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Multidetection, semiquantitative method for determining biogenic amines in foods

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A detection and semiquantitative determination method of eight biogenic amines (histamine, cadaverine, putrescine, phenylethylamine, tyramine, tryptamine, spermine and spermidine) in foods has been described. The complete resolution of the dansylated derivatives of amines from each other and from background interference by one-dimensional TLC was obtained by a multiple development technique. The first developing solvent system was chloroform/benzene/triethylamine (6:4:1 v/v/v) and the second one was benzene/acetone/triethylamine (10:2:1 v/v/v). Identification was achieved with the help of co-developed standards and estimation was achieved visually. As little as 5 or 10 ng of dansylamines could be detected on TLC plates, depending on the type of amine involved. About 2 h are needed for separation and quantitation of amine contents of 14 samples simultaneously. The method has been successfully used to screen fish and meat products and cheese samples for their contents of biogenic amines. The method may be used in the food industry and for routine surveillance of biogenic amines in food.

INTRODUCTION

Histamine, putrescine, cadaverine, tyramine, tryptamine, phenylethylamine, spermine and spermidine are considered to be the most important biogenic amines occurring in foods. The biogenic amine contents of various foods have been widely studied and reported in fish and fish products, cheese and fermented meat products (Shalaby, 1990, 1993; Stratton *et al.*, 1991; Yen & Hsieh, 1991; Tawfik *et al.*, 1992).

Biologically active amines have been implicated as the causative agents in a number of food poisoning episodes, particularly histamine toxicity 'scombroid poisoning' (Taylor *et al.*, 1984) and tyramine toxicity 'cheese reaction' (Smith, 1980). It was reported that 100 mg histamine in a 100 g sample could induce a toxic response in humans (Arnold & Brown, 1978). However, several other compounds, such as putrescine and cadaverine, have been reported to potentiate the biological activity of histamine (Parrot & Nicot, 1966; Bjeldanes *et al.*, 1978) leading to the reduction of the histamine level having toxic effects. Sandler *et al.* (1974) reported that 3 mg of phenylethylamine causes migraine headaches in susceptible individuals, while 6 mg total tyramine intake was stated to be a dangerous dose for patients receiving monoamine oxidase inhibitors (MAOI) (Blackwell & Mabbitt, 1965).

Several methods for estimating biogenic amines in foods have been reviewed (Treptow & Askar, 1990). The complex sample matrix, the presence of potentially interfering compounds and the occurrence of several biogenic amines simultaneously in the same aliquot of an extract are typical problems encountered in the analysis of food for biogenic amines. The methods used to analyse food for amines individually would be extremely difficult and time-consuming, since they require large-scale and laborious purification. Many methods have been published that screen foods for amine content and offer great potential for fully measuring these compounds (Mietz & Karmas, 1978; Staruszkiewicz & Bond, 1981; Chin & Koehler, 1983; Joosten & Olieman, 1986; Tani & Ishihara, 1990; Shalaby, 1994). However, these methods require expensive instrumentation such as HPLC, GLC or spectrophotofluorometry.

The aim of this work was to establish a simple method for biogenic amine analysis in food, independent of expensive instrumentation, and useful as a routine control procedure. Therefore, a thin-layer chromatography method was developed and adapted for visual estimation of eight biogenic amines in foods.

MATERIALS AND METHODS

Source of samples

Twenty-eight samples, consisting of smoked herring (9 samples), cheese (9 samples) and sausage (10 samples)

were collected randomly from a local market in Cairo, Egypt. Samples of about 1 kg each were ground using a domestic meat mincer and thoroughly mixed; 50-g samples were used for analysis.

Chemicals and supplies

Amines (histamine (Him), cadaverine (Cad), putrescine (Put), tyramine (Tyr), tryptamine (Try), β-phenylethylamine (β-phe), spermine (Spm), and spermidine (Spd)) as their crystalline hydrochlorides as well as dansyl chloride (5-dimethylaminonaphthalene-1-sulphonyl) were purchased from E. Merk, Germany. The stock standards were prepared at a concentration of 0·5 mg ml^{-1}. Thin-layer chromatography (TLC) plates (20 × 20 cm aluminium sheets precoated with 0·25 mm silica gel G. 60) were purchased from Macherey-Nagel Duren, Germany. All chemicals were of reagent grade, and double-distilled water and solvents were used.

Amines extraction

Ground food (50 g) was extracted with 5% trichloroacetic acid (TCA) (3 × 75 ml) using a Waring Blender. Each blended mixture was centrifuged and the clean extracts combined. The volume was adjusted to 250 ml with 5% TCA solution. The equivalent of 2 g of samples as the TCA extract (10 ml) was made alkaline with sodium hydroxide and extracted with *n*-butanol/chloroform (1:1 v/v/) (3 × 5 ml). The combined organic phase, after the addition of an equal amount of *n*-heptane (15 ml), was extracted with several 1-ml portions of 0·02 N HCl and the aqueous extract dried.

Derivative formation

The dansylated derivatives of the amines were formed by adding saturated sodium bicarbonate solution (0·50 ml) to the residue, then adding 1ml of dansyl chloride reagent (500 mg in 100 ml acetone) using a Vortex mixer while adding the reagent. After standing for more than 10 h, the dansylamines were extracted by adding water (about 15 ml) and extracting the mixture with several portions of diethylether. The combined ether extracts were evaporated to dryness and the residue redissolved in acetonitrile (2 ml).

Standard solution preparation

The mixture of standard solution as dansyl derivatives was prepared using 50 μl each of tryptamine, spermine and phenylethylamine stock standard solution, and 100 μl each of other stock solutions. Using a current of air and a steam bath, the prepared solution (650 μl) was evaporated to less than 200 μl. The dansyl derivatives were prepared as described above. The residue was dissolved in 5 ml acetonitrile. Afterwards, 1 ml was diluted to 10 ml using acetonitrile (10 μl = 5 or 10 ng of amine as derivatives, according to the amine involved).

Separation of the dansylamines

The chromatographic separation was carried out to separate the eight dansylamines by one-dimensional TLC. On 2 cm from the bottom edge of the TLC plate and at intervals of 1 cm the following samples were applied using a microsyringe: 10, 15, 20, 30, 50 μl of dansylamine standard, and 10 μl each of dansylated food extract.

The plate was developed in chloroform/benzene/triethylamine (6:4:1 v/v/v) for 15 cm. The plate was taken out of the jar and allowed to dry. Then it was developed in the same direction in benzene/acetone/triethylamine (10:2:1 v/v/v) for 15 cm. The plate was allowed to dry at room temperature, then dried with a hair dryer until the excess of solvent disappeared before interpretation.

Interpretation of the chromatogram

The chromatogram after the second development was examined under long-wave (360 nm) ultraviolet light to establish whether or not the dansyl amines of interest are present in the sample. The amine spots from the extract can be identified with the help of co-developed standards. The presumed amine spot should coincide with the reference standard in R_f value and hue.

Quantitation of dansylamines

The intensity of each amine spot of the sample was compared with that of the standard to determine which of the standard spots matched the sample spot. If the intensity of fluorescence given by the 10 μl of extract is greater than that of the 50 μl of standard solution, dilution of the extract should be made with acetonitrile before repeating TLC. The calculation of the concentration of each amine in the sample is made using the following formula:

$$\mu g\ g^{-1}\ (mg\ kg^{-1}) = \frac{SYV}{XW}$$

where S is the amine standard equal to unknown (μl); Y is the concentration of amine standard (μg ml^{-1}); V is the final dilution of sample extract (ml); X is the sample extract given a spot intensity equal to S (μl); and W is the mass of the sample represented by the final extract in grams.

RESULTS AND DISCUSSION

The method described above began with the selection of an appropriate solvent system for separation of the eight biogenic amines by one-dimensional TLC. Separation of dansylated compounds representing any combination of the tested amines is possible only with the multiple development technique described (Fig. 1). The technique used resulted in improved resolution of amines from each other and from interfering materials, and compact and intense spots were obtained. How-

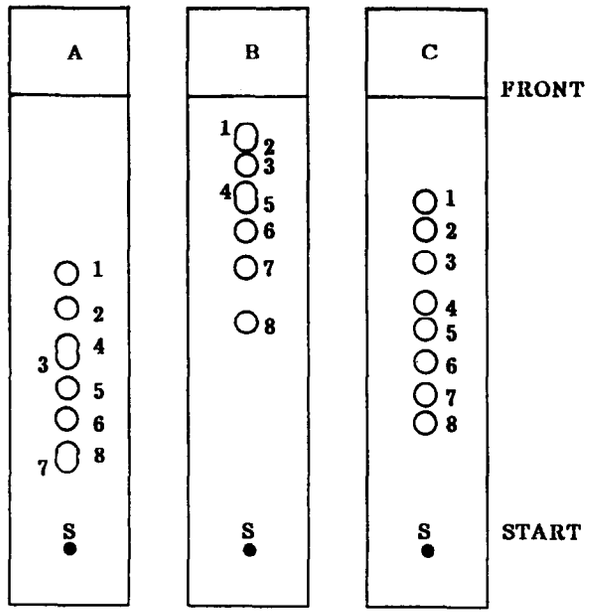

Fig. 1. Schematic of one-dimensional thin layer chromatographic separation of dansylated biogenic amines (1, β-phe; 2, Tyr; 3, Spm; 4, Him; 5, Spd; 6, Cad; 7, Put and 8, Try) spotted as mixture at S. A, single development with chloroform/benzene/triethylamine (6:4:1 v/v/v; B, single development with benzene/acetone/triethylamine (10:2:1 v/v/v) and C, multiple development (twice) with A and B, respectively.

Table 1. R_f Values, fluorescent colours and detection limits (ng/spot) on TLC plate for dansylated amines with pure standard solution and with standard added to 10 μl food extract

Dansylamine	R_f	Minimum detectable quantity (ng)		Spot fluorescent colour[a]
		Standard pure	Standard and 10 μl extract	
Tryptamine	0·45	10	10	Greenish yellow
Putrescine	0·49	5	5	Green
Cadaverine	0·57	5	5	Green
Spermidine	0·62	5	5	Green
Histamine	0·66	5	5	Orange–yellow
Spermine	0·71	10	10	Green
Tyramine	0·78	5	5	Yellow
Phenylethylamine	0·81	10	10	Light blue

[a]Under longwave (360 nm) ultraviolet light.

ever, care must be taken that the diameter of the spot for total volume deposit does not exceed 3 mm.

Table 1 gives R_f values obtained using the multiple development technique along with the fluorescent colours of the developed dansylated amines under long wavelength UV light. The presence on the chromatoplate of amine standard spots developed adjacent to the amine sample spots facilitates the localization and also identification of amines in food commodities.

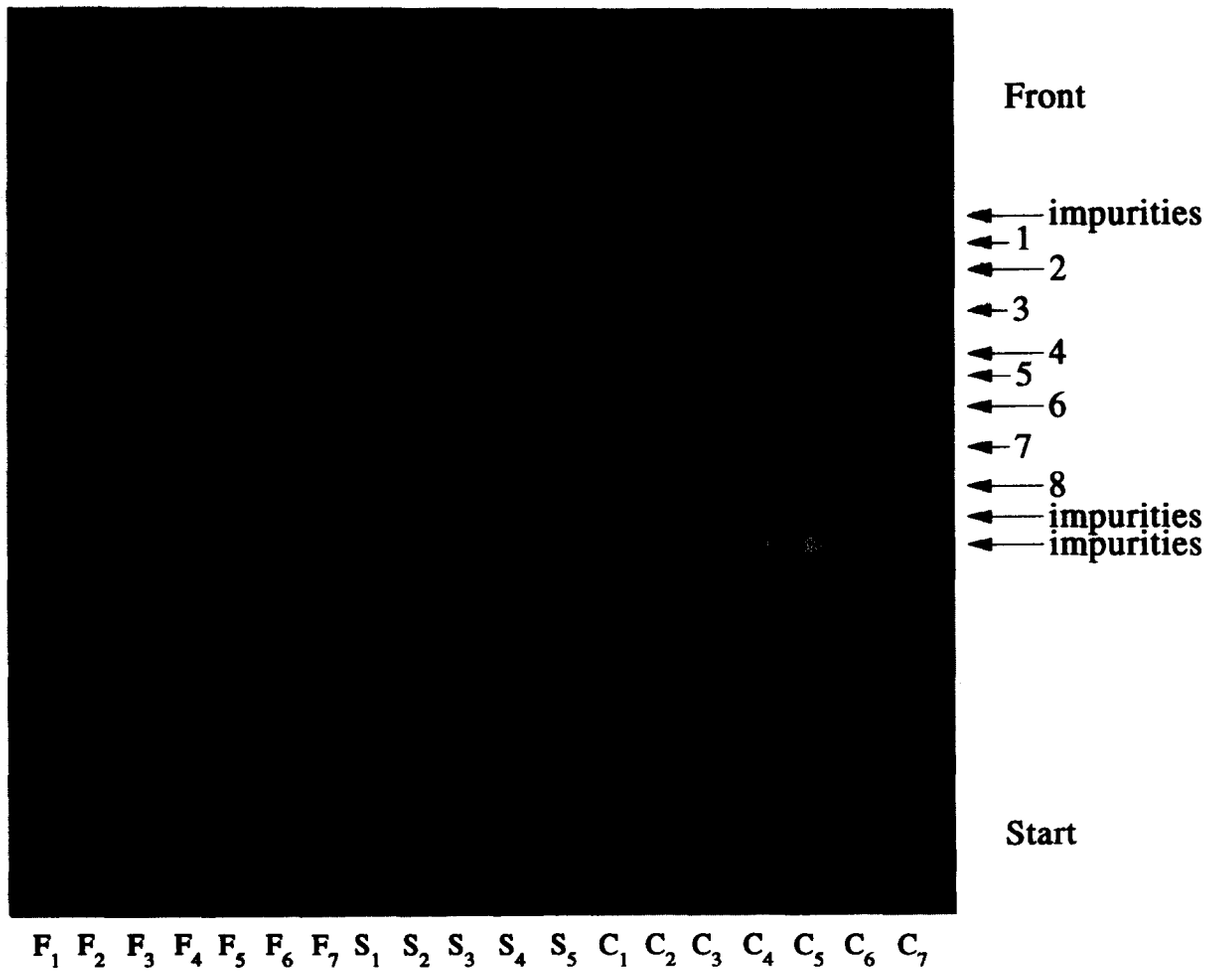

Fig. 2. One-dimensional separation of dansylated biogenic amines using multiple development technique. F_1–F_7 = 10 μl dansylated fish extracts; C_1–C_7 = 10 μl dansylated cheese extracts; S_1, S_2, S_3, S_4 and S_5 = 10, 15, 20, 30 and 50 μl dansylated standard, respectively. For description of the separated spots, see Fig. 1.

Table 2. Biogenic amine contents in various samples of smoked herring

Sample	Biogenic amines ($\mu g\ g^{-1}$)							
	β-phe	Tyr	Spm	Him	Spd	Cad	Put	Try
1	ND	1	2	ND	1	1	1	ND
2	ND	ND	16	8	ND	8	8	ND
3	ND	ND	ND	8	ND	4	4	ND
4	ND	2	2	1	2	2	2	ND
5	ND	ND	2	ND	1	4	2	ND
6	ND	4	8	4	ND	2	8	ND
7	ND	8	6	2	1	4	2	ND
8	ND	ND	16	8	2	1	2	ND
9	ND	2	2	16	ND	8	4	ND

ND, not detectable.

Figure 2 depicts the pattern of separation of various dansylated food extracts on a TLC plate. It seems that the detection of amines present in the extracts does not create any problem, providing the background in the biogenic amines region following TLC is not too intense. This background did not appear to make any contribution to the brightness of dansylamines in any sample extracts. Less polar spots, however, were observed at about R_f 0·40 and 0·42, but these did not interfere with the biogenic amines. It is important to note that experience is not necessary to locate and identify the dansylamine spots developed from sample extracts.

Detection of standards alone on a TLC plate is simple, but detection and measurement in the presence of food extracts is more difficult. The TLC procedure is of value for semiquantitative screening of food. Fortunately, dansylated amines emit the energy of absorbed longwave UV light as fluorescent light, enabling the analyst to detect these compounds at low levels on the chromatogram. The natural fluorescence (under UV light) of the separated spots of dansylated biogenic amines from sample extracts can be compared with that of standard spots by eye. The combination of sample size used for quantitation (10 mg) and the good resolution of dansylated amines provided a lower limit of detection. It was found that spots of about 5 or 10 ng dansylamines can be easily detected on a TLC plate even in the presence of food extract (Table 1), indicating a potentially lower limit of detection of about 0·5 or 1·0 $\mu g\ g^{-1}$, depending on the type of amine involved. The direct assay on the TLC plate avoids the extraction and/or transfer errors of other methods.

The earlier methods of detection and determination of biogenic amines in food rely on sophisticated and expensive equipment such as GLC, HPLC and spectrophotofluorometry, and are time-consuming. Thus they are not suitable for routine monitoring and screening analysis. On the other hand, the proposed procedure is considered the cheapest and fastest, since the time of analysis of one TLC plate (containing 14 samples) is approximately 2 h. Moreover, additional plates can be developed in one run, which increases efficiency in routine analysis.

The proposed procedure has been successfully used as a screening technique for examination of food samples from a limited survey. The amine contents of smoked herring are summarized in Table 2. In general the smoked fish analyzed in this study contained low levels of amines. All samples contained putrescine, cadaverine and spermine, while tyramine and spermidine were found only in a few samples at low levels. Histamine was found in 7 of 9 samples tested. Tryptamine

Table 3. Biogenic amine contents in various samples of sausage

Sample	Biogenic amines ($\mu g\ g^{-1}$)							
	β-phe	Tyr	Spm	Him	Spd	Cad	Put	Try
1	ND	320	2	2	ND	60	4	ND
2	16	160	4	8	8	ND	ND	2
3	ND	2	ND	16	ND	ND	2	ND
4	ND	200	ND	10	2	8	16	8
5	ND	540	8	2	4	16	ND	10
6	ND	2	2	4	ND	ND	8	ND
7	4	4	ND	ND	ND	ND	ND	8
8	ND	210	ND	2	2	2	10	ND
9	40	8	ND	ND	2	20	2	4
10	ND	8	2	8	ND	40	2	2

ND, not detectable

Table 4. Biogenic amines contents in various samples of ripened cheese

Sample	Biogenic amines ($\mu g\ g^{-1}$)							
	β-phe	Tyr	Spm	Him	Spd	Cad	Put	Try
1	320	160	ND	ND	ND	3	ND	ND
2	400	120	ND	4	ND	3	ND	ND
3	8	160	6	ND	ND	2	ND	ND
4	4	8	ND	ND	ND	3	6	ND
5	4	4	ND	8	ND	16	3	ND
6	4	4	ND	ND	ND	8	4	ND
7	32	2	ND	ND	ND	2	ND	12
8	32	8	ND	ND	ND	3	ND	ND
9	12	4	ND	2	ND	ND	ND	12

ND, not detectable

and phenylethylamine were not detected in any samples analyzed. These samples were obtained from a commercial source and their history was not available. Therefore, the variation noticed in concentration of amines among samples might be due to the sources, processing conditions and degree of microbiological contamination. The Food and Drug Administration (FDA, 1982) has established a hazard action level for histamine in fish at a concentration of 500 $\mu g\ g^{-1}$. With this dosage level in mind, it appears that very large amounts of smoked herring would be required to bring about histamine intoxication symptoms.

Table 3 gives the amounts of biogenic amines found in 10 sausage samples. Tyramine was found to be the chief amine present in sausage. The constancy of tyramine formation in sausage was demonstrated by its presence in measurable quantities in all samples examined. Moreover, some samples tested contained a dangerous level (6 mg) for patients receiving MAOI (Blackwell and Mabbitt, 1965) if normal amounts were consumed. On the other hand, none of the tested samples had histamine concentrations above the level of 1 $mg\ g^{-1}$, which has been suggested to be the minimum level necessary to precipitate clinical symptoms of toxicity in humans (Simidu & Hibiki, 1955; Arnold & Brown, 1978). In general there was a great variation among samples with respect to biogenic amine contents. The differences observed between the tested samples might be due to variation in the manufacturing process, implying great variability in the type and quality of the meat used, the proportion of meat content included, and the length of maturation.

The biogenic amine contents of ripened cheese samples are given in Table 4. Tyramine and phenylethylamine were detected in all samples examined (the highest concentrations were 160 and 400 $\mu g\ g^{-1}$, respectively). Spermidine was not found in any sample analysed, but spermine was detected in only one sample. Cadaverine was present in eight samples, 16 $\mu g\ g^{-1}$ was found to be the highest concentration, while putrescine was found in three samples. These two amines can be related to contamination of cheese with of enterobacteriaceae (Joosten, 1987). Histamine was quantifiably present in three samples. Tryptamine was detected in a concentration of 12 $\mu g\ g^{-1}$ in two samples, which may be due to the low tryptophan content of casein and hence the low precursor content in cheese (Joosten, 1987). The formation of biologically active amines in cheese appears to be dependent on several basic factors, including sufficient ripening time for protein degradation to occur with the liberation of amino acids (Foster *et al.*, 1958; Joosten & Olieman, 1986), and the presence of conditions necessary for formation and action of specific decarboxylases.

In conclusion, the technique reported here is rapid: 14 samples can be screened and semiquantitated for biogenic amines within 2 h (unlike earlier methods). The advantage of the present method over the earlier reported methods is its simplicity, rapidity, versatility, applicability to a large number of samples in minimal time and cheapness in terms of reagents and equipment as well as its applicability to food in general. Therefore, the described semiquantitative TLC method may be used as a routine control procedure, and can be put to use by regulatory agencies and food industries for determining the quality of food with respect to biogenic amines.

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