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Quality loss assessment in fish-based ready-to-eat foods during refrigerated storage

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RESUMEN

Determinación de la pérdida de calidad en alimentos marinos precocinados durante su conservación en refrigeración

Se estudió la pérdida de calidad de dos productos marinos elaborados (lasaña de atún, TL; huevas de merluza) conservados en refrigeración (4 °C) durante 35 y 71 días, respectivamente. Ambos alimentos mostraron un desarrollo importante de la oxidación lipídica (formación de peróxidos y compuestos de interacción) durante el periodo de conservación, siendo mayor en el caso de TL; asimismo, el producto TL experimentó un importante desarrollo de hidrólisis lipídica. Sin embargo, ambos alimentos experimentaron un desarrollo bajo en microorganismos mesófilos y aminas volátiles; por otra parte, no maniferstaron presencia de microorganismos patógenos a lo largo del periodo de conservación, a pesar de sobrepasar el tiempo de caducidad. De acuerdo con la creciente demanda de nuevas preparaciones de alimentos refrigerados de calidad alta, se recomienda el estudio de las alteraciones lipídicas en elaboraciones basadas en alimentos de origen marino debido a su fuerte relación con la pérdida de calidades sensorial y nutricional.

PALABRAS CLAVE: Actividad microbiana – Alteración lipídica – Color – Huevas de merluza – Músculo de atún – Refrigeración.

SUMMARY

Quality loss assessment in fish-based ready-to-eat foods during refrigerated storage

The present research addresses the quality loss in two different ready-to-eat (RTE) seafoods. With this aim, chemical and microbiological parameters were measured in tuna lasagne (TL) and hake roe foods which were refrigerated (4°C) for up to 35 and 71 days, respectively. Both foods showed a significant lipid oxidation (peroxide and interaction compound formation) development with storage time, which was especially marked in the case of the TL product, which also underwent an important lipid hydrolysis development. Both RTE products showed a low microbial development, no matter how much time had elapsed since the expiration dates; thus, low total viable count scores and volatile amine formation were attained while the presence of pathogen microorganisms was not detected. In view of the current increasing consumer demand for high quality refrigerated foods, the assessment of lipid damage related to nutritional and sensory values is recommended when fish-based RTE products are encountered.

KEY-WORDS: Colour – Hake roe – Lipid damage – Microbial activity – Refrigeration – Tuna muscle.

1. INTRODUCTION

Seafoods are known to provide relevant contents of different benefitial constituents such as nutritional and digestible proteins, lipid-soluble vitamins, essential minerals and highly unsaturated fatty acids (Piclet, 1987). Among them, the lipid fraction is now the subject of a great deal of attention due to its high content of ω3 polyunsaturated fatty acids, which have been demonstrated to play a positive role in preventing certain human diseases (Knoch *et al*., 2009; Valenzuela, 2009).

In spite of their valuable composition, marine species constitute highly perishable products whose quality and freshness rapidly decline post-mortem. Deterioration of such species begins immediately upon catching or slaughtering, and the degree to which it continues depends directly on processing and storage conditions after death (Whittle *et al*., 1990). In addition, the highly unsaturated lipid composition of marine species has shown to be especially prone to lipid oxidation development, which may cause marked sensory and nutritional quality losses (Aubourg, 1999; Chaouqy *et al*., 2008).

Increasing consumer demand for convenience products is one of the major contemporary trends in marine food consumption. A strategic solution for increasing their distribution according to modern human habits is represented by ready-to-eat (RTE) products (Gilbert *et al*., 2000; Trondsen *et al*., 2003). RTE seaproducts are pre-processed foods that can offer fast meal solutions that solve the problem of home preparation of marine species. Thus, novel and attractive preparations are constantly being provided to the market and

restaurant sectors, with an extraordinary variety of presentations and contents. However, according to the above mentioned high lability of marine species, a strict safety, nutritional and sensory acceptance control is required.

Previous research has focused on the quality control of fish-based RET products from different parts in the world, aiming especially at the microbial activity development and degree of safety (Oh *et al.*, 2007; Meloni *et al.*, 2009; Miya *et al.*, 2010; Kim *et al.*, 2011). However, studies concerning the chemical changes related to quality loss have been scarce and focused on protein changes (smoked salmon product; Guillén-Casla *et al.*, 2011), volatile amine formation (refrigerated vacuum-packaged octopus product; Mendes *et al.*, 2011), lipid composition and oxidation (tilapia fish curry product; Dhanapal *et al.*, 2010) and nutraceutical compound addition (Braida and Gormley, 2008). Additionally, different advanced technologies have been applied in order to enhance the quality and safety of marine RTE products such as E-beam irradiation (Guillén-Casla *et al.*, 2011), tin-free steel can employment (squid masala product; Sreenath Pathiyaparambil *et al.*, 2007), natural antimicrobial compounds (fish burger product; Corbo *et al.*, 2009) and active packaging application (Mendes *et al.*, 2011).

The present research addresses the quality loss in two different RTE seafoods during their refrigeration storage at 4 °C. In it, the quality changes in tuna lasagne (TL) and marinated hake (*Merluccius merluccius*) roe with cooked red peppers (HR), foods related to lipid damage (oxidation and hydrolysis), the microbiological activity development and color evolution were analyzed.

2. MATERIALS AND METHODS

2.1. Starting RTE foods and sampling

Pre-cooked RTE seafood products (TL and HR) were obtained from a local market as soon as they arrived from the factory. Both consisted of vacuum-packed bags (n = 15 of each product), each bag containing 400 g and 240 g of TL and HR products, respectively.

Once in our laboratory, all the bags were placed in a refrigerated room (4 °C). Under such storage conditions, expiration dates announced on the product labels were 23 and 31 days for TL and HR foods, respectively. Accordingly, the present study was designed to reach such expiration dates and over-pass them. Thus, sampling was carried out on days 0, 7, 14, 21 and 35 for the TL product and on days 0, 13, 28, 43 and 71 in the case of the HR product. For each RTE product, three different bags were taken for analysis at each sampling time and analyzed separately in order to achieve the statistical analysis (n = 3). Analyses were carried out on the homogenized RTE food.

2.2. Moisture and lipid contents

Moisture content was determined by the difference between the weight of the homogenized product (1-2 g) and the weight recorded after 16-18 h at 100-102 °C (AOAC, 1990). Results are expressed as g water 100 g^{-1} product.

Lipids were extracted from the RTE product according to the Bligh and Dyer (1959) method, by employing a single-phase solubilization of the lipids using a chloroform-methanol (1:1) mixture. Quantification results are expressed as g lipid 100 g^{-1} product.

2.3. Lipid damage assessment

The peroxide value (PV) was determined in the lipid extract by peroxide reduction with ferric thiocyanate, according to the Chapman and McKay (1949) method. Results are expressed as meq active oxygen kg^{-1} lipids.

The thiobarbituric acid index (TBA-i) was determined according to Vyncke (1970). This method is based on the reaction between a trichloracetic acid extract of the food sample and thiobarbituric acid. The content of thiobarbituric acid reactive substances (TBARS) was spectrophotometrically measured at 532 nm and the results are expressed as mg malondialdehyde kg^{-1} product.

The formation of fluorescent compounds was determined by measurements at 393/463 nm and 327/415 nm as described by Aubourg (2001). The relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the fluorescence measured at each excitation/ emission maximum, and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 µg mL^{-1} in 0.05 M H_2SO_4) at the corresponding wavelength pair. The fluorescence ratio (FR) was calculated as the ratio between the two RF values: $FR = RF_{393/463\ nm} / RF_{327/415\ nm}$. Results are expressed as the FR value and were determined in the aqueous phase resulting from the lipid extraction of both RTE products (Bligh and Dyer, 1959).

Browning development was measured in the lipid extract at 450 nm and 400 nm. The 450 nm/ 400 nm absorbance ratio (browning ratio, BR) was studied according to Hassan *et al.* (1999).

The free fatty acid (FFA) content was determined in the lipid extract according to the Lowry and Tinsley (1976) method based on a complex formation with cupric acetate-pyridine followed by spectrophotometric (715 nm) assessment. Results are expressed as g FFA 100 g^{-1} lipids.

2.4. Color assessment

An instrumental color analysis (CIE 1976 L*, a*, b*) was performed by employing a tristimulus Hunter Labscan 2.0/45 colorimeter. Measurements were made directly on the food sample. For each sample analysis, color scores were obtained as mean values of four measurements obtained by

rotating the measuring head 90º between duplicate measurements per position.

2.5. Microbial analysis

Total viable counts (TVC) were measured following the ISO 4833 (2003) procedure. 25 g of the food sample and 225 mL of plate count agar (PCA, pH 7) were homogenized for 30 s. Decimal dilutions were prepared, inoculated in plates and incubated at 30 ± 1 °C for 72 h. Two consecutive dilutions having between 10 and 300 colonies were counted and mesophile microorganisms were calculated according to the following formula:

$$N = \frac{\sum C}{V \times 1.1 \times d}$$

were ∑C is the sum of the consecutive plates counted, V is the inocula volume (ml) and d is the dilution factor corresponding to the first dilution measured (ISO 7218, 2007).

The presence of β-glucoronidase-positive *Escherichia coli* was determined following the ISO 16649-3 (2005) procedure. Three series of 5 tubes including 10 ml of mineral modified glutamate broth (MMGB) were inoculated with serial decimal dilutions of samples and incubated at 37 ± 1 °C for 24 ± 2 h. After this, the tubes containing broth that had changed their color to yellow were selected and inoculated on tryptone bile X-glucuronide (TBX) agar plates. These plates were incubated at 44 ± 1 °C for 22 ± 2 h to obtain isolated blue or blue-green colonies that indicated the presence of β-glucoronidase-positive *Escherichia coli*.

Staphylococcus aureus counting was determined using the Tempo® System (BioMérieux, Marcy l'Étoile, France) following the manufacturer's instructions. This method is a miniaturized most probable number (MPN) assay.

Listeria monocytogenes and *Salmonella* spp. were analyzed using real-time PCR. For both bacteria, a previous enrichment at 37 °C for 18 ± 2 h was carried out using the two following broths: buffered peptone water (BPW, pH 7) for *Salmonella* and fraser-semi (FS, pH 7.2) for *Listeria monocytogenes.* DNA was extracted following the protocol described by Garrido *et al.* (2012).

Two different real-time PCR reactions were carried out including master mix Kapa™ probefast qPCR (2X) (Kapa Biosystems, Inc., Woburn, Massachusetts, USA), and primers previously described by Cheng *et al.* (2008) targeting the *inv*A gene for *Salmonella*, and by Omiccioli *et al.* (2009) targeting the *hly*A gene for *Listeria monocytogenes*. Also, an internal amplification control (IAC) previously described by Calvó *et al.* (2008) was included in each reaction. Real-time PCR was run in a Stratagene Mx3005p thermocycler (Agilent Technologies, Inc., Santa Clara, CA, USA) including an annealing step at 63 °C.

2.6. Assessment of volatile amine formation

Total volatile base-nitrogen (TVB-N) values were measured using the Antonacopoulos (1960) method, with some modifications. Briefly, the food sample (10 g) was extracted with 6% (w/v) perchloric acid and brought up to 50 mL, determining the TVB-N content–after steam-distillation of the acid extracts rendered alkaline to pH 13 with 2% (w/v) NaOH – by titration of the distillate with 10 mM HCl. The results are expressed as mg TVB-N 100 g^{-1} product.

Trimethylamine-nitrogen (TMA-N) values were obtained by means of the picrate method, as previously described (Tozawa *et al.*, 1971). This involves the preparation of a 5% (w/v) trichloroacetic acid extract of the food sample. The results are expressed as mg TMA-N 100 g^{-1} product.

2.7. Statistical analysis

Data from the different quality indices were subjected to a one-way ANOVA ($p < 0.05$) (Statsoft, Statistica, version 6.0, 2001); comparison of means was performed using a least-squares difference (LSD) method. Correlation analysis among parameters (refrigerated time, microbial values and chemical indices) was carried out by means of the Pearson correlation coefficient (r-value). Linear fitting is expressed; otherwise, the kind of fitting (quadratic or logarithmic) is mentioned.

3. RESULTS AND DISCUSSION

3.1. Moisture and lipid contents

Moisture and lipid contents of the TL product were found in the ranges of 76.9-77.7 and 4.7-5.6 g 100 g^{-1}, respectively. In the case of the HR product, a higher lipid content was observed (8.6-11.3 g 100 g^{-1}) while a lower moisture level was attained (71.3-75.2 g 100 g^{-1} product). Both in TL and HR products, moisture and lipid contents did not provide significant changes as a result of the refrigeration storage; value differences could be explained as a result of sample-to-sample differences and no tendency could be implied as a result of the storage time.

Previous research provides some information concerning the moisture and lipid contents of roes corresponding to different hake species. Thus, fresh *Merluccius hubbsi* roes provided 67 g and 6.6 g 100 g^{-1} of moisture and lipid contents, respectively (Méndez *et al.*, 1992); while fresh *Merluccius merluccius* and *Merluccius mediterraneus* provided 73.5 g and 63.2 g 100 g^{-1}, respectively, for moisture contents (Rincón-Cervera *et al.*, 2009). Such results can be considered as relatively similar to the ones obtained in the present study. However, quite different scores were attained for dried-salted *Merluccius merluccius* roes (28.66 and 14.13 g 100 g^{-1} for moisture and lipid contents, respectively; Rodrigo *et al.*, 1998).

3.2. Lipid oxidation assessment

Lipid oxidation was studied by means of different quality indices corresponding to different steps included in the lipid oxidation development of marine tissues.

The TL product showed (Table 1) a marked primary oxidation increase (peroxide formation) with storage time (r = 0.86; logarithmic fitting); however, a definite tendency could not be observed in the TBARS formation (secondary lipid oxidation) throughout the whole experiment. Interaction compound formation measured by fluorescent and browning properties provided an increasing value throughout the whole experiment (r = 0.86, logarithmic fitting and r = 0.93, quadratic fitting, respectively); this increase is in agreement with the peroxide formation previously mentioned. It is worth pointing out that the values attained for PV and TBA-i parameters can be considered as not very high even at the end of the experiment when the expiration date of the product (23 days under the present storage conditions) had elapsed.

Concerning the HR product (Table 1), a significant primary (PV) and secondary (TBA-i) lipid oxidation development could be observed only at the end of the experiment (day 71), when the expiration date of the product (31 days under the present storage conditions) had greatly elapsed; before this time (13-43-day period), no differences could be attributed when compared to the starting value. Although values can be considered low for both parameters, fair correlation values with storage time were obtained for PV (r = 0.85, quadratic fitting) and TBA-i (r = 0.88, logarithmic fitting). Interaction compound formation measured by the fluorescence properties (FR value) showed a marked increase throughout the refrigerated storage (r = 0.84; logarithmic fitting), according to the peroxide and TBARS formation. All three lipid oxidation parameters showed to be fairly correlated since TBA-i provided fair correlation values with FR and PV parameters (r = 0.85 and 0.86, respectively).

Lipid oxidation development has been recognized as a complex process where different kinds of molecules are produced, most of them unstable, susceptible to breakdown and originate lower weight compounds, or react with other molecules (nucleophilic-type, mostly) present in the fish muscle. Consequently, the determination of each kind of compound cannot always provide an accurate method for quality assessment. In the present research, primary and tertiary lipid oxidation compounds have shown to be the most sensitive values for assessing the degree of oxidation evolution throughout the storage period.

3.3. Lipid hydrolysis assessment

The TL product showed a slightly increasing tendency with storage time for the FFA formation (r = 0.88; logarithmic fitting) (Table 2). However, FFA values can be considered relatively low according to scores previously reported for seafood products related to fatty fish species (Losada *et al*., 2004; Stodolnik *et al*., 2005). In the case of the HR product (Table 2), some differences could be concluded among the different sampling times; however, a general tendency could not be implied, so that it could be assumed that an increasing time did not lead to a marked increase in lipid hydrolysis, in spite of the fact that the expiration date (31 days) was reached before the end of both experiments.

FFA formation during refrigerated storage has been reported to be produced as a result

Table 1
Lipid oxidation assessment* in refrigerated fish-based foods**

Fish-based food	Refrigeration time (days)	PV	TBA-i	FR	BR
Tuna lasagne	0	1.70 a (0.41)	0.89 (0.07)	0.48 a (0.05)	62.67 a (3.28)
	7	3.16 b (0.34)	0.85 (0.06)	0.95 b (0.02)	64.50 a (4.11)
	14	5.62 d (0.97)	0.80 (0.03)	0.99 bc (0.08)	64.74 a (3.95)
	21	4.11 c (0.28)	0.79 (0.10)	1.08 c (0.04)	71.60 b (4.15)
	35	6.09 d (0.83)	0.81 (0.08)	1.04 c (0.05)	78.75 b (3.01)
Hake roe	0	2.35 a (0.51)	0.78 a (0.04)	2.61 a (0.23)	—
	13	2.07 a (0.64)	0.80 ab (0.08)	3.18 b (0.15)	—
	28	2.20 a (0.29)	0.80 ab (0.09)	3.00 b (0.15)	—
	43	2.12 a (0.18)	0.77 a (0.03)	2.97 b (0.09)	—
	71	3.47 b (0.39)	0.86 b (0.02)	3.68 c (0.22)	—

* Mean values of three (n = 3) replicates; standard deviations are indicated in brackets. For each parameter and for each fish-based food, mean values followed by different letters (a-d) denote significant ($p < 0.05$) differences as a result of the refrigeration time; no indication is provided when no differences ($p>0.05$) are found. ** Abbreviations and units: PV (peroxide value; meq active oxygen/ kg lipids), TBA-i (thiobarbituric acid index; mg malondialdehyde/ kg food), FR (fluorescence ratio) and BR (browning ratio).

Table 2
Free fatty acid assessment (g 100 g^{-1} lipids)* in refrigerated fish-based foods

Refrigeration time (days)**	Fish-based food	
	Tuna lasagne	Hake roe
0	0.42 a (0.05)	3.87 ab (0.18)
7 / 13	0.66 b (0.01)	3.66 a (0.23)
14 / 28	0.59 ab (0.20)	4.38 b (0.36)
21 / 43	0.68 bc (0.11)	3.96 ab (0.31)
35 / 71	0.84 c (0.07)	4.18 ab (0.46)

* Mean values of three (n = 3) replicates; standard deviations are indicated in brackets. For each fish-based food, mean values followed by different letters (a-c) indicate significant ($p < 0.05$) differences as a result of the refrigeration time. ** Refrigeration times: The first number indicates the refrigerated time corresponding to the tuna lasagne experiment, while the second one corresponds to the hake roe study.

of endogenous enzyme activity and microbial activity (Whittle *et al.*, 1990). According to the fact that pre-cooked fish products are used in the present research, the formation of FFA during refrigerated storage is expected to be produced mostly as a result of the development of microbial activity.

The formation of FFA itself does not lead to nutritional losses. However, FFA presence has shown an important role in fish muscle texture changes and lipid oxidation enhancement (Mackie, 1993) and as being strongly related to off-odor development (Refsgaard *et al.*, 2000). Additionally, FFA have shown to develop a higher oxidation rate than higher molecular-weight lipid classes such as triacylglycerols and phospholipids as a result of providing a lower steric hindrance to the initial development of the oxidation mechanism. In the present TL study, acceptable correlation values were obtained between the FFA content and the interaction compound formation (r = 0.79 and 0.83 with FR and BR indices, respectively).

3.4. Color assessment

Color plays an important role in the appearance, presentation and acceptability of foods, including fish-based RTE products. In the present research, color changes in both RTE foods were investigated by means of physical (CIE L*, a* and b*) analysis.

No differences could be observed in the lightness (L*) parameter for the TL product (Table 3), and all the values scored in the 66.5-68.0 range. Concerning the HR product, some differences were detected for this parameter, although a clear tendency with time could not be implied as all values fell in the 62.5-64.3 range. Previous research related to the refrigerated storage (4 °C) of raw rainbow trout fillets packed under vacuum did not provide differences either after up to 15 days of storage for the L* parameter (Gobantes *et al.*, 1998). Concerning the greenness/redness assessment (a* value; Table 3), a decrease with time was observed for both RTE products. As a result, TL and HR products showed acceptable correlation values between this parameter and time (r = –0.81; r = –0.78, logarithmic fitting, respectively). This decrease can be explained as a result of the degradation of colored compounds and has already been detected during marine species processing (Ashie *et al.* 1996; Gobantes *et al.*, 1998). Redness (a*) loss has been proposed as a way of following haemoglobin-mediated lipid oxidation in fish, showing an inverse relationship with lipid oxidation development (Wetterskog *et al.*, 2004). In the present research, an accurate correlation value between a* parameter and interaction compound formation was obtained for

Table 3
Color (L*, a* and b* parameters) assessment§ in refrigerated fish-based foods

Fish-based food	Refrigeration time (days)	L*	a*	b*
Tuna lasagne	0	67.54 a (0.35)	14.39 b (0.23)	33.55 a (0.40)
	7	67.37 a (0.30)	14.29 b (0.32)	34.43 ab (0.74)
	14	67.07 a (0.49)	14.23 b (0.20)	34.50 b (0.15)
	21	66.56 a (0.72)	13.32 a (0.27)	34.64 b (0.64)
	35	67.60 a (1.57)	13.45 a (0.14)	37.21 c (0.24)
Hake roe	0	63.30 ab (0.56)	32.55 c (0.17)	49.74 c (0.76)
	13	64.23 b (0.62)	28.98 a (0.48)	46.42 b (0.40)
	28	62.85 a (0.62)	29.78 ab (0.68)	46.09 b (0.74)
	43	62.57 a (0.18)	30.57 b (0.48)	43.03 a (0.97)
	71	63.61 ab (1.12)	28.81 a (0.31)	44.07 a (1.19)

§ Mean values of three (n = 3) replicates; standard deviations are indicated in brackets. For each parameter and for each food product, mean values followed by different letters denote significant ($p < 0.05$) differences as a result of the refrigeration time.

TL (r = –0.85 with BR value) and HR (r = –0.83 with FR value) samples.

Concerning the yellowness/blueness assessment (b* value; Table 3), a marked increase was observed with time (r = 0.92; quadratic fitting) for the TL product. However, b* values underwent marked decreases with refrigeration time in the case of the HR product (r = –0.79). Usually, this color parameter has directly been related to lipid oxidation development. Thus, an important relationship among the b* value and the formation of polymerized Schiff bases and fluorescent compounds (tertiary lipid oxidation compounds) has been proved (Undeland *et al.*, 2003). In the present TL study, this parameter has shown a direct relationship with the development of lipid damage, thus providing fair correlation scores with the BR value (r = 0.88) and FFA formation (r = 0.86). In the case of the HR product, very poor correlation values with lipid damage parameters were obtained; it could be argued that the presence of colored components in this food may have influenced the assessment of this parameter.

3.5. Microbial parameters assessment

Results obtained for TVC in TL samples are shown in Table 4. The content of this group of bacteria remained quite constant throughout storage, so that a definite tendency with time could not be concluded. In the case of the HR product (Table 4), TVC values were found below the limit of detection (1 log CFU g^{-1}) for the 0-43-day period; however, at the end of the experiment a significant ($p < 0.05$) increase was observed. This low TVC value up to day 43 can be explained on the basis of being a marinade product. Thus, marinated seafood products have been reported to be preserved by the simultaneous action of organic acids, such as acetic acid, and salt (Kilinc and Cakli, 2005; Sallam *et al.*, 2007); this combined preservative action would prevent the growth of pathogenic bacteria and most spoilage bacteria.

In the present research, the presence of different kinds of pathogens was analyzed. As a result, none of the pathogens analyzed (*Salmonella, Staphylococcus aureus*, β-glucoronidase positive *Escherichia coli* and *Listeria monocytogenes*) were detected in either RTE products at any of the sampling times in spite of the fact that for both RTE products, expiration dates (23 and 31 days for TL and HR products, respectively) were exceeded.

3.6. Amine formation assessment

Although some significant differences could be detected, the total volatile amine formation (Table 4) did not provide a clear tendency with time in either of the two products under study. Values were included in the 12.3-16.1 and 21.1-23.4 mg TVB-N/100 g ranges for TL and HR samples, respectively. These narrow value ranges are in agreement with the low TVC and FFA formation above mentioned and the non-presence of pathogens. Indeed, an acceptable correlation value (r = 0.83) was observed between TVB-N and FFA parameters in the TL product.

Concerning the trimethylamine formation (Table 4), significant differences were hardly provided by either RTE products throughout the storage time. In the case of the TL samples, values were concentrated in the 0.18-0.21 mg TMA-N 100 g^{-1} range, while those of the HR product were concentrated in the 1.10-1.22 range. As for the TVB-N assessment, a low TMA-N formation is

Table 4
Assessment* of microbial and chemical parameters related to microbial activity in refrigerated fish-based foods**

Fish-based food	Refrigeration time (days)	TVC (log CFU g^{-1} food)	TVB-N (mg 100 g^{-1} food)	TMA-N (mg 100 g^{-1} food)
Tuna lasagne	0	2.23 a (0.21)	12.30 a (0.69)	0.21 a (0.03)
	7	1.57 a (0.25)	16.14 c (0.49)	0.18 a (0.01)
	14	1.70 a (0.40)	14.03 b (0.17)	0.19 a (0.01)
	21	1.67 a (0.25)	14.46 b (0.24)	0.21 a (0.03)
	35	1.50 a (0.56)	15.97 c (0.40)	0.21 a (0.03)
Hake roe	0	< LOD**	23.42 c (1.33)	1.20 ab (0.07)
	13	< LOD	21.14 abc (1.83)	1.11 ab (0.09)
	28	< LOD	21.81 b (0.23)	1.10 a (0.03)
	43	< LOD	20.30 a (0.98)	1.22 b (0.04)
	71	2.50 (0.51)	22.47 abc (1.96)	1.19 b (0.03)

* Mean values of three (n = 3) replicates; standard deviations are indicated in brackets. For each parameter and for each fish-based food, mean values followed by different letters (a-c) indicate significant ($p < 0.05$) differences as a result of the refrigeration time. ** Abbreviations: TVC (total viable counts), TVB-N (total volatile base-nitrogen) and TMA-N (trimethylamine-nitrogen), LOD (limit of detection).

observed with the refrigeration time and agrees with the low TVC and FFA formation above mentioned and the non-presence of pathogens.

For both TVB-N and TMA-N indices, initial values were found higher ($p < 0.05$) in the HR product than in the TL one. According to the effect of storage time on the loss in amine compound formation, the correlation of values between refrigeration time and both amine indices were poor.

4. CONCLUSIONS

The quality changes in two commercial fish-based RTE products were analyzed during refrigerated storage. Both foods showed a significant lipid oxidation (peroxide and interaction compound formation) development with storage time, which was especially marked in the case of the TL product; additionally, TL seafood underwent an important lipid hydrolysis (FFA formation) development. However, both RTE products showed a low microbial development, no matter how far the expiration dates (23 and 31 days for TL and HR products, respectively) were exceeded; thus, low TVC scores were obtained while the presence of pathogen microorganisms was not detected throughout the whole storage period.

Previous research related to quality control in RTE seafoods has especially focused on the microbial development and the assessment of pathogen bacteria presence in order to guarantee consumer safety. On the contrary, research related to the chemical changes produced during storage has been scarce, in spite of the fact that a high content of polyunsaturated fatty acids is well known to be present in such kinds of species. According to the present results, and in view of the current increasing consumer demand for high quality refrigerated foods, the assessment of lipid damage indices related to nutritional and sensory values is recommended in order to produce RTE products including not only safe properties, but also healthy and tasty values in fast meal solutions where marine species are found.

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