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A survey on free biogenic amine content of fresh and preserved vegetables

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Abstract

A survey on free biogenic amine contents in fresh and preserved vegetable products was carried out. A simple extraction method, involving an homogenisation step with 0.1 M HCl, was applied. Two different derivatization procedures (using *o*-phthaldialdehyde and dansyl chloride) were applied on different aliquots of the same acid extracts and HPLC analyses were carried out with the same reversed phase (C18) HPLC column. Results obtained with the two procedures were compared. With the exception of sauerkraut, putrescine (0.2–0.5 mg/100 g fresh weight) and spermidine (0.4–4.5 mg/100 g) were always the most represented amines, generally followed by spermine (maximum 1.1 mg/100 g). Tyramine level was 4.9 mg/100 g in canned sauerkraut while other samples presented levels not exceeding 1.2 mg/100 g. The spinach sample showed the highest histamine content (2.0 mg/100 g).

Keywords: Biogenic amines; Tyramine; Vegetable products; HPLC

1. Introduction

Biogenic amines are low molecular weight organic compounds that can be found in relatively large amounts in some fermented foodstuffs as a consequence of microbial activity (enzymatic decarboxylation of the corresponding amino acids).

Free biogenic amines in fruits and vegetables shape the typical and characteristic taste of mature foods and are precursors of certain aroma compounds (Askar & Treptow, 1989). They can be also exploited for differentiation purposes. Soleas, Carey, and Goldberg (1999) reported cultivar-related differences in biogenic amine concentration of Ontario wines. Their presence at relatively high concentrations in vegetables was also associated with spoilage due to prolonged storage time (Cerutti, Finoli, & Vecchio, 1989; Yen, 1992).

Due to their psychoactive and vasoactive properties, some biogenic amines introduced with the diet (particularly tyramine and histamine) can cause distinctive pharmacological and toxic effects, especially when the detoxifying enzymes monoamino oxidase (MAO) present in the gastrointestinal tract are impaired, as in the case of patients treated with MAO-inhibitors (Stockley, 1973; Ten Brink, Mamink, Joosten, & Huisin'I Veld, 1990). These MAO-inhibitors include painkillers and drugs used for the treatment of stress, depression and Parkinson's disease. Different authors have reported of hypertensive crises following consumption of food rich in tyramine or other pressor amines such as aged cheese, wine, beer and yeast extracts but also vegetables such as sauerkraut, broad bean, banana peel and avocado (McCabe, 1986). Tyramine intake exceeding 6 mg within a 4-h period, has been considered dangerous for these patients (Tailor, Shulman, Walker, Moss, & Gardner, 1994).

Polyamines, such as putrescine, cadaverine, spermidine and spermine, although not exerting a direct toxic effect, can potentiate the toxic effects of tyramine and histamine by competing for the detoxifying enzymes

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(Edwards & Sandine, 1981; Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994) and act as precursors of carcinogenic nitroso-amines (Oliveira, Glória, Barbour, & Scanlan, 1995). Furthermore, some authors (Bardocz, 1995; Eliassen, Reistad, Risøen, & Rønning, 2002) have reported that a polyamine deprivation regime can have beneficial effects in reducing tumour growth.

In plants, polyamines are involved in a number of cell processes (cell division and differentiation, synthesis of nucleic acids and protein, membrane stability, stress responses and delay in senescence) and, in the USA, there is a patent for the use of exogenous polyamines as a method for extending the shelf life of fruits and vegetables (Boucherau, Guénot, & Larher, 2000; Valero, Martinez-Romero, & Serrano, 2002).

Different studies (Kalač, Švecová, & Pelikánová, 2002; Okamoto, Sugi, Koizumi, Yanagida, & Udaka, 1997; Simon-Sarkadi & Holzapfel, 1995) have demonstrated that the polyamines putrescine, spermine and spermidine are practically ubiquitous in all vegetables at level of few a mg/100 g of fresh weight.

Fruits and fruit juices are particularly rich in putrescine (Maxa & Brandes, 1993; Shalaby, 1996), while green vegetables are richer in spermidine (Valero et al., 2002). According to some authors, cooking processes and heat treatments can influence polyamine contents (Cirilo et al., 2003; Shalaby, 2000).

Tyramine and other aromatic amines are less widespread than polyamines, but they can reach particularly high concentrations in some vegetables (e.g. in *Acacia berlandieri*) where they seem to have a defensive role against insects and herbivores (Forbes, Pemberton, Smith, & Hensarling, 1995). Few works deal with the presence of these physiologically active amines in vegetables.

Udenfriend, Lovenberg, and Sjoderma (1959) reported the presence of tyramine and other physiologically active amines (serotonin, tryptamine, dopamine and norepinephrine) in most of the fruits and vegetables analysed. Particularly, they reported mean tyramine contents of 0.7, 2.3, 1.0 and 0.4 mg/100 g, respectively, for banana pulp, avocado, orange pulp and tomato. The high mean levels of tyramine reported by Tarján and Jánnossy (1978) for different vegetables (84.0, 26.6, 25.0 and 67.0 mg/100 g, respectively, for potato, paprika, tomato and cabbage) were not confirmed by following surveys. Simon-Sarkadi and Holzapfel (1994) found, in different leafy vegetables, a tyramine content always lower than 0.3 mg/100 g. Higher amounts of tyramine (ranging from 0.4 to 3.2 mg/100 g) were found by Kalač et al. (2002) in 32 samples of frozen spinach purée. Mean tyramine levels of 0.7 and 3.7 mg/100 g were found, respectively, in concentrated tomato paste ($n = 16$) and ketchup samples ($n = 24$).

Considering the potential adverse effects that biogenic amines (particularly tyramine) can have and the relatively few and sometimes contradictory data available from the literature, the aim of this work was to investigate the amine content of a variety of fresh vegetables (mostly typical products of the Mediterranean diet). Some preserved vegetables were also analysed in order to see if these processed foods had significantly different amine contents with respect to the fresh vegetables. Two different derivatization procedures of the same sample extract, using, respectively, *o*-phthaldialdehyde and dansyl chloride, prior to HPLC analysis, were also compared.

2. Materials and methods

2.1. Sampling

Fresh and preserved vegetable samples were taken from supermarkets and retail shops and extracted soon after purchase. Some of the fresh samples were divided into two parts: one was processed immediately while the other was extracted after 3 weeks of storage at refrigeration temperature (6–8 °C). All samples were frozen before extraction in order to facilitate cell rupture.

2.2. Chemicals

Tryptamine, 2-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, spermine and 1,7 diaminoheptane were purchased from Fluka (Buchs, Switzerland).

A stock standard solution of amines was prepared by adding an accurately weighed amount of each standard (ca. 100 mg) to a 100 ml volumetric flask and diluting to volume with water. A fresh diluted working standard solution was prepared weekly for calibration.

All solvents used were HPLC-grade.

2.3. Analytical procedure

A representative amount of the sample was homogenised with a mixer. As described for other food matrices (Moret & Conte, 1995), a 5-g amount of the resulting slurry was directly weighed in to a centrifuge tube and homogenized with 20 ml of 0.1 M HCl containing a known amount of 1,7 diaminoheptane (internal standard) in a Politron homogenizer (Kinematica, Lucerne, Switzerland) for 2 min. The resulting homogenate was centrifuged at 20,000 *g* for 20 min (4 °C) with a Cryofuge 20-3 centrifuge (Hereaus, Karlruhe, Germany). The aqueous layer was collected and the residue re-extracted with a 20 ml aliquots of 0.1 M HCl. The two combined extracts were filtered and diluted to 100 ml in a volumetric flask.

Two aliquots of the same extract were derivatized and injected into the HPLC following separate procedures.

To prepare dansyl-derivatives, a 1 ml aliquot of the diluted extract (1:4 v/v) was processed as previously described (Moret & Conte, 1995): 0.5 ml of saturated $NaHCO_3$ and 1 ml of dansyl chloride (DCl) reagent (5 mg/ml in acetone) were added to the sample in a test-tube. The test-tube was sealed and the mixture was thoroughly mixed (with a Vortex) for 1 min and left in the dark for 1 h at 40 °C, with occasional shaking. In order to eliminate the excess of DCl (Vallè, Malle, & Bouquelet, 1997), the mixture was treated with 200 μl of a proline solution (100 mg/ml), vortexed for 1 min and left to react in the dark for 15 min (ambient temperature). The sample was then extracted twice with 1 ml-aliquot of diethyl ether. The combined extracts were dried under nitrogen flow and the residue was re-dissolved in acetonitrile for injection.

o-Phthaldialdehyde (OPA) derivatives were prepared by adding 250 μl of OPA reagent solution (Sigma–Aldrich) to a 50 μl aliquot of the diluted extract (1:5 v/v). The mixture was vortexed for 1 min and injected after 2 min.

Both HPLC determinations were performed with the same instrument, consisting of two Pro Star solvent delivery pumps, model 210 (Varian, Palo Alto, CA, USA) equipped with a manual injector and a 20 μl loop. The column was a C18 – Kromasil (LabService Analytica, Bologna, Italy), 250×4.6 mm i.d., 5 μm particle size, thermostatted at 30 °C.

The separation of OPA-derivatives was performed with a mobile phase consisting of 370 ml of water plus 90 ml of a 0.07 M phosphate buffer at pH 7.0 as solvent A, while solvent B was acetonitrile (Beljaars, vanDijk, Bisschop, & Spiegelenberg, 1996). The gradient elution programme was held at 13% of B for 15 min, ramped at 50% (40 min) and then at 85% of B (60 min) and held until the end of the run (62 min) with a flow rate of 0.8 ml/min.

The mobile phase for DCl-derivatives consisted of water (solvent A) and acetonitrile (solvent B). The elution programme was held at 65% of B for 1 min, ramped at 80% (10 min), 90% (12 min), 100% of B (16 min) and held until the end of the run (23 min) with a flow rate of 0.8 ml/min.

OPA-derivatives were detected with a spectrofluorometer, model FP-1520 (Jasco, Tokjo, Japan) set at 330 nm (λ ex.) and 440 nm (λ em.), while dansyl-derivatives where detected with a UV–Vis, model 320 (Varian) set at 254 nm.

For the recovery assays, a known amount of each biogenic amine (6.1–12.0 mg/100 g fresh weight) was added to a cucumber extract. Four replicates were conducted on each fortified sample. The recoveries were calculated by comparing the peak area of the fortified sample with those of the standard.

3. Results and discussion

A simple preparation method, involving direct sample extraction with 0.1 M HCl, was used for free amine and amino acid determination in vegetable samples. The acid extract does not require a further purification step and it can be directly submitted to derivatization prior to HPLC analysis.

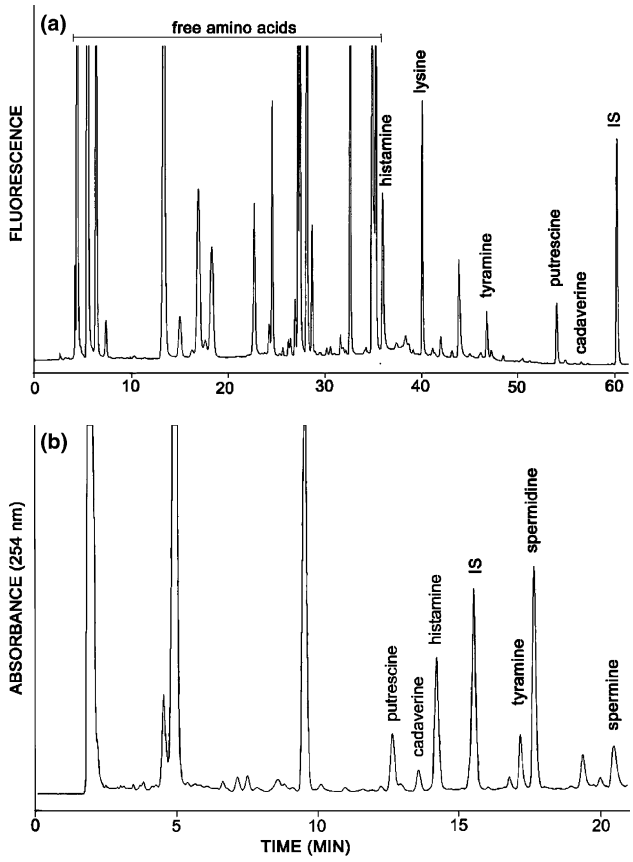

Fig. 1. HPLC chromatograms of a spinach sample derivatized with OPA (a) and DCL (b).

Table 1
Amine recoveries in a cucumber extract using OPA and DCl derivatization procedures

	DCl		OPA	
	Rec. %	RSD%	Rec. %	RSD%
Tryptamine	87.1	2.8	88.1	4.6
2-Phenylethylamine	86.6	4.8	90.5	0.4
Putrescine	88.1	2.6	90.5	2.7
Cadaverine	89.4	3.2	91.3	3.0
Histamine	89.2	4.4	91.8	3.1
1,7 Diaminoheptane (IS)	90.5	0.2	91.1	1.4
Tyramine	94.7	2.8	89.3	4.8
Spermidine	95.9	3.5		
Spermine	95.1	2.8		

Values are means of four replicates.

Table 2
Biogenic amine contents (mg/100 g of fresh weight) of various fresh vegetables

	Tryptamine		2-Phenylethylamine		Putrescine		Cadaverine		Histamine		Tyramine		Spermidine	Spermine
	OPA	DCl	OPA	DCl	OPA	DCl	OPA	DCl	OPA	DCl	OPA	DCl	DCl	DCl
Lettuce	–	–	–	–	1.0	0.7	–	–	–	–	0.1	tr[a]	1.6	0.1
Arugola	–	–	0.1	–	0.2	0.2	–	–	–	tr	1.2	1.0	1.9	0.3
Spinach	–	tr	0.1	tr	0.8	0.6	tr	0.1	2.0	1.6	0.8	0.6	2.3	0.6
Parsley	–	–	0.2	tr	1.1	0.9	–	–	–	–	tr	tr	2.6	0.2
Parsley[b]	–	–	0.2	0.1	0.6	0.5	tr	0.1	tr	0.2	0.2	0.1	1.8	0.2
Broad bean	–	–	–	tr	6.5	6.3	–	–	0.2	0.1	1.0	0.9	0.8	0.3
Potato	–	–	–	–	1.0	0.8	–	tr	–	–	0.7	0.5	1.0	0.7
Carrot	–	0.1	–	–	0.7	0.5	tr	–	tr	–	0.3	0.1	1.2	0.1
Onion	–	–	0.1	tr	0.3	0.2	–	–	–	–	0.2	0.3	0.4	0.1
Fennel	–	–	–	–	1.4	1.1	tr	–	–	–	0.1	tr	0.6	0.1
Pepper	–	–	0.1	tr	0.4	0.5	–	–	–	0.1	0.2	tr	0.4	0.2
Zucchini	–	–	0.2	tr	4.0	3.6	–	–	–	–	tr	tr	1.9	0.2
Zucchini[b]	–	–	0.2	tr	4.9	4.8	–	–	–	–	tr	tr	1.0	0.1
Broccoli	–	–	0.2	0.3	1.4	0.9	–	–	–	–	tr	0.1	4.5	1.1
Broccoli[b]	–	0.1	0.3	0.1	1.2	1.0	–	–	0.2	tr	0.3	0.1	2.4	0.6
Savoy cabbage	–	–	0.1	–	1.9	1.6	–	–	tr	–	0.3	tr	0.4	0.1
Cauliflower	–	–	0.1	tr	1.1	0.9	–	tr	tr	–	0.2	tr	3.8	0.7
Cucumber	–	–	–	–	2.4	2.5	–	–	tr	–	0.2	0.1	1.0	tr
Cucumber[b]	–	–	0.1	tr	2.8	2.9	–	–	–	–	0.1	0.1	0.4	tr
Tomato	–	–	0.1	tr	2.9	2.3	–	tr	0.7	0.6	0.2	tr	0.6	0.1

[a] tr $\leqslant$ 0.05 mg/100 g.
[b] Samples analysed after a 3-week refrigeration.

Figs. 1(a) and (b) show, respectively, the HPLC traces obtained from a spinach sample derivatized with the OPA and the DCl procedure. Although spermidine and spermine are primary amines which react with the OPA reagent, their derivatives were not sufficiently stable to be determinable under the conditions used in this work.

As seen from Table 1 both procedures gave comparable recoveries (around 90%) with low residual standard deviations (RSD). Some trials demonstrated that all amines, except tryptamine, were stable over some days when the acid extract was stored at refrigeration temperature. Tryptamine recovery decreased with storage time and, after a 3-day storage period at 6–8 °C, was less than 50%. Table 1 refers to samples processed immediately after the extraction.

Tables 2 and 3 list, respectively, free biogenic amine contents (mg/100 g of fresh weight) of a large variety of fresh and preserved vegetables obtained by both derivatization procedures. Biogenic amine concentrations obtained with OPA and DCl procedures were generally in good agreement. Free amino acid composition obtained with the OPA procedure will be reported elsewhere.

As seen in Table 2, polyamines putrescine (0.2–6.5 mg/100 g), spermidine (0.4–4.5 mg/100 g), and spermine (maximum 1.1 mg/100 g), were present in virtually all fresh vegetables, while cadaverine was present, in trace amounts, only in few samples. Small amounts of 2-phenylethylamine were also found in most of the samples (less than 0.3 mg/100 g). Higher concentrations of tyramine (not exceeding 1.2 mg/100 g) were found in arugola, broad bean, spinach and potato samples. Relatively high amounts of histamine were found in spinach (2.0 mg/100 g) and tomato samples (0.7 mg/100 g).

With respect to the same samples analysed fresh, vegetables analysed after a 3-week refrigeration period showed a lower spermidine content. Minor differences were found for concentrations of other amines. Further investigation is required in order to confirm these data and to see if different storage conditions can influence results.

With the exception of the sauerkraut sample (4.9 mg/100 g of tyramine), a fermented product often characterised by large amine contents, higher tyramine concentrations of preserved vegetables (Table 3) were found in red beet (0.7 mg/100 g) and tomato paste samples (0.5 mg/100 g). As in the case of fresh vegetables, putrescine and spermidine proved to be the amines present at higher concentrations, but with respect to fresh vegetables, preserved vegetables had a lower spermidine content.

Both derivatization procedures used in this work have already been extensively applied in other food matrices. The OPA procedure has the advantage of allowing the separation of amino acids and amines in the same chromatographic run. On the other hand, OPA reacts only with primary amino-groups, needs spectrofluorometric detection and requires a long elution time.

Table 3
Biogenic amine contents (mg/100 g of fresh weight) of preserved vegetables

	Tryptamine		2-Phenylethylamine		Putrescine		Cadaverine		Histamine		Tyramine		Spermidine		Spermine	
	OPA	DCl	OPA	DCl	OPA	DCl	OPA	DCl	OPA	DCl	OPA	DCl	DCl		DCl	
Peeled tomato	–	0.1	0.2	0.1	0.8	0.6	–	–	–	0.1	0.3	0.2	0.2		0.1	
Tomato souce	–	–	0.2	0.1	2.6	2.0	–	–	tr	0.2	0.4	0.2	0.6		0.2	
Tomato paste	–	–	0.3	0.1	4.3	4.2	0.1	–	0.3	0.5	0.5	0.4	0.3		0.1	
Chick-pea	–	–	tr	–	0.4	0.2	tr	tr	–	–	0.2	tr	2.3		0.7	
Bean (Cannellini)	–	–	0.1	–	0.4	0.3	–	–	tr	–	0.1	0.2	0.6		0.8	
Bean (Borlotti)	–	–	0.1	0.1	0.3	0.1	–	–	tr	–	0.5	0.3	0.6		1.3	
Lentil	–	0.1	0.2	tr	0.5	0.3	–	–	0.1	–	tr	–	1.2		0.5	
Soy-bean sprout	–	0.1	tr	–	1.1	0.7	0.1	0.1	–	–	0.1	tr	0.4		0.1	
Sauerkraut	–	–	0.2	tr	9.6	9.2	4.2	3.9	1.3	1.5	4.9	4.8	0.5		0.2	
Red beet	–	–	0.2	tr	0.6	0.3	–	–	–	–	0.7	0.5	0.7		tr	

Dansyl chloride forms stable derivatives that absorb at 254 nm with both primary and secondary amino-groups (as will be reported in a forthcoming paper, other physiologically active amines such as octopamine, serotonin, epinephrine and dopamine can be separated in the same chromatographic run) and these take shorter analysis times.

Even if the spectrofluorometric detection of OPA derivatives allows lower detection limits, both methods have sufficient sensitivity to detect small amounts of biogenic amines in vegetable samples (at 0.1 mg/100 g of fresh weight). Furthermore, when higher sensitivity is required, spectrofluorometric detection of DCl-derivatives can be used instead of UV detection.

4. Conclusions

In conclusion, the results of this survey confirmed that the biogenic amine content of fresh vegetables does not represent a risk for healthy consumers. With the exception of fermented products (sauerkraut), preserved vegetables also showed a relatively low amine content. Spermidine content of fresh vegetables decreased after a 3-week storage period and was generally lower in preserved products.

Anyway, as in some pathological conditions it is important to introduce the lowest possible amount of amine with the diet, further investigation is needed in order to collect sufficient data to assess the mean contribution of different vegetable products to amine dietary intake. Two simple derivatization procedures were applied on the same sample extracts and results were compared. When free amino acid determination is not of concern, the DCl derivatization procedure is to be preferred rather than the OPA procedure because of the shorter elution time required and the possibility of increasing the number of detectable amines.

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