Aroma profile of different salted dried codfishes

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ABSTRACT

Salted dried codfish is a highly consumed product in Mediterranean and South American countries. The main producers of this commodity are Russian federation, Norway and Iceland, sharing close to 90% of the total catch. One of the first sensorial features concerning perception of the quality of salted dried codfish is aroma and its volatile components. This emphasizes the importance of having qualitative and quantitative information on volatile compounds in order to monitor aroma quality and to relate it to sensorial perception.

The objective of this work was to determine the volatiles profile of salted and air dried codfish obtained from different fishing zones and from different salting and drying processes.

In this work, the volatile profile of different commercially available salted dried codfishes was characterized by HS-SPME/GC-IT-MS. Samples of three species caught in distinct origins (Gadus morhua, Gadus macrocephalus and Theragra chalcogramma) along with two curing processes (Traditional and Yellow curing) and different processing times, were studied.

Fifty-five compounds were identified, including several chemical classes (amines, hydrocarbons, esters, chlorinated compounds, aldehydes, aromatic, alcohols, acids, sulphur compounds, ethers, ketones and terpenes), with 26 of them being reported for the first time in codfish aroma. Principal Component Analysis showed major differences for the yellow cured sample and for T. chalcogramma species. Yellow cured codfish presented a higher content of compounds of almost all classes, possibly due to a higher microbial activity. T. chalcogramma exhibited a high content of terpenes and a low amount of aldehydes.

Differences among fish species and fishing zones were not perceivable, with the exception of T. chalcogramma that presented more terpenes and less aldehydes than the other samples. Microbial activity, known to be much higher in the yellow curing, appears to have a high contribution to the overall production of volatiles.

Keywords: Codfish; curing process; volatile compounds; HS-SPME/GC-IT-MS; PCA.

INTRODUCTION

Salted dried codfish is a highly consumed product in Mediterranean and South American countries. The main producers of this commodity are Russian federation, Norway and Iceland, sharing close to 90% of the total catch [1].

The most consumed codfish species is Gadus morhua, followed, by far, by Gadus macrocephalus and Gadus ogac. Other species are also commercialized as “similar (to codfish) species”, from which the most consumed, is Theragra chalcogramma (known as Alaska pollock) [1].

Several procedures are involved in the traditional manufacturing of this product, but the most common process in Portugal consists of (i) a one to two weeks period of salting in a pile of alternate layers of fish and salt, (ii) a resting period (curing) in piles from less than 1 to 6 months, and (iii) air drying until an overall water content of less than 47% and a salt content higher than 16% are achieved. The difference between yellow cure and traditional manufacturing is the partial desalting of the salt cured codfish before drying, which results in a dried codfish of lower salt content (between 12 and 16%) and less than 45% of water.

Aroma is the first characteristic that influences consumer perception of codfish quality at the selling point and latter at the consumption stage. This emphasizes the importance of having qualitative and quantitative information on volatile compounds, in order to monitor aroma quality and to relate it to sensorial perception.
All stages, from living organism to the catch of the fish and processing, can influence the development of volatiles. In fresh fish, mucus and secretions contain high concentrations of volatiles [2]. After catch, the fish is kept chilled until being salted or further stored in the frozen state before salting. During chilling, the formation of odours is attributed to the development of bacteria, and includes trimethylamine, sulphur compounds, aldehydes, ketones, esters, hypoxantine and other low molecular weight compounds [2, 3, 4, 5]. During chilling and freezing other mechanisms responsible for volatile formation, as the autoxidation of lipids and enzyme mediated conversion of lipids, nitrogen or sulphur containing compounds, have also been reported [2, 6]. Most of the traditional methods used for extraction and pre-concentration of volatile compounds are time consuming and require exhaustive concentration steps. Solid phase microextraction (SPME) is a solventless extraction method and does not induce modifications of the volatile compounds due to temperature or solvent effect. This type of extraction involves the adsorption of analytes onto a fused silica fibre coated with a suitable stationary phase and their subsequent desorption immediately before chromatographic analysis [7]. Head Space-SPME (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) is an important technique for the analysis of volatile compounds and has already been applied to several fish species [4, 8, 9, 10].

The aim of the present study was to evaluate the differences in volatile profile of salted dried codfishes species commercialized in Portugal, caught in different fishing zones, and cured by different processes and for distinct periods. For these purposes HS-SPME/GC IT-MS analysis was performed, data obtained was treated by using a principal component analysis (PCA).

MATERIALS & METHODS

Volatile profiles of three species (G. morhua, G. macrocephalus and T. chalcogramma) caught in distinct origins along with two curing processes (Traditional and Yellow curing) and different processing times, were characterized by HS-SPME/GC IT-MS.

The codfish samples were supplied by Lugrade (Coimbra, Portugal).

Each sample was magnetically stirred (200 rpm) at 40 °C, for 10 min. The fibre coated with divinylbenzene/polydimethylsiloxane (DVB/PDMS - 65 µm) was exposed to the headspace for 30 min, with agitation (200 rpm) at 40 °C. Afterwards the fibre was inserted into the injection port of the GC system for thermal desorption, for 1 min. The fibre was then conditioned in another GC injection port for 10 min at 250 °C.

GC IT-MS analysis was performed with a VARIAN CP-3800 gas chromatograph (USA) coupled to a VARIAN Saturn 4000 mass selective detector (USA) and a Saturn GC/MS workstation software version 6.8. A VF-5 ms 30 mx0.25 mmx0.25 µm (FactorFour) column from VARIAN was used in the analysis. The injector port was heated to 220 °C and injections were performed in splitless mode. The carrier gas was helium C-60 (Gasin, Portugal), at a constant flow of 1 mL/min. Oven temperature was set at 40 °C (for 1 min), then increasing 2 °C/min to 220 °C and held for 30 min. All mass spectra were acquired in the electron impact (EI) mode. The ion trap detector was set at 280, 50 and 180 °C, respectively. Covered mass ranged from 40 to 350 m/z, with a scan rate of 6 scan/s. The emission current was 50 µA, and the electron multiplier was set in relative mode to auto tune procedure. The maximum ionization time was 25,000 ms, with an ionization storage level of 35 m/z. The analysis was performed in FullScan mode. Compounds were identified by comparing their retention times with those of authentic compounds analyzed under the same conditions, and by comparison of the retention indices (as Kovats indices) with literature data. The comparison of MS fragmentation pattern with those of pure compounds and mass spectra database search was performed using the National Institute of Standards and Technology (NIST) MS 05 spectral database, considering fit and retrofit values higher than 70 %.

Principal component analysis (PCA) was carried out using XLSTAT 2010.3.01 software. The PCA method shows similarities between samples projected on a plane and makes it possible to identify which variables determine these similarities and in what way.

RESULTS & DISCUSSION

The analysis by HS SPME/GC-IT-MS of salted cured codfish samples allowed the characterization of fifty-five volatile compounds, which were distributed by distinct chemical classes: two amines, two
hydrocarbons, one ester, one chlorinated compound, ten aldehydes, eight aromatic hydrocarbons, sixteen alcohols, three acids, one sulphur compound, two ethers, three ketones, two terpenes and four other compounds.

Among the identified compounds, dimethylamine, trimethylamine, ethyl acetate, trichloromethane, 3-methyl-butanal, 2-methyl-butanal, hexanal, (Z)-4-heptenal, (E)-2-hexenal, heptanal, benzaldehyde, octanal, 4-ethylbenzaldehyde, toluene, 1-penten-3-ol, 3-methyl-1-butanol, 2-methyl-1-butanol, (Z)-2-penten-1-ol, 1-octen-3-ol, benzyl alcohol, 2-nonen-1-ol, 3-methyl-butanoic acid, dimethyl disulfide, 2-butoxy-ethanol, 3-octanone, 2-nonanone, 2-undecanone, limonene and butylated hydroxytoluene were already reported in codfish [4, 5, 6, 9, 11, 12]. The other twenty six identified compounds are reported for the first time in salted codfish.

The highest diversity of compounds was found in yellow cured codfish sample (MOIY) and traditional cured codfish sample from Canada zone (MOC), with fifty one compounds being determined. Yellow cured codfish (MOIY) showed the highest amount of volatiles, being richer in compounds from all chemical classes excepting amines, terpenes, sulphur compounds and acids. Amongst all, salted dried codfish from Russian/Norway fishing zone (MOR) exhibited the poorer volatiles profile, with thirty four compounds being characterized.

The major class of identified compounds in the analyzed samples was that of alcohols (ca. 29.1% of total identified compounds), followed by aldehydes (ca. 18.2%) and aromatic hydrocarbons (14.5%). Esters, chlorinated and sulphur compounds represented the minor components (<1.8% each).

Amines, namely dimethylamine (DMA) and trimethylamine (TMA), were detected in all of the studied samples. TMA, in particular, contributes to the characteristic “fishy” and ammonia-like off-flavours [11]. The production of TMA from trimethylamine oxide is normally associated to spoilage bacteria, like Shewanella putrefaciens [4, 5]. Unlike TMA, whose formation is related with microbial growth, the formation of DMA normally results from the action of endogenous tissue enzyme(s) on trimethylamine oxide, present in fish muscle.

3-Methylpentane and 3-methylhexane were identified for the first time in codfish samples. The first branched-chain alkene has already been accepted as a volatile of gilthead sea bream. Both hydrocarbons were mentioned as volatile organic constituents of other fish samples [13].

Ethyl acetate was the only ester indentified in this study. It was already found in codfish and in cold-smoked salmon it was associated with Lactobacillus spp. action. The production of this ester was also related with Pseudomonas spp. growth in haddock stored in ice [4, 5].

Trichloromethane was identified in all codfish samples, being important in the yellow cured (MOIY) one. This compound has been previously described in desalted and smoked codfish [9, 12]. Olafsdottir et al. (2005) proposed that trichloromethane found in chilled codfish fillets were originated from styrofoam boxes where fish were kept after being caught.

From the ten detected aldehydes, only (E)-2-octenal was associated to codfish aroma for the first time, although it appears related to other fish volatile profiles, like salmon, mackerel, saithe and redfish. This compound was identified in all studied samples. 3-Methylbutanal, hexanal and (Z)-4-heptenal have been reported as main aldehydes in codfish aroma [11]. 3-Methylbutanal was initially associated to microbial fish spoilage odour. According to several studies, lipid derived aldehydes, like hexanal, seemed to contribute to the fresh fish aroma, decreasing with storage period [5, 12]. However, this volatile is also considered to be a result of oxidation of fatty acids or peroxidation of linoleic acid, contributing to fish rancid off-flavors [4, 11]. Most of the remaining aldehydes detected in this study, namely (E)-2-hexenal, heptanal, benzaldehyde, octanal and 4-ethylbenzaldehyde, have already been described as components of fresh fish aroma and have been determined in desalted codfish [12]. Strecker degradation of amino acids has been proposed as a pathway for the formation of 2-methyl-n-butanal and benzaldehyde from isoleucine and phenylglycine. Benzaldehyde can also be derived from lipid oxidation [9, 11]. T. chalcogramma (TPC) aroma was the poorest one with respect to aldehydes.

Long in the past codfish was classified as “food containing benzene” according to the U.S. Environmental Protection Agency. Aromatic hydrocarbons like benzene and its derivatives were found in the studied
samples, being more represented in yellow cured codfish (MOIY). From the eight compounds found, toluene was the only one previously identified in codfish aroma experiments [5, 12].

Alcohols represented the largest category of identified compounds. Six of them, 1-penten-3-ol, 3-methyl-1-butanol, 2-methyl-1-butanol, (Z) 2-penten-1-ol, benzyl alcohol and (E) 2-decenol, were present in all samples and have been detected before in other codfishes [4, 5, 9, 11, 12]. 1-Penten-3-ol and 1-octen-3-ol are compounds related to fresh fish odour and emerge from lipid oxidation. Duftos et al. (2006) suggested that 1-octen-3-ol and other short-chain volatile carboxyls are derived from the oxidation of arachidonic acid by 12-lipoxygenase. Those two alcohols have also been regarded as key aromas of salted codfish, along with (Z) 4-heptenal, heptanal and some protein degradation resulting compounds, such as 3-methyl-butanal [11]. On the other hand, 3-methyl-butanol had been proposed as a microbial spoilage indicator and as a result of Pseudomonas sp. growth [4, 5, 12]. Recently, 3-methyl-butanol was reported to result from the oxidative deamination of the free amino acids precursors of leucine by the Ehrlich mechanism [4].

The acids found in salted codfish were 2-methyl-propanoic acid (isobutyric acid), 2-methyl-butanolic and 3-methyl-butanolic (isovaleric acid) acids. Isovaleric acid has been identified in most of the salted codfish samples [11]. Short chain acids, particularly isobutyric and isovaleric acids, have been implicated in the unpleasant odor occurring in fish storage and considered as indicative of later spoilage processes [4]. Moreover, volatile acids, like isobutyric acid, are key components of fish sauce aroma. They are thought to be originated from fish and salt bacterial fermentation, and resultant from lipid oxidation [4, 9].

Dimethyl disulfide was the only sulphur compound determined in all studied samples, except in short cure pacific codfish (MAP). This compound was already reported in codfish and is known to be an amino acid derived compound, resulting from the Strecker or thermal degradation of cysteine or methionine. [4, 5, 6, 11, 12].

Two ethers were identified in the analyzed samples: 2-butoxy-ethanol and 1-butoxy-2-propanol. The first was already found in smoked codfish [9] and the second was recently detected in the gilthead sea bream [13].

Ketones found included 3-octanone, 2-nonanone and 2-undecanone. These compounds were absent in dried salted codfish (MOR) sample. Yellow cured codfish (MOIY) sample was the one with highest total amount of this kind of compounds. The same three ketones were described before in desalted codfish [12]. 2-Nonanone and 2-undecanone were recognized as possible markers of spoilage [5].

Terpene compounds were noticed in all studied samples, with dried salted codfish (TCP) exhibiting the highest amount. Limonene was already found in other fish [5].

To assess the variation of volatile composition of the analyzed codfish samples, PCA was performed (Fig. 1).

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**Figure 1** - PCA of the volatile profiles of salted dried codfish samples: projection of volatile compounds into the plane composed by the principal axes F1 and F2 (73.80%).
The above figure shows the loading plots of the chemical groups and the codfish species in the principal axes F1 and F2 containing 73.80% of the total variance. Terpenes are projected into the negative F1 axis and most of the rest of the chemical classes are close to the positive extreme. In F2, chemical classes appear dispersed along this axis. Overlapping the plots, *T. chalcogramma* (TCP) appears located close to the terpenes group, due to its particular high terpene content. Yellow cured *G. morhua* (MOIY) is located in the extreme of F1 axis due to its high content in generally every volatile group, while *G. morhua* from Iceland zone (MOI) and Canadian zone (MOC), with traditional cure, appear on the opposite side due to its low volatile content. The difference between these samples is probably explained by the different microbial activity, much higher in the yellow cured fish, a process where fish results in a lower salt content than that of the traditional cure [14].

CONCLUSIONS

According to what we know, this is the first report comparing the volatile composition of different species of salted dried codfish, from distinct capture zones and different curing processes. Thirty compounds were fully characterized and 25 were tentatively identified, from which 26 are reported for the first time in dried salted codfish aroma, contributing to a greater knowledge of their volatile composition. Differences among fish species and fishing zones were not perceivable, with the exception of *T. chalcogramma* (TCP) that presented more terpenes and less aldehydes than the other samples. Microbial activity, know to be much higher in the yellow curing, appears to have a high contribution to the overall production of volatiles.

REFERENCES