LWT - Food Science and Technology 139 (2021) 110804

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Profiling the occurrence of biogenic amines in different types of tuna samples using an improved analytical approach

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ARTICLE INFO

Keywords:
Biogenic amines (BAs)
Tuna samples
USAμET
DnsCl
UHPLC-FLD
Food safety

ABSTRACT

Food deterioration caused by microbial agents often involve the formation of biogenic amines (BAs), which can have harmful effects on human health. In this study a set of BAs - tryptamine, cadaverine, putrescine, spermine, histamine, tyramine, and spermidine, were simultaneously analysed to monitor their occurrence in different types of tuna samples. An improved extraction approach involving ultrasound-assisted microextraction (USAμET) followed by derivatization with dansyl chloride (DnsCl) and analysis by ultrahigh performance liquid chromatography (UHPLC) with fluorescence detection was validated for BAs quantification. The performance of the USAμET/UHPLC-FLD was assessed by studying the limits of detection (LOD) and quantification (LOQ), linear dynamic range (LDR), precision (intra and inter-day) and matrix effect (ME). Good linearity ($r^2 > 0.98$), LODs (from 0.98 to 8.57 mg kg^{-1}) and LOQs (3.20–25.6 mg kg^{-1}) were achieved for all BAs analysed. Recoveries ranged from 76% to 106%, with relative standard deviations (RSD) lower than 5.0%. ME was determined from 7.52 to 50% and the intra and inter-day precisions ranged from 4.7 to 11.6% and 5.5–14.2%, respectively. BAs levels varied significantly from 4.09 ± 0.8 mg kg^{-1} of putrescine in olive oil canned samples to 577.9 ± 5.9 mg kg^{-1} of cadaverine in natural canned samples. Tryptamine and tyramine were not detected in any of the samples analysed, while spermine and spermidine were found in 85.7% of the assayed tuna samples. Cadaverine was the most dominant BA with concentrations ranging from 54.3 ± 2.5 mg kg^{-1}, in olive oil canned tuna, to 577.9 ± 5.9 mg kg^{-1}, in natural canned samples, whereas putrescine had the lowest concentration (average 6.9 ± 2.5 mg kg^{-1}). The validated methodology revealed important improvements in terms of simplification of the experimental layout, expressed in the low sample and reagent amounts, in addition to less time-consuming and labour-intensive requirements that did not compromise the analytical performance.

1. Introduction

Fish is a food with high nutritional value and easy to digest, making it essential in human diet. However, it is also highly perishable, and its quality can drop very quickly soon after its capture, particularly when improper handling and poor hygiene conditions are used. In such cases, the appearance of undesirable odours and flavours as a result of enzymatic and bacterial reactions may involve the formation of harmful compounds, as biogenic amines (BAs) (Mercogliano & Santonicola, 2019). These basic, non-volatile, low molecular weight organic compounds containing nitrogen atoms (Mercogliano & Santonicola, 2019) can be classified into monoamines, diamines, and polyamines according to the number of amine groups or in aliphatic, aromatic, and heterocyclic considering their chemical structure (Fig. 1A). Although some fishes from the Scombridae family have endogenous high levels of free histidine, the precursor of histamine (Hist), often BAs occurrence in fresh fish is very low as these compounds result essentially from bacteria or yeast proliferation (Đorđević, Buchtová, & Borkovcová, 2016). As food deterioration progresses, bacteria and yeast activities increase boost the levels of different BAs, Hist, tyramine (Tyrm), tryptamine (Try), putrescine (Put) and cadaverine (Cad), which result from the decarboxylation of histidine, tyrosine, tryptophan ornithine and lysine, respectively (Santos et al., 2019). Ultimately, high levels of different BAs determine the loss of organoleptic characteristics, making the fish

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https://doi.org/10.1016/j.lwt.2020.110804
Received 18 August 2020; Received in revised form 27 November 2020; Accepted 19 December 2020
Available online 24 December 2020

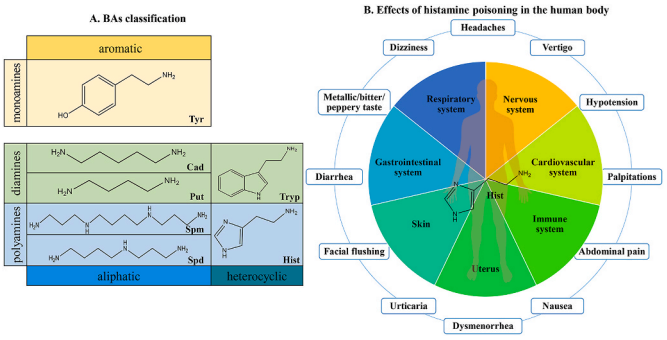

Fig. 1. Classification of selected BAs according to the number of amine groups and structure (**A**) and toxic health effects and symptoms related with Hist poisoning (adapted from Feng et al., 2016) (**B**). Legend: BAs – Biogenic Amines; Try – Tryptamine; Put – Putrescine; Cad – Cadaverine; Spm – Spermine; Hist – Histamine; Tyrm – Tyramine; Spd – Spermidine.

unsafe for human consumption and unsuitable for commercialization. Among BAs, Hist is the most harmful causing a myriad of toxic effects involving diverse biological functions and systems, including the cardiopulmonary, nervous, digestive, and immune systems (Mercogliano & Santonicola, 2019) (Fig. 1B). In addition, it acts synergistically with other amines for fish poisoning and decomposition (Mercogliano & Santonicola, 2019).

Hist poisoning represents therefore, an obvious problem of public health and hence the eventual presence of high levels of BAs has been studied in various foods, including fish (Santos et al., 2019). Moreover, specific legislation establishes legal limits for BAs presence in fish and fishery products to protect consumers from spoiled fish intake (COMMISSION REGULATION (EC), 2005). According to the Food and Drug Administration (FDA), the presence of Hist levels above 500 mg kg^{-1} in tuna fish samples is toxic to human health. European Union (EU), in turn, set a lower limit of 100–200 mg kg^{-1} of Hist in the family Scombridae (Altieri, Semeraro, Scalise, Calderari, & Stacchini, 2016). BAs quantification is very challenging, mainly due to *i*) its strong polar character resulting in greater solubility in water than in most organic solvents; *ii*) the lack of intrinsic properties enabling BAs detection by usual methods (spectrophotometric, fluorometric or electrochemical); *iii*) matrix sample complexity (presence of many interfering compounds); and iv) simultaneous occurrence of several BAs with variable concentration ranges (from very low to very high).

Chromatographic techniques are usually used for BAs separation and quantification in food matrices. The European Food Safety Authority (EFSA) recommends the use of high performance liquid chromatography (HPLC) with pre- or post-column derivatization and fluorescence (FLD), UV or electrochemical detection, pointing it as a high sensitivity and reliable method (Esatbeyoglu, Ehmer, Chaize, & Rimbach, 2016). BAs derivatization adds a detectable group to the free amines, therefore improving the method sensitivity, and so continues to be a good alternative (Milheiro, Ferreira, Filipe-Ribeiro, Cosme, & Nunes, 2019). Several derivatization agents are often used, as benzoyl chloride (BzCl), DnsCl, *o*-phthalaldehyde (OPA) and fluorescein *(Pereira* et al., *2017, pp. 1–23)*. OPA has a short life span, while BzCl only allows the derivatization of primary and secondary amines. DnsCl, however, is able to derivatize also tertiary amines (Pereira et al., 2017, pp. 1–23), being therefore the reagent with broader range of application for BAs derivatization. There are, nevertheless, alternative methodologies for BAs analysis, not involving derivatization, as the ones proposed by Self, Wu, and Marks (2011) or more recently, Dong and Xiao (2017), using UHPLC-MS/MS approaches. However, the high costs necessary to the MS/MS equipment's acquisition constitute an important obstacle to the implementation of this methodology.

Considering the general interest of the risk assessment of BAs presence in foods, we aimed to develop an improved analytical approach to evaluate their occurrence in different types of commercial tuna fish samples (fresh, frozen, and canned). Thus, the concentration of most relevant BAs (tryptamine, cadaverine, putrescine, spermine, histamine, tyramine, and spermidine) was determined using ultrasound-assisted microextraction (USAμET) followed by BAs derivatization with DnsCl and UHPLC-FLD chromatographic separation. Accordingly, several relevant experimental parameters were optimized, namely *i*) the extraction efficiency (ultrasound (US) agitation time, extracting solvent, partitioning salts and buffer solutions); *ii*) the derivatization procedure (concentration of the derivatization reagent - DnsCl, derivatization time and temperature, and US agitation); and *iii*) the chromatographic resolution (mobile phase, column and temperature).

2. Material and methods

2.1. Reagents and standards

All reagents used in this work were used at maximum purity available (analytical or HPLC grade) and the % of purity is mentioned next to each reagent and solvent list, which includes: orthophosphoric acid (H_3PO_4, 85%, AnalaR), formic acid (CH_2O_2, 98%, Panreac; Barcelona, Spain), sodium tetraborate decahydrate ($Na_2B_4O_7{\cdot}10H_2O$, 99.5%, Riedel-de Haën), DnsCl ($C_{12}H_{12}ClNO_2S$, 99%, Sigma-Aldrich; St. Louis, MO, USA), sodium hydroxide (NaOH, 98%, Panreac; Barcelona, Spain), sodium chloride (NaCl, 99.5%, Panreac; Barcelona, Spain), magnesium sulphate anhydrous ($MgSO_4$, 96%, Panreac; Barcelona, Spain), trisodium citrate dihydrate ($C_6H_9Na_3O_9{\cdot}2H_2O$, 99%, Sigma-Aldrich; St. Louis, MO, USA), sodium acetate trihydrate ($CH_3CO_2Na{\cdot}3H_2O$, 99%, Panreac; Barcelona, Spain), sodium hydrogencitrate sesquihydrate ($C_6H_6Na_2O_7{\cdot}1.5H_2O$, 99%, Sigma-Aldrich; St. Louis, MO, USA), acetone, acetonitrile (ACN) and methanol (MeOH) (HPLC grade from Fisher Chemical; Loughborough, UK). BAs (cadaverine, histamine, putrescine, spermidine, spermine and tyramine) and 1,7-diaminoheptane (internal standard, IS) were purchased from Acros Organics (Loughborough, UK), while tryptamine was purchased from Alfa Aesar (Kandel, Germany). Ultrapure water (18 MΩ cm at 23 °C) was prepared using a Milli-Q water purification system (Millipore, Milford, MA, USA). In this work, all the solvents were filtered using a 0.20 μm Grace Membrane nylon filter and all solutions and extracts were filtered using a BGB syringe filter, PTFE, 0.2 μm purchased by SPECANALITICA (ϕ4 mm Lisbon, Portugal).

2.2. Tuna fish samples

Triplicates from seven tuna fish sample types, fresh (N = 7, acquired in the day of analysis and keep at 4 °C) and frozen (N = 7, conserved at −20 °C) tuna (85 g of each, both from *Thunnus obesus* specie acquired in a local fishery market and fished in the Atlantic Ocean, in the Portuguese maritime zone) and triplicates of commercial canned tuna (in oil, olive oil, natural and pate, canned with 85 g of tuna), from unspecified tuna species and purchased in different markets located at Funchal, Madeira Island, Portugal), were analysed in this study. The different parts of the fresh tuna fish were separated and only the white muscle (wm) and dark muscle (dm) were used. After maceration and grounded to a homogenous paste they were aliquoted and stored at −20 °C. The oil, olive oil and water present in the canned samples were discarded before processing the sample. Regardless of the sample type, 1 g of homogenous tuna samples was used in the different assays. All samples were analysed in triplicate (n = 3) and the results were expressed with the mean of the measurements. The experimental layout followed in this study is shown in Fig. 2.

2.3. Standard solutions

BAs stock solutions were prepared in 0.1% formic acid (FA) at 25 mg mL^{-1} and the pooled standard solutions containing all BAs were prepared by aliquot dilution (Concentration Range: Put 5–750 mg kg^{-1},

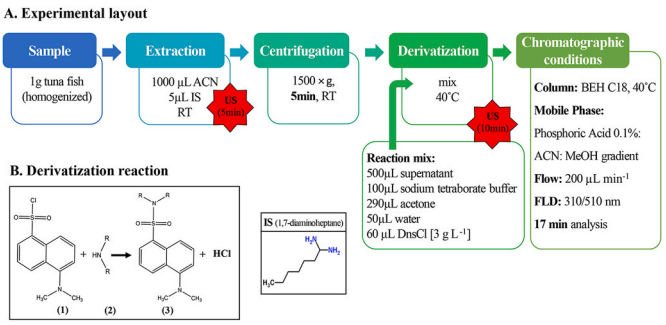

Fig. 2. Summary of the optimized experimental procedure (**A**) and dansyl derivatization (**B**). ACN – acetonitrile; DnsCl – dansyl chloride; FLD – fluorescent detection; IS – internal standard; MeOH – methanol; US – ultrasound.

Try, Cad, Spermine (Spm), Hist, and Spermidine (Spd) 25–750 mg kg^{-1}; Tyrm 125–750 mg kg^{-1} – lower limits for Put and Tyrm are different from remaining BAs to match the detection levels that Put and Tyrm can be detected in food matrices). All BAs solutions were stored refrigerated at 4 °C and prepared from stock solutions every week. The internal standard (IS, 1,7-diaminoheptane) stock solution was prepared at 2 mg L^{-1} in 0.1% FA. The dansyl chloride (DnsCl) solution was prepared at 50 mg mL^{-1} in acetone just before use.

2.4. USAµET optimization

The USAµET optimization was carried out using wm and different experimental conditions, namely the US agitation time (0, 1, 5, 10 min using a Branson 2510, 100 W US bath), the nature of the extracting solvent (ACN and MeOH), the partitioning salts (sodium chloride and magnesium sulphate) and the use of buffers solutions (acetate and citrate buffers as indicated in the Supplementary Table 1.SM, and according to previous work (Porto-Figueira, Camacho, & Câmara, 2015)). The extraction process was performed with 1 g aliquots, 1 mL of ACN and 5 µL of 1,7-diaminoheptane at 2 mg mL^{-1}. The mixture was vortexed and placed 5 min in the US at 23 °C. Finally, it was centrifugated (1500×*g*, 5 min, room temperature) and the supernatant used for the following derivatization procedure. All conditions assayed are detailed in the Supplementary Table 1.

2.5. Derivatization procedure

Several experimental parameters, DnsCl concentration (1, 2, 3 mg mL^{-1}), derivatization time, US agitation (0, 10, 20 min) and temperature (23, 40, 60 °C ± 1 °C), were optimized to simplify BAs derivatization. After the extraction step, 500 mL of the supernatant were removed and added 290 µL acetone, 50 µL ultrapure water, 100 µL tetraborate buffer (0.1 mol dm^{-1}) and 60 µL of DnsCl (Fig. 2A). The reaction mixture was stirred in the vortex and subjected to US for 0, 10 or 20 min, at different temperatures. The conditions assayed are detailed in Supplementary Table 2. Finally, the solution was filtered, diluted in acidified water (0.1% FA, 1:2) and injected in the UHPLC for analysis. The chromatographic conditions assayed are indicated in Fig. 3A.

2.6. Chromatographic conditions

The chromatographic analysis was performed with the Waters UHPLC Chromatograph equipped PDA and FLD. The resolution of the chromatographic separation was evaluated with the columns Acquity UPLC BEH C18 (50 × 2.1 mm, 1.7 µm) and Acquity UPLC CSH C18 (150 × 2.1 mm, 1.7 µm). The PDA and FLD were compared to select the one retrieving greater sensitivity for the detection of derivatized BAs. Upon the initial optimization step, the chromatographic separation was performed with the Acquity UPLC BEH C18 column and a three-phase

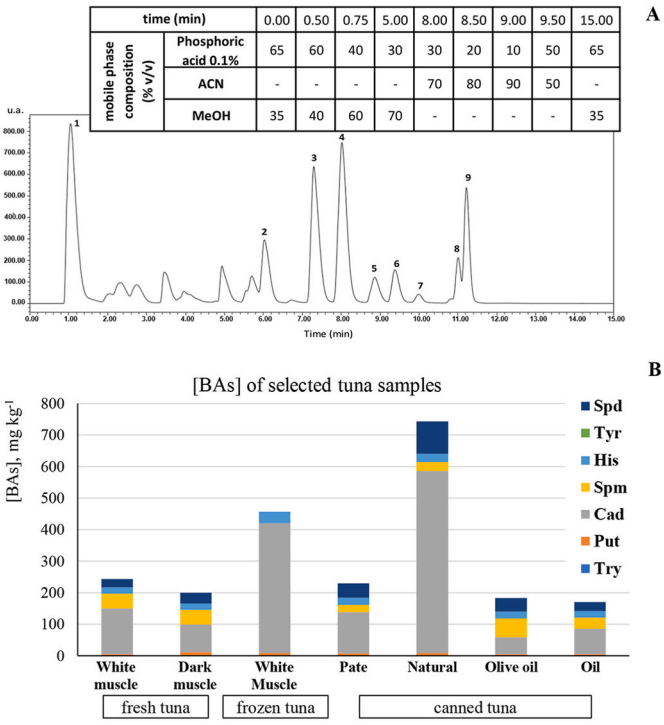

Fig. 3. (**A**) Representative chromatogram of a standard solution of BAs after derivatization (column Acquity UPLC BEH C18 with FLD; λ_{emi} = 500 nm and λ_{exc} = 300 nm). 1 - DnsCl; 2 - Try; 3 - Put; 4 - Cad; 5 - Spm; 6 - Hist; 7 - IS; 8 - Tyrm; 9 - Spd. (**B**) BAs profile found in canned (pate, natural; Olive oil and oil) fresh and frozen tuna fish samples (RSD <5%). Cad - cadaverine, Put - putrescine, Try – tryptamine, Spm - spermine, Hist - histamine, Tyrm - tyramine, Spd – spermidine; wm - white muscle; dm - dark muscle.

gradient (0.1% phosphoric acid, ACN and MeOH) at a flow rate of 0.200 mL/min, with a 15 min-run and a 2 min-reconditioning step (Fig. 3A). The column temperature was 40 °C and the injection volume 2 µL. FLD (300 nm excitation/500 nm emission lengths) was selected to analyse the target BAs. The data obtained was processed using Empower 2 software (Waters).

2.7. Method validation

Validation was performed according to the international guidelines (COMMISSION REGULATION (EC), 2002; FAO/WHO, 2013) and selectivity, linearity (R^2), detection (LOD) and quantification (LOQ) limits, precision, recovery (%) and matrix effect (ME). Selectivity was assessed by comparing chromatograms of solvent, white muscle (wm) and wm with added BAs and IS. To evaluate the method linearity, BAs standard solutions (Table 2) were prepared in 0.1% FA and used to obtain the calibration curve. All solutions were injected 5 times, under repeatability conditions. Sensitivity was evaluated by measuring the detection (LOD) and quantification (LOQ) limits for each analyte using the residual standard deviation ($s_{y/x}$) of corresponding calibration curves. LOD and LOQ were calculated as 3.3 $s_{y/x}$/b and 10 $s_{y/x}$/b, respectively, where $s_{y/x}$ represents the standard deviation of ordinate at origin and b the slope of the regression line, as described in Porto-Figueira et al. (2015). Precision was evaluated by adding to the wm samples three concentration levels of each BAs (low - LL, medium - ML, and high - HL, concentration points of the calibration curve - Table 2). Five replicates (n = 5) were carried out in the same day or in three consecutive days (total n = 15) to calculate intra-day and inter-day precisions, respectively. Recovery was carried out by comparison between a mix of standard solutions (LL, ML and HL) of each BAs and wm samples added with the same amounts of BAs. The concentrations LL,

ML and HL were used to obtain the calibration curves for each BA with five replicates each ($n = 5$). ME were calculated as the ratio of the slopes of the analytical curves obtained for the analytical standard solutions prepared in solvent and in wm, ME = [(y/ × solvent)/(y/ × spiked wm)] × 100 (Perestrelo, Petronilho, Câmara, & Rocha, 2010).

2.8. Quality index

Mietz and Karmas (1977) observed that the presence of Hist, Put and Cad in fresh tuna fish samples is low, increasing concomitantly with sample deterioration. In contrast, Spd and Spm follow the opposite trend, decreasing along with deterioration. Taking this into consideration, these authors implemented a quality index (QI) based on the concentration of the referred BAs in mg kg^{-1}, according to equation (1) (Mietz & Karmas, 1977):

$$Quality\ index = \frac{[histamine] + [putrescine] + [cadaverine]}{1 + [spermidine] + [spermine]} \quad (1)$$

As a result, high levels of Spd and Spm conjugated with low values of Put, Cad and Hist are indicators of good quality, while the inverse indicates tuna decomposition. Thus, QI values below 1 correspond to a good quality of the tuna sample, being safe for human consumption. QI values between 1 and 10 indicate poor quality and decomposition above 10.The grade decomposition is obviously unsafe for human consumption.

2.9. Statistical analysis

Results were expressed as mean ± standard deviation (SD) of the independent experiments. Differences among samples were estimated by analysis of variance (ANOVA) followed by Tukey's 'honest significant difference' test. The statistical significance level was set to p-values<0.05. All statistical analyses were performed using Statistica software (TIBCO Software Inc).

3. Results and discussion

3.1. Definition of the best experimental layout

The absence of natural chromophores in most BAs requires their derivatization to allow a reliable chromatographic analysis. In this work, DnsCl was select as the derivatization reagent since allows the derivatization of primary, secondary, and tertiary amines. Upon a literature survey of chromatographic methods for the analysis of dansylated BAs, we observed a lack of optimization of the derivatization reaction at several levels, particularly in what concerns to the sample amount and reaction conditions (DnsCl concentration, reaction time and temperature; see Table 1).

Our preliminary assays involved the BAs dansylation previously to the acidic extraction. However, this approach did not retrieve satisfactory results, maybe due to the low affinity of the dansylated BAs to the extraction system and was discarded. Therefore, we optimized an experimental layout involving US-assisted extraction under acidic conditions, followed by BAs dansylation, chromatographic separation and data analysis (see Fig. 2). To normalize the chromatographic data obtained and allow an accurate comparison of the experimental conditions assayed, 1,7-diaminoheptane was used as IS. The best conditions found in each optimization step are summarized in the Supplementary Fig. 1 and detailed in the following sections.

3.2. Optimization of extractive conditions by ultrasound-assisted microextraction (USAμET)

To achieve the best extraction conditions, the influence of US extraction time (0, 1, 5 and 10 min) and solvents (ACN, MEOH and ACN: MeOH (1:1)) were thoroughly assayed. The best results were obtained with a 5 min USAμET and ACN as extraction solvent (Supplementary Fig. 1.SM). Additionally, we also assayed the effect of salts (NaCl and $MgSO_4$) and buffers (acetate and citrate buffers) on the extraction efficiency. However, none of these conditions retrieved higher extraction efficiencies (Supplementary Fig. 2.SM).

3.3. Optimization of derivatization conditions with dansyl chloride (DnsCl)

Derivatization was also optimized at several levels, namely DnsCl concentration, reaction time and temperature, and the use of US. This optimization was justified by the disparity in the derivatization conditions reported in the literature involving similar applications (Table 1). According to the obtained results (Supplementary Fig. 1.SM), both the increase of DnsCl concentration and derivatization temperature favour the derivatization procedure. In turn, the effect of US is less obvious as higher relative areas were obtained without the use of US at 60 °C. However, we observed that the use of 60 °C during the derivatization reaction is not recommended since this temperature is very close to the boiling point of acetone, the main constituent (reagent) of this reaction, which is 56 °C. Consequently, part of the acetone evaporates during the derivatization reaction affecting the reproducibility of the results. Possibly the higher relative areas obtained at 60 °C (Supplementary Fig. 3) may have a partial contribution of this concentration effect. Therefore, the assay of 60 °C as a derivatization temperature was abandoned in the following experiments. Overall, the best conditions obtained for the BAs derivatization were 3 mg mL^{-1} of DnsCl, with a US time of 10 min at 40 °C (Supplementary Fig. 3.SM).

3.4. Chromatographic conditions

To obtain the best chromatographic separation of the dansylated BAs, several instrumental conditions were evaluated. Additionally, a preliminary assessment of the suitable detector for the analysis of the derivatized BAs unequivocally shown that the S/N ratio is much higher using the FLD instead of PDA detector (Fig. 4.SM). The selection of the appropriate chromatographic column was limited by the high pH variations that the samples are subject during extraction and derivatization steps. Accordingly, the Acquity UPLC CSH C18 and the Acquity UPLC BEH C18 columns, which can withstand broad pH variations (1–11 and 1–12, respectively) were assayed throughout the optimization of the chromatographic separation. This included the selection of a mobile phase gradient and flow rate able to deliver a fast and efficient chromatographic separation. The best results were obtained using a 200 μL/min of a three-phase gradient composed by 0.1% phosphoric acid, ACN and MeOH, as described in Fig. 3A. Regarding the selection of the best column, the Acquity UPLC BEH C18 allowed a better chromatographic resolution of the dansylated BAs (Fig. 3A).

3.5. Validation of the analytical methodology for the determination of selected BAs in tuna fish samples

The USAμET/UHPLC-FLD methodology developed was validated for the quantification of selected BAs in tune fish samples. This involved the evaluation of selectivity, linearity, LOD, LOQ, precision, recovery, and matrix effect (Table 2). Overall, a good analytical performance is reported for the quantification of the dansylated BAs within the linear range of concentrations indicated in Table 2. The determination coefficients (R^2) obtained were above 0.9993, except for Cad (0.9809), Hist (0.9887) and Tyrm (0.9877) (Table 2). This minor variation was most probably caused by an unidentified error in the several experimental steps involved in the methodology, particularly the derivatization process.

The LODs and LOQs obtained varied between 0.98 mg kg^{-1} and 8.57 mg kg^{-1} and between 3.20 mg kg^{-1} and 25.6 mg kg^{-1}, for Put and Tyrm, respectively, being in the same range of other methodologies reported in

Table 1
Methodologies reported in the literature for the analysis and quantification of BAs in foodstuffs.

Method/Equipment	LDR (mg kg^{-1})	LOQ (mg kg^{-1})	Total time (min)	Reference
AOAC/Fluorometer	1–150	1–5	60–120	FAO/WHO (2013)
Spectrofluorometric/ Spectrofluorometer	0.0015–100	1.5 (μg kg^{-1})	60	
ELISA/Spectrofluorometer	0–500	2–5	60	
Colorimetric/ Spectrofluorometer	0.8–300	20	60	
Chromatographic (LC)	5–2500	1.5–5	60–120	

Chromatographic methods with pre-column derivatization – DnsCl

DnsCl (mg/mL)/ deriv. time (min)	Extraction conditions	Methodology (column, wavelength)	Sample amount	Identified BAs	LDR	LODs/LOQs (units)	Analysis time (min)	References
3/10	**US, 40 °C, SLE**	**UHPLC-FLD (BEH C18, λ_{emi} = 500nm/λ_{exc} = 300 nm)**	**Tuna fish (1 g)**	**Cad, Hist, Put, Spd, Spm, Try, Tyrm**	**5–750 mg kg^{-1}**	**0.98–8.57/ 3.20–25.64 mg kg^{-1}**	**40**	**This work**
-/5[d]	Dark, 60 °C, SLE	HPLC-UV (Kromasil C18, λ = 254 nm)	Fish (5 g)	Cad, Hist, Phe, Put, Spd, Spm, Try, Tyrm	25–250 mg kg^{-1}	–	90	Duflos et al. (2019)
7.5/5[d]	Vortex, D ark, 60 °C; LLE	HPLC-UV (Kromasil C18, λ = 254 nm)	Eggs (3 g yolk, albumen)	Cad, Hist, Phe, Put, Spd, Spm, Tyrm	0.7–22.4 mg kg^{-1}	0.2–0.3/ 0.7–1.0 mg kg^{-1} (yolk) 0.2–0.4/ 0.7–1.1 mg kg^{-1} (albumen)	130	de Figueiredo et al. (2015)
7.5/5[d]	Dark, Bath, 60 °C; SLE	HPLC-UV (Kromasil C18, λ = 254 nm)	Chicken (5 g)	Cad, Hist, Put, Spd, Spm, Tyrm	0.9–94.4 mg kg^{-1}	0.3/0.9–1 mg kg^{-1}	210	Assis et al. (2016)
10/45[d]	40 °C; SLE	HPLC-UV (Spherisorb ODS2 150 A, λ = 254 nm)	Fish (2 g)	Cad, Hist, Put, Try, Tyrm	–	0.005–0.050/ 0.010–0.100 μg mL^{-1}	120	Bilgin and Gençcelep (2015)
10/45[d]	40 °C; SLE	HPLC-UV (COSMOSIL 5C18-PAQ, λ = 254 nm)	Carp (5 g)	Cad, Hist, Phe, Put, Spd, Spm, Try, Tyrm	–	–	170	Li, Li, Qin, Hong, and Luo (2016)
10/30[d]	60 °C; SPE	HPLC-UV (C18, λ = 254 nm)	Salami (5 g)	Cad, Put, Spm, Tyrm	–	0.05–0.1/- mg kg^{-1}	130	Loizzo et al. (2016)
10/10[d]	70 °C; SPE	HPLC-UV (C18 ODS Hypersil, λ = 254 nm)	Food (5 g)	Cad, Hist, Phe, Put, Spd, Try, Tyrm	0.001–50 mg L^{-1}	0.02–0.06/ 0.07–0.13 μg L^{-1}	160	Tameem et al. (2010)
10/45	US-trichloroacetic acid extraction	HPLC-DAD (Zorbax Eclipse plus C18, 254 nm)	skipjack tuna (10 g)	Cad, Hist, Put, Spd, Spm, Tryp	3–30 mg L^{-1}	0.05–0.24/ 0.18–1.23 mg kg^{-1}		Barbosa et al. (2018)
2/45[d]	Shake, 40 °C; SLE	HPLC-UV (Spherisorb ODS2, λ = 254 nm)	Fermented sausages (2 g)	Cad, Hist, Phe, Put, Spd, Spm, Try, Tyrm	–	–	130	Ekici and Omer (2018)
10/45[d]	40 °C; SLE	HPLC-UV (Nova-Pak C18, λ = 254 nm)	Food (5 g)	Cad, Hist, Phe, Put, Spd, Spm, Try, Tyrm	25–1000 mg kg^{-1}	0.01–0.10/ 0.02–0.31 mg kg^{-1}	230	(Yoon et al., 2015)
-/60[d]	45 °C; SLE	HPLC-FLR (ODS-3, λ_{emi} = 525nm/λ_{exc} = 325 nm)	Seafood (10 g)	Hist	0.4–200 mg L^{-1}	2.5/- mg L^{-1}	110	Yoshida et al. (2012)
10/55[d]	40 °C, cooled 10 min, SLE	HPLC-UV (Eclipse XBD C18, λ = 254 nm)	Fish (5 g)	Hist	30–700 mg kg^{-1}	3/10 mg/kg	140	Altieri et al. (2016)
5/60[d]	Vortex, Dark 45 °C, SPE	HPLC-UV (Eclipse XBD C18, λ = 254 nm)	Sausage and cheese (50 g)	Cad, Hist, Phe, Put, Spd, Spm, Try,Tyrm	0.18–500 mg kg^{-1}	0.03–0.36/ 0.11–1.19 mg kg^{-1}	120	Liu et al. (2018)
OPA								
−/−	Post-column, RT, SLE	Ion- pair HPLC-FLR (Nova-Pak C18, λ_{emi} = 450nm/λ_{exc} = 340 nm)	Fish (5 g)	Agm, Cad, Hist, Phe, Put, Ser, Spd, Spm, Try,Tyrm	1–250 mg kg^{-1}	0.03/0.09 mg kg^{-1}	60	Evangelista et al. (2016)
40/-	Post-column, RT, LLE/SLE	UHPLC-FLR (BEH C18, λ_{emi} = 445nm/ λ_{exc} = 340 nm)	Wine, fish, cheese and dry sausage (5–10 g)	Agm, Cad, Dop, Hist, Oct, Phe, Put, Ser Spd, Spm, Try, Tyrm	0.1–50 mg L^{-1}	–	80	Latorre-Moratalla et al. (2009)
BzCl								
36.3/30*	US Bath, SPE	HPLC-UV (Eclipse XDB-C18, λ = 254 nm)	Sausage and cheese (5 g)	Cad, Hist, Phe, Put, Spd, Spm, Try,Tyrm	0.29–500 mg kg^{-1}	0.09–0.38/ 0.29–1.26 mg kg^{-1}	70	Liu et al. (2018)
-/10	Vortex, RT, SLE	UHPLC-MS (ACQUITY HSS T3)	Fish (20 mg)	Agm,Cad, Hist, Phe, Put, Spd, Spm, Try, Tyrm	5–200000 pg mL^{-1}	0.1–10.0/ 1.0–100.0 pg mL^{-1}	50	Lee et al. (2015)

Chromatographic methods without BAs derivatization

(*continued on next page*)

Table 1 (*continued*)

Method/Equipment		LDR (mg kg^{-1})	LOQ (mg kg^{-1})			Total time (min)		Reference
DnsCl (mg/mL)/ deriv. time (min)	**Extraction conditions**	**Methodology (column, wavelength)**	**Sample, amount**	**Identified BAs**	**LDR**	**LODs/LOQs (units)**	**Analysis time (min)**	**References**
	MSPD	UHPLC-HILIC (BEH HILIC)	Canned and frozen tuna (0.5 g)	Agm, Cad, Hist, Phe, Put, Try, Tyr, and urocanic acid	20-100 (ppm)	0.0204–1.57/ 0.0680–5.2 (ppm)	18	Self et al. (2011)
	Modified QuEChERS 10 min US, centrifugation	UHPLC-MS/MS (BEH C18)	Soy sauce (0.500 g)	Hist, Phe, Put, Spm, Spd, Tyr, Tryp	3.0–600 (μg kg^{-1})	4-8/15–30 (μg kg^{-1})	4	Dong and Xiao (2017)
		IC-CD	tuna fish, anchovies, wine, olives, salami, and cheeses, 2 g	Agm, Cad, His, Put, Spd, Spm, triethylamine, trimethylamine	0-200 (mg kg^{-1})	23-65/65–198 (μg kg^{-1})	–	Palermo, Muscarella, Nardiello, Iammarino, and Centonze (2013)
	solvent extraction	ELISA, HPLC (Supelcosil LC-ABZ, 210 nm), CE (210 nm)	Seafood (ELISA 2 g; HPLC, CE, 10 g)	Hist	2.5–200 (mg kg^{-1})	2.5, 10 (μg kg^{-1})	160 (ELISA), 60 (HPLC), 25 (CE)	Muscarella, Iammarino, Centonze, and Palermo (2005)

Legend: DnsCl – dansyl chloride, LDR - IEC-CD - ion-exchange chromatography with conductivity detection, MSPD - matrix solid-phase dispersion. (Assis et al., 2016; Barbosa et al., 2018; Bilgin & Gençcelep, 2015; de Figueiredo et al., 2015; Dong & Xiao, 2017; Duflos et al., 2019; FAO/WHO, 2013; Li et al., 2016; Muscarella et al., 2005; Palermo et al., 2013; Self et al., 2011; Visciano, Schirone, Tofalo, & Suzzi, 2012).

Table 2
Analytical figures of merit of the USAμET/UHPLC-FLD method for the determination of BAs in tuna fish samples.

BAs	RT (min)	LDR (mg kg^{-1})	Linear equation	R^2	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	ME (%)	Spiked level (mg kg^{-1})	Recovery (%)	Precision (RSD,%)	
										Intra-day (n = 5)	Inter-day (n = 15)
Try	5.81	25–750	y = 0.0304x+0.00565	0.9990 ± 0.002	4.97 ± 0.5	14.9 ± 0.3	29.6 ± 0.3	25	87 ± 3	9.8	10.5
								250	93 ± 4	4.7	7.1
								750	99 ± 2	6.3	8.5
Put	7.14	5–750	y = 0.0302x-0.1091	0.9967 ± 0.001	0.98 ± 0.1	3.20 ± 0.2	32.4 ± 0.5	5	76 ± 1	9.3	12.9
								250	81 ± 3	6.3	8.8
								750	99 ± 4	7.9	8.5
Cad	8.06	25–750	y = 0.0487x+0.4876	0.9809 ± 0.003	5.24 ± 0.3	14.9 ± 0.2	42.6 ± 0.2	25	87 ± 3	6.7	8.2
								250	93 ± 1	8.3	10.4
								750	106 ± 2	5.1	7.4
Spm	8.95	25–750	y = 0.0003x-0.0025	0.9929 ± 0.002	7.39 ± 0.2	22.4 ± 0.1	2.01 ± 0.6	25	79 ± 3	7.4	12.8
								250	87 ± 4	8.3	5.5
								750	99 ± 2	4.4	6.9
Hist	9.21	25–750	y = 0.0023x-0.0441	0.9887 ± 0.001	2.71 ± 0.1	8.31 ± 0.2	7.52 ± 0.3	25	80 ± 3	11.6	14.2
								250	95 ± 1	5.4	7.9
								750	103 ± 3	9,1	8.8
Tyrm	10.84	125–750	y = 0.0064x+0.1556	0.9877 ± 0.004	8.57 ± 0.2	25.6 ± 0.3	50.0 ± 0.8	125	94 ± 3	9.1	9.7
								250	91 ± 5	8.7	9.2
								750	103 ± 1	7.1	8.4
Spd	11.22	25–750	y = 0.0037x-0.0456	0.9938 ± 0.002	6.59 ± 0.1	20.1 ± 0.1	15.3 ± 0.7	25	88 ± 2	7.3	11.3
								250	79 ± 3	5.8	9.4
								750	103 ± 4	6,5	6.9

Legend: BAs – Biogenic Amines; **Cad –** Cadaverine; **Hist –** Histamine; **LDR –** Linear dynamic range; **LOD –** Limit of detection; **LOQ –** Limit of Quantification; **ME –** Matrix effect; RSD – relative standard deviation; **RT –** Retention time; **R^2 –** Determination coefficient**; Put –** Putrescine; **Spd –** Spermidine; **Spm –** Spermine; **Tyrm –** Tyramine; **Try –** Tryptamine.

the literature using similar chromatographic approaches (Table 1). The recovery (76 ± 1%-106 ± 4%), intra-day (4.4%–11.6%) and inter-day precision (5.5%–14.2%), are also within acceptable analytical ranges (Table 2). However, the matrix effect, which measures the eventual interference of the sample components in the instrument response and consequently in the analysis and quality of the results obtained, ranged from 2.0% to 50% (Table 2). This suggests an influence of the sample in the extractive process of some of the dansylated BAs, namely Try, Put, Cad and Tyrm. For this reason, the BAs quantification was performed using the standard addition method. Nevertheless, the proposed methodology presents many advantages in comparison with other techniques previously reported in the literature, as a reduced sample and reagent amounts, a lower derivatization temperature and a much faster and simpler derivatization reaction.

3.6. The occurrence of BAs in tuna samples

The validated methodology was applied to different types of tuna fish samples - fresh tuna fish (wm and dm), frozen tuna fish steak containing only wm, and several types of canned tuna fish (pate, oil, olive oil, and natural). Fig. 3B shows the concentration profile of BAs present in the analysed samples. The levels of Cad and Hist are high in wm, while dm is rich in Spd. These results are in agreement with the work of Ruiz-Capillas and Moral (2001) which reported an increase in the concentration of Cad, His, and Tyrm throughout the tuna fish storage and higher concentrations in wm than in dm. The exceptions are Spm and Put which present higher concentrations in dm than in wm. Put, for instance, has been previously found at high concentration in the dm upon 5 days storage at low temperatures (1–2 °C) (Bai et al., 2019). This may be the

cause of the observed discrepancy since the wm and dm samples were analysed after frozen. The results also showed that Cad is the most abundant BA, being consistently present at high levels in all analysed samples. According to Ruiz-Capillas and Moral (2001), the presence of Cad results from an autolytic process that starts promptly with fish death, being then amplified by microorganisms that start to colonize the fish. But these BAs can occur also in other food matrices. Buňka et al. (2012), for instance, found levels of Put, Tyrm and Cad, up to 100 mg/L in beers from the Czech Republic. Novella-Rodríguez, Veciana-Nogués, Roig-Sagués, Trujillo-Mesa, and Vidal-Carou (2004) reported levels of Hist, Tyrm, Put and Cad, in the ripening of goat cheeses from pasteurized and raw milk of 376.6, 1585.4, 257.2, and 2101.4 mg kg^{-1}, while Yongsawatdigul, Choi, and Udomporn (2004) found levels of the same BAs of 574.7, 117.3, 308.2, and 685.5 mg kg^{-1}, respectively. Regarding the canned samples, the naturally preserved tuna fish samples presented the highest concentrations of BAs (except for Spm). This may be due to different reasons related to the media in which samples are conserved. The water, oil or olive oil used to process the canned tuna fish samples certainly elicit a different inhibitory effect on microbial development and, consequentially in BAs generation. Furthermore, the affinity for the extraction solvent (ACN), will be different for each of the media used. It would be required a controlled set of tuna fish samples to discriminate the contribution of such effects in the quantification of the dansylated BAs. Overall, at least in what concerns to the current legislation, the tuna fish samples analysed do not represent any risk as the quantified Hist levels fall below the 50 mg kg^{-1} defined by FDA as safe for human consumption. However, the absence of legislation and conclusive studies regarding the safe levels for other BAs, namely Put and Cad, does not allow us to know whether the amounts we found for these three BAs constitute a risk to human health. We can, however, follow the Quality Index (QI) classification of Mietz and Karmas (1977) to verify the quality of tuna fish samples analysed. The QI was determined for all the samples as previously described in Equation (1), and the results obtained are presented in Table 3. As it can be observed, only the canned tuna fish in olive oil presenting a QI of 0.77 can be considered of good quality. The QI of remaining tuna fish samples ranges between 1 and 4.64, indicating different degrees of deterioration that negatively affect the quality of the respective tuna fish samples. Nevertheless, those samples are still safe for human consumption, at least in what concerns to the presence of BAs.

Table 3
Quality index (QI) of the tuna fish samples analysed[a].

Sample		Storage temperature	QI	Classification
Fresh tuna fish	Dark muscle	4 °C	1.44	Poor quality
	White muscle		2.31	Poor quality
Frozen tuna fish	White muscle	−20 °C	>10	Decomposition
Canned tuna fish	Olive oil		0.77	Good quality
	Oil	RT	1.65	Poor quality
	Natural		4.64	Poor quality
	Pate		2.29	Poor quality

[a] Samples description, including time of storage, are detailed in subsection 2.2 of Materials and Methods, RSD<5%. QI – quality index, RT - room temperature.

4. Conclusions

Monitoring the occurrence of BAs and other hazardous compounds in foods is of utmost importance to ensure high quality and safety of all dietary products. In this context, a sensitive method using an USAμET approach combined with UHPLC-FLD chromatographic analysis was developed to monitor the occurrence of BAs - Try, Put, Cad, Spm, His, Tyrm and Spd, in different types of commercial tuna fish. The methodology was properly validated exhibiting good analytical performance in the concentration range of 5–750 mg kg^{-1}. The USAμET/UHPLC-FLD methodology was used to analyse several tuna fish samples and a great variation in individual and total BAs was observed. Frozen tuna had the highest levels of BAs, followed by samples of canned tuna and fresh tuna. The results obtained demonstrate that the validated USAμET/UHPLC-FLD methodology presents several improvements regarding previous approaches reported in the literature. This includes a simple and fast analytical layout, good sensitivity and robustness, and low sample and reagents requirements.

CRediT authorship contribution statement

Joanna K.G. Pataca: Formal analysis, Investigation, Data curation, Writing - original draft. **Priscilla Porto-Figueira:** Formal analysis, Investigation, Writing - review & editing. **Jorge A.M. Pereira:** Methodology, Data curation, Writing - review & editing, Funding acquisition. **Helena Caldeira:** Resources, Writing - review & editing, Supervision. **José S. Câmara:** Conceptualization, Methodology, Data curation, Writing - review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge FCT-Fundação para a Ciência e a Tecnologia through the CQM Base Fund - UIDB/00674/2020, the Programmatic Fund - UIDP/00674/2020 and the PhD fellowship SFRH/BD/129630/2017 granted to Priscilla Porto-Figueira. The authors also acknowledge Madeira 14–20 Program, project PROEQUIPRAM - Reforço do Investimento em Equipamentos e Infraestruturas Científicas RAM (M1420-01-0145-FEDER-000008), ARDITI - Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação through the project M1420-01-0145-FEDER-000005 - Centro de Química da Madeira - CQM+ (Madeira 14–20 Program) and Project M1420 - 09-5369-FSE-000001 for the Post-Doctoral fellowship granted to JAMP.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2020.110804.

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