Food Chemistry 259 (2018) 245–250

Contents lists available at ScienceDirect

Food Chemistry

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

Analytical Methods

Highly selective colorimetric detection of putrescine in fish products using *o*-phthalaldehyde derivatization reaction

Xuan Qi[a,b,1], Wei-Feng Wang[a,1], Jun Wang[a,b], Jun-Li Yang[a,*], Yan-Ping Shi[a,*]

[a] CAS Key Laboratory of Chemistry of Northwestern Plant Resources and Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences (CAS), Lanzhou 730000, PR China
[b] University of Chinese Academy of Sciences, Beijing 100049, PR China

ARTICLE INFO

Keywords:
High selectivity
Colorimetric detection
Putrescine (PUT)
Fish products

Chemical compounds studied in this article::
Putrescine (PubChem CID: 1045)
o-Phthalaldehyde (PubChem CID: 4807)
Thioglycolic acid (PubChem CID: 1133)
Ethylenediamine (PubChem CID: 3301)
1,3-Propanediamine (PubChem CID: 428)
1,5-Diaminopentane dihydrochloride (PubChem CID: 80282)
1,6-hexanediamine (PubChem CID: 16402)
Alanine (PubChem CID: 5950)
Ornithine (PubChem CID: 6262)
Histamine (PubChem CID: 774)

ABSTRACT

A highly selective colorimetric detection method for putrescine on the basis of an optimized derivatization reaction was established. With the aids of *o*-phthalaldehyde (OPA) and thioglycolic acid (TGA), putrescine was derived to become red derivatives (PUT-RD), the form that can be detected qualitatively and quantitatively by visual inspection and UV-vis spectrophotometer at λ_{max} of 490 nm, respectively. Key parameters affecting the experiment were investigated by one-factor-at-a-time and response surface analysis. At the optimal condition, this colorimetric method achieved good linearity at putrescine concentrations ranging from 0.8 to 200 μM with detection limit of 0.44 μM. The method also has good selectivity when common amino acids and inorganic ions, as well as ethylenediamine, 1,3-propanediamine, 1,5-pentanediamine, and 1,6-hexanediamine were used as interferences. The established colorimetric method was successfully employed for the detection of putrescine in 10 commercial fish products.

1. Introduction

Fish product is an important food in daily diet. According to the 2014 Food and Agriculture Organization of the United Nations (FAO) Yearbook of Fishery and Aquaculture Statistics, global production of fish, crustaceans, molluscs, and other aquatic animals reached 167.2 million tons in 2014, and China was the top-ranking fishing country in terms of quantity with the total output of aquatic products reached 45.5 million tons in 2014 (FAO Yearbook of Fishery and Aquaculture Statistics, 2014). It is worthy to note that fish products are likely to corrupt during transportation and storage due to the richness of nutrients and some active physiological activity of microorganisms (Lehane, 2000).

Putrescine (i.e. 1,4-butanediamine) is one of the biogenic amines (BAs) found in varieties of foodstuffs. There are two common sources of putrescine in foods: endogenous biosynthesis within cells and microbial decarboxylation of amino acids (Straub, Kicherer, Schilcher, & Hammes, 1995). An increase of putrescine in foodstuffs not only has negative effect on flavor, but it also creates potential threats in food safety and human health (Shalaby, 1996). Consumption of food containing high amount of putrescine could lead to serious toxicological consequences. Moreover, putrescine has significant effects in enhancing the toxicological effects of other BAs, especially of histamine and tyramine (Taylor, 1985). Secondary amines such as putrescine can react with nitrite to form N-nitrosamines, which is a carcinogenic agent (Tenbrink, Damink, Joosten, & Tveld, 1990). Putrescine could be used as a marker for food quality (Baixas-Nogueras, Bover-Cid, Veciana-Nogues, Marine-Font, & Vidal-Carou, 2005). Detection methods of putrescine that possess convenient operations and/or result analysis are needed for both customers and regulators.

In recent years, a number of analytical methods have been developed to detect putrescine (Prester, 2011; Visciano et al., 2012), which include pre-column High Performance Liquid Chromatography (HPLC) (Venza, Visalli, Cicciu, & Teti, 2001), post-column HPLC (Triki,

* Corresponding authors.
E-mail addresses: qixuan@licp.cas.cn (X. Qi), wangwf@licp.cas.cn (W.-F. Wang), wangjun@licp.cas.cn (J. Wang), yangjl@licp.cas.cn (J.-L. Yang), shiyp@licp.cas.cn (Y.-P. Shi).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.foodchem.2018.03.131
Received 27 March 2017; Received in revised form 18 November 2017; Accepted 28 March 2018
Available online 29 March 2018

Jimenez-Colmenero, Herrero, & Ruiz-Capillas, 2012), capillary gas chromatography (Chen et al., 2011), capillary zone electrophoresis (Liu et al., 2014), ion-exchange chromatography (Conca, Bruzzoniti, Mentasti, Sarzanini, & Hajos, 2001), and the pre-column derivatization with OPA and detection in an isoindole derivatization form to determine not only putrescine but also N-acetylputrescine (Nguyen et al., 2014).

Although these analytical methods provide good separation with low detection limit, the procedures are complex and require large amount of organic solvents (Onal, Tekkeli, & Onal, 2013). Colorimetric strategy is a low cost analytical approach that not only requires simple operating procedures, but also gives intuitive results, and when couple with UV–vis spectrophotometer, it satisfies both the qualitative (i.e. eye visualization) and quantitative analysis's (Zhang, Li, Sun, & Gao, 2015; Zhang et al., 2015).

O-phthalaldehyde (OPA) derivatization reaction is a classical reaction used in detection of amino acids in the form of OPA derivatives (Dai, Wu, Jia, & Wu, 2014). In this work, a novel highly selective colorimetric detection method for putrescine was established by using OPA derivatization reaction coupled with UV–vis spectrophotometer. We described the design and development of the method, as well as its applications in 10 commercial fish products.

2. Materials and methods

2.1. Chemicals and reagents

Ethylenediamine (Analytical Reagent, AR) was obtained from Tianjin Benchmark Chemical Reagent Co., Ltd, China. 1,4-butanediamine (Putrescine, 98%), 1,3-propanediamine (98%), and 1,5-pentanediamine dihydrochloride (98%) were purchased from Aladdin, (China). 1,6-hexanediamine (99%) and NaOAc (AR) were the products of Kelong Chemical Reagent Co., Ltd, (China). *O*-phthalaldehyde (OPA, Chemically pure), thioglycolic acid (TGA, AR), $NaNO_2$, K_2CO_3, $CaCl_2$, $MgCl_2{\cdot}6H_2O$, $Al(NO_3)_3$, NH_4Cl, $Zn(NO_3)_2$, $FeCl_2$, $FeCl_3$, $CuSO_4$, $HClO_4$ (AR) were the products of Sinopharm Chemical Reagent Co., Ltd (China). HOAc (AR) was purchased from Rionlon Bohua Pharmaceutical & Chemical Co., Ltd. (China). Alanine, Lysine, Valine, Histidine, Glycine, Methionine, Phenylalanine, Ornithine (Orn), Arginine, Threonine, Serine, Isoleucine, Cystine, Tyrosine, Tryptophan, Leucine, Cysteine, Glutamic acid, and histamine were obtained from Sinopharm Chemical Reagent Beijing Co., Ltd (China). All chemicals and reagents were used without further purification.

2.2. Instrumentation

The ultrapure-water was purified by the ultrapure-water system (Lakecore, China), and UV–vis spectrophotometer (Perkin Elmer Lambda 35, USA) was used to collect absorption data. Centrifuge (Shanghai Anting Scientific Instrument Factory TGL-16B, China) and ultrasonic instruments (Kunshan Ultrasonic Instruments Co., Ltd, China) was used to treat real samples. Chemical reagents were weighed by electronic balance (Sartorius BP221S, Germany). Water bath (Zhenzhou Nanbei Instrument Equipment Co., Ltd, China) was used to heat the samples. Buffers were prepared under the help of pH meter (Sartorius PB-10, Germany). Mass spectrum (Bruker MicroTof Q II, USA) was used to determine the molecular weight of the products.

2.3. Preparation of solutions

Series of standard solutions of putrescine were prepared by dissolving putrescine in methanol (MeOH) to concentrations of 1×10^{-7}, 2×10^{-7}, 5×10^{-7}, 1×10^{-6}, 2×10^{-6}, 5×10^{-6}, 1×10^{-5}, 2×10^{-5}, 5×10^{-5}, 1×10^{-4}, 2×10^{-4}, 5×10^{-4}, 1×10^{-3}, 2×10^{-3} and 5×10^{-3} M. 0.2 M OPA and 0.2 M TGA were prepared as follows: 0.1344 g OPA was added to 5.0 mL MeOH and 70.0 μL TGA was added to 5.0 mL MeOH, respectively. The solution was placed in a refrigerator for 24 h. Buffers of various pH were prepared by dissolving NaOAc (50 mM) in ultrapure water and titrated with acetic acid (400 mM).

2.4. Colorimetric reaction procedure

The colorimetric reaction procedure was obtained by optimizing the pH value, concentrations of OPA and TGA, heating temperature and time, and ion strength. Briefly, 20.0 μL OPA (0.2 M), 20.0 μL TGA (0.2 M) and putrescine at a certain concentration were added into buffer (pH 5.5) with the final volume of 2.0 mL. Then the mixture was heated at 60 °C for 12 min.

2.5. Preparation of fish samples

The fresh, dried, and canned fish products that were used to evaluate the concentration of putrescine were purchased from local markets in Lanzhou in China. For fresh fish, sampling with skin and flesh was analyzed instantly when got back from supermarket. Other fish samples were treated immediately after opening package. One gram of each type of fish product was homogenized for 3 min in 5.0 mL of 0.4 M $HClO_4$ solution and subsequently subjected to ultrasonic treatment for 10 min. After centrifugation (1062.55*g*) for 10 min, the supernatant was collected for analysis, and the rest of supernatant was stored at −20 °C for standby. All the treatment and measurement were done at room temperature.

3. Results and discussion

To find optimal reaction conditions of the OPA derivatization, we first systematically investigated the effects of pH, OPA and TGA concentrations, heating time and temperature, and ion strength. We then further investigated the selectivity to putrescine of the method by using various interferences. Finally, the optimized method was applied to analyze the content of putrescine in samples.

3.1. Colorimetric reaction condition optimization

3.1.1. pH value

Eleven buffers with pH values of 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, 8.0, 9.0, and 10.0 were separately prepared by mixing 50 mM NaOAc with 400 mM HOAc and 50 mm NaOH, and their pH were measured by a pH meter. Each of the buffers was mixed with 5.0 μL of 0.2 M putrescine, 10.0 μL of 0.2 M OPA, 10.0 μL of 0.2 M TGA in a 4 mL centrifuge tube, which was then heated at 90 °C in a water bath for 5 min. As shown in Fig. S1, the maximum ultraviolet (UV) absorption value appears at pH 5.5.

3.1.2. Concentrations of OPA and TGA

One-factor-at-a-time experiment was performed to investigate the effects of OPA and TGA concentrations to OPA derivatization reaction. The concentrations of OPA and TGA were both set at 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, and 2.5 mM. The UV–vis absorption values are shown in Fig. S2. We chose the concentrations of OPA and TGA both at 2 mM for a suitable UV–vis absorption value and ensure that putrescine at a lower concentration (0.1 mM) can fully react with OPA and TGA.

3.1.3. Heating temperature and time

Heating temperature and time are two important effective factors in the derivatization reaction, and specially, both of those are interaction. The response surface methodology (RSM) was therefore used to study the influences of reaction temperature and time on UV–vis absorption values of putrescine derivative solution (Table S1). As shown in Fig. S3, the temperature and time of 60 °C and 12 min respectively were the applicable conditions.