

Flavor and Quality Characteristics of Salted and Desalted Cod (*Gadus morhua*) Produced by Different Salting Methods

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ABSTRACT: Flavor characterization and quality of salt-cured and desalted cod (*Gadus morhua*) products was studied using sensory analysis and gas chromatography techniques. The products were produced in Iceland using two different processing methods (filleting and splitting) and three different salting procedures, i.e., the old single-step kench salting or a multistep procedure, and presalting (injection and brine salting or only brine salting), which was followed by kench salting. The main difference observed was between fillets and split fish, where the split fish was darker and had stronger flavor characteristics. Comparison of different salting procedures showed that the use of presalting improved the appearance of the salted products, which can be described as increased lightness and reduced yellowness of the products. In the same products, the intensity of curing flavors was milder, as described by sensory analysis and key aroma compounds. Derivatives from lipid and protein degradation contribute to the characteristic flavor of the salted products.

KEYWORDS: Salted cod, sensory analysis, odorants, gas chromatography—mass spectrometry, gas chromatography—olfactometry

INTRODUCTION

Salted fish, especially cod (*Gadus morhua*), is a traditional product from Iceland and Norway. In Iceland, it is an important export product, often referred to with the Spanish name “bacalao”. The traditional markets for salted cod are the Mediterranean countries, mainly Spain, Portugal, Italy, and Greece, and Latin America. It is a favored product due to its special sensory properties that develop during the salting and storage of the fish. Processing methods have changed rapidly during the last decades, motivated by improved weight yields and appearance of the products. It has been shown that the sensory characteristics of salted cod are influenced by different factors, such as the rigor state of the cod,^{1,2} freshness of the raw material,^{2,3} composition of salt,⁴ and rehydration methods.^{5,6} However, information about the effects of recent developments in salting procedures is lacking.

Stacking or kench salting with alternating layers of piled split cod and dry salt was the original method used. Nowadays, in Iceland, a combination of different salting methods is used, varying between producers. The process involves a presalting step, with pickling, brining, or brine injection. The most common presalting method used is brine salting, but brine injection is increasingly used prior to brine salting. Increasing interest has been in the use of polyphosphates, which is believed to improve the quality and yield of the products. After presalting, the fish is dry (pile) salted for 10–14 days. Then it is packed and stored at refrigerated conditions, until exported. The salted product contains about 20% salt, and therefore, it is necessary to desalt the fish in water prior to consumption, usually for 1–5 days.⁷

During salting and storing, flavor and textural changes occur that are responsible for the ripening of salted cod, and these ripening characteristics remain during desalting and cooking. Few studies have been done on the sensory properties of desalted

cod, but sensory attributes of cooked, newly desalted cod have been described with sensory attributes such as sweet and butter odor, salt taste, and clammy and rubbery texture. With increasing storage time, it has been described by characteristic and boiled potato odors and juicy texture, and then by earthy, sour, and TMA flavors, sour and TMA odors, heterogeneous color, and soft and tender texture.⁸ Furthermore, studies have shown that cod freshness can influence the sensory quality of desalted fish.³

Various volatile compounds have been detected in ripened products such as dry cured ham as a consequence of protein and fat degradation. The formation of the flavor is due to a complex combination of enzymatic or chemical reactions such as lipid oxidation, Maillard reactions, and Strecker degradations.⁹ Similar processes have been reported in ripened seafood products such as ripened roe and ripened anchovy.^{10,11} Methional, 1-octen-3-ol, and 2,6-nonadienal were detected as the most important compounds contributing to ripened roe odor in sugar salted ripened roe products. Moreover, methional together with (*Z*)-1,5-octadien-3-one were identified as the most potent odorants in ripened anchovy.¹¹ Thirty-eight volatile compounds were identified in desalted cod, among them 13 alcohols, 5 ketones, and 12 aldehydes.¹² It is known that degradation plays an important role in the development of texture, aroma, and flavor of salt-cured fish, but information about the volatile flavor compounds in salt-cured cod is, however, lacking. Profiling of the volatile fraction in the muscle is an important factor for understanding the variation in sensory properties of the products due to process related parameters.

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Table 1. Experimental Design for Salting and Desalting of Fillets and Split Fish (S = Salt; P = Phosphate)

group	injected	brine salted (2 days)	dry salted (25–26 days)	stored (13 days)	desalted (4 days)
Inj S + P	x	x	x	x	x
brined		x	x	x	x
kench			x	x	x

The aim of the study presented herein was to identify process dependent alterations in the characteristics of salted cod. This was done by producing salted cod by the most used methods in the Icelandic salt cod industry during the last decades: injection and brining, with phosphate addition and brining; brining only; and the old single step kench salting method as the reference method. The flavor of fully salted and desalted cod (fillets and split fish) was characterized by sensory analysis and analysis of key volatile compounds using gas chromatography–mass spectrometry (GC-MS) and gas chromatography–olfactometry (GC-O). Microbial counts, chemical measurements, TBARS, and instrumental color were additionally performed to characterize the samples. In addition, the objective was to study the effect of different processing and salting methods on the characteristics of the salted and desalted cod.

MATERIALS AND METHODS

Chemicals. TMA, hexanal, butanone, 1-octen-3-ol, 3-methyl-butanol, nonanal, decanal, and C3–C10 ethyl esters were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO), 3-methyl-butanol, 1-penten-3-ol, and dimethyl disulfide from Merck-Schuchardt (Hohenbrunn, Germany), and 4-heptenal and 2,4-heptadienal from Acros Organics (Geel, Belgium). Chemicals used in microbial analysis: Bacto Peptone, agar, and yeast extract were from Difco (Becton, Dickinson and Company, Oxford, UK), Lab Lemco Powder from Oxoid Ltd. (Cambridge, UK), ferric citrate from Sigma, and sodium thiosulphate and NaCl from Merck Chemicals (Darmstadt Germany). Chemicals used in proximate analysis, TBARS, TVN, and TMA analysis, were purchased from Sigma-Aldrich and Merck.

Sample Preparation. Two experiments were done to study the flavor characterization of salted cod (*G. morhua*) in February and March, 2006. The cod was caught by long line from commercial fishing boats near the South West coast of Iceland. The fish was bled and gutted onboard and stored in tubs with ample ice for 4 days in experiment 1 (Exp1) and 5 days in experiment 2 (Exp2), until processed in a salt fish processing plant. In Exp1, the fish was filleted, but split fish was used in Exp2. The reason for the different storage times in Exp1 and Exp2 is that processing was carried out under real life conditions, and normal procedures of the processing plant where variation in supply of raw material influence the timing of processing.

Coarse salt of commercial grade (>99% NaCl) was used for presalting and for dry or kench salting. The phosphate blend used was Carnal 2110 (Budenheim, Germany), which was a combination of sodium and potassium pyrophosphates and sodium and potassium tripolyphosphates.

Salting and Desalting. In both experiments, three salting procedures (Table 1) were used to study process related alterations in characteristics of salted cod products during the last decades. The following salting procedures were used in each experiment: Inj, brine injected, brined, and dry salted; brined, brined and dry salted; and kench, only dry salted. For each procedure, 45–50 fillets (Exp1) or split fish (Exp2) were selected randomly.

Saturated brine (25% w/w NaCl, pH 6.3) was used for injection (FOMACO FMG 64/256F, FOMACO Food Machinery Company,

Koga, Denmark), and for the Inj S + P group, the brine was diluted with dissolved phosphate solution, resulting in 22.5% NaCl and 2.5% of phosphates (pH 6.7). The fish was injected to obtain about 12–15% weight gain of the original weight. All groups were dry salted, where fillets or split fish were piled into tubs with alternating layers of salt and the liquid formed allowed to drain away. One group was only dry salted to simulate the old kench salting method. After dry salting for 26 days, the fillets were packed into waxed cardboard boxes and kept at 0–2 °C for approximately 2 weeks, and then the fish was desalted. The fish was submerged in water with a fish-to-water ratio of 1:5 for 24 h, and the water was replaced with fresh water and then the fish rehydrated for an additional 72 h in a 1:5.5 ratio water bath. The temperature was 4 ± 1 °C.

Sampling. For each treatment, three fillets were collected after salting and three fillets after desalting. Two (approximately 5 cm) pieces were cut from the tail and loin parts and used for chemical analyses, after salting and desalting. Before analysis, the samples were skinned by hand and minced into pooled samples by using three individuals in each group and a Braun mixer (Type 4262; Braun, Kronberg, Germany).

Proximate Analysis and pH. Protein content (g/100 g) was determined by the Kjeldahl method¹³ and calculated using total nitrogen (N) · 6.25. Salt content (g/100 g) was determined using a potentiometric method.¹⁴ Water content (g/100 g) was calculated as the loss in weight during drying at 103 °C for 4 h.¹⁵ The analyses were accredited by Swedish Board for Accreditation and Conformity Assessment (SWEDAC). The measurement uncertainty was ±0.4%, ± 0.4%, and ±0.1% for water, protein, and salt content, respectively. The pH was measured by inserting a combination glass electrode (SE 104, Portamess 913 pH, Knick, Berlin, Germany) directly into the brine/cod mince.⁷ This method was a modification of the procedure by Kramer and Peters who measured pH by directly inserting a glass electrode into fillets.¹⁶

Thiobarbituric Acid Reactive Substances (TBARS). TVN and TMA. TBARS were determined as described by Dulavik, Sorensen, Barstad, Horvli, and Olsen¹⁷ and expressed as μmol/kg sample. Total volatile basic nitrogen (TVB-N) and trimethylamine (TMA) were determined by the methods described by Malle and Poumeyrol.¹⁸ The TVB-N analysis was performed by direct distillation into boric acid using a Kjeldahl-type distillatory (Struer TVN distillatory, STRUERS, Copenhagen, Denmark). The acid was titrated with diluted H₂SO₄ solution. To determine TMA, the same method as that for TVB-N was used, except that 20 mL of 35% formaldehyde was added to the distillation flask to block the primary and secondary amines. The TVB-N and TMA contents were expressed as mg N/100 g salted cod tissue.

Microbial Counts. Total viable psychotropic counts (TVC) and counts of H₂S-producing bacteria were evaluated on iron agar with 0.5% NaCl as described by Gram, Trolle, and Huss.¹⁹ Pour-plating was used and plates incubated at 22 °C for 3 days. TVC and counts on H₂S-producing bacteria were also done on iron agar with 10% NaCl and the plates incubated for 5 days. Bacteria forming black colonies on this medium produce H₂S from sodium thiosulfate and/or cysteine.

Color Measurements. The instrumental color of the muscle surface was determined by using a Minolta Chromameter, CR-200 (Minolta Camera Co.Ltd, Osaka, Japan). Prior to the measurements, excessive salt was carefully removed from the muscle surface. The detector was placed at the dorsal and the ventral side of the central line

Table 2. Evaluation of Salted Fish^a

parameter		description	points
color	lightness	light, fine	0
		light, but has a greyish appearance	1
		gray or red, a dark appearance	2
	yellowness	very dark or red	3
		has no yellow color	0
		tendencies to get yellow color	1
quality defects	discoloration (stains)	yellow main bottom color	2
		none	0
		small red/brownish or yellow stains	1
gaping	in loin part	large, dominating red or yellow stains	2
		none	0
		some in the surface	1
	in tail part	much, but not deep in the fillet	2
		much and deep in the fillet	3
		none	0
odor	curing odor	some	1
		much	2
		characteristic odor of salted fish	0
		a touch of a divergent odor	1
		little sour or divergent odor	2
		strong sour or divergent odor	3

^a Each parameter is given points from 0 to 3 (or 0 to 2) and at the end summed up to a total quality character of the product.

in the muscle surface of the fillet or split fish, and the $L^*a^*b^*$ modulus was recorded, obtaining a mean value for each individual ($N = 5$). The mean values were used to calculate the average and standard deviation presented in Figures 2 and 3.

Sensory Analysis of Raw, Salted Fillets. Sensory analysis of raw fillets was only done in Exp1. The sensory quality of 5 individual fish from each type of salting procedure was evaluated. The sensory attributes are described in Table 2. Each sensory parameter was scored on a scale of 0–2 or 0–3, using a slightly modified sensory scheme previously described by Joensen et al.²⁰ A higher score means that attributes are more intense. Higher scores for yellowness, discoloration, and gaping indicate the worse quality of the product. The importance of lightness and curing odor vary with markets. An experienced sensory panel of 8 persons carried out the tests.

Sensory Analysis of Desalted Cooked Fish. Quantitative descriptive analysis (QDA) was used to assess cooked samples of desalted cod.²¹ Eleven panelists participated in the sensory evaluation. They were all trained according to international standards,²² including the detection and recognition of tastes and odors, and were trained in the use of scales and in the development and use of descriptors. The members of the panel were familiar with the QDA method and experienced in the sensory analysis of cod. Three sessions were used for training prior to the sensory evaluation. The panel was trained in the recognition of sensory characteristics of the samples and in describing the intensity of each attribute for a given sample using an unstructured scale (from 0 to 100%). Most of the attributes were defined and described by the sensory panel during other projects.⁸ The following 27 attributes evaluated were related to appearance (color (light/dark), discoloration (homogeneous/heterogeneous), odor (curing, sweet, sea-like, butter, earth, dishcloth, sour, TMA, and sulfur), flavor (curing, salt, sweet, sea-like, butter, earth, sour, TMA, pungent, and frozen storage), and texture (flakiness, softness, juiciness, tenderness, rubbery, and foamy). Samples weighing approximately 40 g were taken from the loin part of the fillets and placed in aluminum boxes coded with 3-digit random numbers. The samples were cooked for 7 min in a prewarmed oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany) at 95–100 °C with air circulation

and steam, and then served to the panel. Each panelist evaluated duplicates of each sample in a random order, a maximum of four samples in each sensory session. A computerized system (FIZZ, Version 2.0, 1994–2000, Biosystèmes) was used for data recording.

Headspace Solid Phase Microextraction (HS-SPME). The SPME device and fiber (polydimethylsiloxane/divinylbenzene PDMS/DVB) were purchased from Supelco (Bellefonte, Pa, USA). Semipolar (65 μm) fiber was conditioned prior to use in the GC injection port as recommended by the manufacturer. A blank analysis was performed to verify that no extraneous compounds were desorbed from the fiber. A minced sample of salted and desalted cod was weighed (50 g) into a 100 mL vials Erlenmeyer flask. Heptanoic acid ethyl ester was added as an internal standard to all samples by adding 0.5 mL of 10-mg/kg aqueous solution of the standard. Samples were kept at 25 °C for about 15 min before sample collection; the SPME fiber was inserted through the septum placed on the cap of the Erlenmeyer flask and allowed to equilibrate with the headspace volatiles by stirring at 25 °C for 40 min. The fiber was then retracted into the barrel of the syringe and immediately inserted into the injector of the gas chromatograph (GC). Duplicate analyses of each sample were done.

GC-O Measurements. Volatile compounds entrapped on the SPME fibers were thermally desorbed for 2 min in the GC using splitless mode, with helium as the carrier gas at a linear velocity of 22.9 cm/s. Volatiles were separated on a DB 5 ms column (30 m \times 0.25 mm i.d. \times 0.25 μm , J&W Scientific, Folsom, Ca, USA). Measurements were performed on a GC (HP 5890, Hewlett-Packard, Palo Alto, CA). Helium was used as a carrier gas, and the following temperature program was used: 50 °C for 7 min, 50 to 120 °C at 5 °C/min, and from 120 to 220 °C at 10 °C/min. Injector temperature was 250 °C, and the detector temperature was 280 °C. The end of the column was split 1:1 between the flame ionization detector (FID) and an ODO-1 olfactory detector outlet (SGE International Pty. Ltd., Australia). Nitrogen, bubbled through water to add moisture, was used to drive the sample up to the sniffer. Two persons describing the odor sniffed the effluent. The intensity (quality and duration/retention times) of each odor was determined using an intensity from 0–5: 0, not present; 5, very strong. The assessors were trained in recognizing characteristic

Table 3. Proximate Analysis (g/100 g), pH, TBARS ($\mu\text{mol/kg}$), TVN (mg N/100 g), TMA (mg N/100 g), and Microbial Counts (TVC and H_2S -Producing Bacteria (log no./g; IA = Iron Agar) in Salted and Desalted Cod Fillets (Exp 1)^a

group	salted cod			desalted cod		
	Inj S + P	brined	kench	Inj S + P	brined	kench
water ^b	59.5 \pm 0.4	57.8 \pm 0.4	56.2 \pm 0.4	84.9 \pm 0.4	83.3 \pm 0.4	82.5 \pm 0.4
salt ^b	21.4 \pm 0.1	20.4 \pm 0.1	20.6 \pm 0.1	1.7 \pm 0.1	1.4 \pm 0.1	1.3 \pm 0.1
protein ^b	18.9 \pm 0.4	21.3 \pm 0.4	23.3 \pm 0.4	13.2 \pm 0.4	15.1 \pm 0.4	16.1 \pm 0.4
pH	6.1 \pm 0.1	6.3 \pm 0.1	6.1 \pm 0.1	6.2 \pm 0.1	6.4 \pm 0.1	6.3 \pm 0.1
TBARS	2.0 \pm 0.05	3.8 \pm 0.03	3.5 \pm 0.06	2.9 \pm 0.08	3.2 \pm 0.10	2.8 \pm 0.00
TVN	8.3 \pm 0.6	8.9 \pm 0.4	13.1 \pm 0.2	1.6 \pm 0.0	1.7 \pm 0.1	2.1 \pm 0.0
TMA	0.8 \pm 0.1	0.8 \pm 0.0	1.0 \pm 0.2	n.d.	n.d.	n.d.
TVC-0.5% IA	n.a.	n.a.	n.a.	6.48	6.04	6.11
H_2S - 0.5% IA	n.a.	n.a.	n.a.	<1	<1	1.48
TVC-10% IA	3.98	4.15	3.15	4.90	5.11	5.20
H_2S - 10% IA	<1	<1	<1	<1	<1	<1

^a Standard deviation shows measurement uncertainty. n.d. not detectable; n.a. not analyzed. ^b Results for water, salt, and protein content have been published³⁰

oxidatively derived odors by injecting into the GC-O mixtures of standard compounds dissolved in ether and sniffing the effluent. GC-O analysis was only done in Exp1.

GC-MS Measurements. The minced sample of salted and desalted cod was prepared in the same way as for the GC-O measurements except that the volatile compounds were collected for 40 min at 100 mL/min using a Gilian LFS-113D Air sampler on 250 mg of Tenax 60/80 (Alltech, IL, USA) in stainless steel tubes (Perkin-Elmer, Buckinghamshire, UK) for the combined ATD 400 and GC-MS measurements. Volatile compounds were thermally desorbed (ATD 400, Perkin-Elmer, Buckinghamshire, UK) from the Tenax tubes and separated with the same type of column and the same conditions as for the GC-O measurements. The mass detector ion range was 35–300 *m/z*. These measurements were done for the identification of the volatiles.

Identification and Quantification of the Volatile Compounds. Identification of the volatiles was done by matching retention indices (RI), calculated according to Van den Dool and Kratz,²³ on the basis of ethyl esters (i.e., RI of ethyl pentanoate is 500) and verified by the database Flavornet,²⁴ and the mass spectra of samples with authentic standards. Tentative identifications were based on the MS library data in the HP GCD ChemStation software (Hewlett-Packard Co, 1997). Semi-quantitative estimation of concentration of components was done by calculating the peak area ratio (PAR), i.e., the ratio between the total ion count of each peak and internal standard.

Data Handling. QDA data was corrected for level effects (level effects caused by level differences between assessors and replicates removed) by the method of Thybo and Martens.²⁵ Analysis of variance (ANOVA) was carried out on level corrected QDA data, data from sensory analyses of raw salted fish, and data from color measurements in the statistical program NCSS 2000 (NCSS, Utah, USA). Duncan's multiple-comparison test was used for stepwise comparison at the 95% significance level. Multivariate analysis was performed by the Unscrambler 9.7 software package (CAMO AS, Trondheim, Norway). Partial least-squares regression (PLSR) models were calculated with variables based on selected key volatiles obtained by quantification by GC-MS data as X predictors and sensory data as Y response factors. PLS was performed on the data using scores averaged over assessors and replicates, and the sensory data and values of replicates were standardized to equal variance (weighting with 1/standard deviation) for the GC-MS data. Full cross-validation (leave one out at a time) was used as a validation method.

RESULTS AND DISCUSSION

Proximate Analysis and pH. The chemical results together with the microbial results for both salted and desalted fish are shown in Tables 3 and 4 for fish fillets (Exp1) and split fish (Exp2), respectively. The water content in salted fish was 56.2–59.5 g/100 g and the salt content 19.5–21.4 g/100 g. The salt and water content was highest in brine injected fish. The initiation of salt uptake was distinct from other procedures. The injection resulted in a relatively even distribution of salt within the muscle regardless of thickness.²⁶ During brining, the initial mass transfer of salt and water was mainly driven by concentration gradients between the muscle and surrounding brine. At higher concentration (>10%) during dry salting, the muscle proteins aggregated, leading to the loss of water holding capacity and shrinkage of the muscle cells.^{27–29} At that stage, pressure gradients became the main force driving water out of the muscle, whereas concentration gradients were the determinants for the transfer of salt to and within the muscle.^{26,30}

After desalting, the water content was 82.5–84.9 g/100 g in fish fillets. The highest values were found in injected fillets. In split fish, however, the highest water content was found in brine salted cod. The desalting process was slower in split fish, resulting in higher salt content (4.6–6.2 g NaCl/100 g) in comparison to that of fillets (1.3–1.7 g NaCl/100 g). The slower rate of chemical fluxes in the muscle may be due to the longer distance for salt diffusion, i.e., the split fish had a higher thickness.

The pH decreased from 6.6 and 7.0 in fillets and split fish, respectively. During salting, the pH decreased to 6.1–6.3, due to protein aggregation and dehydration of the muscle. Similar values have been obtained in other studies of salted cod.^{2,4,31} In split fish (Exp2), the pH tended to increase to 6.4–6.5, whereas only minor changes were observed in fillets (pH 6.2–6.3). Salting procedures and phosphate addition did not influence the changes.

Thiobarbituric Acid Reactive Substances (TBARS). TBARS values are used as indicators of secondary and tertiary lipid oxidation processes in a food matrix. The TBARS in the raw fillets (before salting) was 2.8 $\mu\text{mol/kg}$. After brining, it was 0.9 $\mu\text{mol/kg}$ in fillets injected with salt and phosphate (Inj S + P) and 2.4 $\mu\text{mol/kg}$ in fillets that were only brine salted. The results presented in

Table 4. Proximate Analysis (g/100 g), pH, TBARS ($\mu\text{mol/kg}$), TVN (mg N/100 g), TMA (mg N/100 g), and Microbial Counts (TVC and H_2S -Producing Bacteria) (log no./g; IA = Iron Agar) in Salted and Desalted Split Cod (Exp 2)^a

	salted cod			desalted cod		
	Inj S + P	brined	kench	Inj S + P	brined	kench
water	59.0 \pm 0.4	57.5 \pm 0.4	56.2 \pm 0.4	76.9 \pm 0.4	78.6 \pm 0.4	76.5 \pm 0.4
salt	21.2 \pm 0.1	20.3 \pm 0.1	19.5 \pm 0.1	6.2 \pm 0.1	4.6 \pm 0.1	4.9 \pm 0.1
pH	6.1 \pm 0.1	6.1 \pm 0.1	6.1 \pm 0.1	6.5 \pm 0.1	6.4 \pm 0.1	6.5 \pm 0.1
TBARS	3.0 \pm 0.07	5.3 \pm 0.28	3.9 \pm 0.28	3.1 \pm 0.11	3.6 \pm 0.08	3.6 \pm 0.08
TVN	14.1 \pm 0.8	16 \pm 0.2	17.1 \pm 0.4	5.0 \pm 0.8	3.4 \pm 0.2	5.3 \pm 0.0
TMA	4.9 \pm 0.6	5.7 \pm 0.2	4.7 \pm 0.0	1.9 \pm 0.2	1.2 \pm 0.0	1.3 \pm 0.2
TVC-0.5% IA	3.86	3.66	3.75	4.92	6.00	4.85
H_2S - 0.5% IA	2.00	2.00	2.08	1.60	1.78	1.78
TVC-10% IA	3.41	2.88	3.11	3.91	4.99	3.65
H_2S - 10% IA	<1	<1	<1	<1	<1	<1

^a Standard deviation shows measurement uncertainty.

Tables 3 and 4 do not indicate a significant oxidation in the salted and desalted fish samples. However, the TBARS were probably not representing the oxidation in the muscle well enough. Results from the GC-analysis show increased levels of lipid degradation compounds as will be discussed later. Further reaction of secondary and tertiary oxidation products in the muscle may limit rises in TBARS values. In studies on salting and curing of ham, TBARS even dropped after 46–74 days of curing.³²

In previous model studies on salting of cod muscle cubes, the TBARS increased from 10 $\mu\text{mol/kg}$ to 25 $\mu\text{mol/kg}$ in dry salted products.³³ The initial values in the raw fish were higher than in our samples after salting. With regard to oxidation during salting, we would like to point out that the relative surface area of the cubes was higher in comparison to that of fillets or split fish, which was likely to facilitate the access of oxygen and increase the oxidation of the muscle.

In our study, slight influences of salting procedures were observed after dry salting. Values tended to be higher in brined and kench salted products, in comparison with the products injected with phosphate and salt. Results obtained in the same trial showed that TBARS were slightly higher for fillets that were only injected with salt (Inj S) compared to those that in phosphate added fillets (Inj S + P): 1.2 vs 0.9 $\mu\text{mol/kg}$ after brining; 3.3 vs 2.0 $\mu\text{mol/kg}$ after dry salting, respectively. However, after desalting, the retarding effects of phosphate were not observed. TBARS were 2.9 vs 1.9 $\mu\text{mol/kg}$ after desalting for (Inj S + P and Inj S, respectively (unpublished results).

Total Volatile Bases (TVN). Chemical determination of TVN is often used as a method for describing product freshness and as an indirect measure of the bacterial growth. Volatile bases such as TMA, trimethylamine oxide (TMAO), and dimethylamine (DMA) contribute to the amount of TVN. In raw material, the TVN values were 10.4 and 13.0 mg N/100 g, and TMA values were 0.6 and 1.9 mg N/100 g in Exp1 and Exp2, respectively. After salting, the TVN value tended to be higher in kench salted fillets than in presalted injected fillets (Tables 3 and 4). The presalting step facilitated stronger extraction of the TMA and other compounds contributing to TVN, such as derivatives from protein degradation.^{7,26} After desalting, the differences in TVN values between the salting procedures leveled off. During desalting, diffusion of the nitrogenous compounds increased due to concentration differences between the muscle and surrounding water. Additionally, the changes in salt

concentration affected the solubility of the nitrogenous compounds. Brining and desalting have shown to be the most effective processes in the extraction of nonprotein nitrogen.⁷

The TVN content was considerably higher in split fish (14.1–17.1 mg N/100 g) than in fillets (8.3–8.9 mg N/100 g). This may be explained by the surface area being proportionally larger and thereby favoring a more efficient solubilization of the muscle compounds from the fillets.

Microbial Analysis. Differences in the total viable count (TVC) on 10% iron agar (IA) were generally small with regard to different salting procedures and processing methods (fillets or split fish), both after salting and desalting (Tables 3 and 4). Slightly higher values were obtained after desalting since microbial growth increased with decreasing salt concentration and increasing water content in the muscle. The H_2S producing bacterial count indicated their sensitivity toward salt. These bacteria were not detectable on 10% iron agar. However, many halotolerant bacteria survive the salting process even though the bacterial composition changes because of their different tolerance toward the high salt content (>20 g NaCl/100 g) in salted cod. Microbial and enzymatic degradation during salting also changes the accessibility to nutritious compounds such as peptides and free amino acids and bacterial growth.³⁴

Sensory Analysis of Raw, Salted Fillets. Sensory analysis of the raw products showed that the curing odor was significantly more intense (higher score) in kench salted cod fillets but the least in the brine injected fillets (Figure 1). The TVN value was also highest in the kench salted product, suggesting that TMA and other volatile bases contributed to the characteristic flavor of salted cod. Correlation between curing odor and TBARS was not found. However, derivatives from lipid oxidation detected by GC-analysis were believed to contribute to the curing flavor of the products.

The color was significantly lighter and less yellow, and the fillets had less gaping in the NaCl and phosphate (Inj S + P) injected samples (Figure 1) compared to that in the other groups. Positive effects of brining on color were also observed in comparison to the kench salted fillets. The addition of polyphosphate is generally believed to brighten salted cod products, although it has not been the case in all studies.³⁵ However, only a tendency toward lighter color (0.31 vs 0.55) and lower yellowness (0.36 vs 0.61) was observed for the phosphate injected fillets in comparison to that in fillets injected with pure salt brine (Inj S) (unpublished data). The higher quality of Inj S + P compared to that of brining only and

kench salting was possibly due to the combined effects of the phosphate addition and injection of the fillets.

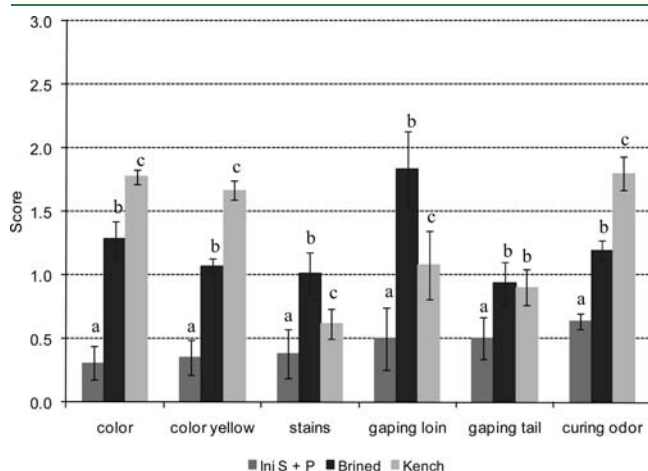


Figure 1. Sensory analysis of raw salted cod fillets (mean \pm std error) in Exp 1.

The appearance of the fillets, especially the lightness, has significant effects on commercial quality and prices of salted cod products.³⁶ Therefore, it can be assumed that the recent development in salting procedures toward a multistep procedure has increased the value of the products compared to the single-kench salting step.

Results of Sensory Analysis of Desalted Products. The sensory characteristics of desalted cod were generally described as curing, sweet, sea, butter, and earth odors and flavors, whereas the raw salted products were mainly described by the curing odor (Table 5). The split fish groups had generally a rather dark and discolored appearance, compared with that of the fillets. Curing odor was strong for all groups, and all groups had evident sweet, sea, butter, and earthy odors, though less evidently in the brine salted split fish group. A hint of dishcloth odor was detected in brine injected split fish (Inj S + P) and obvious dishcloth odor was found in brine salted split fish. Hints of sour and TMA odors were also found in brine salted split fish, but these characteristics were not detected in other groups. An increase in dishcloth and sour odors has mainly been related to spoilage in fresh cod.³⁷ Therefore, higher scores for these attributes may indicate that the

Table 5. Sensory Analysis of Desalted Cod Fillets and Split Cod^a

attribute	<i>p</i> -value	Inj S+P fillets	Inj S+P split	brined fillets	brined split	kench fillets	kench split
<i>appearance</i>							
dark color	0.000	19 c	41 ab	37 b	48 a	35 b	33 b
discolored	0.001	29 c	46 ab	40	50 a	35 bc	39
<i>odor</i>							
curing	0.988	44	47	45	48	44	49
sweet	0.034	29 a	24	29 a	19 b	26	28
sea	0.167	30	33	26	25	26	34
butter	0.054	29 a	24	29 a	18 b	25	26
earth	0.081	20	34	24	37	22	25
dishcloth	0.010	6 b	14 b	11	23 a	9	8 b
sour	0.016	1 b.	8	2	11 a	5	7
TMA	0.002	2 b	8	3 b	12 a	3 b	5 b
sulfur	0.001	0 b	3 b	2 b	8 a	0 b	1 b
<i>flavor</i>							
curing	0.005	46 b	52	58 a	50	56 a	51
salt	0.100	56	63	55	50	47	58
sweet	0.302	14	25	17	21	15	25
sea	0.135	30	40	37	32	31	36
butter	0.087	29	29	31	24	30	30
earth	0.016	10 b	23	20	31 a	24	20
sour	0.016	2 b	12	5	14 a	6	6
TMA	0.036	2 b	7	5	11 a	6	7
pungent	0.001	10 b	25 a	10 b	24 a	10 b	25 a
frozen	0.241	4	9	8	11	10	9
<i>texture</i>							
flakes	0.043	61	54	45 b	61 a	55	53
soft	0.199	55	49	56	51	52	45
juicy	0.115	55	50	54	50	52	49
tender	0.575	44	45	43	52	41	42
rubbery	0.202	35	33	30	20	27	27
foamy	0.077	23	26	27	23	34	21

^a Letters a–c are used to show significant differences. Samples with different letters within each line are different ($p < 0.05$).

decrease in water activity took a longer time in injected and brined fillets compared to that in kench salted fish, favoring the prolonged activity of spoilage organisms. In addition, injection alone spreads microbes through the muscle, whereas in fresh fish, these are only present on the surface of the fillets.

The curing flavor was strongest in brine salted and kench salted fillets, but less in brine injected split fish. Sweet, sea, and butter-like flavors were evident in all groups. Earthy flavor was evident in most groups, especially in brine salted split fish, but hardly detectable in brine injected fillets. A hint of sour flavor was detected in brine salted split fish, but TMA flavor was hardly detectable in any of the groups. Pungent flavor was evident in all split samples, but barely

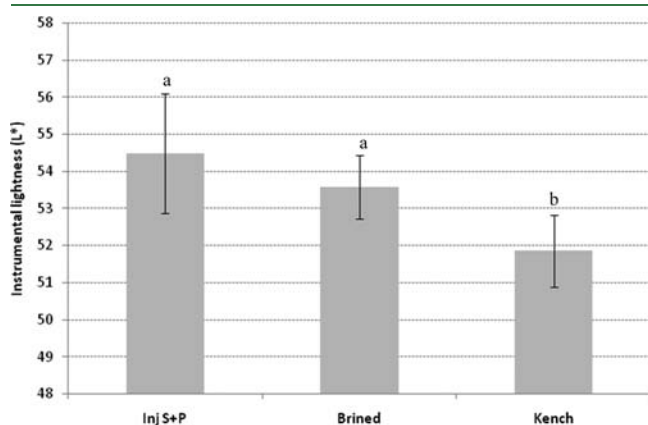


Figure 2. Mean instrumental lightness values and standard deviations of fully salted fillets ($N = 5$) in Exp1.

detected in fillets, as the low scores indicated. All groups had a flaky texture, and brine salted split fish was more flaky than brine salted fillets. The groups were all rather soft, juicy, and tender, with somewhat rubbery and foamy texture. The brine salted split fish differed from other products in having a stronger spoilage character.

Instrumental Color Determinations. The instrumental color determinations on raw salted fillets (Figures 2 and 3) were in accordance with the sensory results. Injected and brined fillets (Inj S + P) and brined fillets (brined) were lighter on the muscle surface than kench salted fillets ($p < 0.05$). Significant difference ($p < 0.05$) was found in yellowness between the injected and kench salted fillets. This may be due to a more efficient washing of blood out of the muscle tissue early in the salting process compared to that in the brine and kench salted fillets. The phosphates may also have given metal chelating effects on free iron ions in the muscle tissue

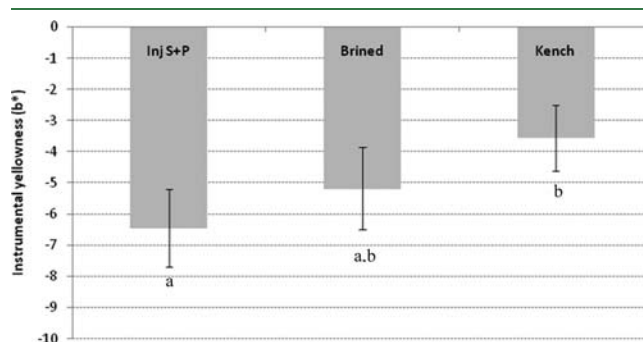


Figure 3. Mean instrumental yellowness values and standard deviations of fully salted fillets ($N = 5$) in Exp1.

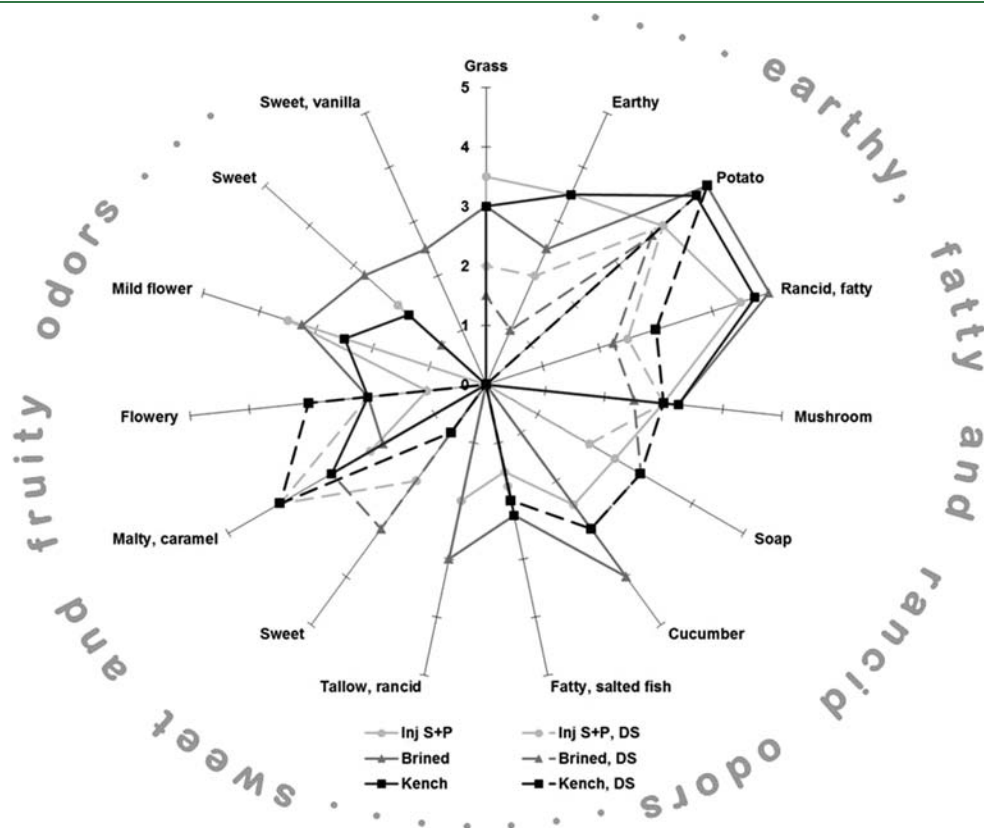


Figure 4. Intensity of aroma-active compounds in salted (—) and desalted (---) cod fillets measured using GC-O in Exp1.

Table 6. Volatile Compounds of Salted and Desalted Cod Fillets (Exp1), Odor Evaluation by GC-O and Quantification by GC-MS Expressed As the Mean Peak Area Ratio (stdv)

Compound	RI DB-5 ms ^a	ID means ^b	odor description (GC-O) ^c	Inj S+P		brine salted		kench salted					
				salted	desalted	salted	desalted	salted	desalted				
ethanol	<165	MS, 1	n.d.	49.5	±32.7		117.1		31.8	±22.9	1.1		
trimethylamine	200	MS, 1	n.d.										
2-methyl-propanal	204	MS	n.d.	11.6	±9.6	17.0	±6.4						
2-butanone	211	MS, 1	sweet	12.7									
2-methyl-1-propanol	232	MS	n.d.										
3-methyl-butanal	255	MS, 1, 2	malty, caramel			68.2	±4.7		16.3	33.6	±4.4	26.4	
1-Penten-3-ol	265	MS, 1, 2	butter	56.9	±39.0	3.9	±0.6	22.3	±7.9	21.2	82.0	3.6	
3-pentanone	287	MS	n.d.										
3-hydroxy-2-butanone	300	MS, 1,	n.d.				13.9	±1.3					
3-methyl-1-butanol	325	MS, 1, 2	flowery	5.6		5.5	±0.2					1.3	
dimethyl disulfide	330	MS, 1	n.d.	1.7									
2-penten-1-ol	355	MS	n.d.										
unknown	364		mild, sweet										
hexanal	397	MS, 1, 2	grass	7.2	±3.9	2.1	±0.4	11.0	±2.0	7.6	11.4	±2.7	3.2
(Z,Z)-3,5-octadiene	404	MS	n.d.	0.5				2.1					
unknown	412		earthy										
unknown	419		sulfur, onion										
3-methyl-butanoic acid	430	MS	n.d.	2.6									
unknown	445		fruity, sweet										
unknown	467		earthy, mushroom										
unknown	486		cardboard										
cis-4-heptenal	497	1, 2	potato,										
heptanal	500	1	rancid, fatty										
benzaldehyde	569	MS	n.d.										
phenol	591	MS	n.d.										
1-octen-3-ol	577	MS, 1, 2	mushroom	7.9	±3.7								
6-methyl-5-hepten-2-one	590	MS, 2	mild flower	23.1	±10.4	6.3	±1.3	24.3	±8.6	11.1	30.7	±2.1	8.0
2,4-heptadienal	612	MS, 1, 2	soap										
1-octanol	621												
3-carene	620			2.7	±1.3	3.1	±1.2						
D-limonene	634	MS	n.d.						0.9				
eucalyptol	632	MS	n.d.	2.0	±1.2	1.2							
acetophenone	666	MS	n.d.	1.1	±0.4	0.4	±0.02	1.5	±0.2	0.8	2.0	±0.8	
unknown	690		sweet										
3,5-octadien-2-one	711	MS	n.d.										
unknown	718		sweet, vanilla										
nonanal	724	MS, 1	n.d.										
nonanal/2-nonen-1-ol	726	MS	n.d.										
2-ethyl-hexanoic acid	738	MS	n.d.										
unknown	750		mild, sweet										
unknown	759		cucumber										
unknown	780		mild, cucumber										
octanoic acid	797	MS	n.d.										
naphthalene	806	MS	n.d.										
decanal	809	MS, 1	n.d.	12.7	±5.0	4.3	±0.6	18.5	±5.2	7.8	21.0	±2.6	5.4
unknown	848		fatty, salted fish										
nonanoic acid	893	MS	n.d.										
unknown	896		sweet, tallow										
unknown	930		tallow, rancid										
hexadecanal	938	MS	n.d.										

^a Calculated ethyl ester retention index on the DB-5 ms capillary column. ^b Identification: MS = mass spectra; 1 = authentic standards; 2 = odor identification. ^c n.d. = odor not detected by GC-O.

Table 7. Volatile Compounds of Split Salted and Desalted Cod (Exp2): Quantification by GC-MS Expressed As the Mean Peak Area Ratio (stdv)

compound	RI DB-5 ms ^a	ID means ^b	Inj S+P		brine salted		kench salted	
			salted	desalted	salted	desalted	salted	desalted
ethanol	<165	MS, 1	31.6 ±3.3	2.7 ±1.8	55.5 ±2.7	6.3 ±0.5	283.7 ±22	1.5 ±0.5
trimethylamine	200	MS, 1	9.8		22.3			
2-methyl-propanal	204	MS	0.9 ±0.2	1.2	1.0		3.7	0.7
2-butanone	211	MS, 1	19.5 ±14.9	4.1 ±3.7	45.5 ±17.9	10.5 ±5.0	129.0 ±9	3.1 ±0.9
2-methyl-1-propanol	232	MS	1.1 ±0.0		1.4 ±0.1		2.5	
3-methyl-butanal	255	MS, 1	7.7 ±5.5	4.1 ±3.6	20.8 ±16.9	14.7		2.92 ±1.7
1-penten-3-ol	265	MS, 1	87.6 ±51.4	10.5 ±7.1	68.7 ±8.1	31.9 ±2.3	92.9 ±8	7.21 ±2.2
3-pentanone	287	MS	20.6 ±5.4	6.3 ±4.7	38.2 ±0.8	23.8 ±1.2	62.4	5.17 ±2.4
3-hydroxy-2-butanone	300	MS, 1	18.9 ±14.9	1.2 ±0.9	19.6 ±13.4	6.7	9.3	
3-methyl-1-butanol	325	MS, 1	5.7 ±5.2	0.9 ±0.5	3.7 ±0.6	2.3	6.4	0.44 ±0.1
dimethyl disulfide	330	MS, 1	0.9	0.3 ±0.2	0.4	2.1		0.8 ±0.1
2-penten-1-ol	355	MS	53.9		12.3 ±1.3			
hexanal	397	MS, 1	6.9 ±6.6	2.0 ±1.4	6.6	5.0	3.6	1.2 ±0.5
(Z,Z)-3,5-octadiene	404	MS						
3-methyl-butanoic acid	430	MS	0.5		1.0			
cis-4-heptenal	497	1	2.9 ±1.9	1.2 ±0.7	3.2 ±2.1	1.3	1.2	0.4
heptanal	500	1	1.4	0.7 ±0.6	1.1			
benzaldehyde	569	MS	4.2 ±4.5		2.0 ±0.0	1.2		
phenol	591	MS	1.0		1.4			
1-octen-3-ol	577	MS, 1	4.3 ±4.9	1.1 ±0.7	2.2 ±1.3		3.5	0.4 ±0.1
6-methyl-5-hepten-2-one	590	MS						
2,4-heptadienal	612	MS, 1	0.4		0.2			
1-octanol	621	MS		0.7 ±0.6		1.2		0.1
3-carene	620	MS	3.6	0.3 ±0.2	0.9 ±0.7	0.9		
D-limonene	634	MS	1.3 ±1.52	0.1 ±0.1	0.4	1.5		0.6
eucalyptol	632	MS						
acetophenone	666	MS	0.3 ±0.26		0.1			
3,5-octadien-2-one	711	MS	1.2 ±1.26		0.9		0.4	
nonanal	724	MS, 1	5.2		2.5			
nonanal/2-nonen-1-ol	726	MS		1.6	1.6			0.1
2-ethyl-hexanoic acid	738	MS	0.8		0.8			
octanoic acid	797	MS	6.5		4.6 ±4.3			
naphthalene	806	MS	0.8	0.1	0.4			
decanal	809	MS, 1	1.1 ±0.94	1.1 ±0.8	0.9 ±0.3		0.9	0.3
nonanoic acid	893	MS	1.7					
hexadecanal	938	MS		2.4	0.7 ±0.4	1.7		

^a Calculated ethyl ester retention index on the DB-5 ms capillary column. ^b Identification: MS = mass spectra; 1 = authentic standards.

and thereby inhibiting the autoxidation processes. In addition, the phosphates may have resulted in chelating effects on metal ions that are present in commercial grade coarse salt.³³

The color values fit well with the sensory evaluation of raw fillets and TBARS values of salted products showing most oxidation in the brine and kench salted fish. High oxidation levels may give rise to Maillard type of reactions which in turn may develop yellow discoloration of the muscle tissue.

Characteristic Odors and Identification of Key Volatile Compounds. Characteristic odors in the salt-cured cod fillets (Exp1) are grouped into two classes according to their odor characteristics in Figure 4, that is, earthy, fatty, and rancid odors, and sweet and fruity odors. GC-O analysis was only done on salt-cured fillets in salted and desalted forms.

The volatile compounds of fillets and split cod (Exp2) are listed in Tables 6 and 7, respectively, to demonstrate which odors are most dominating in the aroma profile. About 50 volatile compounds were detected in salted and desalted fillets and split cod. Quantifications (peak area ratio, PAR) of the GC-MS data based on comparison to the internal standard are only semiquantitative. The odor descriptions are listed in Table 6 according to retention times with corresponding compounds identified by GC-MS, but some of the components detected by GC-O were not identified. GC-O analysis was only done on salt-cured fillets. It must be pointed out that the most abundant compounds quantified by GC-MS do not necessarily contribute to the most intense odors as can be explained by different odor thresholds. In addition, higher PAR could be the result of higher detector sensitivity for that compound and not necessarily the higher concentration of the corresponding compound.

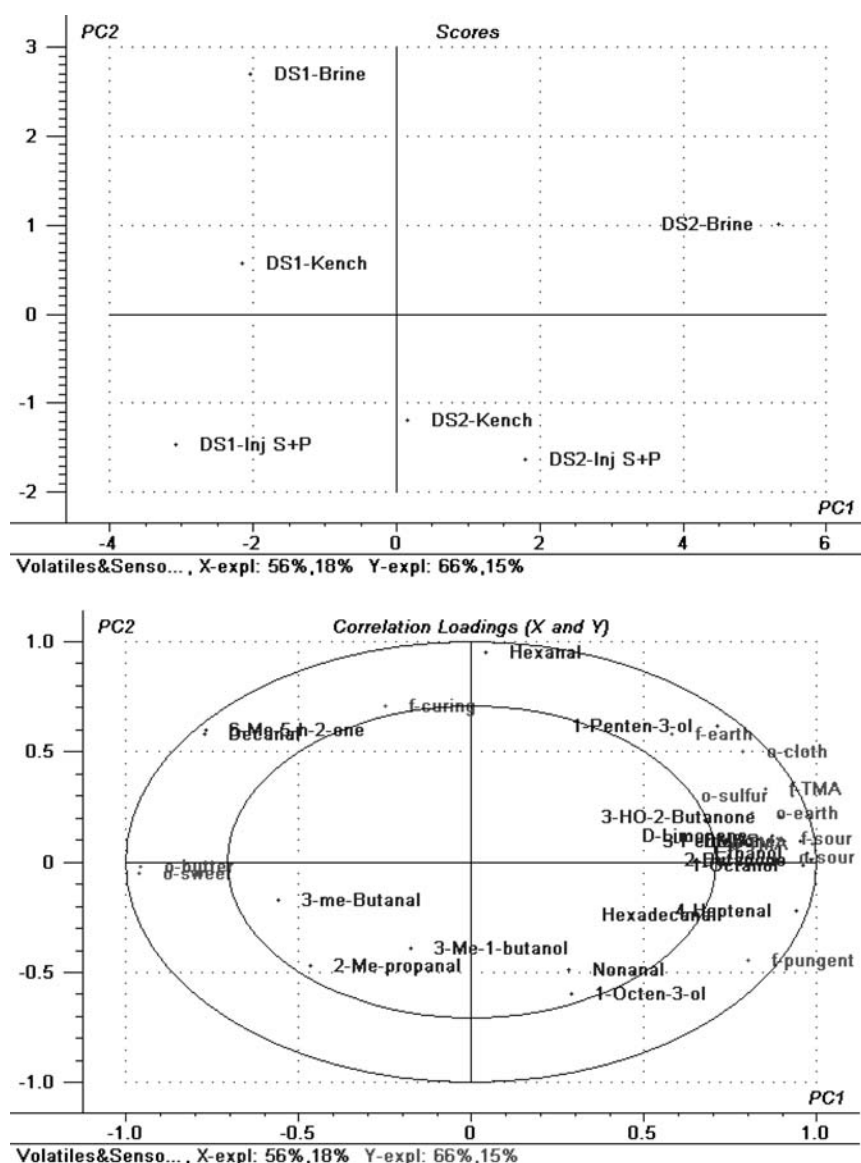


Figure 5. Correlation loading plot from a partial least-squares regression (PLSR) model based on key volatile compounds as predictors (x -variables) and significant sensory odor (o) and flavor (f) attributes as response variables (y -variables) in desalted cod (DS), fillets (1), and split (2) fish. o = odor; f = flavor. The outer and the inner ellipses indicate 100% and 50% explained variance, respectively.

Earthy, Fatty, and Rancid Odors. These characteristic odors are mainly oxidatively derived compounds. The intensity of the odors was much lower in desalted fillets. Potato-like and fatty, rancid-like odors contributed by *cis*-4-heptenal and heptanal were the most potent odors, especially in brine and kench salted fish (Figure 4 and Table 6). Methional, formed via Strecker degradation of methionine and eluting at a similar time as *cis*-4-heptenal and heptanal, could also be responsible for the boiled potato-like odor, although not detectable with the static head-space sampling method. Heptanal and *cis*-4-heptenal were only detected by GC-MS in low concentration in split fish. But because of their low odor threshold (3 and 0.04 ppb, respectively), they can have high sensory impact as seen by the GC-O results (Figure 4). The odor of *cis*-4-heptenal, which is derived from lipid oxidation of *n*-3 unsaturated fatty acids (PUFA), has been described as having boiled potato-like odor.³⁸ In fact, this aldehyde does not exhibit a fishy-type aroma by itself, but it rather participates in the expression of the overall fishy odor. Its odor

has been described both as cardboardy, paint-like,³⁹ and as well as boiled potato-like.⁴⁰ The intensity of the cucumber odor was high in brine salted fish, but this compound could not be identified by GC-MS. Other key volatile compounds in the salt-cured cod with earthy, fatty, and rancid characteristics were also the lipid oxidation derived compounds hexanal, 1-octen-3-ol, and 2,4-heptadienal. The grass odor is contributed by hexanal and is derived from oxidation of *n*-6 unsaturated fatty acid. Mushroom odor is a characteristic odor of 1-octen-3-ol, an oxidatively derived compound from polyunsaturated fatty acid.⁴¹ Fatty and soap-like odors explained by 2,4-heptadienal, are also oxidation end products.

Sweet and Fruity Odors. Malt-like odor contributed by 3-methyl butanol was one of the most potent odors in desalted fish fillets. It was also detected in salted and desalted split fish, especially in kench and brine salted fish. This compound is probably originating from the amino acid leucine. It has an odor threshold of 0.06 ppm and therefore moderate flavor impact. This compound has been suggested as an indicator for the ripening process of ripened

anchovy.⁴² Sweet-like odor by 2-butanone, butter-like odor contributed by 1-penten-3-ol,²⁴ sweet, and flower-like odor by 3-methyl-1-butanol were also among key aroma compounds. 3-Methyl-1-butanol can be produced by microbial spoilage.

Other Key Volatile Compounds. Acetoin (3-hydroxy-2-butanone), giving a butter-like odor, is a lipid oxidation product, or it can be derived from amino acids. This compound was only in detectable concentration in brine salted fish fillets using GC-MS but in all treatments of split fish, especially in injected fish and brine salted fish. The compound was not detectable with GC-O. Normally, it characterizes the spoilage of chilled cod fillets packed in styrofoam boxes caused by the growth of *Photobacterium phosphoreum*.⁴³ The flower-like odor was contributed by 3-methyl-1-butanol that is derived from amino acid leucine. Compounds like acetoin, 3-methylbutanal, and 3-methyl-1-butanol can act as substrates for Maillard reactions⁹ and therefore influence the flavor of salt-cured cod. TMA is a potent odorant with a characteristic fishy, dried fish, and ammonia-like odor. Additionally, TMA has been noted for intensifying fishiness by a synergistic action with certain volatile unsaturated aldehydes derived from the autoxidation of polyunsaturated fatty acids.⁴⁴ The intensity of TMA odor was not high in the salted fillets and was not detected by GC-MS in the fillets (Table 6), but it was detected in brine injected and brine salted split fish (Table 7). Because of its volatility, TMA is difficult to quantify with GC-MS.

Correlation between Flavor Compounds and Sensory Analysis. The PLSR model was calculated to illustrate the relationships between key volatile compounds (x -variables), and significant odor and flavor sensory attributes (y -variables) assessed for fillets and split fish. The key volatiles selected for the PLSR model were compounds present in high levels in the headspace of either fillets or split fish, and some of them contributed to the characteristic earthy, fatty, and rancid odors, and sweet and fruity odors as discussed before. The model was based on all desalted samples ($N = 6$). The PLSR score and loading plots (Figure 5) show that the two first principal components explained 74% of the x -variables (key volatiles) and 81% of the y -variables (sensory attributes). The loading plot illustrates contributions of the scaled variables to the PCs. The first principal component appears to be discriminating between fillets and split fish as seen on the score plot, with higher sensory scores for split fish (DS2), except for the butter and sweet odors. This is in accordance with the TBARS values that were also higher in split fish (Table 4). TMA and sour odors and flavors, together with earthy and sulfur odors, correlated with the presence of high levels of volatile compounds such as 2-butanone, ethanol, 3-hydroxy-2-butanone, 1-octanol, and 3-pentanone. Earthy flavor and dishcloth odor correlated with 1-penten-3-ol which can be produced by microbial spoilage. Pungent flavor correlated with *cis*-4-heptenal and hexadecanal, and it was negatively correlated with decanal and 6-methyl-5-hepten-2-one. Curing flavor correlated with hexanal and was negatively correlated with nonanal and 1-octen-3-ol. On the opposite side of the split fish samples are the fillets with lower sensory scores except for butter and sweet odors that correlated positively with 3-methylbutanal. PLSR with volatiles as predictors for significant sensory attributes explained the different contribution of volatiles to odors and flavors of the differently processed fish.

The practical meaning of higher sensory scores for the split fish products can be negative and positive depending on markets according to the experience of producers. In Spain, juiciness and light color are important quality attributes,³⁶ whereas in Portugal, consumers like products which are stronger in flavor, darker, and not

as juicy due to drying of the products after salting. Preferences are also changing with generations. Products with strong flavor characteristics are more likely to be preferred by older consumers, whereas the young generation favors milder products.

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