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Determination of biogenic amines in fresh unpacked and vacuum-packed beef during storage at 4°C

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Abstract

Biogenic amines, putrescine, cadaverine, and histamine were determined in unpacked and vacuum-packed beef; during storage at 4°C for 12 and 35 days, respectively, under the same conditions. Analysis by High Performance Liquid Chromatography in both kind of samples, showed variation of cadaverine and histamine with an increasing tendency to a definite increase of putrescine. In unpacked samples, maximum mean contents for each amine reached 10.4, 5.2 μg/g for putrescine and cadaverine, respectively (12th day) and 2.2 μg/g for histamine (5th day). The total amine content reached 16.5 μg/g (12th day). After 8 days of storage, organoleptic fall-off began and, on the 12th day, all samples were disqualified. In vacuum packs, amine levels increased after 19 days. Maximum levels were determined for putrescine on the 35th day (36.3 μg/g), for cadaverine on the 26th day (28.9 μg/g) and for histamine on the 35th day (19.0 μg/g). Although the vacuum-packed beef was organoleptically acceptable up to 35 days, the total amine levels were greater than *5* μg/g after the 12th day, indicating a leveling off of quality.

1. Introduction

An essential criterion of meat quality is its hygiene status, which is determined decisively by the presence and activity of microbes. During maturity and storage, flavour and odour substances, as well as biogenic amines may be formed.

Biogenic amines are organic bases of low molecular weight of aliphatic, aromatic or heterocycic structure. They occur as natural components of many foods, including meat and meat products. Their presence in food is attributed to protein metabolism (exogenous and endogenous enzymatic action) in the product (Brink, Damink, Joosten, & Huis in't Vela, 1990; Shalaby, 1993; Wortberg & Woller, 1982). Dainty, Edwards, Hibbard, and Ramantanis (1986) have shown that enterobacteriaceae, especially *Hafnia alvei* and *Serratia liquefaciens,* play a major role in the accumulation of diamines during storage of vacuum-packed meat at low temperatures.

It is well known that biogenic amines at high levels are considered to be unhealthy. (Luthy & Schlatter, 1983). They can cause distinctive pharmacological, physiological and toxic effects in organisms. Moreover, they are precursors of carcinogenic nitroso-amines (Patterson & Mottram, 1974). Exogenous biogenic amines constitute a potential health risk, especially when coupled with additional risk factors, such as monoamine oxidase inhibitors (Stratton, Huntkins, & Taylor, 1991).

The Food and Drug Administration (FDA) has established a tolerance level of histamine in fish, of 100 mg/1 kg of flesh (FDA, 1990). High levels of putrescine and cadaverine have been recognized as potentiators of histamine or tyramine toxicity, but no recommendations about levels have been suggested.

Since little information is available on the biological active amine content in meat compared with fish and cheese, the following study was performed to provide data on the presence of biogenic amines in unpacked and vacuum-packed meat, during storage at 4°C. Furthermore, information has been obtained on the variation of total amine content during storage.

2. Materials and methods

2.1. Sample preparation

Meat samples of the throat, from five different animal slaughters of young bulls (20–24 months old) were taken

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for analysis. Each part was divided into nine pieces of 250 g weight and 3 cm thickness. One such piece from each slaughter was left unpacked and stored at 4°C. The other eight pieces from each slaughter were vacuum-packed in bags made of high-barrier film (Nylon/Binding layer/L.LDPE, 300×250 mm, 0.07 mm thick) and stored under the same conditions. The unpacked samples were analyzed at the 1st, 5th, 8th and 12th day of storage, while the vacuum-packed were analyzed at the 1st, 5th, 8th, 12th, 15th, 19th, 26th and 35th day of storage. On the appropriate sampling day, 5 g of core were removed. This plug of meat was a complete cross section of the sample and included both exterior surfaces.

2.2. Chromatographic conditions

Amines were analyzed according to the method described for fish by Malle and Valle (1996). It involves derivatization of the amines with dansyl chloride and chromatographic separation of the dansyl amines with a modification in the elution. As little as 10 ng of these compounds can be detected with relatively insensitive UV (HPLC) detectors.

Conditions of chromatographic separation are reported in detail in Table 1. The mobile phase consisted of acetonitrile and water (55:45 v/v).

Table 1
Conditions of chromatographic separation

Static phase	Nucleosil 100-5 C18
Particle size	5 μm
Column dimensions	250×4.6 mm
Mobile phase	Acetonitrile: Water (55:45)
Flow rate	1.8 ml/min
Temperature	ambient
Loop volume	20 μl
Detector (wavelength)	254 nm

3. Results and discussion

3.1. General

The biogenic amines were well resolved on the C18 column with the chromatographic conditions described in Table 1. Fig. 1 shows the typical chromatogram of putrescine, cadaverine and histamine in standard solutions of 0.5 μg/g, 0.6 μg/g and 1.2 μg/g, respectively.

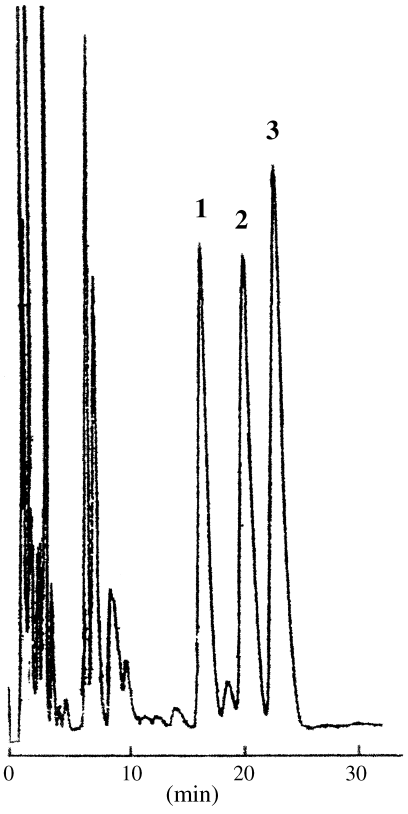

Fig.1. I. HPLC chromatogram of standard solution (1) putrescine (0.5 μg/ml). (2) cadaverine (0.6 μg/g/ml) and (3) histamine (1.2 μg/ml).

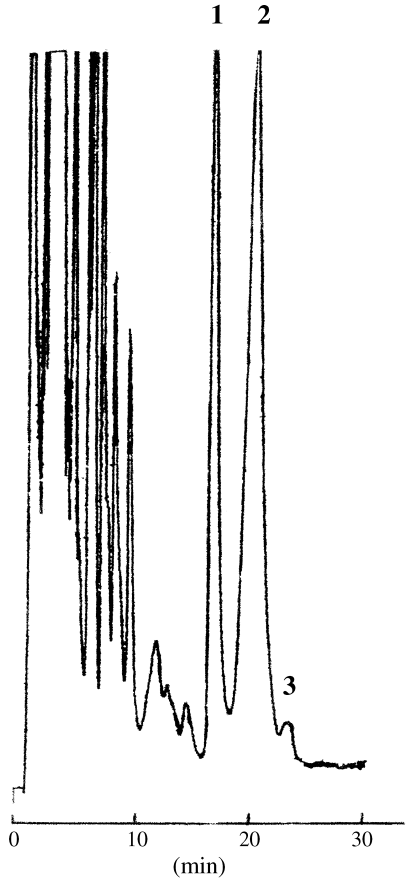

Fig. 2. Separation of dansyl derivatives of biogenic amines by isocratic elution. See text for isocratic elution conditions. Peak identification and retention time (1) putrescine. 17.6 min, (2) cadaverine, 21.3 mm, (3) histamine, 24.4 min.

Amines were identified on the basis of retention time by comparison with standard solutions. Retention times of the amines were stable and consistently reproducible. Major side-products of the dansyl reaction on the left side of the chromatogram, were eluted by 15 min and were separated very clearly from derivatized polyamines. The amines were separated with very good peak resolution and sharpness. Dansyl polyamines are completely eluted from the column after 26 min. (Fig. 2)

Standard curves of the amines were separately prepared in the range 0–5 μg/ml and peak area versus concentration of amine was plotted. Data for calibration curves were subjected to linear regression analysis. The correlation coefficient in every curve was >0.998 ($P<0.01$), indicating a definite linear relationship between amine concentration and detector response. We concluded that the modified isocratic elution program, which had been used, was satisfactory.

Tables 2 and 3 show, in an exemplary manner, the means and the precisions of the six-fold determined amines in unpacked and vacuum-packed samples, respectively. The precision ranged from 0.5 to 8.1%, while recovery ranged from 78 to 89%. Detection limits were 0.01, 0.14 and 0.26 μg/ml for putrescine, cadaverine and histamine, respectively.

3.2. Unpacked samples

In Table 2 mean values of amine concentrations are given, as they result from five unpackaged beef samples. Amine development in these samples is shown in Fig. 3.

Putrescine was detected in most samples from the beginning of storage (0.8 μg/g, 24 h after slaughtering). (All concentrations referred to, represent mean values of all five different slaughter samples). As can be seen from Fig. 3, there is a definite increasing tendency until the end of the study (12th day), when putrescine appears at its maximum concentration of 10.4 μg/g. Similar behaviour was observed by Daher and Simard (1985), studying ground beef, stored under the same conditions (4°C for 12 days). Trevino, Beil, and Steinhart (1997) also found increases of putrescine during 12 weeks of storage of sausages.

Cadaverine was first detected after the 8th day; it then increased, reaching a maximum level of 5.2 μg/g at the end of storage (12th day), while histamine was detected only on the 5th day (2.2 μg/g). From other studies carried out in food products, some factors have been found which affect histamine level. For example, low temperature of storage (4°C) does not favour histamine formation, which is determined by mesophilic bacteria that require temperatures higher than 15°C. Optimum

Table 2
Biogenic amine concentrations (μg/g)[a] in unpacked meat samples, during storage at 4°C

Day[b]	Putrescine (μg/g)		Cadaverine (μg/g)		Histamine (μg/g)		Amine sum (μg/g)
	Mean (S.D.)	RSD	Mean (S.D.)	RSD	Mean (S.D.)	RSD	
0	LOQ[c]	–	<LOQ	–	<LOQ	–	<2.5
1	0.8 (0.42)	52.5	<LOQ	–	<LOQ	–	3.2
5	1.3 (0.84)	64.6	<LOQ	–	2.2 (1.55)	53.4	4.3
8	2.3 (0.95)	41.3	2.9 (2.56)	88.2	<LOQ	–	6.8
12	10.4(0.78)	7.5	5.2 (3.16)	60.7	<LOQ	–	17.1

[a] Concentrations represent mean values of all five different slaughter samples.
[b] Day is referred to the time after animal slaughter.
[c] LOQ = Limit of Quantification (0.084 μg/g for putrescine, 0.84 μg/g for cadaverine and 1.56 μg/g for histamine).

Table 3
Biogenic amine concentrations (μg/g) in vacuum-packed meat samples, during storage at 4°C

Day	Putrescine (μg/g)		Cadaverine (μg/g)		Histamine (μg/g)		Amine sum (μg/g)
	Mean (S.D.)	RSD	Mean (S.D.)	RSD	Mean (S.D.)	RSD	
0	<LOQ		<LOQ		<LOQ		<2.5
1	0.8 (0.22)	LOQ–1.1	<LOQ	<LOQ	<LOQ	LOQ–2.4	3.2
5	0.9 (1.26)	LOQ–2.7	<LOQ	<LOQ	<LOQ	<LOQ	3.3
8	1.1 (0.61)	0.4–1.7	1.5(1.26)	0.7–3.3	<LOQ	LOQ–2	4.2
12	1.1 (1.34)	0.7–3.2	3.1 (3.13)	1.6–4.2	<LOQv	<LOQ	5.8
15	2.8 (1.27)	1.1–4.5	25.3 (23.7)	10.8–49.5	2.0 (0.35)	1.7–2.5	30.1
19	3.9 (2.39)	1.4–7.6	11.0 (8.62)	4.8–20.2	3.1 (0.88)	2.1–4	18.0
26	32.9 (10.18)	20.2–42.3	28.9 (26.08)	17.9–32.2	1.5 (1.54)	DL–5.2	63.3
35	36.3 (2.43)	33.7–38.4	13.3 (6.88)	9.1–25.3	19 (4.25)	15.5–24.9	68.6

temperature for histidine decarboxylase enzyme activity is 30°C (Joosten & Van Boekel, 1988).

In Fig. 5, total variance of amine content in unpacked samples is illustrated. Amine content was 0.8 μg/g (1st day), which increased and reached a maximum level of 15.6 μg/g (12th day). The sum of all three amines, leads to a useful biogenic amine index to evaluate meat freshness. Hernandez, Izquierdo-Pulido, Veciana-Nogues, Marine-Font, and Vida-Arou (1997) reported that when the sum of putrescine, cadaverine, histamine and tyramine is below 5.0 μg/g, this indicates meat of high hygienic quality. In the present study, this limit is passed after 8 days of storage, indicating low quality, although organoleptic characteristics were acceptable. After 12 days, however, tenderness appeared to level off and samples were disqualified. That was the reason why the study lasted only 12 days.

3.3. Vacuum packed samples

Development of biogenic amine content in vacuum-packed beef, during storage, is illustrated in Fig. 4.

Putrescine was detectable from the first day of storage (after 24 h). Until the 19th day it was at levels lower than 3.9 μg/g, showing a continual increase, which became rapid after that day. Maximum level was 36.3 μg/g after 35 days of storage.

Cadaverine content varied greatly during storage, with an increasing tendency. It was first detected on the 8th day and, after a fluctuation, it reached the maximum level of 28.9 μg/g on the 26th day. Histamine showed a fluctuation too. It ranged from non detectable to low levels during storage, with an increasing tendency, reaching the maximum level of 19 μg/g on the 35th day. Great histamine and cadaverine variations were also reported by Hernandez-Javer et al. (1997) in raw beef.

During storage, samples did not show abnormal organoleptic characteristics due to vacuum-packing. The total amine content ranged from 0.8 μg/g (first day) to 74.0 μg/g (35th day), showing an increasing tendency with some fluctuations (Fig. 5). After 12 days, amine content reached 4.4 μg/g, indicating good quality of the samples up to then. However, it did not exceed 100 μg/g.

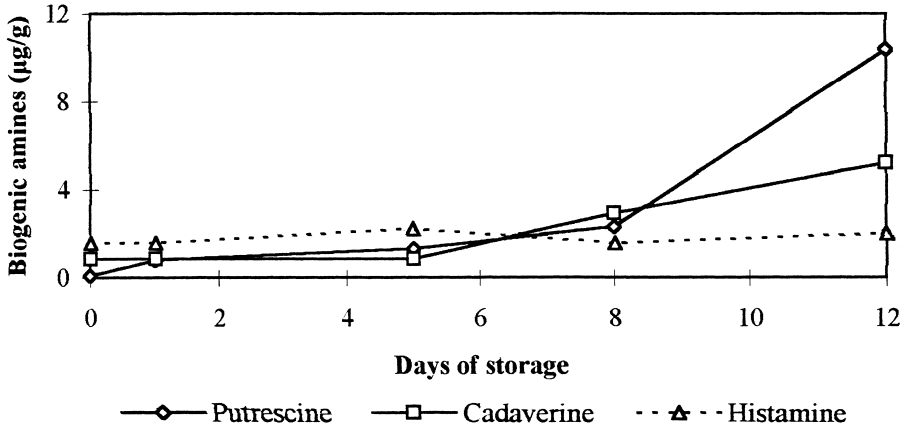

Fig. 3. Development of biogenic amines in unpacked raw beef, during storage at 4°C.

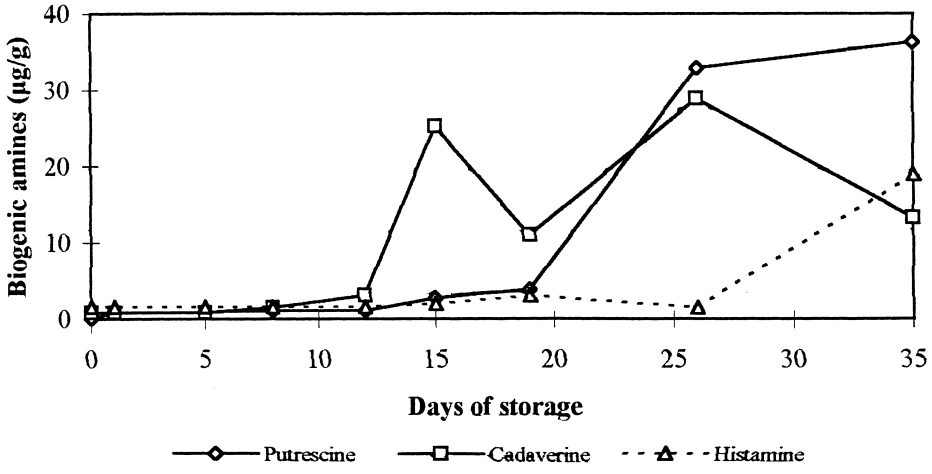

Fig. 4. Development of biogenic amines in vacuum-packed meat, during storage at 4°C.

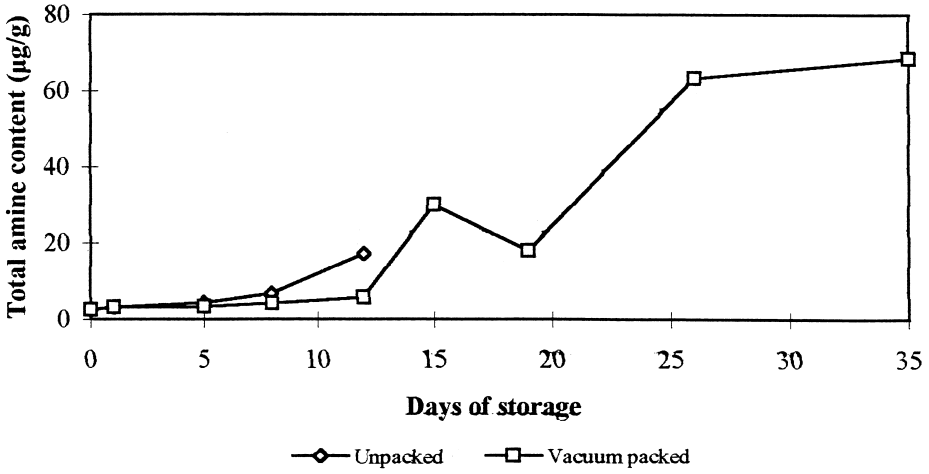

Fig. 5. Changes of total amine level in unpacked and vacuum-packed samples, during storage at 4°C.

Yano, Kataro, Watanabe, Nakamura, and Asano (1995), studying vacuum-packed beef stored at 5 and 0°C, observed decreased tenderness and level-off after about 10 days at 5°C and 28 days at 0°C, well before putrefaction was detected.

4. Conclusions

Unpacked and vacuum-packaged beef was stored at 4°C and biogenic amines were measured over time. In unpacked samples, total amine content increased over time, reaching 5 μg/g (after 8 days). On the 12th day it reached 16.5 μg/g, showing abnormal organoleptic characteristics, and then samples were disqualified.

Although vacuum-packed samples appeared organoleptically acceptable until the end of storage (35th day), total amine content approached 5 μg/g from the 12th day, without exceeding 100 μg/g. This indicates decrease of hygiene quality in the product.

Finally, it was observed that vacuum-packing triples storage time of meat, maintaining organoleptic characteristics at a very good level. A simple procedure to lower the amine levels, alleviating any potential problem, is to wash the subprimals as they are removed from the package. Washing may remove substantial amounts of amines, as they are presumably located mainly on the surfaces.

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