Microbiologie et Hygiène Alimentaire
March 2009 ; Volume 21 (60) : Pages 35-43

Sensory and aromatic characteristics of tongue sole by-products hydrolysates (*Cynoglossus senegalensis*)

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Abstract :

Tongue sole by-products coming from fish-filleting plant were hydrolyzed by Protamex® protease. To identify the future application of hydrolysates, a sensory analysis was carried out.The sensory profile was performed with a jury of 14 specialized judges.11 profiles were found by this panel of tasting.
In addition, the aromatic characterization revealed that 57 molecules are responsible for these odours described in sensory analysis.The description of these aromatic compounds opens potential way of valorization of these hydrolysates in human and animal consumption.

Keywords: Enzymatic hydrolysis; by-products; aroma.

Resumé :

Les coproduits de sole tropicale provenant d'une usine de filetage ont été hydrolysés par une enzyme de type protease vendu sous le nom commercial de Protamex®. Pour identifier les futurs domaines d'application, une analyse sensorielle a été effectuée. Le profil sensoriel a été réalisé avec un jury de 14 personnes. 11 profils ont été identifiés par ce panel de dégustation.
Par ailleurs, la caractérisation aromatique a révélé que 57 molécules sont responsables de ces odeurs décrites en analyse sensorielle. La mise en évidence de ces composés aromatiques ouvre des pistes potentielles de valorisation de ces hydrolysats en alimentation humaine et animale.

Mots clés: hydrolyse enzymatique; co-produits; arôme.

1. Introduction

By-product utilization is an important issue for the seafood industry. Fish processing by-products are about 40 to 60% of the whole fish weight and they have been commonly regarded as low-value resources. The by-products from the filleting line are head, viscera, bones, tail, fin and skin. They are an important environmental contamination sources **(Ravallec and al., 2000)**. However, fish by-products contain valuable protein and lipid fractions as well as vitamins and minerals. Therefore, many researches have been carried out in order to transform these by-products into useful products. There has been an increasing interest in fish by-products during the past years. Today these by-products are considered as a potential resource instead of a waste **(Rustad, 2003).**

The main part of by-products was used in the production of fish meal, fish oil and fish silage. However, fish by-products are also used to produce protein hydrolysates. Hydrolysates can be defined as proteins that are broken down to peptides of varying sizes.
Fish by-products are now used for human consumption and another profitable way of valorization is animal feeding. Nevertheless the highest profitability relates to the bioactive compounds like some peptides and lipids for biotechnological or pharmaceutical application. Chemical compositions of the fractions obtained from the hydrolysis of fish by-products were also reported **(Kristinsson, 2000; Sathivel, 2003 and 2006; Liaset, 2003, Thiansilakul, 2006)**. The protein solubilisation of fish by-products was also studied **(Benjakul, 1997; Sathivel, 2005; Aspmo, 2005; Slizyte, 2005)**. Many authors reported the molecular weight distribution of peptides **(Hoylen, 1994; Guérard, 2001; Slizyte, 2005)**.
In Senegal, fish by-products are used to produce meal destinated generally to poultry feed. The aim of this study is to find another way of valorization of the whole biomass of tongue sole by-products. In a specific objective, sensory and aroma profiles were studied in order to use them in human or animal industry. For that, sensory descriptors were described after 3 and 6 hours enzymatic hydrolysis by using Protamex® enzyme.

2. Material and methods

2.1 Raw material and hydrolysis

Tongue sole *(Cynoglossus senegalenis,* picture 1*)* was caught in the Atlantic Ocean (FAO area number 34), stored in ice and brought to the seafood processing plant “Pirogue bleue” in Dakar-Sénégal. Raw fish were filleted less than 36 hours after catch.
16 kg of sole (*Cynoglossus senegalensis)* by-products (carcasses, viscera, heads, skin) were mixed to 8 litres of distilled water. 1% of Protamex® (a bacteria proteases complex) was added to the mixture and poured in a bioreactor (picture 1). Various hydrolysis parameters were studied: the hydrolysis temperature (T= 50°C or 40°C) and the hydrolysis time (t= 3 or 6 hours). The enyme was inactivated during 15 minutes at 85 and 95°C. Hydrolysates were centrifuged at 30000g during 30 minutes and the supernatrant was filtered using filters of 0,7 and 0,2 µm. Samples were stored at -80°C ans were presented for a sensory analysis. Then, an aroma analysis was done on the different samples in order to correlate both methods.

Picture 1: Tongue sole

Picture 2: Bioreactor

2.2 Enzyme

Protamex® is a *Bacillus* protease complex developed for the hydrolysis of food proteins. This enzyme is produced by Novozyme A/S (Bagsvaerd, Denmark). Protamex® has a declared activity of 1.5 Anson Unit (AU)/g. The optimal working conditions for Protamex® are temperatures between 35 and 60^0C and pH values between 5.5 and 7.5. Protamex® can be inactivated in 10 minutes at 85^0C when the pH is 8.

2.3. Sensory analysis of the hydrolysates

The sensory analysis was done by a trained panel of 14 panelists belonging to IFREMER staff and specialized on sea-products characterization. Sessions were performed in individual partitioned booths according to the AFNOR V-09-105 requirements and equipped with a computerized system (Fizz, Biosystème, Dijon-France). Two sensory sessions were organized. The first one, a descriptive test on the perceived odours of the different hydrolysates was done in order to create and select a list of relevant, and if possible discriminating descriptors. Then a sensory profiling test was carried out to characterize the main odours of each hydrolysate and the level of olfactory intensity of each descriptor. During the test the panel was asked to rate sensory attributes on a continuous unstructured linear scale from “weak intensity” (score o) to “strong intensity” (score 10).
The data obtained were analysed using a two factors Analysis of Variance (product effect and judge effect). A standardized PCA (Principal Component Analysis) was performed on the mean of the group score of each product and each descriptor. The PCA shows the main sensory characteristics of each product (sample hydrolysed under different conditions of time and temperature) and the ANOVA treatment gives, for each attribute the statistical level of the difference.

2.4 Aroma analysis of the hydrolysates

Aroma extraction was performed using SPME (picture 2) fibres coated with 75µm of carboxen-Polydimethylsiloxane (CAR-PDMS). Extraction time was 30 minutes and extraction and desorption temperatures were respectively 30°C and 260°C. Prior to analysis, the CAR-PDMS fibre was conditioned 120 minutes at 260°C and samples were maintained at 30°C during 20 minutes. Each extraction was repeated 3 times.
All the Gas-chromatography-Mass spectrometry analyses were performed using HP 5890 series II GC with a HP 5971 series mass selective detector (picture 3). SPME fibre wasthermally desorbed at 260°C in the injection port for 2 minutes in the spitless mode. During desorption the oven was held at 50°C. A DB-5MS colum (30m x 0,32 Id, 0,5 µm film thickness, J & W Scientific, US) was used to separated the volatile compounds of the hydrolysates. After desorption the oven was heated from 50°C to 250°C at 5°C/minute and held for 5 minutes at this temperature. A series of n-alcalanes (C6-C20) was injected under the same conditions in order to obtain the volatile compounds retention indices (RI). A fixed quantity of an internal standard (1,2,4-Trimethylbenzene) was added to the samples in order to lead a quantitative analysis.
The volatile compounds were first identified by comparing their mass spectra with those of th NIST/WILEY mass spectra database. Identities were confirmed by comparison of RI values with those of published values and standard injection. Data were subjected to analysis of variance (ANOVA) with two factors. A PCA was done using the mean quantity of each volatile compound expressed in micro-grams of equivalent of internal standard by litre of hydrolysate (µg eq.I.S/L).
All characterization have been done into ENITIAA (France) laboratory.

Picture 3: SPME extraction

Picture 4: CPG-SM HP 5890 series II

3. Results and discussion

3.1 Sensory characterization of hydrolysates

The data obtained during the sensory profiling test were analysed using a two factors analysis of variance (judge effect and product effect). The following attributes were chosen: global intensity, iodine-like, dried shellfish, dried alga, fat fish, rancid, wet dog, dried fish, feet-cheese, sulphur and faecal. The results can be seen on table 1.

Table 1 : Mean of the notes (out of 10) obtained during sensory profiling if probability results. p> 5% : non significant ; * :significant difference with a 5% threshold ; ** :significant difference with a 1% threshold ; *** :significant difference with a 0,1% threshold. The mean values followed by the same letter are not significantly different.

Odeurs	3h/40° 85°	3h/50° 85°	6h/40° 85°	6h/50° 85°	3h/40° 95°	3h/50° 95°	6h/40° 95°	6h/50° 95°	F calc.	Probabilité
Global intensity	**5.45 bc**	**5.23 c**	**8.19 a**	**5.71 bc**	**5.49 bc**	**5.63 bc**	**7.84 a**	**6.50 b**	**10.95**	**<0.0001 *****
Marine/iodised	**2.46 a**	**2.53 a**	**1.26 b**	**2.72 a**	**2.59 a**	**2.26 a**	**1.24 b**	**2.84 a**	**4.16**	**0.0005 *****
Dried shellfish	**2.56 b**	**2.59 b**	**2.45 b**	**3.41 ab**	**2.70 b**	**2.28 b**	**2.19 b**	**4.63 a**	**3.18**	**0.0047 ****
Dried algae	2.07	1.85	1.59	2.74	2.63	2.58	1.68	2.25	1.02	0.42
Fatty fish	**1.85 ab**	**2.09 a**	**0.41 c**	**1.73 ab**	**1.66 ab**	**1.74 ab**	**0.64 bc**	**1.26 abc**	**2.45**	**0.02 ***
Rancid	0.76	1.03	0.64	0.97	1.38	1.32	1.07	0.52	1.14	0.34
Wet dog	0.96	0.61	0.78	0.56	0.82	1.06	0.92	0.59	0.63	0.72
Dried fish	1.81	2.59	2.94	2.36	1.77	2.16	2.34	2.51	0.93	0.49
Feet/cheese	**0.31 b**	**0.26 b**	**2.67 a**	**0.29 b**	**0.35 b**	**0.53 b**	**2.51 a**	**0.29 b**	**8.38**	**<0.0001 *****
Sulphurous	**0.89 b**	**0.78 b**	**4.31 a**	**0.76 b**	**0.74 b**	**0.80 b**	**4.10 a**	**0.59 b**	**16.90**	**<0.0001 *****
faecal	**0.41 b**	**0.12 b**	**3.19 a**	**0.19 b**	**0.29 b**	**0.37 b**	**2.44 a**	**0.15 b**	**10.35**	**<0.0001 *****

A standardized principal component analysis, performed on mean scores for each sensory descriptor and all the hydrolysis conditions (fig. 1), shows a good discrimination between samples. The first plane of this PCA describes 99.7% of the variance. First axis is the mainly defined on one side by descriptors, “feet/cheese”, “faecal”, “sulphurous” and on the other side by the attribute “marine/iodised”. Axis 2 is created by two descriptors from opposite direction, “fatty fish” and “dried fish”. This map allows separating three groups of products with specific sensory characteristics.

-the first one is constituted by the product (T40, t6: hydrolysis temperature =40°C, hydrolysis time =6 hours) which develops an intense global odour and “feet/cheese”, “faecal”, “sulphurous” olfactory notes;

-the two other groups are separated on the second axis: on the top of the figure 1, a group of two samples, with 3 hydrolysis hours, have light olfactory notes, a “marine/iodised” odour and mainly a “fatty fish” characteristic. The sample T50, t6 (hydrolysis temperature =50°C, hydrolysis time =6 hours) represents the third group with “marine/iodised” and “dried shellfish” notes.

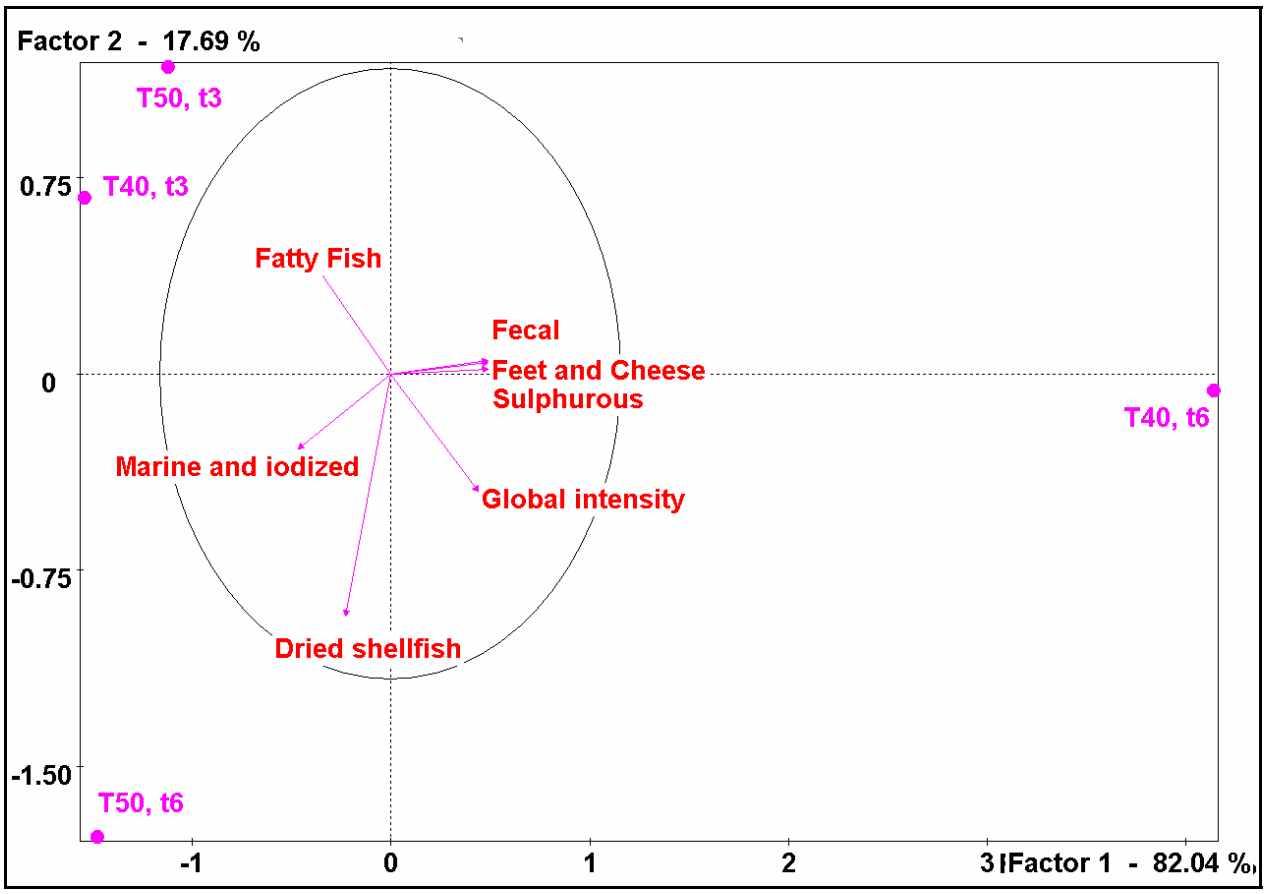

Figure 1: Simultaneous representation of samples and sensory descriptors

It's interesting to note the interaction between time and temperature of hydrolysis. This interaction appeared in the results from analysis of variance (table 1). Indeed, aroma characteristics developed after 6 hours of hydrolysis are in a rather large extend, different according to the temperature applied, 40°C or 50°C. The correlation circle represented in figure 1 is useful in interpreting the meaning of the axis. It shows a projection of the initial variables in the factors space. This figure permits, at the same time, to look at the data on a two dimensional map, and to identify trends.

3.2 Volatile compounds in hydrolysates

57 molecules were separated and 37 were identified. The analysis of these results was performed in an original way: olfactory notes of each compound as well as their formation pathway were searched in scientific publications. All these data were used for PCA in order to relate them with the sensory analysis results.
The table 2 shows the differences between the samples and how the link between the molecules and their olfactory note and origin was done.

Table 2 : Example of the identified molecules in the sole hydrolysates and their olfactory note and possible origin. +++ : quantity of molecule > 50µg eq.IS/L ; ++ : 20µg eq.IS/L < quantity of molecule < 50µg eq.IS/L ; + : 4µg eq.IS/L < quantity of molecule < 20µg eq.IS/L ; - : absence of the molecule

Molecule	Hydrolysates				Olfactory note	Origin
	T40, t3	T40, t6	T50, t3	T50, t6		
Hexanal	+	++	+	+++	Green (12, 14, 21), Grass (21), fatty	PUFA Oxydation (ω6) (9, 21)
3-Methyl Butanal	-	-	+	+	Almond, cacao, green, coffee (34)	Fat Oxydation (9)
Heptanal	+	+	-	+	Green (12, 14), fatty, roasted, lemon (12, 14)	PUFA Oxydation (ω3) (9)
1-Octen-3-ol	+	++	+	++	Mushroom (21)	PUFA Oxydation (ω6) (9, 20)
S-Methyl ester ethanethioic acid	-	++	-	+++	sulphurous	Protein degradation (18)
Dimethyl disulphide	-	+++	-	+++	Butter, sulphurous (13), cabbage (22)	Protein degradation (18)
Dimethyl trisulphide	-	+	-	+	Sulphurous	Protein degradation (18)

3.2.1 Compounds with a fatty acids origin

Fatty acids omega 3 and omega 6 degradation allows the formation of many volatile molecules. Compared to earthly animals that have plenty of 18 carbon fatty acids, marine products have a lot of fatty acids with 20 or 22 carbons. These fatty acids can be transformed into volatile compounds in many ways.

By action of lipoxygenases which are very active and which have a big activity in skin and viscera. Molecules resulting from this degradation are primarily alcohols and ketones with 8 carbons which bring fresh, vegetable and metal notes to sea products although these compounds have individually mushroom and geranium notes. In sole by-products hydrolysates several molecules which could result from degradation of fatty acids by lipoxygenases are found:

- 3-pentanone (ether note)
- pentenal (almond, malted, spicy notes)
- 1-penten-3-ol (marine (3), butter, spicy, fatty, chemical (34) notes)

- 1-pentanol (wine, ether, lemon, acids notes)
- (E)-2-pentenal (green, apple notes)
- (E)-2-penten-1-ol (mushroom (13,14, 19) notes
- (Z)-2-penten-1-ol (green, plastic, rubber notes)
- 1-hexen-3-ol (oxidized, fatty notes)
- hexanal (green (12, 14, 21), grass (21), fatty notes)
- (Z)-3-hexenal (green, apple, grass, grape notes)
- (E)-2-hexenal (green, burnt notes)
- 1-hexanol (resin, flower, green, fatty notes)
- 3-octonone and 2,3 octanedione (burnt, caproic, metallic notes)
- 1-octen-3-ol (earthy, mushroom (21) notes)
- octanal (fatty, lemon (12, 14, 19), soap notes)
- (E)-2-octenal (cucumber (21), almond (20), green, nutty, fatty notes)
- (E)-2-octen-1-ol (mushroom notes)
- 1-octanol (moss, nutty, mushroom notes)
- (E, E)-3,5-octadien-2-one (geranium, metallic notes)
- 2-Nonanone (hot milk, soap, green notes)
- nonanal (vegetables, fatty (22), lemon, green (3) notes)
- nonanol (fatty, grass notes)
- (E)-2-nonenal (fatty, cucumber notes)
- (E, Z)-2,6-nonadienal (cucumber (19), green, fatty, melon notes)

6 carbon volatile compounds need 15-lipoxygenases to transform poly unsaturated acids into their hyperperoxidated intermediary which is degraded, either directly or helped by an adapted lyase, in corresponding aldehyde with 6 carbonnes. Then, an isomerase is needed to transform molecules like (Z)-3-hexanal into (E)-2-hexenal. Formation of corresponding alcohols requires the reduction of precursors via a deshydrogenase.
For compounds with 9 carbons, the mechanisms of formation of the volatile molecules are homologous with those with 6 carbons. It was show that for these molecules, aldehydes are present in greater quantity than the corresponding alcohols.
By auto-oxidation, we can note that historically fish taste and odours were associated with polyunsaturated fatty acids (PUFA) auto-oxidation, especially ω3 fatty acids. In sole by-products hydrolysates several molecules resulting from this degradation were found:
Propanal (solvent and pungent notes)
Hexanal (green (12, 14, 21), grass (21), fatty notes)
Heptanal (green (12, 14), burnt, fatty, rancid, lemon (12, 14) notes)
(E)-2-heptenal (soap, almond, fatty (12) notes)
2,4-heptadienal (acid, fatty, putrid notes)
Decanal (marine (20), soap, orange peel notes)
3-dodecen-1-al
(E)-2-decenal (tallow note)
2,4-decadienal (fatty note)

Polyunsaturated lipids are not only lipid precursors of seafood flavor. Indeed, volatile compounds derived from carotenoids strongly contribute to the characteristics seafood flavors, especially to algae ones. These volatile compounds give to the product several olfactive notes like green, grass, violet, geranium notes. In sole by-products hydrolysates only, the 6-methyl-2-heptanone derived from carotenoid was identified.

3.2.2 Compounds with a sulphurous origin

Traditionally flavours resulting from sulphur molecules were associated with seafood degradation, but this kind of molecules has been found in sea animals that were alive. This fact shows that

finding these molecules does not imply spoilage of the fish or seafood. In the samples, many sulphurous molecules were found:
Thiophen (garlic note)
S-methyl ester ethanetioic acid
Dimethyl disulphide (sulphurous (13), rubber, green, butter notes)
Dimethyl trisulphide (sulphurous, cabbage)
S-methyl ester hexanethioic acid

3.2.2 Compounds derived from Maillard reaction

As it can be seen on several publications, volatile products of Maillard reaction can be classified into the following 3 groups:
“simple” sugar dehydratation/fragmentation products. By this way many different compounds are obtained: furan, pyrones, cyclopentenes, carbonyls and acids. Two molecules in sole by-products hydrolysates were found which could have these origins: acetic acid and 2-ethyl-furan.
“simple” amino acid degradation products. Because of the Strecker degradation many aldehydes are produced during the Maillard reaction. Molecules like 3 or 2-methyl butanal were found in sole by-products hydrolysates. These molecule can come from the lipids degradation or from Strecker reaction of the leucine and Isoleucine amino-acids.
Volatiles produced by further reactions like adol condensation. Molecules like pyroles, pyridines, imidazoles, pyrazines, oxazoles, thiazoles, thiophenes are produced by this kind of reactions. Compounds like 3-methyl thiopene are found in the sole by-products hydrolysates.

3.3. Relation between sensory criteria and analysed volatile compounds

In order to find relations between sensory characteristics and volatile compounds analysed by GC-SM, a principal component analysis was performed on volatile compounds. Most discriminant compounds were used as active variables and sensory descriptors were added as illustrative variables (fig. 2).

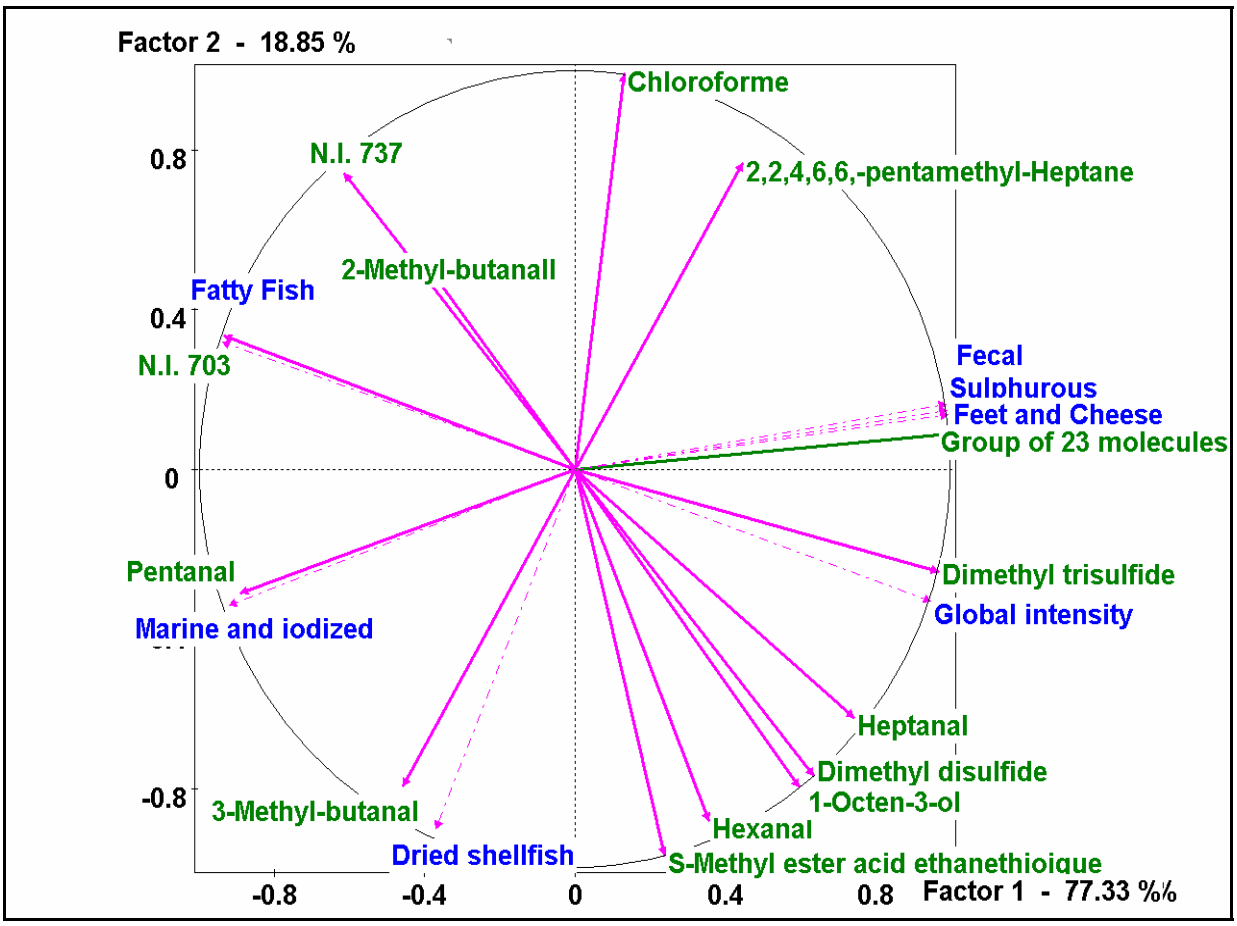

Figure 2: PCA correlation circle representing the sensory descriptors and the identified molecules: → continuous active variables (molecules); - - - continuous illustrative variables (descriptors)

Horizontal axis which represents 77,3% of variability is linked with strong descriptors like feet and cheese, faecal, sulphurous and global intensity opposed to fresh descriptors like marine and iodized. Vertical axis which represents only 18.9% of the variability is linked to the dried shellfish descriptor.

Some correlations appear between descriptors of the sensory analysis and the molecules identified by GC-SM:

Global intensity of samples is linked to suphurous molecules

Descriptors like feet and cheese, faecal and sulphurous are linked to a groupe of 23 molecules (table 3) which gather a large range of molecules (ketones, aldehydes, alcanes) and therefore can explain a complex sensory perception.

Marine and iodized are linked to the pentanal

Fatty fish is linked to the Non Idebtified molecule RI 703

Dried shellefish is linked to the 3-methyl-butanal

Table 3: Group of 23 molecules with their retention index on DB-5 colum and theur olfactory notes. NI: non identified molecules

Molecules	Retention index (DB-5 colum)	Theoretical olfactory notes
Methane thiol	674	unknown
3-methyl-butanal	697	Green, coffee (34)
NI	777	
(E)-2-hexanal	819	Green (23)
NI	820	
Stryrene	847	
1-(methylthio)-pentane	902	
Benzaldehyde	913	Almond (22)
1-heptanol	918	Mushroom (34)
NI	935	
6-methyl-2-heptanone	957	
Octanal	965	Citrus fruit (12, 14, 19)
NI	1001	
(E)-2-octenal	1044	Cucumber, almond (20)
Methanethiol caproate	1063	
2-nonanone	1070	Rot, fruity (23)
Undecane	1076	Alkane (34)
Nonanal	1081	Green (3), fatty (22)
NI	1152	
decanal	1196	Marine, cucumber (20)
Dodecane	1200	Alkane (34)
2-undecanone	1298	Fruity, citrus fruit (23)
(E,E)-2,4-decadienal	1338	Fat, fishy (34)

3.4. Relation between sensory criteria and theoretical aromatic notes

The quantity of each molecule was associated with olfactory notes. A PCA was done with the data obtained. Horizontal axis which represents 67.55% of the variability is linked with the global intensity and to strong descriptors like feet and cheese, faecal and sulphurous opposed to fresh descriptors like fatty fish.

3.5. Relation between sensory criteria and the origin of the molecules

The possible origins of each molecule found in the hydrolysates were studied and the quantity of each molecule was associated with the different origins to make a PCA in order to have an overview on the possible origins of the sensory criteria (fig. 3).

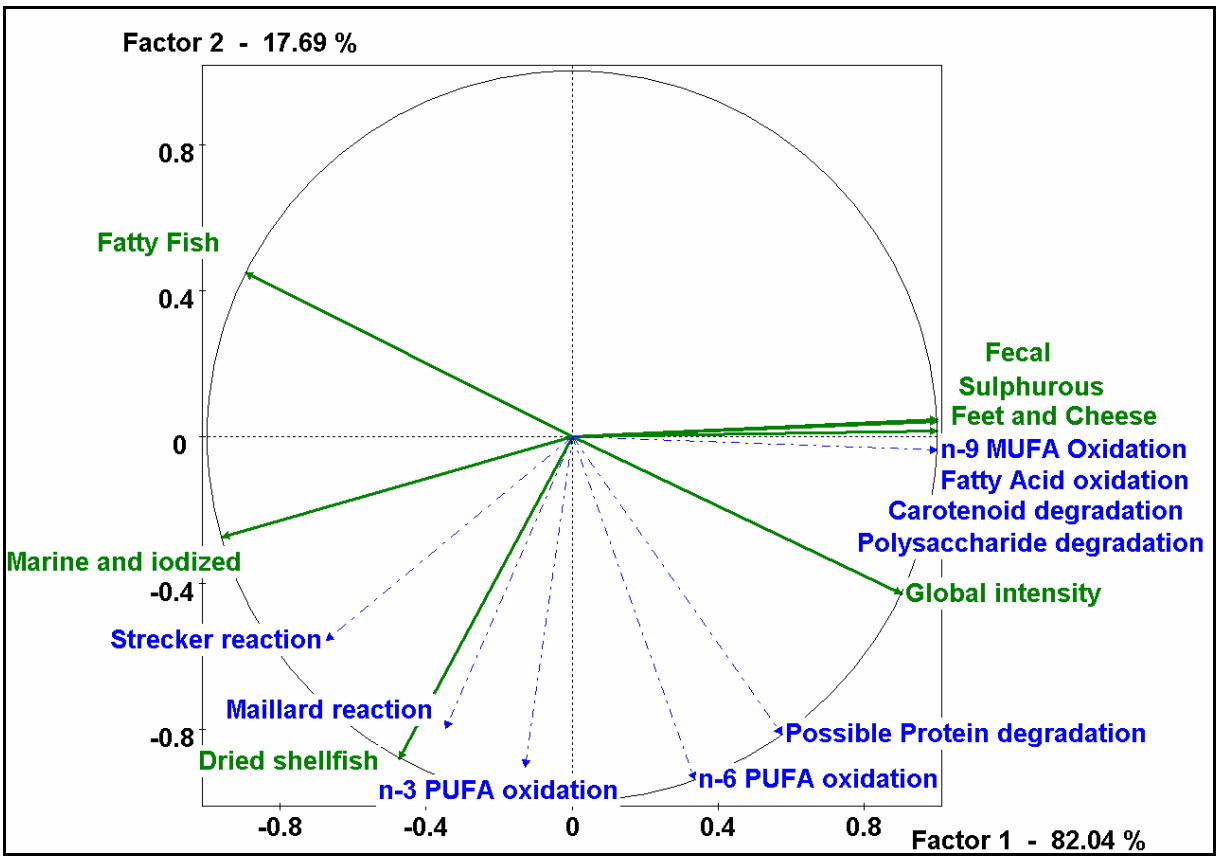

Figure 3: PCA correlation circle representing descriptors and the origins of the molecules: → continuous active variables (origins); - - - continuous illustrative variables (descriptors)

Horizontal axis which represents 82.04% of variability is linked with strong descriptors like feet and cheese, faecal and sulphurous and the global intensity opposed to fresh descriptors like marine and iodized.
Vertical axis Vertical axis which represents only 17.6% of the variability is linked to the dried shellfish descriptor.
The correlation between sensory analysis descriptors and origins of the different compounds was observed:
Dried shellfish descriptor is correlated with the Maillard and PUFA n-3 origin mechanisms
Faecal, sulphurous and feet/cheese descriptors are linked to the fatty acid oxidation, polysaccharides and carotenoids degradation.

This study authorized the determination of the parameters of hydrolysis which should be used depending on the olfactory note wanted. If marine sea-product note (marine, iodized, dried crustacean notes) is searched, time and temperature of hydrolysis must be optimized. Instead of that, if sulphur not must be prioritized, hydrolysis temperature must be around 40°C and hydrolysis time must be long (around 6 hours).

However, this work shows that the choice of hydrolysis parameters has an important role on the aromatic and sensory properties of the final product. Today these by-products are considered as a potential resource instead of a waste **(Dumay, 2006)** and the way of valorization by controlling the parameters make possible to obtain hydrolysates with targeted properties. So, it may be compatible with the future use as aromatic ingredients.

4. Conclusion

This work demonstrated that by-products from tongue sole were solubilised by hydrolysis with Protamex. The results of study permit to consider utilization of sole hydrolysates as aromatic ingredients. Existence of many typical marine aromatic molecules in hydrolysates consent to distinguish many possible applications under their liquid form, as well in human food industry as in Pet food industry. In human industry these products would be used as enhancer for marine aroma in fish based preparations such as soups or quiches. Another possible utilization is as aroma basis for the ready-cooked dishes. Otherwise these hydrolysates can be used in Pet food industry as palatability enhacers for dogs, cats or ornamental fishes which are strongly attracted by products that have a strong smell.
A different possible use of these hydrolysates is their use after stabilization by atomization,that is to say under their powder form, as coating aroma for alimentary products for human consumption like chips or extruded crackers. This kind of powder can be used as enhancer for Pet food industry using it as a coating product for biscuits or dry-food for pets.

Acknowledgement

The authors are grateful to the seafood processing company “Pirogue bleue” (Dakar-Sénégal) for providing the raw materials for this research.

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