Damage Detection in Horse Mackerel (*Trachurus trachurus*) During Chilled Storage

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ABSTRACT: Sensory and chemical analyses were performed during the chilled storage of whole and filleted horse mackerel (*Trachurus trachurus*), an underutilized medium-fat fish species. For both kinds of fish products, satisfactory correlations with the storage time were obtained for amine formation (total volatile base-nitrogen and trimethylamine-nitrogen), lipid damage (free fatty acid formation), and formation of interaction compounds (fluorescence detection in the aqueous phase). Sensory analyses showed a gradual lower grading with time, with a shelf life of 14 d for whole-fish samples and 12 d for fillet samples. Correlation and multivariate analyses between the sensory attributes and the chemical indices showed that trimethylamine-nitrogen detection was the most accurate chemical method for damage assessment during the chilled storage of whole and filleted horse mackerel.

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KEY WORDS: Chilling, damage, fillets, sensory and chemical analysis, underutilized fish, whole fish.

Fish and other marine species are the raw materials for products of great economic importance in many countries. The fish industry is currently suffering from dwindling stocks of traditional species as a result of drastic changes in their availability. As a result, fish technologists and the fish trade have turned their attention to some unconventional sources of raw material (1,2).

One such species is horse mackerel (*Trachurus trachurus*), a medium-fat species abundant in the northeast Atlantic (3,4). Efforts have been made to utilize it in the manufacture of smoked (5), canned (6), chilled and frozen (7,8), and restructured (9) fish products. The effects of cryoprotectants and antioxidants on stability of frozen products have also been investigated (10,11).

During chilled storage of fish, important changes are known to take place. Thus, significant deterioration of sensory quality and loss of nutritional value have been detected as a result of changes in the protein and lipid fractions, formation of amines (volatile and biogenic) and hypoxanthine, and changes in the physical properties of the muscle (12–14). Indeed, prolonged chilled storage has proved to have a rather negative effect if further processing is to be carried out on the fish material (15,16).

Like most fish species, horse mackerel has to be kept in ice before it is consumed as such or otherwise processed. The present work was undertaken to determine the preservability of whole and filleted horse mackerel during ice storage and to evaluate the validity of fast chemical methods to assess changes in both kinds of fish products during normal trading practice.

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MATERIALS AND METHODS

Raw material, processing and sampling. Fresh horse mackerel (*T. trachurus*) were obtained 10 h after being caught; during this interval, the fish had been kept on ice. The length of the horse mackerel was in the range 18–24 cm; the weight was in the range 250–280 g. Upon arrival in our laboratory, some of the fish were carefully dressed and filleted by hand; the remainder were kept intact. Both the filleted and the whole-fish samples were divided into three batches that were stored (on ice) in isothermal rooms at 0°C and studied separately for statistical analysis. Samples were taken for analysis on days 0, 2, 6, 9, 12, 14, and 19.

Sensory analyses. Sensory analyses were conducted by a taste panel consisting of five experienced judges according to the guidelines presented in Table 1 (17). Four categories were ranked: highest quality, good quality, fair quality, and rejectable. Sensory assessment of the whole-fish samples included the following parameters: skin, eyes, gills, flesh odor, consistency, and flesh appearance. In the case of fillets, the following attributes were considered: skin, flesh odor, consistency, and flesh appearance.

At each sampling time and for both kinds of fish products (whole fish and fillets), two individual fishes from each of the three batches were taken for analysis. Scores among panelists were averaged.

Chemical analyses were then carried out on the homogenized white muscle of the individual fishes employed for the sensory assessment.

Composition analyses. Water content was determined by the difference between the weight of fresh homogenized muscle (1–2 g) and the weight recorded after 24 h at 105°C. Results were calculated as g water/100 g muscle. Lipids were extracted by the Bligh and Dyer method (18). Quantification results were calculated as g lipid/100 g wet muscle.

Lipid damage measurements. Free fatty acid (FFA) content was determined by the Lowry and Tinsley method (19) based on complex formation with cupric acetate–pyridine. Results are expressed as g FFA/100 g lipids.

Conjugated diene (CD) formation was measured at 233 nm (20). The results are expressed according to the formula $CD = B \times V/w$, where B is the absorbance reading at 233 nm, V

TABLE 1
Scale Employed for Evaluating Freshness of Chilled Horse Mackerel

Attribute	Highest quality	Good quality	Fair quality	Rejectable
Skin	Very intense pigmentation transparent mucus	Insignificant pigmentation losses; slightly turbid mucus	Pigmentation discolored and without shine; milky mucus	Important pigmentation losses; opaque mucus
Eyes	Convex; transparent cornea; bright and black pupil	Convex and slightly sunken; slightly opalescent cornea; black and cloudy pupil	Flat; opalescent cornea; opaque pupil	Concave and milky cornea; grey pupil
Gills	Bright red; without odor; lamina perfectly separated	Rose-colored; without odor; lamina adhered in groups	Slightly pale; fishy odor; lamina adhered in groups	Grey-yellowish color; intense ammonia odor; lamina totally adhered
Flesh odor	Sharply seaweedy and shellfish	Weakly seaweedy and shellfish	Slightly sour and rancid	Sharply sour and rancid
Consistency	Presence or partial disappearance of rigor mortis symptoms	Firm and elastic; pressure signs disappear immediately and completely	Presence of mechanical signs; elasticity notably reduced	Important shape changes due to mechanical factors
Flesh appearance	Strongly hydrated and pinky; myotomes totally adhered	Still hydrated and pinky; myotomes adhered	Slightly dry and pale; myotomes adhered in groups	Yellowish and dry; myotomes totally separated

denotes the volume (mL), and *w* is the mass (mg) of the lipid extract measured.

The thiobarbituric acid index (TBA-i) (mg malondialdehyde/kg fish sample) was determined according to Vyncke (21).

Formation of protein-oxidized lipid interaction compounds. Formation of interaction compounds was investigated by measurements at 393/463 nm and 327/415 nm, as described earlier (16,22), performed using a PerkinElmer LS 3B fluorimeter. The relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the fluorescence measured at each excitation/emission maximum, and F_{st} is the fluorescence intensity of a quinine sulfate solution (1 μg/mL in 0.05 M H_2SO_4) at the corresponding wavelength. The fluorescence ratio (δF) was calculated as the ratio between the two RF values: $\delta F = RF_{393/463}$ nm/$RF_{327/415}$ nm. The δF value was determined for the aqueous (δF_{aq}) and organic (δF_{or}) phases obtained from the lipid extraction (18).

Assessment of volatile amines. Ten grams of fish muscle was extracted with perchloric acid (6%) and made up to 50 mL.

Total volatile base-nitrogen (TVB-N) values were measured by the Antonacopoulos method (23) with some modifications (24). TVB-N content was obtained by steam distillation of the acid extracts made alkaline to pH 13 with NaOH (20%), followed by titration of the distillate with 10 mM hydrochloric acid. Data are expressed as mg TVB-N/100 g muscle.

Trimethylamine-nitrogen (TMA-N) values were obtained by the picrate method performed according to Tozawa *et al.* (25) by employing the same acid extracts as in the case of the TVB-N detection. Data are expressed as mg TMA-N/100 g muscle.

Statistical analyses. Data from the different chemical quality measurements were subjected to one-way analysis of variance ($P < 0.05$); comparison of means was performed using a least-squares difference method (26). Correlation analyses and the Spearman test for nonparametric correlations were performed ($P < 0.01$). Factor analysis (principal components) was carried out with all parameters measured; a Varimax normalized rotation was employed for factor rotation (26).

RESULTS AND DISCUSSION

Composition analyses. Water contents ranged between 75 and 80% for the whole-fish samples and 76 and 84% for the fillets. A slight increase in water content during the chilled storage was observed in both kinds of fish products. This increase could be explained as the result of contact with ice (27) and was greater in the case of fillet samples.

Lipid contents ranged between 1.20 and 2.70% in both fillets and whole fish: these are typical values for a medium fat content species. For each kind of fish product, lipid content variations measured during chilled storage were attributed to differences between individual fishes rather than to effects of storage.

Comparison with previous research showed a higher water content than in fattier species (24) and a lower content than in lean fish species (27,28) in accordance with an inverse relationship between the two constituents (29).

Lipid damage assessment. FFA contents of the raw material were rather similar to those of fatty fish species (tuna, sardine) (24,30) and lower than those of lean fish (blue whiting, haddock, cod) (27,28). In whole-fish samples, lipid hydrolysis increased gradually during chilled storage (Table 2); a significant increase was observed at day 6 compared to the unstored sample, and the FFA content at the end of the experiment (day 19) reached 5.3%. A gradual increase in FFA content was also observed for fillet samples, although a significant increase was only obtained at day 14 (Table 3); the value at the end of the experiment (day 19) showed a large increase and was higher ($P < 0.05$) than the value for the whole- fish samples, in accordance with the greater access of microorganisms in the fillet samples.

CD formation did not show any significant trend during the storage process for both kinds of fish products (Tables 2 and 3). In initial stages of oxidation, the measurement of CD formation has been used satisfactorily for damage assessment (31,32). However, in the present experiment, this index was not suitable because dienes are relatively unstable and capable of interacting with other constituents (22,33).

TABLE 2
Lipid Damage and Fluorescence Measurements During Chilled Storage of Whole Horse Mackerel[a,b]

Storage time (d)	FFA	CD	TBA-i	δF_{aq}	δF_{or}
0	1.39 a	0.90 a	0.10 a	1.52 a	0.74 a,b
2	2.14 a,b	1.04 a	0.09 a	1.52 a	0.57 a
6	2.63 b,c	0.91 a	0.34 a,b	1.28 a	0.62 a
9	2.81 b,c	1.03 a	0.37 a,b	1.20 a	0.76 a,b
12	2.74 b,c	1.18 a	0.32 a,b	4.79 a,b	0.93 a,b
14	3.14 c	0.98 a	0.64 b,c	5.47 b	0.89 a,b
19	5.28 d	1.09 a	0.85 c	58.85 c	1.08 b

[a]Abbreviations: FFA, free fatty acids; CD, conjugated dienes; TBA-i, thiobarbituric acid index; δF_{aq} and δF_{or}, fluorescence ratio values in the aqueous and organic phases, respectively.
[b]Values are means of three independent determinations. Values in the same column followed by different letters are significantly different ($P < 0.05$).

Secondary oxidation measured by the TBA-i showed a gradual increase during the chilled storage in the case of the whole-fish samples (Table 2); compared to the raw sample, a significant increase was obtained at day 14. In the case of fish fillets, a gradual increase in the TBA-i was observed up to day 12, followed by a decrease (Table 3); molecules that contribute to this index may have interacted with other fish constituents in the last stage of the chilled storage (12–19 d) (20,22), so that a continuous increase was not served throughout the experiment. TBA-i values were higher ($P < 0.05$) in the fillet samples than in the whole-fish ones for the initial 2–12-d period, which can be attributed to a greater access of oxygen and trace metals from melted ice and the presence of higher levels of blood contaminants in the fillet samples (34).

Formation of interaction compounds. Analysis of the organic phase showed little significant differences in the δF_{or} values of whole-fish samples (Table 2) and a gradual increase up to day 14 followed by a decrease at day 19 in the values of fillet samples (Table 3). Accordingly, this analysis was unable to afford damage assessment throughout the storage period investigated.

In the case of the aqueous phase, δF_{aq} showed an increase tendency with storage time in both kinds of fish products (Tables 2 and 3). This increase was especially high after day 12 and was greater in the fish fillet samples than in the whole-fish ones. The large increase obtained for fish fillets at days 14 and 19 agrees with the decrease found in the TBA-i values for the same days.

The relative formation of fluorescent compounds that are lipid- and water-soluble ($\delta F_{or}/\delta F_{aq}$ ratio) was studied in previous research on fatty (24) and lean fish (27). It was observed that, as long as lipid damage increased, fluorescence detected in the aqueous phase predominated and was more accurate for assessing quality changes. It can be argued that fluorescent substances formed from oxidized membrane lipids remain attached to the amino constituent, resulting in compounds that are quite insoluble in organic solvents (35,36).

Formation in volatile amines. The TVB-N value showed a gradual increase throughout the chilled storage in the case of the whole-fish samples (Fig. 1); compared to the raw sample, a significant increase was observed at day 12, followed by a large increase at the end of the storage period. This increase agrees with previous results for fatty (12,13) and lean (27,37) fish species, which showed a sharp increase in total volatile amine content after 9–10 d of storage, as a result of the end of the lag phase of microorganisms.

In the case of filleted fish, a clear tendency could not be discerned for the TVB-N values (Fig. 1). Compared to unstored samples, lower values were obtained in the 2–14 d period, whereas a higher value was obtained at the end of the storage period. It could be argued that contact with ice could produce a loss of the volatile amine content present in the muscle, especially ammonia, which is highly water soluble. Throughout the storage period, lower values ($P < 0.05$) were obtained for the fillet samples than for the whole-fish ones.

The TMA-N value showed a gradual increase with storage time for both the whole-fish and fillet products (Fig. 2); compared to unstored samples, a significant increase was observed at day 9 in both cases. Higher TMA-N contents ($P < 0.05$) were obtained for the whole-fish samples than for the fillets throughout the storage period. Values obtained for the

TABLE 3
Lipid Damage and Fluorescence Measurements During Chilled Storage of Horse Mackerel Fillets[a,b]

Storage time (d)	FFA	CD	TBA-i	δF_{aq}	δF_{or}
0	1.39 a	0.90 a	0.10 a	1.52 a	0.74 a
2	1.82 a	1.00 a	0.33 a	1.57 a	0.68 a
6	1.97 a	1.24 a	1.10 b	1.20 a	1.99 a,b
9	2.46 a,b	0.92 a	1.56 c	2.14 a	2.73 b,c
12	2.47 a,b	1.03 a	1.76 c	5.08 a	6.95 d
14	3.80 b	1.09 a	0.74 b	27.65 b	6.88 d
19	10.09 c	1.26 a	0.92 b	283.80 c	3.89 c

[a]See Table 2 for abbreviations.
[b]Values are means of three independent determinations. Values in the same column followed by different letters are significantly different ($P < 0.05$).

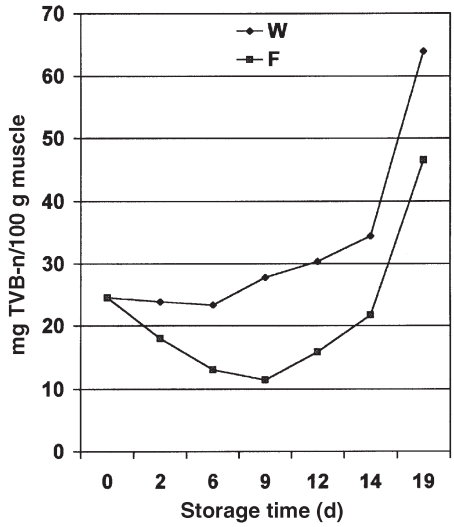

FIG. 1. Total volatile base-nitrogen (TVB-N) measured during the chilled storage of whole (W) and fillet (F) samples of horse mackerel.

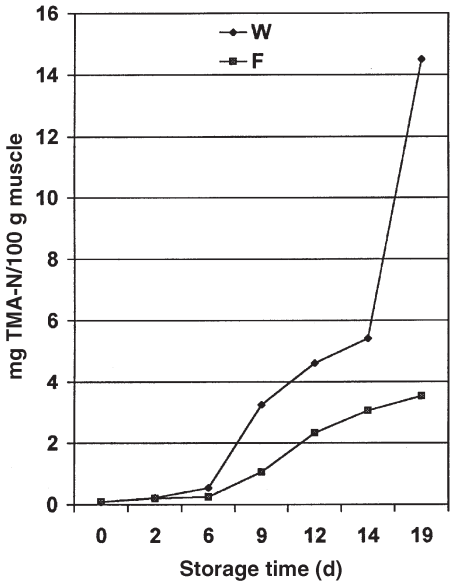

FIG. 2. Trimethylamine-nitrogen (TMA-N) measured during the chilled storage of whole (W) and fillet (F) samples of horse mackerel.

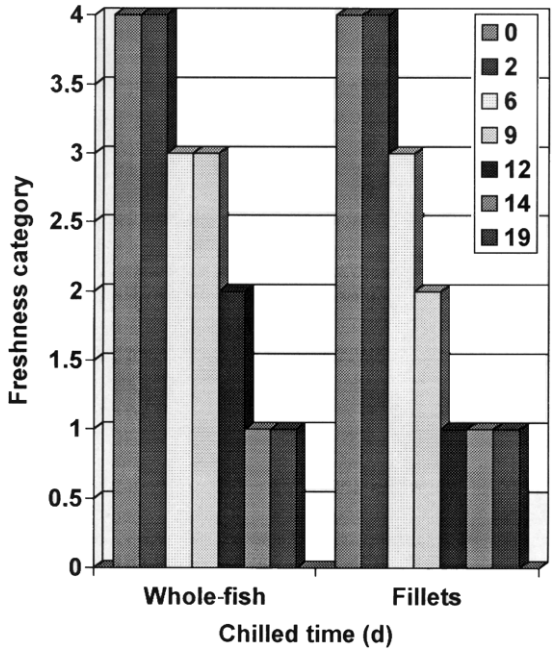

FIG. 3. Sensory acceptance of whole and filleted horse mackerel during chilled storage (0, 2, 6, 9, 12, 14, and 19 d). Freshness category marks: 4 (highest quality), 3 (good quality), 2 (fair quality), and 1 (rejectable).

whole-fish samples agreed with previous results for chilled fish species (13,38). The relatively lower formation of TMA-N in fillets could be explained by the absence of viscera and other parts of the fish in which microorganisms that are able to convert trimethylamine oxide into trimethylamine are known to be concentrated (37). In this regard, Smith *et al.* (7) found that during a short iced storage of horse mackerel, gutted fish had lower formation of TMA-N than whole fish.

Correlation of chemical indices with storage time. Correlations of all the chemical indices tested with the storage time were studied (Table 4). Results for the whole-fish samples showed satisfactory correlation with all the damage indices except for the CD value; quadratic equations yielded the best fits in all cases, especially for TMA-N ($r^2 = 0.97$) and TVB-N ($r^2 = 0.92$).

In the case of the fish fillets, correlations of the chemical indices tested with the storage time showed satisfactory values for most indices, except CD and TBA-i; with quadratic fitting, good correlations were obtained for TMA-N ($r^2 = 0.93$), FFA ($r^2 = 0.90$), and δF_{aq} ($r^2 = 0.89$).

TABLE 4
Linear Correlations Between the Chilled Storage Time and Chemical Indices for Whole-Fish and Fillets[a]

Chemical index[b]	Whole-fish	Fillets
CD	0.24 (0.25)	0.37 (0.37)
δF_{or}	**0.59 (0.62)**	**0.69** (0.55)
δF_{aq}	**0.66 (0.83)**	**0.75 (0.89)**
FFA	**0.81 (0.86)**	**0.78 (0.90)**
TBA-i	**0.74 (0.74)**	0.47 (0.24)
TVB-N	**0.78 (0.92)**	0.59 **(0.80)**
TMA-N	**0.89 (0.97)**	**0.93 (0.93)**

[a]Significant values ($P < 0.01$) are in bold type. Values in parentheses are for quadratic fits.
[b]TVB-N, total volatile base-nitrogen; TMA-N, trimethylamine-nitrogen. See Table 2 for other abbreviations.

Sensory analyses. A gradual decrease in grading was observed with time for whole-fish and fillets (Fig. 3); the fish material was judged unacceptable for consumption at day 14 (whole-fish) and day 12 (fillets). All attributes studied showed a satisfactory Spearman correlation with storage time (Table 5).

Comparison with previous research carried out on commercial fish species showed that shelf life in ice was shorter than for lean fish species but longer than in the case of fattier species (12,37,38). The lipid fraction in fish species has been shown to be highly unsaturated (39) and thus very susceptible to damage during processing (40), and a good correlation has been found between lipid damage and shelf life time (41,42).

Correlation and multivariate analyses. In the case of the whole-fish samples, significant values were obtained for Spearman correlations between the sensory attributes considered and four of the chemical indices investigated (TMA-N, TVB-N, TBA-i, and δF_{aq}) (Table 6); the best results were obtained in the case of the TMA-N index.

TABLE 5
Coefficients of Spearman Correlations Between the Chilled Storage Time and Sensory Attributes for Whole-Fish and Fillets[a]

Sensory attribute	Whole-fish	Fillets
Skin	0.96	0.93
Eyes	0.94	—
Gills	0.89	—
Flesh odor	0.97	0.97
Consistency	0.95	0.95
Flesh appearance	0.91	0.94

[a]Significant values ($P < 0.01$) were obtained in all cases.

TABLE 6
Coefficients of Spearman Correlations Between Chemical Indices and Sensory Attributes for Whole-Fish[a]

Chemical index[b]	Skin	Eyes	Gills	Flesh odor	Consistency	Flesh appearance
FFA	**0.78**	**0.73**	**0.78**	**0.72**	**0.80**	**0.71**
CD	0.16	0.15	0.18	0.17	0.14	0.11
TBA-i	**0.83**	**0.83**	**0.76**	**0.81**	**0.82**	**0.71**
δF_{or}	**0.61**	**0.64**	**0.56**	**0.68**	**0.58**	**0.57**
δF_{aq}	**0.63**	**0.59**	0.48	0.56	0.52	0.51
TVB-N	**0.87**	**0.82**	**0.74**	**0.86**	**0.81**	**0.77**
TMA-N	**0.94**	**0.92**	**0.90**	**0.94**	**0.91**	**0.87**

[a]Significant values ($P < 0.01$) are in bold type.
[b]See Tables 2 and 4 for abbreviations.

Spearman correlations were also studied in the case of fish fillets. Significant values were obtained between the four sensory attributes considered and four of the indices studied (TMA-N, δF_{or}, δF_{aq}, and FFA) (Table 7). The best values were obtained again for the TMA-N index.

In order to segregate the different parameters (chemical and sensory indices) into different factors, principal component analysis (PCA) was carried out. In the case of the results obtained for whole-fish samples, 80.1% of the variability of the variables under study could be explained with two factors. Following a Varimax rotation (Table 8), it was found that factor 1 alone accounted for 69.1% of the variability. Factor 1 had relatively high loadings (>0.85) for four sensory attributes (skin, eyes, flesh odor, and consistency) and three chemical indices (TMA-N, FFA, and TVB-N); very high loadings (>0.92) were obtained in the case of consistency, TMA-N, and flesh odor. Factor 2 accounted for only 11.0% of the variability. The results obtained by PCA indicated that TMA-N detection would be the most accurate chemical method to check the grading decrease of horse mackerel during chilled storage as whole pieces.

The results for fillet samples were also studied by PCA. With this method, 84.0% of the variability of all the parameters under study could be explained with two factors. Following a Varimax rotation (Table 9), it was found that factor 1 alone accounted for 63.0% of the variability. Factor 1 had relatively high loadings (>0.92) for the four sensory attributes and TMA-N. Factor 2 accounted for only 21.0% of the variability. As in the case of whole-fish samples, it was concluded again that measurement of the TMA-N content would be the best chemical method to employ for assessment of damage and grading decrease during chilled storage.

TABLE 7
Coefficients of Spearman Correlations Between Chemical Indices and Sensory Attributes for Fillets[a]

Chemical index[b]	Skin	Flesh odor	Consistency	Flesh appearance
FFA	**0.71**	**0.77**	**0.82**	**0.76**
CD	0.20	0.33	0.14	0.18
TBA-i	0.48	0.44	0.51	0.47
δF_{or}	**0.88**	**0.86**	**0.85**	**0.89**
δF_{aq}	**0.89**	**0.86**	**0.88**	**0.89**
TVB-N	0.19	0.25	0.15	0.19
TMA-N	**0.94**	**0.93**	**0.94**	**0.93**

[a]Significant values ($P < 0.01$) are in bold type.
[b]See Tables 2 and 4 for abbreviations.

TABLE 8
Factor Loadings from Principal Component Analysis of Chemical and Sensory Parameters Measured in Whole-Fish Samples[a]

Parameter	Factor 1	Factor 2
FFA	0.90	–0.25
CD	0.18	–0.34
TBA-i	0.82	0.01
δF_{or}	0.76	–0.61
δF_{aq}	0.69	0.01
TVB-N	0.87	–0.48
TMA-N	0.94	–0.30
Skin	0.91	0.34
Eyes	0.90	0.34
Gills	0.80	0.40
Flesh odor	0.93	0.26
Consistency	0.96	–0.01
Flesh appearance	0.85	0.32

[a]See Tables 2 and 4 for abbreviations.

TABLE 9
Factor Loadings from Principal Component Analysis of Chemical and Sensory Parameters Measured in Fillet Samples[a]

Parameter	Factor 1	Factor 2
FFA	0.78	–0.56
CD	0.44	–0.07
TBA-i	0.41	0.72
δF_{or}	0.75	–0.62
δF_{aq}	0.73	0.57
TVB-N	0.62	–0.75
TMA-N	0.94	–0.04
Skin	0.93	0.27
Flesh odor	0.97	0.02
Consistency	0.94	0.25
Flesh appearance	0.94	0.25

[a]See Tables 2 and 4 for abbreviations.

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