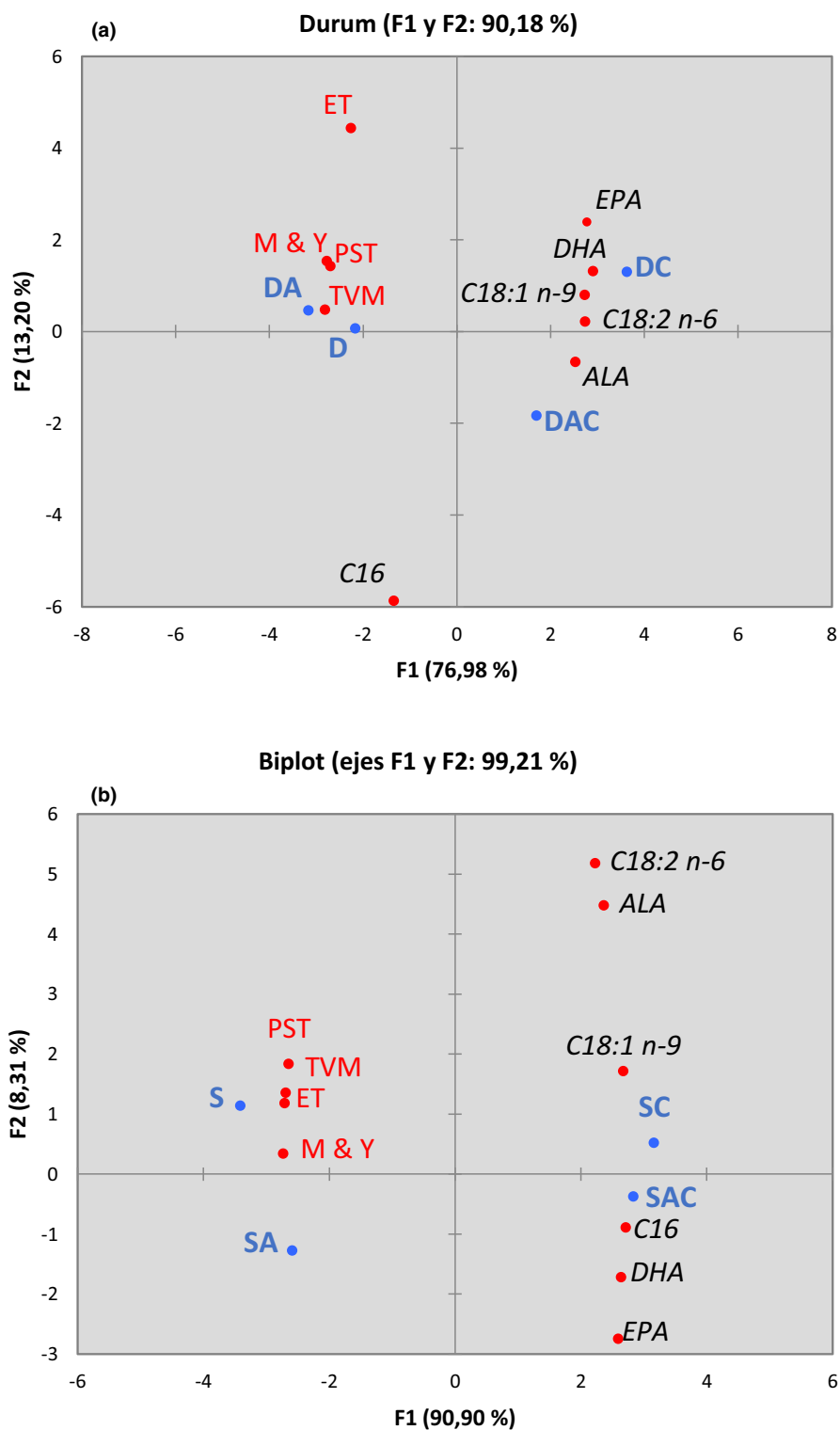
4.1 | Fatty acid stability

Values after cooking confirmed a greater stability of saturated fatty acids when compared to unsaturated. As double bonds appear, fatty acids become more unstable and highly oxidizable. In lipids with a double bond, there are two isomeric possibilities: *cis* and *trans*. It should be noted that positional isomerism is related to the location of double bonds, and in this sense, the most common is to find unconjugated systems, but the heat treatment applied to pasta causes them

to become conjugated, giving rise to chemical compounds with alternating single and double bonds, where the electrons that form the double bonds are delocalized and are located uniformly around the compound. When this occurs, the substrates are more reactive and more easily oxidizable (Badui, 2006; Castro & Carrillo, 2015). Therefore, its availability increases, and this results in a higher proportion of unsaturated fatty acids in cooked pasta than in raw pasta.

As enriched pasta were made with a high quantity of fish concentrate (10%), the increase of fatty acids in all types of developed pasta, shown in Table 2, is to be explained by the behavior of fatty acids from sea bass that was the species used as raw material. Other studies have already indicated an increase, mainly in ALA, EPA, and DHA, for sea bass (Türkkan et al., 2008) and for other species (Ågren & Hänninen, 1993; Gall et al., 1983). On top of that, performances of ALA and EPA & DHA could be explained by the conversion of the first one into these second acids (EPA and DHA) which could have happened in Durum and Spelt without antioxidant, so improving the availability of these fatty acids to offer health benefits (Ainsa et al., 2021; Harper & Jacobson, 2001). However,

FIGURE 2 Principal Component Analysis of pasta with and without antioxidant before and after cooking. (a) Durum Pasta (b) Spelt Pasta TVM, Total viable mesophilic count; PST, psychrotroph count; ET, enterobacteriaceae count; M&Y, mold and yeast count; D, durum; DA, durum with antioxidant; DC, durum cooked; DAC, durum with antioxidant cooked; S, spelt; SA, spelt with antioxidant; SC, spelt cooked; SAC, spelt with antioxidant cooked



just as the bioavailability increases, the tendency to oxidation increases too, since its speed doubles for every 15°C of increase in pasta cooking (Badui, 2006). For this reason, if pasta is cooked and is not consumed at the moment, its oxidation will occur at a higher speed, causing possible rancidity of pasta (Badui, 2006; Castro and Carrillo, 2015).

Chemical indices (TBARS and acidity index) related to fat stability demonstrated in general a satisfactory result. TBARS

values were <2 mg MDA/kg pasta indicating that there would probably be no sensory rejection for unpleasant perceptions related to rancidity of lipids (Marcuse & Johansson, 1973). Regarding acidity index, expressed as oleic acid/100 g pasta, showed significant decreases ($p < .05$) in values of all cooked pasta. The above could be due to the fact that oleic acid tends to break free from triacylglycerides but being exposed to oxidize to free radicals and peroxides, and consequently, a

certain reduction may follow pasta cooking. This is related to the catalytic lipases capacity. The temperature has two effects: firstly, a thermic increase implies an increment of the kinetic constant of the catalytic reaction, and thus, an increase in reaction speed where ester bond is hydrolyzed and fatty acids are liberated resulting in product acidity (Arteaga et al., 2010). Secondly, a higher increase implies an increase in enzyme deactivation constant and so a substrate decrease per unit time. This is why at the beginning a maximum value of activity is reached, from which, a higher increase causes a denaturation effect that exceeds that of increase in the reaction speed, so the activity decreases (Guillén, 2012) and a reduction in acidity content occurs.

4.2 | Microbiological quality

It should be noted that before applying the cooking treatment, most counts were below recommendations, except for mold and yeast—M&Y, but they fell below the established legal limits after cooking. On top of that, some authors have studied the antimicrobial effect of *R. officinalis* and the results obtained showed greater antibacterial activity in those samples in which rosemary extract had been added (Fernández-López et al., 2005). The inhibitory effect of rosemary is due to the action of its rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic, rosmanol, and isorosmanol. They interact with the cellular membrane, leading to changes in genetic material and nutrients, altering electron transport and causing changes in the production of fatty acids. Furthermore, it is capable of producing an interaction with the protein membrane, causing a loss of functionality (Nieto et al., 2018).

4.3 | Global analysis

It seems reasonable to affirm that cooking had a positive influence on fatty acids, since the highest values of MUFA and PUFA corresponded to cooked pasta. Fatty acids in durum pasta (Figure 2) were located in the plot close to each other, in agreement with their homogeneous origin from fish, and they showed no differences caused by the addition of antioxidant. In spelt pasta, in which fatty acids come both from fish and bran, they were separated into two groups by the second principal component, associating EPA and DHA to pasta with antioxidant and ALA, oleic, and linoleic acids to pasta without antioxidant. This demonstrated the effectiveness of the antioxidant in this type of pasta. As a consequence, the bioavailability of those fatty acids would increase. Besides this, it appears evident that cooking time was sufficient to improve the microbiological quality of pasta since cooked pasta had significantly lower microbial counts.

5 | CONCLUSION


The developed pasta showed a higher concentration of fatty acids after cooking and the balance in the ratio $\omega 6/\omega 3$ improved, thus contributing to achieving a healthier diet. Cooking had also a positive effect in their microbiological quality, due to the reduction in microbial counts and the increase in the bioavailability of polyunsaturated fatty acids as nutrients. The fatty acid profile remained stable after cooking, which suggests a possible synergistic effect of the use of rosemary extract as antioxidant. The enriched pastas developed in this research showed a general behavior related to cooking similar to that of fresh sea bass when cooked, as their fatty acid profiles were more similar to fresh fish profile than that of conventional wheat-based pasta.

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