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Original Article

Detection of 3-N-oxalyl-L-2,3-diaminopropanoic acid in thermally processed foods by reverse phase high performance liquid chromatography

Rohini Misra [a], Asha Martin [a], Lalitha R. Gowda [b,*]

[a] Food Safety and Analytical Quality Control Laboratory, Central Food Technological Research Institute, Council of Scientific and Industrial Research (CSIR), Mysore 570 020, India
[b] Department of Protein Chemistry and Technology, Central Food Technological Research Institute, Council of Scientific and Industrial Research (CSIR), Mysore 570 020, India

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ABSTRACT

Grasspea (*Lathyrus sativus*), because of its low cost and ease of cultivation, is often an adulterant of other legumes. The seeds—albeit rich in protein (~20–32% of dry seed weight)—contain a neurotoxin, 3-N-oxalyl-L-2,3-diaminopropanoic acid (β-ODAP). An efficient and sensitive RP-HPLC method for detecting β-ODAP by pre-column derivatisation with *O*-phthalaldehyde (OPA) was developed in our laboratory. The usefulness of this method for measuring the β-ODAP content of thermally processed legume-based foods with various substitution levels of grasspea flour is demonstrated. The β-ODAP content of dehulled grasspea flour used was 5.26 ± 0.11 mg/g. The method shows a clear resolution between β-ODAP and its non-toxic α-isomer. At 1% (w/w) substitution it was possible to detect and distinguish the two isomers. Among the unit heat operations deep-frying results in the β-ODAP content being lowered by ~80% irrespective of the substitution level. The decrease in β-ODAP content by fermentation was ~58%, which was further decreased on steaming. The results indicate that levels as low as 0.01 mg of β-ODAP/g of product are measurable. Therefore this method is a useful tool in detecting the unwarranted use of grasspea flour in processed foods that pose health risks to unsuspecting customers.

1. Introduction

Adulteration of food with cheaper base ingredient(s), masking the ability to distinguish one ingredient from another, is a widespread problem (Woolfe and Primrose, 2004). Increasing consumer awareness and concern regarding food quality and safety has rendered detection of adulterants in final food products an important but difficult task for food control laboratories. This difficulty is compounded by physical and biochemical similarities between the adulterant and food ingredient as well as the complex nature of food matrices.

Grasspea (*Lathyrus sativus*), commonly known as khesari, belonging to the family Fabaceae and tribe Vicieae, is the third most important legume after chickpea (*Cicer arietinum*, Bengal gram) and pigeon pea (red gram, *Cajanus cajan*) (Pandey et al., 1997). It is rich in proteins (~20–32% of dry seed weight) and therefore consumed as a staple food by economically poor population groups (Grela et al., 2000, 2001). The low cost and ease of cultivation under adverse environmental conditions renders it as a common adulterant of other legumes (Hartman et al., 1974; Padmanaban, 1980). The seeds, however, contain a neurotoxin called 3-N-oxalyl-L-2,3-diaminopropanoic acid (β-ODAP) or its synonym L-2-amino-3-oxalylaminopropanoic acid (IUPAC-rules), a non-protein amino acid, which causes spastic paraparesis of legs and general weakness of skeletal muscles together with increased stiffness, leading to a degenerative condition referred to as neurolathyrism in humans (Hanbury et al., 2000; Tekle-Haimanot et al., 1993).

Chickpea flour, locally called besan, is a popular culinary ingredient in households across the Indian subcontinent. Grasspea flour looks very much like it and is often admixed with food for human consumption along with powdered chickpea flour. A large number of popular traditional oriental convenience and confectionary food products, both at household and industrial scale, are prepared from dehulled chickpea flour.

Many of the legume-based foods are traditionally prepared, using various unit operations of roasting, deep-frying, fermentation and steaming which involve heat transfer (Tekle-Haimanot et al., 1993). These domestic cooking processes have been reported to decrease β-ODAP to a significant degree, but they do not eliminate it completely (Padmajaprasad et al., 1997; Parada and Aguilera, 2007). Food processing results in multicomponent,

* Corresponding author. Tel.: +91 821 2515331; fax: +91 821 2517233.
E-mail addresses: lrg@cftri.res.in, lrgowda@yahoo.com (L.R. Gowda).

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microstructured matrices where the individual components are reassembled by unit heat operations and/or application of shear (Parada and Aguilera, 2007). It is plausible that in the multi-component structure of a food matrix, β-ODAP can bind to other components or get entrapped in the complex macromolecular matrix of swollen starch granules and protein on heating and/or cooling.

This stresses the need for a sensitive and rapid analytical technique to detect and monitor the β-ODAP content in processed food products. The most commonly used spectrophotometric method is non-selective, detecting both isomers α- and β-ODAP collectively (Rao, 1978). We have earlier reported a RP-HPLC method for detecting β-ODAP by an instantaneous pre-column derivatisation using o-phthalaldehyde (OPA). The sensitivity of this method for the detection of β-ODAP was 3.5 ± 0.1 μg/g (Thippeswamy et al., 2007). This method can therefore be extended to monitoring the β-ODAP content in thermally processed foods.

In the present study we have extended this RP-HPLC method to quantify the β-ODAP and its non-toxic α-isomer content in medium and highly thermally processed foods prepared from the mixtures of dehulled chickpea flour containing grasspea. The proportion of grasspea admixture ranged from 1 to 100%. Foodstuffs which were deep-fried (boondi) or roasted (besan laddoo) were considered as medium processed, and a preparation that involved fermentation followed by steaming (dhokla) was considered as highly processed. We further demonstrate that in all the unit heat operations β-ODAP content was detectable and quantifiable.

2. Materials and methods

2.1. Materials

The chemicals o-phthalaldehyde (OPA), β-mercaptoethanol and triethylamine (TEA) were obtained from Sigma–Aldrich Co. St. Louis, MO, USA. Glucose was from Himedia, Mumbai, India. Acetonitrile and methanol were HPLC grade from Ranbaxy Fine Chemicals Ltd., New Delhi, India. β-ODAP, α-ODAP and diaminopropionic acid (DAP) were obtained from Lathyrus Technologies, Hyderabad, India. Boric acid was obtained from Amresco, OH, USA and sodium acetate trihydrate from SD Fine Chemicals, Mumbai, India. *L. sativus* seeds were a gift from Ms. Usharani, Mysore, India. Dehulled chickpea (Bengal gram) flour was obtained from the local supermarket. All solutions were prepared with water purified with a MilliQ system (Millipore, Bedford, MA, USA).

2.2. Preparation of grasspea admixtures

Dehulled chickpea flour shown to be free of β-ODAP was used to prepare the admixtures. One gram of grasspea flour was mixed uniformly with 99 g of chickpea flour in triplicate. The mixture was sieved three times to ensure uniform distribution of the grasspea flour. This provided 1% (w/w) admixture. Similar admixtures were prepared to a composition in proportion reflecting 15, 25 and 50% (w/w) grasspea flour.

2.3. Preparation of traditional chickpea-based processed products

2.3.1. Deep-fried boondi

Dehulled chickpea flour and the mixtures containing grasspea flour were used to prepare a batter of solid concentration 44% (w/w) (in triplicate) in distilled water. The lump-free batter was allowed to pass through a perforated (~3 mm in diameter) ladle known as boondi jhara and dropped into hot refined groundnut oil preheated and maintained at 175 ± 5 °C (Bhat and Bhattacharya, 2001). Frying was continued until the globules developed a golden yellow colour. Boondi was then removed from the hot oil and placed on a stainless steel mesh to drain surface oil and cooled to room temperature.

2.3.2. Steamed dhokla

A thick batter of uniform consistency was prepared (100 g in 50 mL water) to which 5 mL curd (a product of natural fermentation of milk by lactic acid bacteria) and 2 g sodium bicarbonate was added and allowed to ferment overnight (16 h) at 27 ± 2 °C. At the end of fermentation the batter was steamed for 15 min and allowed to cool. In addition, aliquots of fermenting batter were removed at 2 h intervals and cooled on ice to evaluate the effect of fermentation time on β-ODAP content.

2.3.3. Roasted besan laddoo

Dehulled chickpea flour with admixed grasspea flour was mixed with equal proportions of ghee and finely ground refined sugar (50% w/w). This mixture free from lumps was then roasted at 115 ± 5 °C for 10–15 min, when the mixture developed a golden brown colour. Flour was then removed from the pan and cooled to room temperature.

2.4. Maillard reaction

The effect of a Maillard reaction involving the complexation of β-ODAP and free sugar present in the food preparations was studied. A fixed amount of β-ODAP was added to different concentrations of glucose (1.7–6.8 mM). These solutions were then heated at 100 °C for 30 min. The resulting solution was analysed for the residual β-ODAP by RP-HPLC as described later.

2.5. Sample preparation

The processed food products were defatted using petroleum ether (60–80 °C) at 37 ± 2 °C, and subsequently ground to a fine powder. Sulfosalicylic acid (2.5 mL, 3% w/v) was added to 500 mg finely powdered samples and agitated for 1 h at 25 ± 2 °C in a rotary shaker set at 200 rpm. The solution was centrifuged at 15,000 × *g* for 15 min at 4 °C and the clear supernatant was subsequently filtered through a 0.45 μm membrane.

2.6. RP-HPLC analysis

Derivatisation reagent (OPA reagent) was prepared fresh every day. OPA (5 mg) dissolved in 0.05 mL methanol was added to 0.45 mL of 0.4 M sodium borate buffer (pH 10.5) followed by 0.025 mL β-mercaptoethanol. A 20 μL aliquot of the sample prepared as described above was mixed with an equal volume of OPA reagent and incubated at 25 ± 2 °C for exactly 2 min and then subjected to HPLC analysis as described earlier (Thippeswamy et al., 2007). The analysis was performed using a Discovery 5 μm C18 (250 mm × 4.6 mm) column, Shimadzu Model LC-10AT VP HPLC system (Shimadzu, Japan) equipped with a Rheodyne injector, LC-10A separation module and SPD-M10A VP PDA detector set to monitor the derivatised β-ODAP at 340 and 420 nm.

A standard solution of 2.5 mM β-ODAP was serially diluted and used to construct a calibration curve. Mobile phase A was 0.14 M sodium acetate containing 0.05% (v/v) TEA adjusted to pH 6.8 with glacial acetic acid and methanol (90:10) and mobile phase B was 60:40 acetonitrile in water. The optimised binary gradient traversing from 0 to 75% B in 15 min followed by a 5 min wash in 100% B and 5 min reequilibration in 100% A was operated at a flow rate of 1 mL/min (Thippeswamy et al., 2007).