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Influence of different preservation treatments on the volatile fraction of desalted cod

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Abstract

In the present study, desalted cod underwent a water blanching treatment and the incorporation of additives (citric acid and potassium sorbate), combined with different types of packaging (air, vacuum and modified atmosphere), to achieve an improvement of the shelf life of ready-to-use desalted cod. The purpose of this work was to evaluate the effect of these combined methods of preservation on the volatile fraction of desalted cod during 42 days in cold storage. The volatile compounds identified in all samples were those mainly related to fresh odor in whitefish, although with some exceptions. Untreated desalted cod showed a high increase of 3-methyl-1-butanol (described as a microbial spoilage index) during the storage period; a higher increase in air packaging than in vacuum and modified atmosphere packaging. The slow increase of this compound, as well as the evolution of ketones and aldehydes observed for the rest of the samples submitted to treatment, demonstrated the effectiveness of the combined treatments applied. The most efficient treatment was the combination of additives together with modified atmosphere packaging.
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Keywords: Desalted cod; Preservation; Water blanching; Potassium sorbate; Citric acid; Air, vacuum and modified atmosphere packaging; Volatile compounds

1. Introduction

Salting is a traditional method to improve the shelf life of cod. The main producers of salted cod are Norway and Iceland and the traditional consumers are Spain, Portugal, and Latin America, with the United States rapidly becoming an increasingly growing market (Rodríguez, Ho, López-Caballero, Vaz-Pires, & Nunes, 2003; Thorarinsdottir, Arason, Bogason, & Kristbergsson, 2001; Vilhelmsson, Hafsteinsson, & Kristjánsson, 1996).

After the salting process is completed, the product is relatively shelf-stable and may be stored for months or even years under the right conditions, since the salt concentration in the salted cod reaches approximately 20% (w/w) (Bjørkevoll, Olsen, & Skjerdal, 2003; Pedro et al., 2002; Thorarinsdottir, Arason, Geirsdottir, Bogason, & Kristbergsson, 2002). Due to this high salt content, the fish has to be desalted for at least 24 h before consumption. Traditionally, the re-hydration process was home-made, which does not harmonize with the present "fast food" trends, in which time saving considerations are of the utmost importance. Accordingly, the industry has developed a product of ready-to-use desalted cod that is usually sold frozen and with a relatively long shelf life. However, the chilled product has a short shelf life due to the favorable conditions for bacterial growth in this product (Bjørkevoll et al., 2003; Fernández-Segovia, Garrigues, Carot, & Escriche, 2003; Skjerdal, Lorentzen, Joensen, & Akse, 1997), as well as to sensory spoilage (Akse & Joensen, 1996), which implies the development of undesirable aroma and flavor.

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Therefore, the use of some kind of preservation method becomes necessary to develop a product of desalted cod suitable to be commercialized refrigerated for up to one month.

In this sense, the application of mild thermal treatments has been studied and the results have shown that some of these treatments slow down the microbial growth (Fenández-Segovia et al., 2000; Fernández-Segovia, Guevara, Escriche, Díaz, & Serra, 2003), although causing an alteration of the original features of the recently desalted cod due to heat (Escriche, Fernández-Segovia, Serra, Andrés, & Barat, 2001; Fernández-Segovia, Camacho, Martínez-Navarrete, Escriche, & Chiralt, 2003).

On the other hand, the use of additives could be interesting for the preservation of this product. Several studies report the use of oxygenated water on desalted cod (Gimeno, Rodríguez-Barona, Barat, & Andrés, 2001; Martínez-Álvarez, 2002) obtaining an increase of the product's shelf life; however and depending on the application conditions, some disadvantages such as abnormal coloring of the skin – turning from grayish to brown – or unpleasant changes in the appearance and texture of muscle were observed. These problems and the lack of legislation concerning oxygenated water make it a hardly advisable alternative. The incorporation of additives authorized by most food regulations, such as potassium sorbate, sorbic acid, citric acid, etc., has been studied for the preservation of cod (Ampola & Keller, 1985; Licciardello, Ravesi, & Entremont, 1986; Osthold & Leinstner, 1983; Shaw, Bligh, & Woyewoda, 1983) and other types of fish (Ravindranathan, George-Joseph, Unnikrishnan, & Mathen, 1990; Dorsa, Marshall, & Semien, 1993; Chinnamma & Perigreen, 1999; Hassan, Khallaf, Abd-El-Fattah, & Yasin, 1999; Hattula, 1999; Gelman, Glatman, Drabkin, & Harpaz, 2001; Shalini, Jasmine, Shanmugam, & Ramkumar, 2001). A previous study of the effect of different concentrations of citric acid and potassium sorbate on the microbial growth of chilled desalted cod (Fernández-Segovia, Garrigues, et al., 2003) showed that these additives could be a suitable choice for the preservation of this product from a microbiological point of view.

The basis of hurdle technology (Leistner & Gorris, 1995) is the combination of traditional and innovative preservation techniques in small doses with the aim of establishing a series of factors that interact cumulatively or sinergically to control the microbial population in food. In this way, the same inhibitory effect is reached with a lower intensity treatment, inhibiting or delaying the multiplication of the microorganisms surviving to the treatment. This fact contributes to a better preservation of the sensory properties after the treatment. The factors that are frequently combined are: heat, pH and a_w reduction, chemical additives, vacuum or modified atmosphere packaging, electric pulses, high pressures, etc.

The effectiveness of the different preservation methods can be studied through different parameters such as microbial growth, sensory analysis, changes in lipids or in proteins, volatile compounds, etc. Aroma is one of the most important parameters in the evaluation of fish freshness, since the spoilage of fish during storage and the loss of quality imply the development of an unpleasant odor and aroma. Therefore, volatile compounds contributing to this odor changes can be measured to evaluate the freshness and spoilage of fish (Ólafsdóttir & Fleurence, 1997).

The aim of this study was to evaluate the effect of different combined methods of preservation: water blanching and the incorporation of additives (citric acid and potassium sorbate), combined with different kinds of packaging (air, vacuum and modified atmosphere), on the volatile fraction of desalted cod during 42 days in cold storage.

2. Materials and methods

2.1. Sample preparation

The raw material used was entire pieces of Spanish salted cod (*Gadus morhua*) supplied by a local factory (Valencia, Spain). All tests were performed with cod from a single batch and with a similar size.

A rectangle sized piece was obtained from the central part of each cod by removing the fish tail and the sides of the main piece. This rectangle was cut into portions of 6.5×2.5 cm, these being randomly divided into three batches (C, control samples; B, blanching; A, additives) that were desalted and treated differently (Table 1). The desalting process in the three cases was carried out for 48 h by submerging the samples into distilled water (weight:volume ratio 1:6) at 4 °C and water changes were carried out at 2, 7, 24 and 36 h. Water in the third and fourth water change had 7% (w/v) of NaCl to assure that all portions of the desalted samples had a previously established salt concentration.

Samples in batch C were desalted without additives and the portions of desalted cod in this group were used as control samples.

After performing the desalting process as previously described, portions in batch B were submitted to a blanching treatment by introducing the samples into boiling water for 1 min. The thermal treatment conditions were chosen based on previous studies (Fenández-Segovia et al., 2000).

In the desalting process of samples in batch A, NaCl (7% w/v, in solution) and citric acid (CA) (0.2% w/v, in solution) were incorporated in the third water change, and NaCl (7% w/v, in solution) and potassium sorbate (KS) (0.45% w/v, in solution) in the fourth (Table 1). CA, KS and NaCl concentrations were chosen based

Table 1
Experimental design of the treatments carried out with the raw material

Treatment	Desalting process						Kind of packaging
	2 h	7 h	24 h	36 h	48 h		
Control (C)	Water change	Water change	Water change + NaCl (7%)	Water change + NaCl (7%)	End of process		Air Vacuum MAP
Blanching (B)	Water change	Water change	Water change + NaCl (7%)	Water change + NaCl (7%)	End of process	Water blanching (1 min)	Air Vacuum MAP
Additives (A)	Water change	Water change	Water change + NaCl (7%) + CA (0.2%)	Water change + NaCl (7%) + KS (0.45%)	End of process		Air Vacuum MAP

CA, citric acid; KS, potassium sorbate; MAP, modified atmosphere. Concentrations of NaCl, CA and KS are expressed in % (w/v).

on previous experiments (Fernández-Segovia, Garrigues, et al., 2003).

Immediately after desalting, samples in the three batches were each randomly divided into three new groups that were packaged in plastic bags, one group with air, another one in vacuum and the remaining one in a modified atmosphere (60% CO_2, 30% N_2, 10% O_2), and they were stored for 42 days at 4 °C (Table 1).

2.2. Extraction of volatile compounds

Volatile components analyses were carried out in all samples immediately after desalting, as well as during the 42 days of storage, at 7-day intervals. The combined simultaneous distillation–extraction (SDE) technique was used to isolate the volatile compounds (Godefroot, Sandra, & Verzele, 1981) in a J&W Simultaneous Steam Distillation–Extraction Apparatus obtained from Fisher Scientific UK Ltd. (Loughborough, Leics., England) and according to the methodology described in previous papers (Escriche, Chiralt, Moreno, & Serra, 2000; Talens, Escriche, Martínez-Navarrete, & Chiralt, 2003). In each analysis, 45 g of sample homogenized in an Ultra-Turrax T 25 model and 225 mL of bi-distilled water were put into a 500 mL round-bottom flask, and 50 μL of 2,4,6-trimethylpyridine (internal standard) aqueous solution at 150 μg/mL were added. The flask was held in an ultrasonic bath for 2 min to totally disintegrate the sample and it was then introduced into the oil bath of the extraction equipment and heated to boiling point. A 50 mL heart flask containing 3 mL of pentane was introduced into a water bath at 40 °C. The steam of both flasks was condensed in the common refrigerated "U-tube" of the equipment. After 1 h distillation, the content of the U-tube was collected in an airtight sealed tube and frozen at −18 °C to facilitate the separation of the organic phase (which is liquid and has lower density at −18 °C), where volatile compounds were dissolved. This phase was concentrated under nitrogen stream up to a final volume of approximately 50 μL.

2.3. GC–MS analysis

The analysis was conducted on a gas chromatograph/mass spectrometer (GC–MS) Finnigan TRACE MS (TermoQuest, Austin, USA). Eight μL of each extract were injected in split mode (split ratio 1:10) into a DB-WAX fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness; J&W Scientific INC., Folsom, CA, USA). Helium gas (ultrahigh purity grade, 99.999%) was used as the carrier gas at a constant flow rate of 0.8 mL/min. Injector temperature was 250 °C; oven temperature was programmed from 50 to 230 °C at a ramp rate of 10 °C/min; being the initial and final hold times 5 and 25 min, respectively. The MS interface temperature was set at 250 °C, the ion source temperature was set at 200 °C, and the ionization voltage was 70 eV; the m/z range was 35–450 and the scan rate was 2.5 scans/s.

2.4. Identification and quantification of compounds

Positive identifications were based on comparison of retention indices (RI) (van den Dool & Kratz, 1963) and mass spectra of unknowns with authentic compounds (Acros Organics, Geel, Belgium). Tentative identifications were based on comparison of RI and mass spectra of unknowns with those in the literature (Cha & Cadwallader, 1995; Chung & Cadwallader, 1993; Kondjoyan & Berdagué, 1996) and NIST mass spectral library, respectively.

Positively identified compounds were quantified using calibration curves of peak area ratios (compound/internal standard) vs. concentration ratios (compound/internal standard) under identical chromatographic conditions. A total of 3 extracts were obtained for each sample.

2.5. Statistical analysis

Statistical treatment of the data was performed using the Statgraphics Plus version 4.0 (Manugistics, 1999).

The data of each variable (volatile compounds concentration expressed as ng/g) were analyzed with a multifactor analysis of variance (ANOVA), considering the interactions amongst factors. The treatment, packaging procedure and time of storage were the factors for this analysis. The method used for multiple comparisons was the LSD test (least significant difference) with a significance level of $\alpha = 0.05$. A stepwise discriminant analysis was also performed using Wilks' lambda as the statistical selection criterion for the variables.

3. Results and discussion

3.1. Volatile fraction composition of desalted cod

The identification of the compounds in the volatile fraction was carried out in recently desalted cod (control), as well as in treated cod immediately after treatments (blanching and additives). Thirty-eight volatile compounds were identified: 13 alcohols, 5 ketones, 12 aldehydes, 1 aromatic compound, 1 sulphur-containing compound, 2 furans, and 4 halogen-containing compounds (Table 2). Among these thirty-eight compounds identified, eighteen were quantified, due to the fact that several authors have considered these components to be very important in the spoilage of fish (Josephson, Lindsay, & Ólafsdóttir, 1986; Ólafsdóttir & Fleurence, 1997).

According to several studies, whitefish volatile compounds can be related to quality depending on their structural characteristics. Long chain alcohols and carbonyls (C6–C9) are indices of freshness; on the other hand, short chain alcohols, sulphur compounds, amines or aromatics, are suggested as indicators of spoilage (Josephson et al., 1986). Taking this classification into account, the volatile compounds identified in this study are mainly compounds related to fresh odor (1-octen-3-ol, 3,6-nonadienol, hexanal, (*E*)-2-hexenal, (*E*,*Z*)-2,6-nonadienal, etc.) with some exceptions such as 3-methyl-1-butanol, 3-methylbutanal or dimethyltrisulfide that have been associated to microbial spoilage odor (Ólafsdóttir & Fleurence, 1997).

Table 2 illustrates the concentrations of the 18 quantified compounds in the samples submitted to the different treatments (control, blanching and additives) before packaging (time 0). The content of all compounds was not significantly different amongst the samples, which implies that neither the water blanching nor the incorporation of additives affected the quantified compounds of the volatile fraction in recently desalted cod.

3.2. Evolution of volatile compounds during cold storage

To study the influence of the three factors on the volatile fraction of cold desalted cod, a multifactor analysis of variance (ANOVA) was carried out taking into consideration the type of treatment, packaging and storage time applied in each case, as well as the interactions among these factors. Table 3 shows the F-ratio obtained in this analysis for the 18 volatile compounds quantified. The F-ratio represents the quotient between variability due to the effect considered and the residual variance. The F-ratio values are also comparable in each column, because the number of observations was the same in all cases. A higher value of F-ratio means a more marked effect of that factor on a variable. According to this, variables in general were most affected by the treatment applied, followed by storage time and packaging. The interactions amongst the three factors took place in most of the cases, which indicates that the evolution of the volatile compounds throughout time was different depending on the treatment–packaging combination.

Comparing the F-ratio in each column, it can be deduced that the effect of all factors and their interactions was much more important for 3-methyl-1-butanol. As above mentioned, this compound has been associated to microbial spoilage odor (Ólafsdóttir & Fleurence, 1997). For this reason, the evolution of this compound has been chosen amongst the 18 volatile compounds to be represented in the different samples packaged in air, vacuum and modified atmosphere, during 42 days of storage (Fig. 1).

The rest of the data obtained in this study, corresponding to the whole storage period for all the samples (control, blanched and with additives, packaged with air, in vacuum and in modified atmosphere) have not been shown because of their quantity, but they are discussed grouped by chemical class.

3.2.1. Alcohols

The alcohols quantified in this study were: 3-methyl-1-butanol, 1-penten-3-ol and 1-octen-3-ol.

With regards to 3-methyl-1-butanol (Fig. 1), an important increase in the concentration of this compound can be observed in the control samples, where the initial level was 9.47 ng/g and at the end of the storage period it reached values of 7663.92 ng/g for samples packaged with air, 2000 ng/g for samples packaged in vacuum and 4213.98 ng/g for samples packaged in modified atmosphere. In the samples submitted to blanching treatment and in those with additives, this increase was much lower, both having a similar evolution, although concentrations in samples with additives were slightly lower during the 42 days of storage (Fig. 1 with amplified scale). This short chain alcohol has been described by several authors (Ahamed & Matches, 1983; Lindsay, Josephson, & Ólafsdóttir, 1986; Josephson, Lindsay, & Stuiber, 1987; Ólafsdóttir & Fleurence, 1997; Jensen, Refsbaard, & Ólafsdóttir, 1997) as a microbial spoilage index, reaching high levels during the storage, in the

Table 2
Volatile compounds identified in the different samples of desalted cod, as well as those quantified in the samples of recently desalted cod (control, blanching and additives at day 0 of storage)

Compound name by class	RI	Methods of detection	Mean[a] (ng/g)			ANOVA
			Control	Blanching	Additives	
Alcohols						
1-Penten-3-ol	1158	RI, MS	81 (±44) a	66 (±18) a	47 (±27) a	ns
3-Methyl-1-butanol	1202	RI, MS	9 (±13) a	14 (±20) a	18 (±2) a	ns
1-Pentanol*	1241	RI, MS				
Cyclopentanol*	1300	RI, MS				
(*Z*)-2-penten-1-ol*	1304	RI, MS				
1-Hexanol*	1345	RI, MS				
1-Octen-3-ol	1442	RI, MS	50 (±9) ab	95 (±2) a	42 (±25) b	ns
1-Heptanol*	1447	RI, MS				
2-Ethyl-1-hexanol*	1480	RI, MS				
(*E*)-3-hepten-1-ol*	–	MS				
1-Octanol*	1558	RI, MS				
3,6-Nonadienol*	–	MS				
2-Octen-1-ol*	–	MS				
Ketones						
3-Octanone	1250	RI, MS	40 (±13) a	55 (±7) a	33 (±28) a	ns
(*Z*)-6-octen-2-one*	–	MS				
2-Nonanone	1387	RI, MS	14 (±20) a	47 (±28) a	15 (±11) a	ns
(*E*,*E*)-3,5-octadien-2-one*	1570	RI, MS				
2-Undecanone	1599	RI, MS	6 (±2) a	21 (±2) b	9 (±7) ab	ns
Aldehydes						
3-Methylbutanal	916	RI, MS	51 (±6) a	96 (±28) a	23 (±32) a	ns
Hexanal	1080	RI, MS	155 (±53) a	143 (±29) a	65 (±50) a	ns
(*E*)-2-pentenal*	1128	RI, MS				
Heptanal	1182	RI, MS	105 (±16) a	35 (±10) a	38 (±42) a	ns
(*E*)-2-hexenal	1215	RI, MS	160 (±9) a	124 (±25) a	105 (±83) a	ns
(*Z*)-4-heptenal	1236	RI, MS	172 (±31) a	220 (±32) a	136 (±100) a	ns
Octanal	1286	RI, MS	49 (±8) a	51 (±4) a	23 (±19) a	ns
Nonanal	1392	RI, MS	13 (±18) a	22 (±32) a	17 (±11) a	ns
(*E*,*E*)-2,4-heptadienal	1493	RI, MS	53 (±16) a	64 (±3) a	55 (±47) a	ns
Benzaldehyde	1521	RI, MS	27 (±33) a	23 (±23) a	34 (±21) a	ns
(*E*,*Z*)-2,6-nonadienal	1590	RI, MS	23 (±1) a	51 (±8) a	41 (±35) a	ns
4-Ethylbenzaldehyde	1730	RI, MS	20 (±8) a	19 (±16) a	19 (±13) a	ns
Aromatics						
Toluene	1036	RI, MS	49 (±10) a	21 (±30) a	54 (±31) a	ns
S-containing compounds						
Dimethyltrisulfide*	1378	RI, MS				
Furans						
2-Ethylfuran*	955	RI, MS				
2-Pentylfuran*	1229	RI, MS				
Halogen-containing compounds						
Chloroform*	1021	RI, MS				
Bromodichloromethane*	–	MS				
Dibromochloromethane*	–	MS				
Tribromomethane*	–	MS				

[a] Mean concentration from three SDE extractions. Numbers in parentheses represent standard deviation. Means followed by the same letter within the same row are not significantly different. ns, Non significant.
* Compound tentatively identified.

region of μg/g when the fish is spoiled. This fact would confirm the effectiveness of the two preservation treatments applied in this study.

Concerning the kind of packaging, the higher concentrations of this compound corresponded to the samples packaged with air for the three treatments (control, blanching or additives), as it has been reported in several studies carried out in whitefish (Lindsay et al., 1986; Josephson et al., 1987). These differences amongst the three types of packaging were significantly higher in the control samples (ANOVA data not shown), which indicates that there is a

combined effect of the two preservation treatments (blanching and additives) with the oxygen reduction in the vacuum and MAP packaging.

The other two alcohols (1-penten-3-ol and 1-octen-3-ol) relating to fresh fish odors (Josephson, Lindsay, & Stuiber, 1984; Josephson et al., 1986; Hirano,

Table 3
ANOVA F-ratio for each of the 3 factors and their respective interactions in the 18 variables observed (volatile compounds)

	F-ratio						
	P	*T*	*t*	*P* × *T*	*P* × *t*	*T* × *t*	*P* × *T* × *t*
3-Methyl-1-butanol	43.45***	116.29***	38.69***	36.45***	13.08***	34.38***	10.67***
1-Penten-3-ol	0.46ns	6.02**	9.76***	0.40ns	2.38*	4.55***	1.46ns
1-Octen-3-ol	8.33***	20.08***	13.03***	4.04**	3.37***	6.76***	2.76***
3-Octanone	3.16*	41.36***	9.58***	3.42*	2.58**	8.22***	5.70***
2-Nonanone	0.29ns	6.85**	4.37**	0.73ns	0.64ns	3.15**	0.84ns
2-Undecanone	3.51*	21.32***	4.58***	6.03***	4.62***	7.25***	4.35***
3-Methylbutanal	16.34***	14.87***	20.25***	13.98***	7.16***	6.91***	5.73***
Hexanal	14.80***	8.63***	23.11***	3.62*	2.20*	8.87***	2.26**
(*E*)-2-hexenal	10.72***	20.78***	12.91***	1.97ns	2.03*	4.94***	2.25**
Heptanal	8.26***	4.69*	3.11**	3.29*	3.24**	6.36***	3.20***
(*Z*)-4-heptenal	5.68**	20.78***	9.58***	1.29ns	3.42***	4.06***	5.62***
(*E*,*E*)-2,4-heptadienal	1.46ns	14.14***	6.65***	2.83*	3.74***	1.71ns	3.25***
Octanal	2.60ns	18.75***	18.62***	7.15***	4.43***	9.41***	6.90***
Nonanal	0.26ns	5.23**	2.36*	1.10ns	1.70ns	3.06**	2.37**
(*E*,*Z*)-2,6-nonadienal	8.03***	59.88***	5.81***	2.33ns	2.74**	5.03***	3.21***
Benzaldehyde	4.59*	21.77***	9.60***	0.78ns	2.58**	1.50ns	2.91***
4-Ethylbenzaldehyde	1.39ns	1.27ns	1.90ns	3.20*	1.53ns	1.78ns	1.67ns
Toluene	0.56ns	1.92ns	18.30***	2.26ns	1.29ns	2.21*	2.11**

P, packaging; *T*, treatment; *t*, time of storage.
ns, Non significant.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

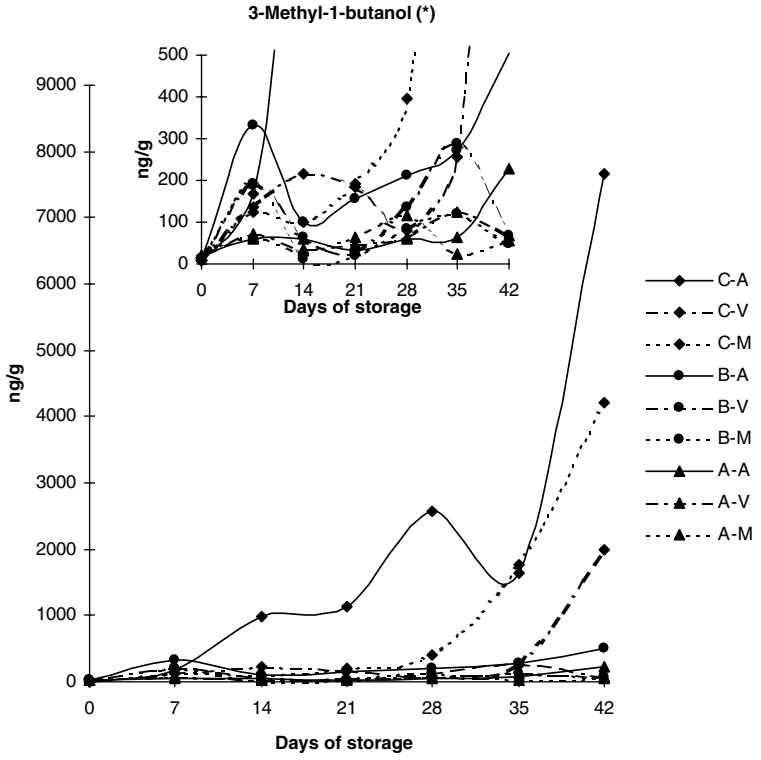

Fig. 1. Evolution of 3-methyl-1-butanol in the different treatments and types of packaging during 42 days of storage. C-A, control-air; C-V, control-vacuum; C-M, control-modified atmosphere; B-A, blanching-air; B-V, blanching-vacuum; B-M, blanching-modified atmosphere; A-A, additives-air; A-V, additives-vacuum; A-M, additives-modified atmosphere. *Figure with amplified scale in the *y*-axis.

Zhang, Morishita, Suzuki, & Shirai, 1992) were found in concentrations between 60 and 120 ng/g, and in general, no significant differences were found amongst treatments, types of packaging or storage times.

3.2.2. Ketones

The ketones quantified were: 3-octanone, 2-nonanone and 2-undecanone. The highest concentrations of these compounds were found in the additive treatment group mainly at the end of the storage time, although in general there were few significant differences amongst treatments. Concerning the type of packaging, there were only significant differences at the end of the period studied, with the samples packaged in modified atmosphere showing the highest concentrations of these compounds and with no significant differences found between air and vacuum packaging. These results could indicate that the additive treatment and modified atmosphere packaging were more effective in the preservation of the original organoleptic characteristics of the product, since these ketones have been described as components of the aroma of fresh fish (Josephson et al., 1984, 1986; Hirano et al., 1992).

3.2.3. Aldehydes

The following were quantified: 3-methylbutanal, hexanal, (*E*)-2-hexenal, heptanal, (*Z*)-4-heptenal, (*E*,*E*)-2,4-heptadienal, octanal, nonanal, (*E*,*Z*)-2,6-nonadienal, benzaldehyde, 4-ethylbenzaldehyde. It was observed that the majority of these aldehydes showed high concentrations in vacuum packaging and even higher in modified atmosphere when compared to air. With respect to the differences amongst treatments, the concentrations of aldehydes were slightly higher in the blanching and additive treatment samples than in the control samples.

With regard to the evolution through the storage time, a decrease in the concentration of hexanal, (*E*)-2-hexenal, heptanal, octanal, and 4-ethylbenzaldehyde was observed in the control and blanched samples during the 42 days of storage. However, in the additives treatment samples, the concentration of these compounds was almost constant, with no significant changes during the storage period.

These results would confirm again the higher effectiveness of the additives treatment and the vacuum and modified atmosphere packaging, because these aldehydes have also been described as components of the aroma of fresh fish (Josephson et al., 1984, 1986; Hirano et al., 1992).

3.2.4. Aromatics

Toluene was the only aromatic compound quantified (besides benzaldehyde and 4-ethylbenzaldehyde which have been analyzed within the aldehydes chemical class), and no significant differences were observed amongst treatments, packaging or storage times.

3.3. Discriminant analysis

Due to the difficulty in evaluating the behavior of the volatile fraction considering each compound individually, the global effect of the type of treatment, packaging and storage time was analyzed throughout three discriminant analyses (one for each factor: treatment, packaging and storage time), using the concentrations of the quantified compounds as variables.

In the discriminant analysis carried out with the factor treatment, two discriminant functions were obtained. Fig. 2 shows the distribution of the three treatments in the discriminant space. Function 1 determined the separation of samples with additives, while no differences were observed between the control and the blanching treatment. Taking into account that the spoilage in the control samples took place much faster than in the treated samples (blanching and additives), as shown in Fig. 1 and in other studies on the microbial quality of desalted cod (Fernández-Segovia, Escriche, Guillem, & Serra, 2004), the separation of the additives treatment samples in Fig. 2 would imply a higher effectiveness in the preservation of the volatile fraction of the desalted cod than in the blanching treatment. Table 4 lists the standardized discriminant function coefficients of the variables. The variables contributing most to the separation of the additive treatment according to F1 were 3-octanone and (*E*,*Z*)-2,6-nonadienal, whereas for F2, 1-octen-3-ol, *E*-2-hexenal and 3-methyl-1-butanol were most responsible.

Two discriminant functions were obtained in the discriminant analysis performed with the factor packaging. The distribution of the samples packaged with air in the discriminant space was very similar to the one of the samples packaged in vacuum (Fig. 3), the samples packaged in modified atmosphere

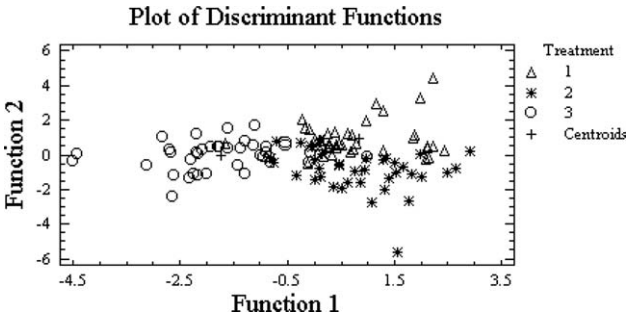

Fig. 2. Discriminant functions plot for the three treatments: control (1); blanching (2); additives (3).

Table 4
Standardized discriminant function coefficients of the variables using the factor treatment

Compound	Function (% variance)	
	F1 (70.58)	F2 (29.42)
3-Methyl-1-butanol	−0.176774	0.702239
1-Penten-3-ol	0.292903	−0.0696017
1-Octen-3-ol	−0.0606209	−0.885927
3-Octanone	−1.00047	0.290302
2-Nonanone	0.129739	−0.668483
2-Undecanone	0.244591	0.119098
3-Methylbutanal	0.309041	0.0986463
Hexanal	0.24735	0.537464
(*E*)-2-hexenal	−0.0489176	0.859688
Heptanal	−0.104887	−0.168193
(*Z*)-4-heptenal	0.441001	−0.41435
(*E*,*E*)-2,4-heptadienal	−0.0438783	0.363414
Octanal	0.706303	−0.3751
Nonanal	0.355883	−0.240577
(*E*,*Z*)-2,6-nonadienal	−0.865774	−0.0219565
Benzaldehyde	−0.569195	−0.446014
4-Ethylbenzaldehyde	0.255475	0.377755
Toluene	−0.161563	−0.46945

being slightly separated from the other two according to Function 1. The variables with more weight in this function were 1-octen-3-ol and 3-methylbutanal with positive coefficient, and (*E*,*E*)-2,4-heptadienal and 3-octanone with negative coefficient (Table 5).

In the discriminant analysis carried out with the factor time of storage, six discriminant functions were obtained, the two first functions (F1 and F2) explaining the 75.17% variance (F1 55.48% and F2 19.60%). Fig. 4 shows that Function 1 determined the separation of samples at day 0 from the samples at 28, 35 and 42 days of storage. Function 2 determined a slight separation of samples at 42 days of storage. The variables with more weight in Function 1 were benzaldehyde, (*Z*)-4-heptenal and hexanal, and in Function 2 they were (*Z*)-4-heptenal, 3-methyl-1-butanol and 2-undecanone (Table 6).

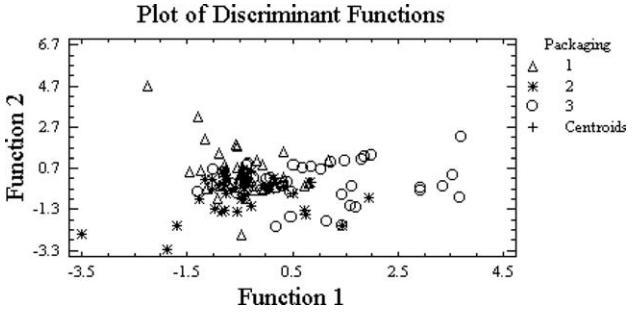

Fig. 3. Discriminant functions plot for the three kind of packaging: air (1); vacuum (2); modified atmosphere (3).

Table 5
Standardized discriminant function coefficients of the variables using the factor packaging

Compound	Function (% variance)	
	F1 (65.32)	F2 (34.68)
3-Methyl-1-butanol	−0.338337	0.498549
1-Penten-3-ol	−0.384788	0.152335
1-Octen-3-ol	1.09778	−0.319085
3-Octanone	−0.761423	−0.356779
2-Nonanone	−0.305685	0.172972
2-Undecanone	−0.0586707	0.142388
3-Methylbutanal	0.997668	−0.236942
Hexanal	−0.563568	−0.676973
(*E*)-2-hexenal	0.621564	−0.203584
Heptanal	−0.164271	−0.415509
(*Z*)-4-heptenal	−0.199262	0.598453
(*E*,*E*)-2,4-Heptadienal	−1.41098	−0.316795
Octanal	0.925661	1.09664
Nonanal	−0.381495	−0.38926
(*E*,*Z*)-2,6-nonadienal	0.526347	0.431364
Benzaldehyde	0.861183	0.238075
4-Ethylbenzaldehyde	−0.0940049	−0.589365
Toluene	0.394926	−0.0696013

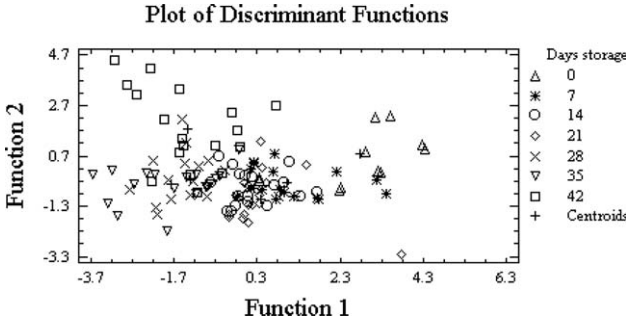

Fig. 4. Discriminant functions plot for the days of storage.

Table 6
Standardized discriminant function coefficients of the variables using the factor time of storage

Compound	Function (% variance)	
	F1 (55.48)	F2 (19.60)
3-Methyl-1-butanol	0.141949	0.828668
1-Penten-3-ol	−0.346978	−0.150964
1-Octen-3-ol	−0.353348	−0.127308
3-Octanone	0.316949	−0.101424
2-Nonanone	0.0631685	0.342398
2-Undecanone	0.131984	−0.667665
3-Methylbutanal	−0.363254	0.227972
Hexanal	0.669797	0.0328831
(*E*)-2-hexenal	0.21391	−0.336766
Heptanal	−0.0826601	−0.201508
(*Z*)-4-heptenal	0.944982	1.78711
(*E*,*E*)-2,4-heptadienal	0.252061	−0.579061
Octanal	0.100096	0.357045
Nonanal	−0.50257	−0.190758
(*E*,*Z*)-2,6-nonadienal	−0.290459	0.258248
Benzaldehyde	−1.25276	−0.25009
4-Ethylbenzaldehyde	0.0823615	−0.515936
Toluene	0.459125	−0.287356

4. Conclusions

The application of the blanching treatment or the incorporation of additives did not alter the volatile fraction composition of recently desalted cod. However, the evolution of the volatile compounds throughout 42 days of storage at 4 °C showed a different behavior depending on the kind of treatment applied as well as on the type of packaging. Untreated desalted cod exhibited a high increase of 3-methyl-1-butanol (compound described by several authors as a microbial spoilage index) during the storage period, higher in air than in vacuum and modified atmosphere packaging. The low increase of this compound in the desalted cod submitted to blanching treatment and to additive incorporation, as well as the results obtained in the study of ketones and aldehydes, demonstrated the effectiveness of these treatments combined with the vacuum and modified atmosphere. As a result of this, the best choice to develop a chilled product of ready-to-use desalted cod with a shelf life longer than 1 month is the additives treatment combined with modified atmosphere packaging. This conclusion is supported by a parallel microbiological study to this work, carried out in our laboratory.

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