Available online at www.sciencedirect.com
SCIENCE DIRECT
Talanta 69 (2006) 548–555
Talanta
www.elsevier.com/locate/talanta

Evaluation of two different extraction methods for chromatographic determination of bioactive amines in tomato products

E. Chiacchierini, D. Restuccia, G. Vinci *

University "La Sapienza", Dipartimento di Controllo e Gestione delle Merci e del loro Impatto sull'Ambiente, Via del Castro Laurenziano 9, 00161 Rome, Italy

Received 20 September 2004; received in revised form 12 September 2005; accepted 25 October 2005
Available online 29 November 2005

Abstract

Bioactive amines are organic bases originating from corresponding amino acid which have undergone decarboxylation by putrefactive bacteria or lactic acid bacteria. When formed by microbial enzymatic decarboxylation of amino acids, they are called "biogenic" and can produce detrimental effects on human health. Many techniques have been developed for extraction and/or clean up of bioactive amines in food, including acidic or organic extraction as well as solid phase extraction. This study deals with the comparison of two different extraction methods, homogenizing and matrix solid phase dispersion, for the chromatographic determination of eight non-volatile bioactive amines in tomato-based products (mashed tomato, biological mashed tomato, concentrated tomato pasta and ketchup) very popular in Italian alimentary habits. In both cases, perchloric acid has been used for analytes extraction and the influence of different parameters affecting amine recoveries have been evaluated. After a derivatization step with dansyl-chloride, samples were analyzed for amines quantitative determination using 1,7-diaminoheptane as internal standard on a C_{18}-RP-HPLC-UV system. Method performances were tested and good results of linearity, repeatability and recovery were obtained for all the considered amines. The collected data have shown that ketchup contains the highest levels of amines followed by concentrated tomato pasta, biological mashed tomato and common mashed tomato. Moreover, it has been found that in all samples, putrescine is the most abundant amine followed by tyramine, spermidine and tryptamine.

Keywords: Bioactive amines; Tomato products; Extraction methods; RP-HPLC-UV

1. Introduction

Bioactive amines are biologically ubiquitous nitrogen-containing compounds of cationic nature and of low molecular mass comprising aliphatic, mono-, di- and polyamines. Polyamines such as putrescine, spermidine, spermine and also cadaverine are indispensable components of living cells and are important in the regulation of nucleic acid function and protein synthesis and probably also in the stabilization of membranes [1–3]. Putrescine, spermidine and spermine, probably occur universally in animals and plants, and at least putrescine and spermidine are found in most bacteria [4]. As their formation and metabolism occurs widely in living organisms, they are also present in a variety of different foods, primarily as a consequence of amino acid decarboxylation. When these amines are formed by the microbial action through the decarboxylation process of amino acids, they are designed as biogenic. Proteolytic processes generally take place during preparation, ripening and storage of food high in protein content. Increased and high biogenic amines levels have been observed in proteinaceous and/or fermented foods (sgombroid fish, cheeses, ripened salami, sauerkraut, wine and beer) [5] while different opinions and very few data exist about biogenic amines content in non-fermented vegetables and fruit products [6,7]. Although low levels of biogenic amines in food are not considered a serious risk, if the amount consumed is high enough or normal routes of amine catabolism are inhibited, various physiological effects may be the consequence such as hypotension (in the case of histamine, putrescine and cadaverine), hypertension (in the case of tyramine), nausea, headache, rash, dizziness, cardiac palpitation and emesis, and even intracerebral haemorrhage, anaphylactic shock syndrome and death in very severe cases [8]. It follows that analysis of certain biogenic amines if food is a necessity to assess potential health hazards before consumption and several

* Corresponding author. Tel.: +39 0649766514; fax: +39 064452251.
E-mail address: giuliana.vinci@uniroma1.it (G. Vinci).

0039-9140/$ – see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.talanta.2005.10.027

criteria for food quality assessment are based on biogenic amines determination.

Several methods to analyze biogenic amines in food have been described so far, including thin layer chromatography [9], the use of amino acid analyzers [10], liquid chromatography [11–13], gas chromatography [14] and several biochemical assays [15,16]. All the chromatographic techniques involve two main steps: the compounds extraction from the matrix, including additional purification of the raw extract [17], and the analytical determination of the amines. The first phase is the most critical in terms of obtaining an adequate recovery for each amine. The extraction of free amines from a solid matrix can be carried out with room temperature [18] or higher temperature water [19], but generally the extraction with an acid medium is preferred so that amines linked to other matrix components can be also extracted. The most common acids used are HCl [20,21], $HClO_4$ [7,11,22] and trichloroacetic acid [23,24], as well as organic or organic-acid mixtures [12,25]. However, this approach require repeated extractions of the homogenized matrix, replacing the solvent with fresh each time, remixing, centrifuging and pooling the supernatant fractions, using large amounts of solvent further to be evaporated and disposed. Moreover, the formation of emulsions can further complicate the efficiency of extraction and adds greatly to required time for the analyst to complete the protocol. Another approach known as matrix solid phase dispersion (MSPD), involves the use of abrasives, such as sand, blended with the sample by means of a mortar and pestle. The shearing forces generated by the blending process disrupt the sample architecture and provide a more finely divided material for extraction. It follows that MSPD is a sample preparation technique that combines both sample homogenization and extraction of the analytes in one step thus offering useful applications in food analysis [26,27].

The objective of the present work is the analytical determination of eight bioactive amines (tryptamine, β-phenylethylamine, putrscine, cadaverine, histamine, tyramine, spermidine and spermine) in commercial non-fermented tomato-based products commonly consumed in Italy, using RP-HPLC-UV, dansyl-chloride as derivatizing agent and 1,7-diaminoheptane as internal standard. Optimization and comparison of two different methods, i.e. homogenization and MSPD, applied for analytes extraction will be also presented.

2. Experimental

2.1. Sampling

In total 48 samples of four product typologies were taken from supermarkets and retail shops: 15 mashed tomato samples in glass bottles, 10 biological mashed tomato samples in glass bottles, 8 concentrated tomato pasta samples in tins and 15 ketchup samples all produced by Italian manufacturers. Harvest year was not clearly declared and nearly all products were sampled at the beginning of their shelf life. Samples were stored at −20 °C and prior to analysis were thawed in original packages using warm water.

2.2. Chemical and reagents

The eight amines studied were: tryptamine (TRP), β-phenylethylamine (β-PEA), putrescine (PUT), cadaverine (CAD), hystamine (HIS), tyramine (TYR), spermidine (SPD) and spermine (SPM) all of which were supplied by Sigma Chemical (St. Louis, USA) as well as 1,7-diaminoheptane and dansyl-chloride. For MSPD experiments, sand (acid purified; 40–200 mesh) was purchased by Fluka (AG, Switzerland). The extraction solvent was $HClO_4$ from Carlo Erba (Milan, Italy) as well as all the other reagents. For chromatographic analysis, acetonitrile of HPLC grade Merck (Darmstadt, Germany) and purified water Millipore (Bedford, MA, USA) were used throughout.

2.3. Amine standard solutions

An individual standard solution of about 1 mg ml^{-1} of each amine were prepared in purified water and stored in darkness at 4 ± 1 °C. A water solution with the same concentration of 1 mg ml^{-1} was also prepared for the internal standard.

To perform calibration experiments, six standard solutions containing all the amines were obtained with different aliquots of each water solution, all diluted to 25 ml and added with $HClO_4$ 10.3 M to reach a final acid concentration of 0.6 M. The final amine concentration injected were 0.1, 0.4, 0.8, 4.0, 8.0 and 16.0 mg l^{-1}. All the solution contained the internal standard at the same concentration of 0.8 mg l^{-1}.

To perform recovery experiments, other standard solutions have been prepared depending on the extraction procedure used. In particular, for homogenization, 1 ml of each individual water solution (amine and IS) were pooled and diluted to 25 ml with water (concentration 0.04 mg ml^{-1}) (H1 + IS). One millilitre of this last solution was further diluted to 10 ml and added with $HClO_4$ 10.3 M in such a manner to obtain an acid concentration of 0.2, 0.4 or 0.6 M depending on the experiments to perform (H2 + IS). After dilution to 10 ml, the amine and IS concentration was 0.004 mg ml^{-1} each. Also, a water single solution of 0.04 mg ml^{-1} of the internal standard was prepared (ISH) by diluting to 25 ml with water, 1 ml of the water solution of 1 mg ml^{-1}.

In the same way, for the MSPD recovery experiments, 1 ml of each individual water solution (amines and IS) were pooled together reaching the final volume of 9 ml (concentration 0.111 mg ml^{-1} for each amine and IS) (S1 + IS). 0.25 ml of this solution was further diluted to 15 ml and added with $HClO_4$ 10.3 M for reaching the final acid concentration of 0.6 M (S2 + IS). After dilution to 15 ml, concentration was 0.0019 mg ml^{-1} for both amines and IS. A water solution of IS 0.111 mg ml^{-1} was also obtained by diluting to 9 ml with water, 1 ml of a solution 1 mg ml^{-1} (ISS).

2.4. Derivatization protocol

The dansylated derivatives of the amines were formed by adding to 1 ml standard solution (H2 + IS, S2 + IS or sample extract), 200 μl of NaOH 2 M, 300 μl of saturated $NaHCO_3$

solution, and 2 ml of dansyl-chloride solution (10 mg ml^{-1} in acetone). Fresh dansyl-chloride solutions were prepared each time just before use. After shaking, samples were left in the dark at room temperature for 20 min [11]. After reaction time has passed, the residual dansyl-chloride was removed by addition of 100 μl of NH_4OH 25% (v/v). Finally, the volume was adjusted to 5 ml with acetonitrile. After filtration with 0.45 μm filters (Polypro Acrodisc, Pall Gelmann Laboratory), a volume aliquot of 50 μl was injected (loop 50 μl) for the HPLC analysis.

2.5. Homogenization procedure

To an amount of 25 g of sample were added 75 ml of $HClO_4$ 0.6 M. The sample was homogenized and then centrifuged (3000 × *g* for 15 min). Supernatant was filtered through a 0.20 μm membrane Millipore filter and sediment was added with 15 ml of $HClO_4$ 0.6 M and centrifuged again (3000 × *g* for 10 min). The second extract was then filtered and added to the first. The final volume was adjusted to 100 ml with $HClO_4$ 0.6 M. An aliquot of 1 ml of the final extract was then used for analysis after derivatization while the remaining volume was stored at 4 °C for no more than one week.

Recovery experiments were performed by adding 10 ml of the H1 + IS solution to 25 g of tomato product (added sample) while the not added sample was obtained by mixing 10 ml of the ISH solution to the same sample weight (25 g); after the homogenization/extraction procedure previously described, 1 ml of the final acid extract was derivatized and 50 μl injected for HPLC analysis. The standard solution used for recovery experiments was H2 + IS, 1 ml of which were directly derivatized and then 50 μl injected.

To evaluate the possible interference of other matrix compounds on the amine recovery, another experiment was performed by adding the same aliquots of the H1 + IS, H2 + IS and ISH solutions after the whole extraction procedure just before adjusting the final volume to 100 ml.

2.6. MSPD procedure

To an amount of 5 g of sand was added 1 g of sample in an agate mortar and blended with a pestle for about 5 min. Once the MSPD blending process was complete, the material was transferred to a column constructed from a syringe barrel (10 ml) containing a paper frit for retaining the entire sample. The sample was then compressed to form a column packing by using a modified syringe plunger, placing a second frit on the top of the material before compression. Three extractions were then performed by gravity flow, using 5 ml $HClO_4$ 0.6 M each. The extracts were filtered, pooled and adjusted to 15 ml with $HClO_4$ 0.6 M. Finally, 1 ml of extract was derivatized and analyzed while the remaining volume stored at 4 °C.

In this case, recovery experiments were performed by adding 0.25 ml of the S1 + IS solution to 5 g of tomato product (added sample) while the not added sample was obtained by mixing 0.25 ml of the ISS solution to the same sample weight (5 g); after the MSPD procedure, 1 ml of the final extract was derivatized and 50 μl injected for HPLC analysis. The standard solution used for these experiments was S2 + IS, 1 ml of which were directly derivatized and then 50 μl injected.

As already described, also in this case, another experiment were performed by adding the same aliquots of the S1 + IS, S2 + IS and ISS solutions after the whole extraction procedure and just before adjusting the final volume to 15 ml.

2.7. Equipment

All measurements were performed with a liquid chromatograph Shimadzu (Tokyo, Japan) LC-10 ATVP, equipped with a UV–vis detector SP-10 AVP (Shimadzu) operating at λ = 254 nm and a loop of 50 μl. The analytical column was Supelcosil LC-18 (250 mm × 4.6 mm × 5 μm) with a Supelguard LC-18 (Supelco Inc., Bellefonte, PA 16823) pre-column. The analysis were performed to a fixed temperature of 25 °C. A homogenizer Universal Laboratory Aid MPW-309 and a centrifuge ALC 4236 were also employed.

2.8. Chromatographic conditions

Two solvent reservoirs containing (A) purified water and (B) acetonitrile were used to separate all the amines with an HPLC elution programme which began with 3 min of isocratic programme A–B 50:50 (v/v) reaching after 20 min A–B 10:90 (v/v). Then 3 min of isocratic elution were carried out and 4 min further were necessary to restore again the starting conditions (A–B 50:50, v/v). Flow was kept constant at 1.2 ml min^{-1}, for a total analysis time of 30 min and a time interval of 10 min between two injections was applied.

3. Results and discussion

3.1. Choice of acid concentration

Some authors have compared the extraction capability of several acid systems for different amines in different foods but they did not obtained the same results because these choices have to be related to the characteristics of the matrix to be analysed [28,29]. Kalač et al. [7] report the use of $HClO_4$ 0.6 M for the analysis of biogenic amines in ketchup and concentrated tomato pasta sample extracted by homogenization and determined by micellar electrokinetic chromatography. On the other hand, Lange et al. used trichloroacetic acid 5 and 10% for the extraction of the analytes from tomatoes in oil. After homogenization, amines were determined by HPLC and capillary electrophoresis. On the base of the available data, it was decided to use only perchloric acid at different concentrations (0.2, 0.4 and 0.6 M) and in Fig. 1, the influence of acid concentration on the amine recovery is reported. To a conventional mashed tomato sample, a known quantity (10 ml) of H1 + IS has been added. The standard solutions for these experiments were H2 + IS with the corresponding acid concentration (0.2, 0.4 and 0.6 M). The analysis was repeated six times and relative standard deviations ranged from 1.5% for PUT ($HClO_4$ 0.6 M) to 4.0% for SPD ($HClO_4$ 0.2 M). As can be seen, best recoveries are achieved using perchloric acid 0.6 M for all the considered

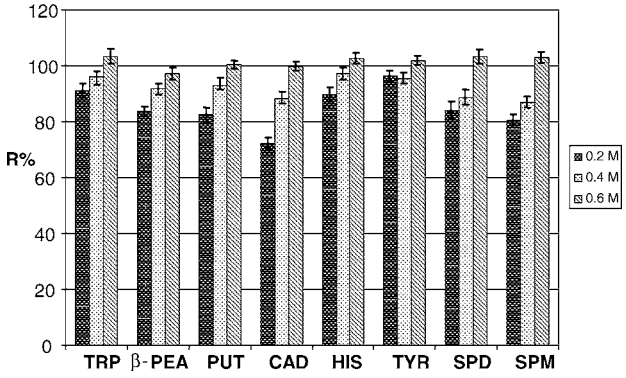

Fig. 1. Influence of $HClO_4$ concentration (0.2, 0.4 and 0.6 M) on amine percentage of recovery in a homogenized mashed tomato sample. Data are the mean value of six independent analysis and spiking (0.4 mg of each amine) has been accomplished before extraction procedure.

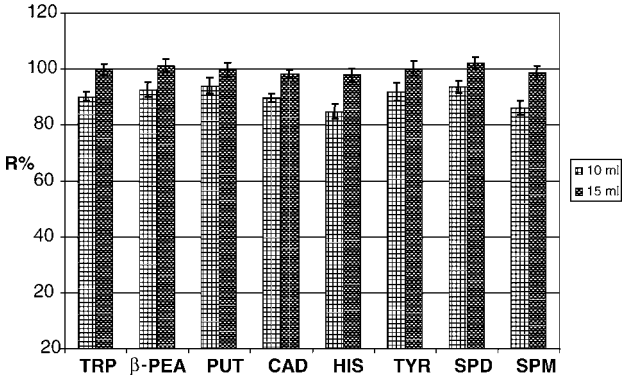

Fig. 2. Influence of final volume (10 and 15 ml) on amine percentage of recovery in a mashed tomato sample treated by MSPD technique. Data are the mean value of six independent analysis and spiking (0.028 mg of each amine) has been accomplished before sample treatment.

amines. Extraction has been accomplished by homogenization and the addition was carried out before the extraction procedure. The same experiment with post-extraction spiking (10 ml of H1 + IS to the acid extract) has been also performed (only for 0.6 M acid concentration) showing very small differences ranging from −3.1% for SPD to 2.1% for CAD (Table 1). The good recovery values obtained indicated that the applied extraction procedure did not produce any loss of analytes in the extract to be injected.

3.2. Choice of final volume (MSPD)

One of the parameters affecting MSPD performance is the nature and sequence of elution solvent addition. Most applications have utilized 8 ml of solvent to perform an elution starting from 0.5 g of sample and 2 g of bonded-phase solid support [26] and evidence from some studies indicates that most target analytes are eluted in the first 4 ml [30–32]. On the basis of these data, four final volumes were tested (5, 10, 15 and 20 ml) in order to obtain best amine extraction from the matrix. Fig. 2 shows the influence of this parameter on amine recovery for a conventional mashed tomato sample. Values come from six replicated analysis with relative standard deviations ranging from 1.5% for CAD (15 ml) to 3.6% for TYR (10 ml). As can be seen from Fig. 2, a final volume of 15 ml appear to me more suitable for a complete extraction of all amines while data for 5 and 20 ml are not reported because the first was not sufficient to perform any elution and a final volume of 20 ml just produced a further dilution of the sample with same recovery values of 15 ml final volume.

As already described, also in this case, the same addition of S1 + IS (0.25 ml at concentration 0.111 mg ml^{-1}) were carried out also after the extraction procedure. The standard solutions for these experiments were S2 + IS at acid concentration of 0.6 M. Obtained data (Table 1) showed that, for each final volume, comparison of recovery values for pre- and post-extraction spiking produced values ranging from −2.3% for TYR to 2.8% for CAD. Also in this case, the recovery values obtained from the two kinds of experiments, demonstrated that not substantial loss of analytes occurred during MSPD procedure resulting in good extraction yields for all the considered amines.

Table 1
Comparison of percentage of recovery and relative standard deviations in conventional tomato samples spiked either before or after the two different extraction methods

Amine	Homogenization				MSPD		
	0.2 M	0.4 M	0.6 M		10 ml	15 ml	
	R% (R.S.D.%) before	*R*% (R.S.D.%) before	*R*% (R.S.D.%) before	*R*% (R.S.D.%) after	*R*% (R.S.D.%) before	*R*% (R.S.D.%) before	*R*% (R.S.D.%) after
TRP	91.4 (2.9)	95.7 (2.4)	103.6 (2.8)	101.7 (1.8)	90.1 (1.8)	100.1 (2.2)	98.3 (2.1)
β-PEA	84.3 (2.6)	91.5 (2.4)	98.6 (2.2)	99.1 (2.0)	92.3 (3.1)	100.6 (2.6)	102.3 (2.8)
PUT	82.9 (3.5)	92.9 (2.3)	100.7 (1.5)	99.5 (2.3)	93.0 (3.1)	99.9 (2.7)	99.0 (1.7)
CAD	72.9 (3.0)	87.9 (2.5)	99.9 (1.6)	102.1 (1.9)	89.9 (1.6)	97.4 (1.5)	100.2 (2.3)
HIS	89.0 (2.9)	96.1 (2.2)	101.4 (2.1)	101.9 (2.4)	84.3 (3.5)	96.7 (2.4)	95.4 (1.8)
TYR	95.7 (2.3)	96.0 (2.3)	94.2 (1.6)	96.0 (2.2)	91.6 (3.6)	100.1 (2.8)	97.8 (2.0)
SPD	84.6 (4.0)	88.6 (3.2)	102.9 (2.7)	99.8 (2.6)	92.9 (2.4)	101.7 (2.2)	100.1 (2.7)
SPM	80.7 (2.7)	87.1 (2.5)	102.7 (2.1)	100.9 (1.8)	85.8 (3.1)	98.7 (3.0)	96.8 (2.8)

For homogenization treated samples, acid concentration was 0.2, 0.4 and 0.6 M; 0.4 mg of each amine were added before extraction. Only 0.6 M acid concentration was tested for experiments of spiking after sample treatment. For MSPD treated samples, final volume was 10 and 15 ml (addition before MSPD) and addition of 0.028 mg of each amine was carried out. Only a final volume of 15 ml was tested in experiments of addition after extraction.

Table 2
Method performances

	R_t (min)	Concentration range (mg l^{-1})	Regression Eq.	R^2	LIN (%)	AS (μg l^{-1})	DL (μg l^{-1})	DL_{S1} (mg kg^{-1})	DL_{S2} (mg kg^{-1})
TRP	11.8	0.1–16.0	$A = 694743 + 244642C$	0.999	98.99	23.1	42.8	1.3	1.2
β-PEA	12.7	0.1–16.0	$A = 53548 + 223994C$	0.998	99.20	37.3	64.2	0.4	0.6
PUT	14.2	0.1–16.0	$A = 72630 + 88316C$	1.000	99.70	3.3	8.0	0.8	0.8
CAD	15.1	0.1–16.0	$A = 28733 + 373251C$	0.999	99.25	8.2	17.1	0.7	0.6
HIS	15.8	0.1–16.0	$A = 73162 + 460595C$	0.999	99.85	27.1	50.4	0.5	0.4
TYR	20.2	0.1–16.0	$A = 28705 + 473490C$	0.999	99.10	30.3	61.2	1.1	0.9
SPD	21.4	0.1–16.0	$A = -86715 + 591162C$	1.000	99.97	11.1	20.4	0.8	0.7
SPM	25.8	0.1–16.0	$A = -75535 + 416653C$	0.999	98.96	14.7	27.0	0.4	0.5

R_t: retention time; A: amine to IS peak area ratio; C: concentration injected; R^2: square of regression coefficient; LIN linearity on-line; AS: analytical sensitivity; DL: detection limit in the injected solution; DL_{S1}: detection limit in food sample obtained by homogenization; DL_{S2}: detection limit in food sample obtained by MSPD.

3.3. Performance characteristics

Table 2 shows the performance characteristics of the method. Data for calibration curves were collected, using triplicate responses, for six different concentrations ranging from 0.1 to 16.0 mg l^{-1}. Calibration graphs were constructed by plotting the amine to IS peak areas ratios (A) against the amine concentrations (C). Linearity was observed into the considered concentration range for each compound with good regression coefficients values. The linearity 'on-line' is defined as $LIN\% = 100\,(1 - S_b)$ [33] where S_b is the slope standard deviation, and indicates the greater or lesser dispersion of the data around the calibration line. On the other hand, the analytical sensitivity (AS), was calculated following the expression: $AS = S_{A,C}/b$ where $S_{A,C}$ is the regression standard deviation of A versus C, and b is the slope of the regression line. Data reported show good values obtained for all amines. The limits of detection were calculated from the amount of amines required to give a signal-to-noise ratio of 3. The detection limits recalculated with respect of sample preparation were obtained following the method of Křížek and Pelikánová [34] resulting in DL_{S1} and DL_{S2}, where DL_{S1} and DL_{S2} means the detection limit in food sample obtained by homogenization and MSPD technique, respectively.

Repeatability of the analytical procedure was also tested by six parallel analysis of a conventional mashed tomato sample, treated by both extraction techniques. Using homogenization, relative standard deviations were 3.6, 6.1, 2.8, 5.9, 3.4, 2.9, 4.1 and 7.1 % at mean concentrations of 5.54, 2.23, 10.81, 4.21, 1.76, 20.37, 8.34 and 1.28 mg kg^{-1} for TRP, β-PEA, PUT, CAD, HIS, TYR, SPD and SPM, while the same values for MSPD technique were 3.8, 5.5, 3.5, 6.6, 3.0, 3.2, 4.8 and 8.2% with mean concentrations of 5.45, 2.13, 10.90, 4.52, 1.81, 19.81, 8.63 and 1.52 mg kg^{-1}.

3.4. Amine determination

In Fig. 3, chromatograms of H2 + IS and of a mashed tomato sample are shown while data of bioactive amines concentrations of each class of products are reported for homogenization and MSPD techniques in Tables 2–5 and in Table 6, respectively. As can be seen, data achieved by these techniques are comparable either in terms of mean concentrations or in terms of standard deviations showing that both procedures can be used interchangeably for extraction of amines from tomato-based products.

In non-fermented foods, the presence of biogenic amines above a certain level is considered as indicative of undesired microbial activity. Therefore, the amine level could be used as an indicator of microbial spoilage. For all class of analyzed tomato products, PUT was the most abundant amine, followed

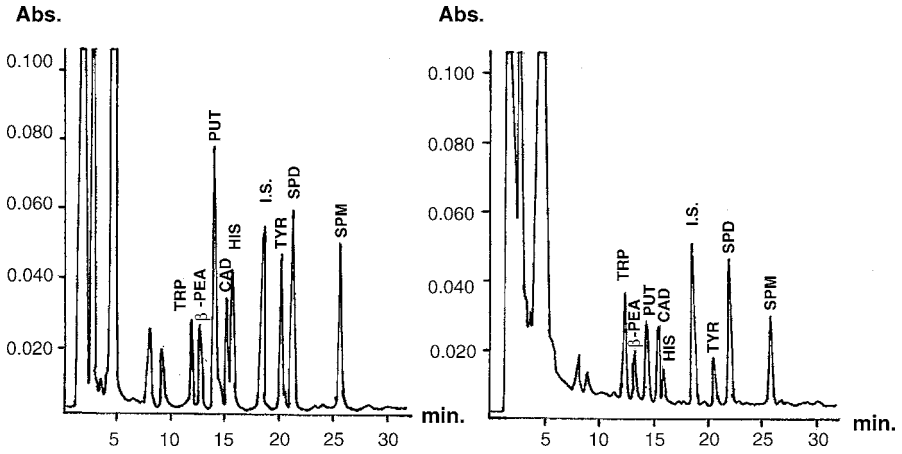

Fig. 3. Chromatograms of: amine standard solution at concentration 0.8 mg l^{-1} each with internal standard (left); homogenized mashed tomato sample with internal standard (right).

Table 3
Analytical quantities of bioactive amines in conventional mashed tomato samples ($n = 15$) treated by homogenization and MSPD

Amine	$n > DL_{S1}, DL_{S2}$[a]	Homogenization				MSPD			
		$x_{mean} > DL_{S2}$[b]	sx[c]	x_{min}	x_{max}	$x_{mean} > DL_{S1}$[b]	sx[c]	x_{min}	x_{max}
TRP	13	6.25	4.30	1.92	15.90	7.43	5.82	1.66	16.41
β-PEA	4	2.05	1.15	0.88	3.24	1.82	1.32	0.74	3.36
PUT	15	14.27	6.28	8.39	30.43	16.32	5.65	9.32	35.31
CAD	9	2.76	2.36	1.08	9.05	3.00	2.36	0.91	9.77
HIS	3	2.83	1.92	1.10	4.89	2.65	1.61	1.00	4.21
TYR	12	7.73	6.36	2.50	25.12	8.62	5.51	2.16	23.85
SPD	14	6.78	3.37	2.13	13.66	7.21	3.37	2.41	13.21
SPM	5	1.44	0.87	0.98	3.15	1.53	1.44	1.14	3.86

Analysis was repeated six times.
[a] Number of samples with concentrations (mg kg^{-1}) above detection limits DL_{S1}, DL_{S2}.
[b] Mean values for samples with concentrations (mg kg^{-1}) above detection limits DL_{S1}, DL_{S2}.
[c] Standard deviation calculated using values of the $x_{mean} > DL_{S1}$ or DL_{S2}.

Table 4
Analytical quantities of bioactive amines in biological mashed tomato samples ($n = 10$) treated by homogenization and MSPD

Amine	$n > DL_{S1}, DL_{S2}$[a]	Homogenization				MSPD			
		$x_{mean} > DL_{S1}$[b]	sx[c]	x_{min}	x_{max}	$x_{mean} > DL_{S2}$[b]	sx[c]	x_{min}	x_{max}
TRP	8	9.26	7.20	3.31	26.90	10.05	7.20	3.91	28.00
β-PEA	3	2.15	1.49	1.15	3.86	1.86	1.93	1.27	4.01
PUT	10	23.31	8.54	9.38	46.77	22.71	8.54	8.33	48.21
CAD	4	1.48	0.69	0.70	2.92	1.54	1.40	0.62	3.21
HIS	3	1.98	1.63	1.35	2.70	1.87	0.64	1.17	2.44
TYR	8	14.35	12.50	5.82	36.90	13.81	10.01	4.39	38.21
SPD	9	9.50	5.76	2.95	25.69	9.92	6.71	3.06	24.98
SPM	2	2.32	1.36	1.36	3.28	2.80	1.70	1.60	4.00

Analysis was repeated six times.
[a] Number of samples with concentrations (mg kg^{-1}) above detection limits DL_{S1}, DL_{S2}.
[b] Mean values for samples with concentrations (mg kg^{-1}) above detection limits DL_{S1}, DL_{S2}.
[c] Standard deviation calculated using values of the $x_{mean} > DL_{S1}$ or DL_{S2}.

Table 5
Analytical quantities of bioactive amines in concentrated tomato pasta samples ($n = 8$) treated by homogenization and MSPD

Amine	$n > DL_{S1}, DL_{S2}$[a]	Homogenization				MSPD			
		$x_{mean} > DL_{S1}$[b]	sx[c]	x_{min}	x_{max}	$x_{mean} > DL_{S2}$[b]	sx[c]	x_{min}	x_{max}
TRP	8	6.95	5.41	1.34	18.68	7.71	4.18	1.28	19.32
β-PEA	5	1.08	2.10	0.47	3.85	1.21	2.31	0.54	4.21
PUT	8	33.35	10.44	9.65	54.73	32.09	8.32	9.11	53.61
CAD	7	3.06	2.51	1.29	5.81	2.88	3.00	1.20	6.21
HIS	2	1.99	2.00	0.57	3.40	1.89	2.09	0.41	3.37
TYR	8	21.01	10.16	2.15	38.23	22.18	8.21	1.92	37.53
SPD	6	11.02	6.24	2.45	30.41	10.43	4.92	2.23	31.92
SPM	3	1.39	0.79	0.63	2.21	1.19	0.87	0.54	2.17

Analysis was repeated six times.
[a] Number of samples with concentrations (mg kg^{-1}) above detection limits DL_{S1}, DL_{S2}.
[b] Mean values for samples with concentrations (mg kg^{-1}) above detection limits DL_{S1}, DL_{S2}.
[c] Standard deviation calculated using values of the $x_{mean} > DL_{S1}$ or DL_{S2}.

by TYR, SPD and TRP while HIS, SPM and β-PEA were often not detectable. Particularly high levels of TRP have been found in all class of products, as previous papers reported [4]. Considering that this amine is present at very low concentrations in almost all other foods [5,6], TRP could be a quality marker specific for tomato and tomato products.

Ketchup shows the highest level for all amines followed by concentrated tomato pasta, biological mashed tomato and conventional mashed tomato. This could be related with the higher complexity of technological tomato processing, passing from mashed tomato to ketchup, which could responsible of the observed increased values. It has been reported that the

Table 6
Analytical quantities of bioactive amines in ketchup samples ($n = 15$) treated by homogenization and MSPD

Amine	$n > DL_{S1}, DL_{S2}$[a]	Homogenization				MSPD			
		$x_{mean} > DL_{S1}$[b]	sx[c]	x_{min}	x_{max}	$x_{mean} > DL_{S2}$[b]	sx[c]	x_{min}	x_{max}
TRP	14	12.21	5.41	2.34	32.88	13.05	4.77	2.21	33.98
β-PEA	4	1.88	2.89	0.85	5.00	2.03	1.98	0.90	4.77
PUT	15	42.21	8.82	15.71	77.29	42.95	10.15	14.78	75.97
CAD	7	4.53	1.21	2.93	8.71	4.71	3.01	3.00	9.15
HIS	5	5.21	2.60	3.58	7.90	5.01	3.10	3.76	8.32
TYR	15	26.95	10.83	19.71	51.23	27.86	13.01	19.29	50.23
SPD	11	19.31	6.24	5.45	30.41	19.69	5.34	4.93	31.07
SPM	5	3.09	1.70	1.93	5.28	2.84	1.23	1.87	5.06

Analysis was repeated six times.
[a] Number of samples with concentrations (mg kg^{-1}) above detection limits DL_{S1}, DL_{S2}.
[b] Mean values for samples with concentrations (mg kg^{-1}) above detection limits DL_{S1}, DL_{S2}.
[c] Standard deviation calculated using values of the $x_{mean} > DL_{S1}$ or DL_{S2}.

original concentration of amines in food can also be changed as a result of storage conditions, which is a parameter to be controlled [28,29]. On the other hand, ketchup also shows the broader range of concentrations observed for each amine, maybe because the analysed products are quite different in composition and in ingredient proportions producing very variable data for the same amine. Also, raw materials are related to the widely variable amine concentration ranges. This is particularly true for natural polyamines such as PUT and SPD and to a lesser extent to CAD and SPM. This is in agreement with data obtained by Kalač et al. [7] who determined biogenic amines in ketchup and concentrated tomato pasta among others. However, it must be said that the variability of the obtained concentrations in the latter class of products could be also related to the small number of samples commercially available.

Finally, the higher levels of analytes found in biological mashed tomato in comparison with its conventional counterpart is quite surprising. European Community establishes [35] very strict agronomic and processing rules for biological products. Maybe the absence of additives, generally limiting the accumulation of amines [28], could be responsible of the higher amine levels determined in these tomato products.

It has been reported that an intake higher than 40 mg biogenic amines per meal can be considered potentially toxic [36]. However, not all amines are equally toxic; consequently, HIS, TYR and β-PEA are of concern. More recently, Nout [37] proposed for fermented foods 50–100 ppm, 100–800 ppm and 30 ppm for HIS, TYR and β-PEA, respectively, or a total of 100–200 ppm. Such levels could be regarded as acceptable also for non-fermented foods. Data obtained show that none of the analyzed samples represent a possible risk for consumer health, although additional risk factors such as amine oxidase-inhibiting drugs, alcohol and gastrointestinal diseases may play an important role in determining the threshold for bioactive amines toxicity.

4. Conclusions

Homogenization and matrix solid phase dispersion techniques have been exploited for determining eight bioactive amines in tomato products by RP-HPLC-UV with dansyl-chloride as derivatizing agent and 1,7-diaminoheptane as internal standard. Extraction procedure have been optimized (extracting acid concentration, extract final volume) and compared, showing similar results for all the samples analyzed. The most abundant amines were PUT, TYR, SPD and TRP found at highest levels in ketchup, followed by concentrated tomato pasta, biological mashed tomato and conventional mashed tomato. According to the toxicity levels reported in literature and regarded as acceptable, all the sample analyzed not represent a potential health hazard for consumers, although a better understanding of the mechanism by which biogenic amines are being produced is necessary to prevent their formation. Generally, biogenic amines in food can be controlled by strict use of good hygiene in both raw material and manufacturing environments with corresponding inhibition of spoiling microorganisms.

References

[1] S. Bardócz, G. Grant, D.S. Brow, D.S. Ralph, A. Putsztai, J. Nutr. Biochem. 4 (1993) 66.
[2] R.L. Maijala, S.H. Eerola, Meat Sci. 35 (1993) 387–395.
[3] A. Halàsz, A. Baràth, L. Simon-Sarkadi, W. Holzapfel, Trends Food Sci. Technol. 5 (1994) 42.
[4] T.A. Smith, Food Chem. 6 (1980) 169.
[5] D.M. Beutling, Biogene Amine in der Ernährung, Springer-Verlag, Berlin, 1996.
[6] T. Davidek, J. Davidek, in: J. Davidek (Ed.), Natural Toxic Compounds in Foods, Boca Raton, CRC Press, 1995, pp. 108–123.
[7] P. Kalač, S. Švecová, T. Pelicánová, Food Chem. 77 (2002) 349.
[8] D.D. Rawles, G.J. Flick, R.E. Martin, Adv. Food Nutr., Res. 39 (1996) 329.
[9] A.R. Shalaby, Food Chem. 49 (1994) 305–310.
[10] L. Simon-Sarkadi, W.H. Holzapfel, Z. Lebensm, Unters. Forsch. 198 (1994) 230.
[11] G. Vinci, M.L. Antonelli, Food Control 13 (2002) 519.
[12] J. Lange, K. Thomas, C. Wittman, J. Chromatogr. B 779 (2002) 229.
[13] Z. Loukou, A. Zotou, J. Chromatogr. A 996 (2003) 103.
[14] P.L. Rogers, W. Staruszkiewicz, J. AOAC Int. 80 (1997) 591.
[15] M. Hall, D.B. Heldridge, R.D. Saunders, D.L. Fairclough, R.C. Bateman Jr., Food Biotechnol. 9 (1995) 39.
[16] K. Zeng, H. Tachikawa, Z. Zhu, V.L. Davidson, Anal. Chem. 72 (2000) 2215.
[17] S. Moret, R. Bortolomeazzi, G. Lercker, J. Chromatogr. 591 (1992) 175.
[18] D.L. Ingles, J.F. Back, D. Gallimore, R. Tindale, K.J. Shaw, J. Sci. Food Agric. 36 (1984) 402.

[19] M.N. Voigt, R.R. Eitenmiller, P.E. Kowhlerand, M.K. Handy, J. Milk Food Technol. 37 (1973) 377.
[20] R. Romero, M.G. Bagur, D. Gàzquez, M. Sànchez-Viñas, L. Cuadros-Rodriguez, M. Ortega, LCGC North Am. 22 (2) (2004) 150.
[21] B. Fontaniella, J.L. Mateos, C. Vicente, M.-E. Legaz, J. Chromatogr. A 919 (2001) 283.
[22] S. Eerola, R. Hinkkanen, E. Lindfors, T. Hirvi, J. AOAC Int. 76 (1993) 575.
[23] S. Moret, L.S. Conte, J. Chromatogr. A 729 (1996) 363.
[24] S. Suzuki, K. Kobayashi, J. Noda, T. Suzuki, K. Takama, J. Chromatogr. 508 (1990) 225.
[25] K. Takeba, T. Maruyama, M. Matsumoto, H. Nakazawa, J. Chromatogr. 504 (1990) 441.
[26] S.A. Barker, J. Chromatogr. A 880 (2000) 63.
[27] O. Busto, Y. Valero, J. Guash, F. Borull, Chromatographia 38 (1994) 571.
[28] A.R. Shalaby, Food Res. Int. 29 (7) (1996) 675.
[29] M.H. Silla Santos, Int. J. Food Microbiol. 29 (1996) 213.
[30] S.A. Barker, J. Chromatogr. A 885 (2000) 115–127.
[31] M.E. Hines, A.R. Long, T.G. Snider, S.A. Barker, Anal. Biochem. 195 (1991) 197.
[32] S.A. Barker, A.R. Long, in: V.K. Agarwal (Ed.), Analysis of Antibiotic Drug Residues in Food Products of Animal Origin, Plenum Press, New York, 1992, p. 119.
[33] L. Cuadros Rodrìguez, A.M. Garcìa Campanã, J.M. Bosque Sendra, Anal. Lett. 29 (1) (1996) 1231.
[34] M. Křížek, T. Pelikánová, J. Chromatogr. A 815 (1998) 243.
[35] European Commission. Reg. CEE 209/91, Bruxelles, 1991.
[36] J.C. Ayres, J.O. Mundt, W.E. Sandine, Microbiology of Foods, W.H. Freeman and Co., San Francisco, CA, 1980, p. 543.
[37] M.J.R. Nout, Food Res. Int. 27 (1994) 291.