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Food Chemistry 86 (2004) 85–91

Food Chemistry

www.elsevier.com/locate/foodchem

Free amino acids in muscle of Norway lobster (Nephrops novergicus (L)) in controlled and modified atmospheres during chilled storage

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Received 17 June 2003; received in revised form 18 August 2003; accepted 18 August 2003

Abstract

Changes in free amino acids (FAAs) in relation to freshness were examined in the muscle of Norway lobster during bulk storage in controlled and modified atmospheres containing two different gas mixes (1: $60/15/25$ and 2: $40/40/20$, $CO₂%/O₂%/N₂%$). The essential free amino acids in the highest concentrations were threonine, leucine, valine, lysine and arginine, all over 40 mg/100 g. Threonine, valine, lysine and arginine concentrations decreased significantly $(p < 0.05)$ during storage. These decreases were more pronounced in the lots kept in the CO_2 -rich mix (1) than the lots kept in the O_2 -rich mix (2) and this was independent of whether the storage was under controlled (C) or modified (M) atmosphere. The dipeptide anserine showed decreases with the same trend. The most abundant non-essential FAAs were glycine, alanine and glutamic acid (57.9, 57.2 and 31.2 mg/100 g, respectively), along with the dipeptide anserine (52.9 mg/100 g). The non-essential FAAs presented differences attributable to the gas mixes used. No differences were detected in any of the FAA groups studied with respect to the different types of atmospheres (C or M). Only ornithine and tryptophan levels increased significantly $(p < 0.05)$ in the course of storage. These increases were significantly $(p < 0.05)$ different only in the control lot. These FAAs would appear to be suitable as freshness indices for Norway lobster stored in ice. Moreover, the significant $(p < 0.05)$ decrease of the dipeptide anserine could be a suitable freshness index for Norway lobster stored in either ice or in atmospheres (C or M).

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Keywords: Anserine; Controlled and modified atmospheres; Chilled storage; Free amino acids; Norway lobster

1. Introduction

Free amino acids are one of the most important fractions of non-protein nitrogen in crustaceans. Many of these FAAs, such as alanine, glutamic acid and glycine, are responsible for flavour and taste. Alanine and glycine have sweet tastes, and glutamic acid has the ''umami'' taste typical of crustaceans (Yamanaka & Shimada, 1996). Free amino acids have also been used as quality indices in various fish and crustacean species. Ruiz-Capillas and Moral (2001a) reported that the FAAs b-alanine and 1-methylhistidine could be used, in combination with the dipeptide anserine, and tryptophan as

quality indices in both ice- and atmosphere-stored hake. In the case of molluscs and crustaceans, most authors recommend ornithine as a freshness index (Matsumoto & Yamanaka, 1992; Matsumoto & Yamanaka, 1990; Miyagawa et al., 1990; Otsuka, Tanaka, Nishigaki, & Miyagawa, 1992; Yamanaka & Shimada, 1996).

Free amino acids undergo considerable modification during processing and storage of fish, and therefore the different technologies used decisively influence the FAA profile in the course of storage (Matsumoto & Yamanaka, 1990; Ruiz-Capillas & Moral, 2001a). In the Spanish market, crustaceans, such as Norway lobster, are commercialized either frozen or chilled in boxes of ice. Commercialization of chilled Norway lobster has been increasing in recent years in response to growing consumer demand for the fresh product. Protective

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^{0308-8146/\$ -} see front matter © 2003 Elsevier Ltd. All rights reserved. doi:10.1016/j.foodchem.2003.08.019

atmospheres are now being successfully used as a coadjuvant to refrigeration to prolong the shelf life of many fish species, both in retail packaging and bulk storage (Lauzon, Stefasson, Jonsson, & Sveinsdottir, 2002; Olafsdottir, Xiuchen, Lauzon, & Jonsdottir, 2002; Ozogul, Taylor, Quantick, & Ozogul, 2000; Randell et al., 1999; Ruiz-Capillas, Morales, & Moral, 2001; Ruiz-Capillas & Moral, 2001b). In most of these cases, a modified atmosphere (M) has been used. This procedure consists of replacing the air in the container around the fish with a gas or a gas mixture and the modifications which occur inside the container are left unaltered during the storage period. On the other hand, for controlled atmospheres (C), which have been used less, as well as replacing the air inside the container, the mixture is maintained and regulated accordingly. In other words, the controlled atmospheres regulate the gas used and the humidity very closely and a monitoring system keeps these parameters within very strict margins (Ruiz-Capillas & Moral, 2001b; Stammen, Gerdes, & Caporaso, 1990).

The object of the present study was to assess changes in free amino acid contents in the muscle tissue of Norway lobster resulting from the use of controlled and modified atmospheres in bulk refrigerated storage, and also to assess the possibility of using the target amino acids as quality indices for this species.

2. Materials and methods

2.1. Material

The crustacean species used for this work was Norway Lobster (*Nephrops novergicus* (L)) chosen for its particular commercial importance in Spain. The fish were caught by trawling at Grand Sole (between the parallels 52–53 $^{\circ}$ north and 12 $^{\circ}$ east). The batch of this lobster (100 kg) was handled on board as usual. It was selected, washed and treated with a commercial antimelanotic (Bacterol from Hispanoquimica SA, Spain) and then placed in boxes with ice. When the vessel reached the port in Vigo, the lobsters were taken in an isothermal lorry in a day to the research centre Instituto del Frío (Madrid, Spain). Then, the batch was divided into five lots. Four of these lots were placed inside different hermetic stainless steel containers (78 \times 48 \times 56 cm), as described by Ruiz-Capillas and Moral (2001b). The containers with the boxes inside were sealed and the respective mixtures were injected from pressurised bottles. Two of the four containers were kept in the controlled atmosphere (C) or modified atmosphere (M) with the gas mixture (1): 60/15/ 25 $(CO_2\%O_2\%/N_2\%)$ and the other two with the gas mixture (2): $40/40/20$ (CO₂%/O₂%/N₂%). The containers were placed in a forced ventilation chamber at 1 ± 1 °C together with the fifth lot, the control lot T.

2.2. Measurement of temperature

Temperatures were measured every 6 h with copper thermocouples connected to a Yokogawa Hokushin Electric meter, model 3087, Tokyo (Japan). For this propose, thermocouples were placed in the container through the access ports, inside the Norways lobster and the chill room temperature was also taken. The temperatures taken were the average three measurements.

2.3. Measurement of gases

The determination of the $CO₂$ and $O₂$ concentrations was done with Abiss Pack 12 meter equipment (France). which determines the concentrations of both gases at the same time. The gas sample for analysis was taken by extraction from the container through a hole (orifice) in its frontal part with a syringe of 50 ml connected to the set by a neoprene tube (diam 3–4 mm) (Ruiz-Capillas & Moral, 2001b).

2.4. Quality chemical parameters

For these tests, at least 20 individual lobsters were taken from each lot and they were shelled and cut into pieces and a homogeneous sample was prepared. These analyses were normally run periodically in triplicate. The results were the averages of these three determinations.

2.5. Determination of free amino acids (FAAs) and peptides

Muscle tissue (5 g) of Norway lobster was homogenised with 6% (v/v) perchloric acid in a 1:2 ratio (v/w) in an Ultraturrax homogenizer and prepared in accordance with the technique described by Yamanaka (14). The chromatographic determination was done with a liquid chromatograph model 1022 with a Pickering PCX 3100 post-column system (Pickering Laboratories, Mountain View, Ca (USA)) using a chromatography column of lithium cationic exchange (Li^+ , 3×150 mm) and a pre-column, also of lithium (Li^+ , 3×20 mm). Ortho-phthalaldehyde (OPA) was used as a postcolumn derivative reagent and it was prepared daily. The detection was done in a fluorescence spectrometer LC 240 (Perkin Elmer, Spain) at 330 nm excitation and 465 emission (Ruiz-Capillas & Moral, 2001a).

2.6. Statistical analysis

Statistical analysis was performed using analyses of variance (ANOVA), followed by a least significant difference (LSD) test at $p \le 0.05$ using SPSS 11.0 (SPSS Inc., Chicago, Ile).

3.1. Measurements of the gas mix

The evolution of modified and control atmosphere gases in the containers is shown in Figs. 1 and 2. Gas concentrations were higher in the controlled atmospheres (C), where gas mixes can be regulated and maintained throughout storage. Nonetheless, in this experiment, $CO₂$ and $O₂$ levels in the modified atmospheres (M) were also high in both gas mixes because, every 7 days, more of the gas mix was injected to restore the atmosphere after sampling. The opening of the containers registers in the figures as a sharp drop in the concentrations of the gases, followed by a rise when more gas was injected (Figs. 1 and 2). As noted in previous studies (Ruiz-Capillas & Moral, 2001b), the $CO₂$ levels inside the containers were consistently below the levels of $CO₂$ injected in the mixes, as the $CO₂$ was dissolved in the aqueous medium and the lobster tissue liquids, thus reducing the levels of injected $CO₂$.

Such loss and dissolution of $CO₂$ in the tissues enhances and preserves $CO₂$ levels in the atmosphere (Lannelongue, Hanna, Finne, Nickelson, & Vanderzant, 1982; Ruiz-Capillas & Moral, 2001b). However, the concentrations of O_2 only remain constant during storage when the levels of a gas in the mix are low, as in the case of mix 1 (60/15/25). In the case of mix 2 (40/40/ 20), containing high levels of O_2 , the levels of this gas behaved similarly to $CO₂$ and were lower than the levels injected with the mix. Gas mixes are more effective when the use of atmospheres is combined with strict temper-

Fig. 1. Evolution of concentrations of CO₂ (%) and O₂ (%) in the containers of lots C1 and M1 storage under controlled (C) and modified (M) atmospheres with the mixture 1: $60\%/15\%/25\%$ (CO₂/O₂/N₂).

Fig. 2. Evolution of concentrations of $CO₂$ (%) and $O₂$ (%) in the containers of lots C2 and M2 storage under controlled (C) and modified (M) atmospheres with the mixture 2: $40\frac{\%}{40\%}$ (CO₂/O₂/N₂).

ature control (Stammen et al., 1990). In the present case, the cold store temperature was programmed at 1 ± 1 °C and the mean temperature recorded in the container was 1.11 ± 0.4 °C, while the inside temperatures of the lobsters were -0.40 ± 0.3 °C and 0.45 ± 0.3 °C, respectively. It has also been found that deterioration of Norway lobster can be partially delayed by strict temperature control alone (IIF, 1979; Moral, 1996).

3.2. Evolution of essential free amino acids

Table 1 shows the essential free amino acids determined in Norway lobster. Concentrations of threonine, leucine, valine, lysine and arginine were greater than 40 mg/100 g and as high as 50 mg/100 g in the case of lysine. The concentrations of these essential amino acids were similar to those found by other authors in various crustacean species (Matsumoto & Yamanaka, 1992; Otsuka et al., 1992; Yamanaka & Shimada, 1996) and in some cephalopods, such as pota and volador (Ruiz-Capillas, Moral, Morales, & Montero, 2002). However, these levels are not especially high when compared to other fish species. Ruiz-Capillas and Moral (2001a) reported essential amino acid levels lower than 2 mg/100 g in hake, except for threonine and lysine, which exceeded 11.9 and 9 mg/100 g but were still much lower than the levels found in Norway lobster (Table 1).

In the course of storage, all lots presented significant $p < 0.05$ decreases in the concentrations of threonine, valine, lysine and arginine. These decreases were more pronounced $(p < 0.05)$ in the lots kept in the CO₂-rich mix (1) than the lots kept in the O_2 -rich mix (2),

T, control lot, stored in air throughout the experiment.

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Each value is the mean score of at least 20 individuals of Norway lobster. The same letter in each column means no significant difference between the samples ($p \le 0.05$). The same number after the letter in each row means no significant difference along days of storage ($p \le 0.05$).

independently of atmosphere types (M or C). The reason for this could be that the gas mix with lower concentrations of $CO₂$, such as mix 2, less effectively inhibited spoilage than the O_2 -rich mix 1 (Figs. 1 and 2). A less effective atmosphere allows more microbial growth and hence greater consumption of FAAs. In the cases of lysine and arginine, the microorganisms can cause decarboxylation of the amino acids to produce spoilage compounds such as the biogenic amines: cadaverine and putrescine, respectively (Ruiz-Capillas & Moral, 2001c; Wendakoon, Murata, & Sakaguchi, 1990; Yamanaka, 1989). Otsuka et al. (1992) also observed decreases of arginine in snow crab muscle during ice storage.

The concentrations of the other essential amino acids, except for tryptophan, generally decreased up to the 7th day of storage, remaining stable up to the end of storage, at which time there was also a clear decrease in all lots (Table 1). Of these essential amino acids, only tryptophan increased over storage in all the lots. Levels were highest in the control, followed by the lots stored in

Table 2

Not-essential free amino acids (mg/100 g) in muscle of Norway losbter (Nephrops novergicus (L.)) in controlled (C) and modified (M) atmospheres with 2 mixes of gases (1): $60/15/25$, and (2): $(49/49/20)$ $(CO_2/O_2/N_2%)$ during chilled storage

Free amino acids	Lots	Days of storage				
		$\overline{0}$	τ	14	21	28
Tyrosine	T	16.6 a/1	18.2 a/2	14.3 a/3	17.7 a/2	$\overline{}$
	C1		18.7 a/2	21.2 b/3	18.6 a/2	17.1 a/2
	C2		18.6 a/2	$18.7 \text{ c}/2$	17.2 a/2	15.4 b/3
	M1		16.2 a/1	$16.8 \text{ c}/1$	19.4 a/1	19.0 a/2
	M ₂		19.3 a/2	20.1 b/2	$15.3 \frac{\text{b}}{1}$	12.8 c/3
Glutamic acid	$\mathbf T$	31.2 a/1	47.2 a/2	87.9 a/3	84.4 a/4	$\overline{}$
	C1		51.7 b/2	70.9 b/3	68.8 b/3	59.2 a/4
	C ₂		$65.8 \text{ c}/2$	71.3 b/3	65.5 b/2	50.8 b/4
	M1		46.0 a/2	44.1 c/2	68.5 b/3	74.7 c/4
	M ₂		46.1 a/2	69.7 b/3	54.8 c/4	42.2 d/5
Glycine	$\mathbf T$	57.9 a/1	45.1 a/2	42.8 a/3	43.1 a/3	$\qquad \qquad -$
	C1		64.8 b/2	53.1 b/3	55.1 b/1	55.7 a/1
	C2		76.5 c/2	$61.2 \text{ c}/1$	58.7 c/1	54.1 a/3
	M1		60.5 d/1	57.5 d/1	60.2 c/1	58.5 b/1
	M ₂		53.3 e/2	45.9 a/3	47.6 d/3	41.3 c/4
Alanine	$\mathbf T$	57.2 a/1	48.1 a/2	56.3 $a/1$	55.5 a/1	$\overline{}$
	C1		66.0 b/2	55.1 a/1	58.6 b/1	52.7 a/3
	C2		66.4 b/2	56.8 $a/1$	68.7 c/2	48.2 b/3
	M1		$60.1 \text{ c}/2$	56.0 $a/1$	56.1 a/1	59.1 c/2
	M ₂		51.5 d/2	56.3 a/l	57.5 b/1	40.1 d/3
Ornithine	T	7.69 a/1	13.0 a/2	28.0 a/3	31.7 a/4	
	C1		6.35 b/1	$7.35 \text{ b}/1$	8.76 b/1	10.7 a/2
	C ₂		$8.67 \text{ c}/1$	$11.7 \text{ c}/2$	$12.0 \text{ c}/2$	8.95 b/1
	M1		7.43 b/1	$10.8 \text{ c}/2$	6.19 a/1	13.0 c/2
	M ₂		6.51 b/1	$10.9 \text{ c}/2$	9.14 b/2	9.17 b/2
ß-Alanine	T	0.94 a/1	1.08 a/1	0.68 a/2	0.81 a/1	$\qquad \qquad -$
	C1		0.97 a/1	0.68 a/1	0.70 a/1	0.70 a/1
	C2		0.89 a/1	0.70 a/1	0.69 a/2	0.54 b/2
	M1		0.88 a/1	0.71 a/1	0.65 a/2	0.74 a/1
	M ₂		1.07 a/1	0.78 a/1	0.79 a/1	0.34 c/2
1-methyl-histidine	$\mathbf T$	1.69 a/1	2.01 a/1	1.65 a/1	2.20 a/1	
	C1		4.65 b/2	1.78 a/1	1.36 b/1	1.50 a/1
	C2		2.13 a/1	1.52 a/1	1.49 b/1	1.39 a/1
	M1		2.36 a/1	1.74 a/1	1.14 b/1	1.49 a/1
	M ₂		1.99 a/1	1.65 a/1	1.29 b/1	1.79 a/1
Anserine	T	52.9 a/1	50.1 a/2	45.7 a/3	43.1 a/4	
	C1		50.2 a/2	45.9 a/3	42.1 a/4	36.2 a/5
	C2		51.1 a/2	45.2 a/3	42.3 a/4	33.6 b/5
	M1		49.1 a/2	$42.6 \text{ b}/3$	41.4 a/4	38.3 a/5
	M ₂		45.4 b/2	43.1 b/3	36.0 b/4	30.4 b/5

T, control lot, stored in air throughout the experiment.

Each value is the mean score of at least 20 individuals of Norway lobster. The same letter in each column means no significant difference between the samples ($p \le 0.05$). The same number after the letter in each row means no significant difference along days of storage ($p \le 0.05$).

atmospheres with the mix 2 (Table 1). However, this increase was only significant $(p < 0.05)$ in the control lot. Other authors have likewise reported increases of free amino acids during spoilage, associated with the proteolysis that occurs in crustacean and fish muscle in the spoilage process (Murata & Sakaguchi, 1986; Ruiz-Capillas & Moral, 2001a; Sakaguchi, Murata, & Kawai, 1982). On the other hand, Otsuka et al. (1992) found no increases of free amino acids in invertebrates during storage in ice. Increases in tryptophan, in the course of storage, both in ice and atmospheres, have also been reported in hake, for which tryptophan has been proposed as a quality control index, thanks to this increase (Ruiz-Capillas & Moral, 2001a). However, in Norway lobster, tryptophan could only be used as a quality index for lobster stored in ice.

3.3. Evolution of non-essential free amino acids

The most abundant of the non-essential amino acids shown in Table 2 were glycine, alanine and glutamic acid, with respective concentrations of 57.9, 57.2 and 31.2 mg/100 g. These amino acids are important in that they give crustaceans and fish their characteristic taste and flavour (Hayashi, Yamaguchi, & Konosu, 1981; Konosu & Yamaguchi, 1982). Yamanaka and Shimada (1996) detected comparable levels of these amino acids in spiny lobster. The Norway lobster muscle also had high concentrations of the dipeptide, anserine, initially 52.9 mg/100 g. Higher levels of anserine (76 mg/100 g) have been detected in hake (Ruiz-Capillas & Moral, 2001a). However, as in the case of essential free amino acids, the FAA levels found in Norway lobster were much higher than in fish species such as hake although comparable to levels found in other crustaceans and molluscs (Otsuka et al., 1992). It is this high concentration of free amino acids that gives Norway lobster its particular flavour as distinct from fish.

During storage, the evolution of most of the nonessential FAAs presented a saw-tooth profile, with scarcely any significant $(p < 0.05)$ changes in the various amino acids. Only in the case of ornithine was there a significant increase of concentration in the control, which reached 31.7 mg/100 g by the end of storage and was significantly ($p < 0.05$) different from the levels in the atmosphere lots. In the atmosphere lots, on the other hand, evolution was similar to the other free amino acids. However, the lowest levels of ornithine were observed in the lots kept in the atmosphere with the mix 2 with lower concentrations of $CO₂$ as was observed with the other FAAs. Matsumoto and Yamanaka (1990) reported higher ornithine concentrations in spiny lobster than we found in Norway lobster. Ornithine is formed in crustacean muscle from arginine and arginase (Matsumoto & Yamanaka, 1990), which would account for the observation of decreased arginine (Table 1) and increased ornithine (Table 2).

Many authors have noted that ornithine is useful as an index for the freshness of some crustaceans, such as kuruma prawn, snow crab, tanner crab, spiny lobster and kuro shrimp (Matsumoto & Yamanaka, 1992; Matsumoto & Yamanaka, 1990; Miyagawa et al., 1990; Otsuka et al., 1992; Yamanaka & Shimada, 1996). In this study, ornithine appeared to be useful as an index of freshness and decomposition of Norway lobster in storage, but only for the control lot. In the atmospherestored lots, there were no significant differences in the concentrations.

In general, the same behaviour was reported in the essential FAAs, the highest levels of non-essential FAA being observed in the lots kept in the gas mix 1.

Anserine concentrations in Norway lobster also decreased significantly $(p < 0.05)$ during storage. It was this that enabled Ruiz-Capillas and Moral (2001a) to use anserine evolution as a quality control index in atmosphere-stored hake. In that study, it was found that decreases of anserine were accompanied by increases in its components, β -alanine and 1-methylhistidine. In the present study, however, we found that anserine decreased in Norway lobster without any changes in the β alanine and 1-methylhistidine concentrations (Table 2). Therefore, in this species, anserine could be used as a quality control index, both in ice and in modified or controlled atmospheres.

4. Conclusions

The concentrations of free amino acids in Norway lobster were very high compared to other species. Essential and non-essential FAAs differed significantly, depending on the gas mixes used. The gas mix 1 (60/15/25) seemed more effective than the mix 2 (40/40/20). However, no significant differences were observed between the two types of atmospheres used, controlled and modified.

The concentrations of most of the FAAs decreased during storage. On the other hand, ornithine and tryptophan concentrations increased in all the lots. However, this increase was only significant $(p < 0.05)$ in the control. The FAAs would appear to be suitable as a freshness index for Norway lobster stored in ice, but would, however, be useless for the atmosphere-storage lots. Moreover, the significant $(p < 0.05)$ decrease of the dipeptide anserine could be a suitable freshness index for Norway lobster stored in either ice or atmospheres (C or M).

Acknowledgements

The experiment reported here is a part of the project ''Maintaining quality of fresh fish aboard ship and on land by modified atmosphere. Development of equipment, containers and procedures'' contract nos. AIR1 CT92-0273 EU and ALI93-1004-CE (C.I.C.Y T.).

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