



## Review

## Identification of a novel umami compound in potatoes and potato chips



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## ABSTRACT

The influence of frying time on the taste profile of potato chips was characterized. Direct comparison of isolates from potato chip samples fried for 170 s and 210 s indicated longer frying time increased the perceived umami intensity and decreased the sour intensity. The compounds responsible for the greater umami intensity were identified as monosodium L-pyroglutamate (L-MSPG) and monosodium D-pyroglutamate (D-MSPG). The reduction in sour intensity was attributed to the degradation of D-chlorogenic acid. MSPGs were endogenous in raw potatoes and also thermally generated from glutamic acid during frying. Taste recombination studies further confirmed the contribution of both compounds to the umami character of potato chips. Furthermore, time-intensity taste analysis revealed that topical addition of both L- and D-MSPG enhanced the perceived intensity of the umami taste and the overall flavor characteristic of the potato chips.

## 1. Introduction

Potato chips are a popular salted snack worldwide. As with most savory snacks, the flavor profile of potato chips is a key driver of consumption. A current focus of the food industry is reduced fat and salt-content snacks to provide choices that help promote a healthy lifestyle. However, manufacturing these reformulated products are typically challenged by lower flavor quality. For example, regular potato chips exhibit a more desirable flavor profile with greater consumer liking and acceptability when compared to their reduced-fat counterparts. Moreover, the level of salt in a product has been found to positively influence the taste profile and enhance overall flavor intensity (Gillette, 1985). Consequently, the successful development of lower fat- or salt-content snacks is challenged by the lack of technical know-how in maintaining positive flavor attributes.

Flavor studies on potato chips have been predominantly focused on aroma. Very little is known about the taste profile and how it might be affected by different processing treatments (Majcher, & Jelen, 2005; Oruna-Concha, Bakker, & Ames, 2002; Oruna-Concha, Duckham, & Ames, 2001). A better understanding of the taste profile of potato chips can facilitate the development of reformulation approaches as well as the discovery of desirable tastants, such as compounds with umami attributes typically associated with fried potato products. Additionally, with the increasing demand for reduced sodium alternatives, the discovery of novel food-derived umami enhancing compounds is desirable because they have been shown to improve overall flavor perception of savory foods and snacks (Dermiki, 2013; Morris et al., 2007).

Umami, described as savory or meaty, is one of the primary tastes (Hofmann, Ho, & Pickenhagen, 2003). This pleasant savory taste has been discovered in a wide range of foods such as meat, seafood, soy sauce and some processed foods. As early as 1909, monosodium glutamate (MSG) was identified in seaweed as the key compound responsible for meaty and savory taste (Winkel et al., 2008). Since then more molecules have been discovered that demonstrate umami characteristics. These compounds include small organic acids (e.g. succinic acid, aconitic acid, gluconic acid) (Buckholz, 1994), L-amino acids (L-glutamic acid, L-aspartic acid, L-aromatic amino acids (Lioe, Apriyanton, Takara, Wada, & Yasuda, 2005), peptides (Shiga et al., 2014), nucleotide derivatives, as well as Maillard-reaction products (Suess et al., 2014). In addition to savory characteristics, umami compounds have shown synergic taste effects (Hofmann et al., 2003). For example, a mixture of L-glutamate and purine-5'-ribonucleotides exhibits a more than additive effect on the umami taste intensity when compared to just L-glutamate or purine-5'-ribonucleotides alone. Studies have also revealed that overall potato flavor is enhanced when umami enhancing compounds are added (Morris et al., 2007).

The main objective of this work was to investigate how different processing treatments affect the taste profiles of potato chips. More specifically, the goal was to focus on the influence of frying time on the taste compounds of potato chip samples in order to provide guidelines for product flavor optimization.

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## 2. Materials and methods

L-glutamic acid (L-GA), D-glutamic acid (D-GA), L-pyroglutamic acid (L-PGA),  $\alpha$ -chasolanine,  $\alpha$ -chaconine, chlorogenic acid, and D-quinic acid were purchased from Sigma Aldrich, Inc (St. Louis, MO, USA). D-pyroglutamic acid (D-PGA) was obtained from TCI Co, Ltd (Portland, OR, USA). HPLC grade ethanol and methanol, USP sodium hydroxide and sodium chloride were obtained from Fisher Scientific (Pittsburgh, PA, USA). Unsalted and salted potato chips fried for either 170 s (termed shorter fry time potato chip or 'SFT-PC') or 210 s (termed regular fry time potato chip or 'RFT-PC') were obtained from Frito-Lay (Plano, TX, USA). L- and D-MSG plus L- and D-MSpG were prepared using aqueous solutions of GA or PGA that were neutralized with 0.1 mol/L NaOH to yield a high purity powder liberated by freeze-drying (purity verify by LC/MS).

### 2.1. Taste and astringency profiling of potato chip isolates and HPLC fractions

#### 2.1.1. Sample preparation

Unsalted potato chips (200 g) fried for 170 s (SFT-PC) and 210 s (RFT-PC) were individually ground and extracted with hexane to remove fat (200 mL  $\times$  2). The hexane extracts for each sample were separately pooled together and the solvent was removed under vacuum. The resulting isolate (Isolate I) was rehydrated, frozen and freeze dried twice to flash off residual hexane prior to taste activity screening. The defatted potato chips (Isolate II) were allowed to air dry in a fume hood and were further extracted (2  $\times$  200 mL) using an ethanol: water mixture (1:1) for a total extraction time of 6 h (2  $\times$  3 h). The aqueous ethanol solvent extracts from each sample were separately pooled (Isolate III) and centrifuged. The supernatant was collected and the organic solvent was removed under vacuum. The resulting aqueous isolate was then loaded onto a C-18 solid phase extraction cartridge, and eluted with 70:30 methanol: water (Isolate V). The resulting eluent isolate was subsequently filtered using a 0.22  $\mu$ m Nylon tip filter and stored at  $-80^\circ\text{C}$  prior to preparative LC fractionation (see Supplemental Fig. A). Extracted potato chips (Isolate IV) were also collected for each sample separately in order to screen for residual taste activity. The solvent was removed under vacuum and potato chips were then frozen and freeze dried twice prior to tasting. When reconstituted in water, Isolates I and IV were found to be practically tasteless. Isolate V (Supplemental Fig. A) was found to be taste-active and was subsequently selected for HPLC fractionation and sensory evaluation.

#### 2.1.2. Preparative HPLC fractionation-sensory screening

Fractions were collected using a LC binary pump system (Shimadzu, MD) configured with a autosampler, a variable-wavelength UV–VIS detector at 254 and 220 nm, a fraction collector and a Zorbax RP-18 column (150 mm  $\times$  21.2 mm, Varian, USA). The sample injection volume was 5 mL. A binary mobile phase system was used consisting of 100% water (solvent A) and 100% ethanol (solvent B), the column flow rate was 10 mL/min. The gradient elution started with 5% B (0–3.5 min), then linearly increased to 50% B (3.5–12 min), then increased to 100% B (12–13 min), then held at 100% B (13–16 min), then finally decreased to 5% B (16–16.5 min), and held at 5% B (16.5–20 min) in order to equilibrate to initial gradient conditions. Fourteen fractions (F1–F14) were collected (Fig. 1) and screened for taste activity. Prior to sensory analysis, the organic solvent was removed under vacuum (rotary evaporation) and subsequently fractions were frozen and freeze-dried twice. The resultant powdered isolates from each fraction were rehydrated in 5 mL of water for tasting and initial sensory screening. The panelists rated the intensity of all taste attributes perceived as well as astringency using a categorical scale (not present, threshold, weak, moderate, and strong). Fractions with the greatest observed intensities, namely those with moderate to strong ratings, were selected for further purification using secondary

dimension HPLC fractionation. Second dimension fractionation was conducted using a Zorbax SB-AQ column (150 mm  $\times$  21.2 mm, Agilent, USA) and a binary mobile phase system consisting of 100% water (solvent A) and 100% ethanol (solvent B) at a flow rate of 10 mL/min. Three different taste attributes with moderate to strong intensities were identified among the first dimension fractions and were further separated in order to isolate and identify tastants of interest. Fractions F1 and F2 (Fig. 1) were umami and were each further separated into ten additional fractions using the following mobile phase gradient conditions: 0% B (0–5 min) initially, then increased to 100% B (6–9 min), and finally held at 100% B (9–13 min). Fractions F3 and F4 (Fig. 1) were sour and were each further separated into ten additional fractions using the following mobile phase gradient conditions: 0% B (0–3.75 min) initially, then increased to 2% B (3.75–10 min), then linearly increased to 100% B (10–11 min), and finally held at 100% B (11–13 min). Lastly, fractions F11–F14 (Fig. 1) were bitter and were each further separated into twelve additional fractions using the following mobile phase gradient conditions: 10% B (0–4.49 min) initially, then linearly increased to 100% (4.5 min–16 min), then held at 100% B (16–20 min), and finally decreased to 10% (20–21 min), and held at 10% B (21–26 min). The resultant fractions containing the corresponding tastants were subsequently analyzed by LC-MS-ToF and NMR analysis for structural characterization and identification.

**Accurate Mass Analysis (LC/MS-ToF).** Samples were analyzed on a Waters 2D UPLC Acquity iClass system consisting of a binary solvent delivery module, autosampler, and column heater and coupled with a Xevo G2 QToF system (Waters, Milford, MA, USA). Injections were performed on Agilent Zorbax UPLC SB-AQ RRHD column (2.1  $\times$  100 mm, 1.8  $\mu$ m) maintained at a flow rate of 0.3 mL/min using a binary solvent system of 0.1% formic acid in water (solvent A) and 0.1% formic acid in methanol (solvent B). The elution gradient started at 5% B, linearly increased to 100% B (1–6 min), held at 100% B (6 min–7 min), and then held at 5% B (7–9 min). Mass spectrometric ionization conditions were as follows: desolvation temperature of  $400^\circ\text{C}$ ; source temperature of  $110^\circ\text{C}$ ; capillary voltage of 1.1 kV; leucine enkephalin was used as the lockspray mass calibrant. Samples were analyzed in both positive and negative mode, with a scan range of 50–1000 Da.

### 2.2. Preparation of fried potatoes for targeted umami analysis

Russet potatoes, Crisco pure vegetable oil (J.M. Smucker Co, USA), and a potato slicer were purchased from local grocery store (Minneapolis, MN, USA). The potatoes were washed, peeled and sliced with a thickness of less than 0.1 inches. The potato slices were fried in vegetable oil at  $173^\circ\text{C}$  for 170 s for reduced fat potato chips and 210 s for regular-fat potato chips. The moisture content of the raw potatoes, low-fat potato chips, and regular-fat potato chips were 74.6%, 25.1%, and 6.1%, respectively, based on the method previously reported by Ghadge, Britton, and Jayas (1989). The fat content of the raw potatoes, low-fat potato chips, and regular-fat potato chips were 0.17%, 25.4%, and 30.1%, respectively, based on the method previously reported by Krokida, Oreopoulou, and Maroulis (2000).

### 2.3. Quantification of taste compounds and sodium in Potato, potato chips and in saliva during potato chip mastication

#### 2.3.1. Potato and potato chip taste extraction

Raw potato, salted SFT-PC, and salted RFT-PC were individually ground and extracted with an ethanol: water mixture (1:1) for three hours. The final extracts were centrifuged and the supernatant was run through solid phase extraction (C-18 cartridge) and eluted with a 70:30 methanol:water solution for LC/MS/MS analysis.

#### 2.3.2. Saliva collection and preparation

Three subjects were instructed to chew 2 g of potato chips for 20 s

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