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Effect of different treatments on the quality of cuttlefish (*Sepia officinalis* L.) viscera

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Abstract

The effects of different preservation times (cuttlefish auction), transformation factory treatments, different times of frozen storage at −20 °C and various defrosting temperatures were investigated with respect to the chemical changes of the viscera of cuttlefish (*Sepia officinalis*). During auction storage, pH and TCA (trichloroacetic acid)-soluble protein concentration decreased, whereas intracellular (cathepsins, total acid proteases and acid phosphatases) and extracellular (amylase, chymotrypsin, trypsin and total alkaline proteases) enzymatic activities increased. In cuttlefish transformation factories, pH value and TCA-soluble protein concentration increased. In transformation factory conditions, lipase and amylase levels varied, while other assayed enzymes were stable. Moreover, during transformation factory treatment, the molecular weight of proteins/peptides decreased and the oil composition evolution reflected hydrolysis of cuttlefish viscera. Thus, cuttlefish viscera fatty acid composition differed between fresh viscera and factory viscera. Despite changes in the fatty acid chain composition during factory transformation, the percentage of poly-unsaturated fatty acids remained high. Frozen storage implied major changes in viscera quality such as an increase in pH and a decrease in the quantity of high molecular weight protein. Moreover, the higher the defrosting temperature, the higher was the pH. During storage at −20 °C the quantities of proteins, carbohydrates and lipids decreased slightly.

Keywords: Auction; Cuttlefish *Sepia officinalis*; Defrosting; Digestive enzymes; Factory transformation; Gel filtration; Molecular weight; Oil composition; Storage; TCA-soluble proteins

1. Introduction

Cephalopods are an important economic resource for global fisheries. Traditionally, viscera have been considered as waste and have been utilized only to a minor extent (Gildberg & Almas, 1986). Nevertheless, cuttlefish viscera represent an important part of the cuttlefish mass (15–25%), and thus their waste represents an important commercial loss. Moreover, the digestive gland, which corresponds to 15% of viscera, contains about 10% of lipids rich in poly-unsaturated fatty acids (Boucaud-Camou, 1973). Because of their biochemical composition, viscera could be the basis of marine autolysates for use as feed in aquaculture diets. In order to make cuttlefish viscera autolysate it is essential to study the activities of hydrolytic enzymes in viscera during different treatments (auction, transformation factories, freezing and defrosting) to reveal mechanisms of autolysis and viscera quality.

Although frozen storage can inhibit microbial spoilage, tissue proteins undergo a number of changes, which modify their structural and functional properties (Mackie, 1982). These changes include protein insolubility, formation of aggregates and mechanical damage during frozen storage (Badii & Howell, 2001, 2002). Enzymes and other components are released (Nilsson & Ekstrand, 1993). Moreover, lipid oxidation is one of the major problems in frozen animals, since cuttlefish contain significant levels of poly-unsaturated fatty acids, especially eicopentaenoic acid (20:5$n-3$) and docosahexaenoic acid (22:6$n-3$). Different parameters influence viscera autolysis such as endogenous enzymes, which determine the autolysate quality, pH,

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which is an essential parameter to enzymatic activity, and temperature, which influences the degree of autolysis reached after storage and the degree of hydrolysis of the amide groups (Haaland & Njaa, 1989). Nevertheless, storage conditions determine the viscera quality and enzymatic degradation. Then, the effects of different preservation times (cuttlefish auction), transformation factory treatments, different times of freezing at −20 °C and various defrosting temperatures were investigated on chemical changes of cuttlefish (*Sepia officinalis*) viscera. Therefore, we assayed enzymatic activity, pH, the TCA (trichloroacetic acid)-soluble protein level (which reflects the degree of peptide hydrolysis bonding), the quantities of proteins, lipids, carbohydrates and the molecular weights of proteins and peptides.

2. Material and methods

2.1. Biological material

Viscera were obtained from cuttlefish *S. officinalis* caught in the English Channel during autumn 2003 and were removed from anaesthetized live cuttlefish. Auction cuttlefish viscera came from cuttlefish caught in the English Channel during autumn 2003 by fisherman and purchased in the Port en Bessin cuttlefish auction room. Factory viscera came from cuttlefish purchased in the fishing port auction, which were subsequently frozen for 4 months at −20 °C, then defrosted in seawater at 10°C for two days and eviscerated.

Auction cuttlefish were stored at −20 °C for different times and defrosted at 4, 25 or 40 °C with the aim of determining the quality of frozen viscera and the impact of defrosting temperature.

After harvesting, all viscera were immediately disrupted, frozen in liquid nitrogen and lyophilized until analyses.

2.2. Analysis

2.2.1. pH

One gramme of dry tissue was added to 8 ml of double distilled water. After vortexing, the pH was estimated with a pH-meter.

2.2.2. TCA-soluble protein and protein content

The TCA-soluble protein concentration was assayed according to the Benjakul et al., method (Benjakul, Visessanguan, & Tueksuban, 2003). Nine milliliters of TCA 5% (w/v) were added to 1 g of dry tissue. After homogenization, the mixture was stored for 1 h at 4 °C, and then centrifuged at 5000*g* for 5 min at 4 °C. Protein content was estimated using the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951).

The amount of protein in each extract (in extraction buffer) was determined by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

2.2.3. Molecular weight of proteins and peptides

Dry tissue was added to Tris buffer containing 10 mM Tris–HCl and 150 mM NaCl, pH 8 (0.1 g of tissue to 10 ml). The mixture was stored at 4 °C for 1 h, then centrifuged at 10,000*g* for 10 min at 4 °C. The supernatant contains the Tris soluble proteins. The protein content was assayed according to the Lowry et al. (1951) method using BSA as standard. The protein concentration in the supernatant was then adjusted to 10 mg/ml. The molecular weight of the extract obtained was estimated using a Sephadex G25 column (Pharmacia G25M PD 10 Sephadex), which had been calibrated with the following molecular weight standards: β-amylase (200,000), alcohol deshydrogenase (150,000), albumin (66,000), carbonic anhydrase (29,000), vitamin B12 (1350). The amount of protein in the different fractions (500 µl) was measured by absorbance at 280 nm.

2.2.4. Enzyme extraction and measurements

2.2.4.1. Extraction. For total alkaline proteases, trypsin, chymotrypsin, lipases, carboxypeptidases A and B, and amylase assays, the extraction was carried out in buffer (1 ml of buffer per 60 mg of sample) containing: 0.09 M TRIS-base, 0.08 M boric acid, 3 mM EDTA, 0.5 mM mercapto-ethanol, glycerol 10%, pH 8.3 (Koueta, 1983; Le Bihan, Zatylny, Perrin, & Koueta, 2006; Perrin, 2004; Perrin, Le Bihan, & Koueta, 2004). The crude extract was centrifuged for 30 min at 10,000*g* at 4 °C, and the supernatant used for assays.

For total acid proteases, total acid phosphatases and cathepsin assays, the extraction was carried out in 2.5 volume of the extraction buffer (1% KCl containing 1 mM of EDTA) (Le Bihan, Perrin, & Koueta, 2004).

The homogenate was centrifuged for 60 min at 10,000*g* at 4 °C. The supernatant was used for the assays.

2.2.4.2. Enzymatic assays. The amylase activity was assayed using starch as substrate. Substrate buffer contained starch at 1% in a monobasic phosphate buffer at 20 mM and 6.7 mM of sodium chloride, pH 6.9. 100 µl of substrate buffer were added to 100 µl of extract (Le Bihan et al., 2006). The incubation was for 3 min at 20 °C, after which 100 µl of revelator (tartrate sodium potassium 0.94 M, NaOH 0.4 M and 3-5-dinitrosalicilic acid 48 mM) was added and incubated for 15 min in boiling water. The absorbance was recorded at 640 nm. Enzyme activity was expressed as specific activity (U mg^{-1} protein).

Carboxypeptidases A and B activities were assayed using the Folk methods and enzyme activities were expressed as specific activity (U mg^{-1} protein) (Folk & Schirmer, 1963).

Trypsin activity was measured using the Tsunematsu method (Tsunematsu, Nishimura, Mizusaki, & Makisumi, 1985; Villanueva, Koueta, Riba, & Boucaud-Camou, 2002), and chymotrypsin using the Delmar method (Delmar, Largman, Brodick, & Geokas, 1979). Enzyme activity was expressed as specific activity (U mg^{-1} protein) where

one enzymatic unit corresponds to 10 mmoles L^{-1} of pNa min^{-1}.

Total acid and alkaline proteases were measured using the Charney method (Charney & Tomarelli, 1947; Van Wormhoudt & Sellos, 1980). Enzyme activity was expressed as specific activity (U mg^{-1} protein), where one unit correspond to the variation of one unit of O.D. min^{-1}.

Lipase activity was assayed according to a modified method (Versaw, Cuppett, Winters, & Williams, 1989) using β-naphtyl caprilate as substrate. The assay mixture contained: 50 μl of 200 mM sodium taurocholate; 450 μl of BES 50 mM pH 7.2, 100 μl of extract and 5 μl of substrate at 200 mM (dissolved in DMSO). The mixture was incubated for 30 min at 40 °C, then 5 μl of fast Blue BB 100 mM (dissolved in DMSO) was added. The second incubation was for 5 min at 40 °C. The reaction was stopped by addition of 50 μl of trichloroacetic acid 0.72 N and 675 μl of 95% ethanol/ethylacetylate solution (V/V). The colored product was read at 540 nm. The lipase activity was expressed as specific activity (U mg^{-1} protein).

Cathepsin activity was measured using hemoglobin as substrate (Bonete, Manjon, Llorca, & Iborra, 1984; Le Bihan et al., 2004). Appropriate blanks were used, and the products were evaluated by using the Folin-Lowry reaction employing tyrosine as standard (Barrett & Kirschke, 1981). The activity was expressed as specific activity (U mg^{-1} protein) where one enzymatic unit corresponds to one micromole of tyrosine mg^{-1} protein.

Total acid phosphatase activities were determined using *p*-nitrophenyl-phosphate 2% as substrate (Moyano, Diaz, Alarcon, & Sarasquete, 1996). Total acid protease activity was expressed as specific activity (μU mg^{-1}.prot) where one enzymatic unit corresponds to 1 mmole L^{-1} of *p*-nitrophenol.

2.2.5. Viscera oil composition

Fatty acids were extracted using the Bligh and Dyer method (Bligh & Dyer, 1959). They were methylated in order to eliminate glycerolipids and phospholipids. Next, they were separated using liquid chromatography to obtain fatty acids methylated extract (FAME). FAME were run through a FAREWAX gas chromatography column (Restek), 30 m * 0.32 mm, with polyethylene glycol.

2.2.6. Total protein concentration

Dry tissue (10 mg) was digested in NaOH 1 M (10 ml) during 12 h at 20 °C. Then, total protein concentration was determined by the method of Lowry et al. (1951).

2.2.7. Total carbohydrate concentration

Total carbohydrates were extracted by the methods of Staats (Staats, De Winder, Stal, & Mur, 1999) and assayed according to the method of Dubois (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.2.8. Total lipid concentration

Total lipids were extracted by the methods of Bligh and Dyer (1959) and assayed according to the method of Marsh and Weinstein Marsh and Weinstein (1966).

2.3. Statistical analysis

Results are given as mean ± standard deviation ($n = 6$ for each treatment). Data between treatments were compared with an ANOVA followed by a Tukey's test when significant differences ($p < 0.05$) were found (Sokal & Rohlf, 1981).

3. Results and discussion

3.1. pH

In auction cuttlefish, viscera pH was significantly lower ($p < 0.05$) to that in viscera from live cuttlefish, whereas pH in viscera from live cuttlefish was significantly lower ($p < 0.05$) to that in viscera from cuttlefish transformation factories (Table 1). Moreover, we can observe, Table 2, that freezing cuttlefish caused a significant increase of pH. Subsequently, pH increased slowly during the storage of cuttlefish at −20 °C. Therefore, we observed a decrease in pH a

Table 1
Effect of different storage treatments on cuttlefish viscera

	Live	Auction	Factories
pH:	6.42 ± 0.01	6.33 ± 0.01[a]	6.55 ± 0.01[a]
TCA-soluble proteins:	48.7 ± 8	35.6 ± 11[a]	58.9 ± 5[a]
Total alkaline proteolytic activity (U[a] 10^{-5}/mg proteins):	4.8 ± 0.01	8.3 ± 0.005[a]	6.1 ± 0.01[a]
Amylase activity (U[a] 10^{-4}/mg proteins)	2 ± 0.008	2.7± 0.003[a]	0.96 ± 0.004[a]
Carboxypeptidases A activity (U[a] 10^{-4}/mg proteins)	2.4 ± 0.001	2.2 ± 0.005	2.6 ± 0.001[a]
Carboxypeptidases B activity (U[a] 10^{-4}/mg proteins)	2.3 ± 0.008	2.3 ± 0.001	2.9 ± 0.005[a]
Chymotrypsin activity (U[a] 10^{-9}/mg proteins)	9.8 ± 0.1	13 ± 0.05[a]	9.9 ± 0.1
Trypsin activity (U[a] 10^{-9}/mg proteins)	7.9 ± 0.8	14 ± 0.3[a]	15± 0.4[a]
Lipases activity (U[a] 10^{-4}/mg proteins)	1.9 ± 0.01	1.9 ± 0.05	1.3± 0.01
Cathepsin activity (U[a] 10^{-3}/mg proteins)	7.2 ± 0.8	10.9 ± 0.11[a]	15 ± 0.5[a]
Total acid proteolytic activity (U[a] 10^{-5}/mg proteins)	9 ± 0.1	18 ± 0.5[a]	40 ± 0.1[a]
Acid phosphatases activity (U[a] 10^{-3}/mg proteins)	6 ± 0.8	7.7 ± 0.3[a]	8.4 ± 0.4[a]

[a] Significantly different from viscera from live cuttlefish ($p < 0.05$).

Table 2
Impact of storage time and defrosting temperature on cuttlefish viscera

	Time storage at −20 °C									
	0 days (auction)	15 days			30 days			60 days		
Temperature of defrosting	–	4 °C	25 °C	40 °C	4 °C	25 °C	40 °C	4 °C	25 °C	40 °C
pH	6.35 ± 0.05	6.51 ± 0.01	6.55 ± 0.05	6.62 ± 0.02	6.53 ± 0.01	6.61 ± 0.01	6.7 ± 0.015	6.58 ± 0.02	6.64 ± 0.01	6.73 ± 0.01
Total proteins (g/100 g)	85.79 ± 5	52.66 ± 8	42.6 ± 11	44.37 ± 9	24.85 ± 5	40.82 ± 6	36.37 ± 4	21.79 ± 4	15.66 ± 9	14.3 ± 6
Total carbohydrates (g/100 g)	2.14 ± 0.2	1.26 ± 0.1	1.05 ± 0.05	1.2 ± 0.1	1.43 ± 0.1	1.17 ± 0.05	0.76 ± 0.02	1.16 ± 0.03	1.22 ± 0.04	0.69 ± 0.02
Total lipids (g/100 g)	1.86 ± 0.5	4.4 ± 0.8	4 ± 0.3	5.15 ± 0.8	6.13 ± 0.4	6 ± 0.6	7 ± 0.4	3.54 ± 0.6	5.6 ± 0.9	4.7 ± 0.5

few hours after the animal death. The death of cuttlefish implied lysosome breaking, which enhances the liberation of acidity. In previous published assays of pH in pre-rigor fillets of Atlantic salmon (Einen, Guerin, Fjaera, & Skjervold, 2002), it has been observed that pH of fresh fillets decreased rapidly from pH 6.6–6.3 after 10 h at refrigerated storage (3–4 °C). The death of an animal initiates major changes in the biochemical features of tissue leading to its disintegration, which include a decrease in pH and a reduction in the integrity of the cell membranes (Delbarre-Ladrat, Verrez-Bagnis, Noel, & Fleurence, 2004). These changes interact with other biochemical processes occurring after death, especially proteolysis.

Nevertheless, during frozen storage in cuttlefish transformation factories (Table 2), the pH increases as observed by other authors (Hultmann & Rustad, 2004) in their experiments on Atlantic salmon, where the pH value increased during the storage period.

3.2. TCA-soluble proteins

In auction cuttlefish, viscera concentrations of TCA-soluble proteins were significantly lower ($p < 0.05$) than TCA-soluble proteins measured in viscera from live cuttlefish, whereas they were significantly higher ($p < 0.05$) in transformation factory viscera (Table 1). So, we observed a decrease of TCA-soluble proteins a few hours after the animal's death. The death of animals leads to the lysosomes breaking, which enhanced liberation of acidity and the decrease of TCA-soluble protein concentration. During factory storage pH increases, which is linked to the increase of TCA-soluble proteins.

Benjakul and Bauer (2001) used the TCA-soluble proteins measured as an index of autolytic degradation products. Using this method, they observed an increase of autolytic degradation products after a freeze–thaw cycle and storage at 2–4 °C for 3 days. Therefore, freeze-thawing may affect the distribution of proteases in tissues, leading to the hydrolysis of proteins. As reported by Benjakul et al. (2003) TCA-soluble proteins in cuttlefish viscera increased during iced storage, suggesting the autolytic degradation of cuttlefish proteins.

In factories, cuttlefish are maintained frozen during several months. Ruiz-Capillas, Moral, Morales, and Montero (2002) observed that 5% NaCl protein solubility in the muscle of volador (*Illex coindetii*) was higher than 60% throughout frozen storage and exhibited an increasing solubility in the initial months. Moreover, an initial increase of protein solubility was also observed during frozen storage of sardine muscle (Montero, Gomez-Guillen, & Borderias, 1996). This could be because in the early stages of storage, there is, as a result of the formation of ice crystals, a redistribution of hydrogen bridges and hydrophobic interactions, leading to denaturation (Matsumoto, 1980). This phenomenon could also result from the intense activity of cephalopod enzymes, which is several times greater than in fish (Ruiz-Capillas et al., 2002).

3.3. Molecular weight of proteins and peptides

Changes in the molecular weight of proteins and peptides in viscera according to their storage are presented in Figs. 1 and 2. The quantity of high molecular weight proteins (greater than 20 kDa) decreased during freezing, while, amounts of low molecular weight proteins (lower than 6.5 kDa) stay similar during storage. Thus, freezing implied major changing in the molecular weight distribution, because of the denaturation of high molecular weight proteins (Privalov, 1990).

3.4. Extracellular enzyme specific activity

In viscera from auction, specific activity of amylase was significantly higher ($p < 0.05$) to activity measured in viscera from live cuttlefish, while it was significantly lower ($p < 0.05$) to factory viscera (Table 1). Therefore, a few hours after the animal death, we observed an increase of amylase activity in cuttlefish viscera. Nevertheless, amylase activity was lower in factory viscera, in which we observed a degradation of this activity during factory transformation due to the instability of amylase in these storage conditions.

In factory viscera, specific activities of carboxypeptidases A and B were significantly higher ($p < 0.05$) to activity measured in viscera from live cuttlefish (Table 1). Thus, carboxypeptidases are very stable under the tested storage conditions.

In viscera from auction, specific activity of chymotrypsin was significantly higher ($p < 0.05$) to activity measured in viscera from live cuttlefish (Table 1). However, chymotrypsin activity was lower in factory viscera than in auction viscera, which demonstrated a slight degradation of this activity during factory transformation.

In auction and factory viscera, the specific activity of trypsin was significantly higher ($p < 0.05$) than the activity measured in viscera from live cuttlefish (Table 1). Moreover, this activity stays high in factory viscera. Thus, trypsin was stable in the tested conditions. Chymotrypsin and trypsin are quantitatively important in the digestive system of carnivores due to their high proteolytic activities (Heu, Kim, & Pyeun, 1995). They actively participate in viscera autolysis.

In auction and factory viscera, specific total proteolytic alkaline activity was significantly higher ($p < 0.05$) than activity measured in viscera from live cuttlefish (Table 1). The death of cuttlefish leads to the degradation of zymogene vesicles. Then, a few hours after the animal death, we observed an increase in total proteolytic alkaline activity in cuttlefish viscera. Nevertheless, total proteolytic alkaline activity was lower in factory viscera than in auction viscera, which demonstrated a little degradation of this activity during factory transformation.

Viscera contain a variety of digestive proteases, which play a role in the softening of abdominal tissue during post-mortem storage (Haard, 1992). Animal death results in major changes in the biochemical features of the tissue, leading to its disintegration (Delbarre-Ladrat et al., 2004). This suggests that zymogene vesicles present in cells, containing secreted proteolytic alkaline enzymes, break down a few hours after of the cuttlefish death inducing the increased alkaline protease activity (Le Bihan et al., 2006).

There were no significant differences between live cuttlefish viscera and other viscera with respect to specific lipase activity (Table 1). Thus, lipases were very stable in the tested storage conditions. Lipases are essential in autolysis because they determine the lipid quality in the final product. Their high stability over time could result in the

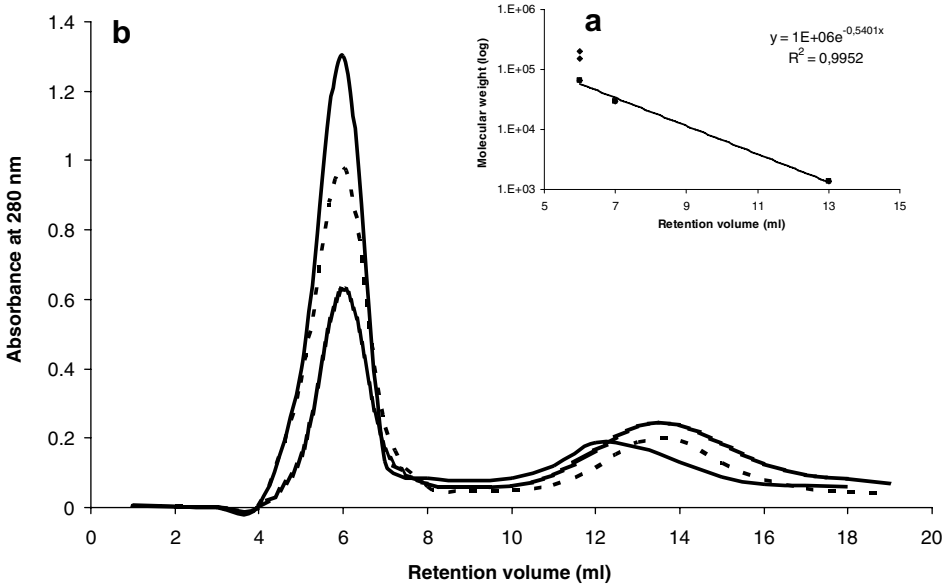

Fig. 1. (a) Calibration graph of molecular weight for Pharmacia G25M PD 10 Sephadex. (b) Elution profile of peptides and proteins in viscera from live cuttlefish. —, viscera from auction ---, viscera from factories ···.

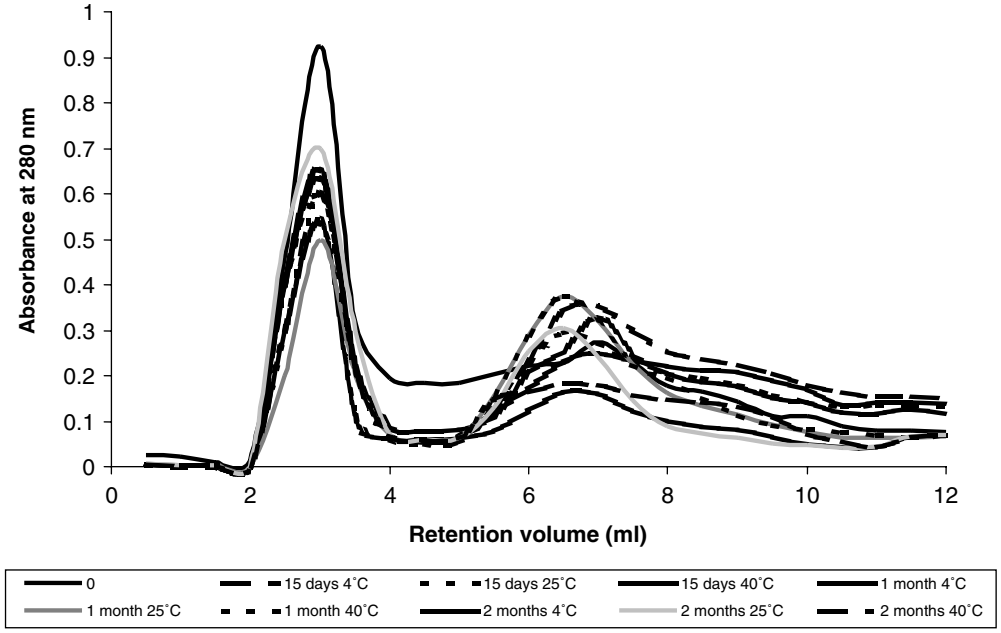

Fig. 2. Elution profile of peptides and proteins in viscera (absorbance at 280 nm).

accumulation of poly-unsaturated fatty acids in high quantity in the viscera autolysate.

Benjakul and Bauer (2001) used activities of enzymes such as glucosidase to observe the effect of freeze–thaw cycles on cell integrity of catfish fillets. They detected an increase in enzymatic activities in correlation to the disintegration of membrane structure. They concluded that freeze–thaw processing potentially disrupts the cells, leading to the release of enzymes from intracellular organelles into the sarcoplasm. Thus, the freezing resulted in the loss of membrane integrity. Moreover, Benjakul and Bauer (2001) observed that after 3 days of storage at 2–4 °C enzymatic activities decreased, probably due to the denaturation or instability of these enzymes during storage.

3.5. Specific activity of intracellular enzymes

In auction and factory viscera, the specific activity of cathepsins, total proteolytic acids and phosphatase acids were significantly higher ($p < 0.05$) than the activity measured in viscera from live cuttlefish (Table 1). Thus, we observed an increase of total proteolytic acids and phosphatase acids activities a few hours after animal death. The death of cuttlefish implied the lysosomes breaking, which improved the liberation of intracellular enzymes. Moreover, these activities were higher in factory viscera than in auction viscera. Thus, cuttlefish transformation in factories does not alter these activities.

Lysosomes are cytoplasmic granules that contain several acid hydrolases within the lysosomal membrane (Aoki & Ueno, 1997; Duve, Pressman, Gianetto, Wattiaux, & Appelmans, 1955). These hydrolases, such as catheptic enzymes, are considered to be the main cause of post-mortem tissue softening. The most important post-mortem change with regard to tissue degradation is considered to be the liberation of cathepsin from lysosomes (Aoki & Ueno, 1997; Haard, 1992). These authors observed the gradual decrease of cathepsins activities in the lysosomal fraction and the parallel increase in the supernatant fraction as the tissue aged at 0 °C. Thus, it appears that the cathepsins were liberated from lysosomes during ageing.

3.6. Fatty acid composition

Cuttlefish viscera fatty acid composition (Table 3) significantly ($p < 0.05$) differed between fresh viscera, auction viscera and factory viscera. The prominent fatty acids were C16:0, C22:6–3 and C20:5–3 in fresh viscera. In auction viscera, most important fatty acids were C16:0 and C22:6–3. Whereas in factory viscera the major fatty acid was C22:6–3. During auction and frozen storage the percentage of C22:6–3 increased, but that of C20:5–3 decreased. Despite changes in fatty acid composition during factory transformation, the percentage of poly-unsaturated fatty acids remained high. Our results are in accord with other published data, such as those of Jeong Jeong, Ohshima, and Koizumi (1999) who observed that the prominent fatty acids in muscle of giant scallop during frozen storage were poly-unsaturated fatty acids such as 20:5$n-3$, 22: 6$n-3$ and 20:4$n-6$ (Jeong et al., 1999).

As observed by Pirini, Gatta, Testi, Trigari, and Monetti (2000) in sea bass, we note that the high unsaturated fatty acid content is typical of oil of marine origin (20:5$n-3$; 22:6$n-3$). The $n-3$ poly-unsaturated fatty acid content was five times higher than that of $n-6$ fatty acids, which emphasizes the high quality of viscera fat from a cardiovascular point of view (Garcia-Arias, Alvarez Pontes, Garcia-Linares, Garcia-Fernandez, & Sanchez-Muniz, 2003; Pirini et al., 2000).

Table 3
Cuttlefish viscera oil composition (% of total oil extracted)

	Live	Auction	Factories
C14:0	3.74 ± 0.03	5.95± 0.02[a]	3.66 ± 0.01
C15:0	0.8 ± 0.004	1.02 ± 0.009[a]	0.68 ± 0.01[a]
C16:0	15.39 ± 0.05	15.42 ± 0.03	15.55 ± 0.04
C17:0	1.33 ± 0.01	1.45± 0.006[a]	1.59 ± 0.01[a]
C18:0	5.56 ± 0.12	6.14 ± 0.01[a]	6.37 ± 0.05[a]
C19:0	0.09 ± 0.005	0.09 ± 0.003	0.10 ± 0.0046
C20:0	0.16 ± 0.01	0.16 ± 0.03	0.13 ± 0.02
C22:0	0.08 ± 0.03	0.079 ± 0.01	0.09 ± 0.01
C24:0	0.53 ± 0.02	0.6 ± 0.04[a]	0.65 ± 0.01[a]
C14:1n9	0.21 ± 0.007	0.2 ± 0.005	0.17 ± 0.002[a]
C14:1n7	0.29 ± 0.002	0.25 ± 0.007	0.15 ± 0.003[a]
C15:1n8	0.06 ± 0.003	0.06 ± 0.001	0.05 ± 0.003
C16:1n7	7.05 ± 0.02	7.01 ± 0.08	5.73 ± 0.06[a]
C16:1n5	0.30 ± 0.02	0.32 ± 0.007	0.26 ± 0.004
C17:1n10	0.57 ± 0.01	0.58 ± 0.03	0.50 ± 0.004[a]
C17:1n8	0.10 ± 0.02	0.12 ± 0.01	0.18 ± 0.004
C18:1n9	8.07 ± 0.05	8.5 ± 0.07	9.23 ± 0.1[a]
C18:1n7	5.09 ± 0.1	5.01 ± 0.09	3.64 ± 0.04[a]
C18:1n5	0.33 ± 0.01	0.3 ± 0.007	0.32 ± 0.0014
C19:1n10	0.20 ± 0.01	0.18 ± 0.009	0.16 ± 0.01
C19:1n8	0.11 ± 0.02	0.12 ± 0.008	0.15 ± 0.02
C20:1n11	0.76 ± 0.05	0.7 ± 0.007[a]	0.39 ± 0.01[a]
C20:1n9	1.54 ± 0.05	2.01 ± 0.08[a]	2.54 ± 0.06[a]
C20:1n7	0.49 ± 0.005	0.4 ± 0.002[a]	0.36 ± 0.005[a]
C20:1n5	0.05 ± 0.01	0.04 ± 0.007	0.03 ± 0.002
C22:1n13+11	0.29 ± 0.05	0.33 ± 0.04	0.42 ± 0.05
C22:1n9	0.15 ± 0.04	0.17 ± 0.01	0.22 ± 0.03
C22:1n7	0.10 ± 0.01	0.1 ± 0.003	0.06 ± 0.01
C22:1n5	0.16 ± 0.02	0.16 ± 0.01	0.19 ± 0.01
C24:1n9	0.15 ± 0.01	0.16 ± 0.009	0.22 ± 0.01
C24:1n7	0.04 ± 0.005	0.04 ± 0.007	0.03 ± 0.01
C24:1	0.04 ± 0.01	0.04 ± 0.001	0.05 ± 0.008
C16:2n6	0.06 ± 0.02	0	0
C16:2n4	0.25 ± 0.02	0.1 ± 0.001[a]	0.07 ± 0.003[a]
C18:2n7	0.04 ± 0.01	0.05 ± 0.002	0.05 ± 0.002
C18:2n6	0.49 ± 0.02	0.5 ± 0.04	0.57 ± 0.01
C18:2n4	0.37 ± 0.01	0.3 ± 0.03[a]	0.18 ± 0.003[a]
C20:2n6	0.32 ± 0.002	0.30 ± 0.008	0.40 ± 0.002[a]
C22:2n6	0.04 ± 0.01	0.05 ± 0.009	0.05 ± 0.0007
C16:3n6	0.42 ± 0.01	0.5 ± 0.01[a]	0.63 ± 0.01[a]
C16:3n4	0.05 ± 0.002	0.025 ± 0.003[a]	0.02 ± 0.001[a]
C16:3n3	0.10 ± 0.003	0.1 ± 0.001	0.11 ± 0.001
C18:3n6	0.10 ± 0.01	0.08 ± 0.0008	0.05 ± 0.0007[a]
C18:3n3	0.28 ± 0.01	0.3 ± 0.003	0.35 ± 0.003[a]
C20:3n6	0.19 ± 0.002	0.15 ± 0.005	0.12 ± 0.003[a]
C20:3n3	0.09 ± 0.002	0.11 ± 0.003	0.11 ± 0.003
C16:4n3	0.11 ± 0.001	0.12 ± 0.007	0.11 ± 0.0007
C18:4n3	0.58 ± 0.01	0.6 ± 0.05	0.36 ± 0.01[a]
C20:4n6	3.87 ± 0.08	4.53 ± 0.08[a]	4.83 ± 0.04[a]
C20:4n3	0.41 ± 0.01	0.43 ± 0.001	0.41 ± 0.005
C22:4n6	0.61 ± 0.01	0.5 ± 0.004[a]	0.71 ± 0.003[a]
C20:5n3	13.90 ± 0.16	12 ± 0.03[a]	10.95 ± 0.04[a]
C21:5n3	0.43 ± 0.03	0.33 ± 0.001[a]	0.21 ± 0.004[a]
C22:5n6	0.44 ± 0.05	0.39 ± 0.004[a]	0.64 ± 0.004[a]
C22:5n3	2.88 ± 0.04	2.9 ± 0.03	2.78 ± 0.02
C22:6n3	14.91 ± 0.19	16.11 ± 0.05[a]	18.23 ± 0.03[a]

[a] Significantly different from viscera from live cuttlefish ($p < 0.05$).

3.7. Biochemical composition

Cuttlefish viscera from auction contain 86 g/100 g of proteins, 12 g/100 g of lipids and 2.1 g/100 g of carbohydrates (Table 3). These data are in accordance with other authors (Blanchier & Boucaud-Camou, 1982). The freezing of cuttlefish caused a significant decrease of proteins, lipids and carbohydrates viscera (Table 2). Moreover, the storage at −20 °C provokes a small decrease of protein quantity in cuttlefish viscera. Nevertheless, the proportions of proteins, lipids and carbohydrates stay similar during storage at −20 °C. The temperature of defrosting implied negligible modifications on viscera quality. Then, major changes were observed during the freezing of cuttlefish.

4. Conclusion

Different phenomena take place during the auction and factory storage of cuttlefish. Primarily, our results show a breakdown of lysosomes, which induces an increase in acid enzyme activities and a decrease of pH and TCA-soluble protein concentration. Secondly, the degradation of zymogene vesicles, which contain secreted enzymes, implied an increase in the concentration of secreted enzymes. Finally, we observed a slight hydrolysis of viscera. Cuttlefish viscera fatty acid composition differs between fresh viscera, auction viscera and factory viscera. The prominent fatty acids were C16:0, C22:6–3 and C20:5–3 in fresh viscera. In auction, major fatty acids are C16:0 and C22:6W3. In factory viscera, the main fatty acid was C22: 6–3. During frozen storage the percentage of C22:6–3 increased, but that of C20:5–3 decreased. Despite changes in fatty acid composition during factory transformation, the percentage of poly-unsaturated fatty acids was maintained high and the proportion between proteins, lipids and carbohydrates varied little. Thus, factory viscera conserved good capacities to be used for the production of autolysates.

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