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Research report

Sensory attributes of dishes containing shrimp paste with different concentrations of glutamate and 5′-nucleotides

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ABSTRACT

The shrimp paste called *belacan* is a traditional umami taste condiment extensively used in Malaysia that is rich in glutamate and 5′-nucleotides. The aim of this study was to determine the concentration of glutamate and 5′-nucleotides of various types of foods prepared with *belacan* and to measure their sensory attributes. The concentration of free glutamic acid found in different brands of *belacan* was 180–530 mg/100 g and in local dishes 601–4207 mg/100 g. The total amount of 5′-nucleotides in *belacan* samples ranged from 0.85 to 42.25 μg/g. A Quantitative Descriptive Analysis (QDA) using a list of 17 sensory attributes showed a good correlation between *belacan* concentration in the final food and a range of positive sensory attributes, except for bitter, sweet, sour taste and astringency. *Belacan* also contains bitter, sweet and sour compounds that change the positive attributes of belacan at higher concentrations. The highest aroma attributes were linked to *nasi goreng belacan* (*belacan* fried rice) while the highest flavour attributes were found in *sambal belacan*. There was a 32 folds significant increase of umami attributes with the addition of *belacan* to final foods. The optimum amount of *belacan* was 0.45% for *asam pedas* (tamarind flavoured dish with belacan), 18% for *sambal belacan* (chilli *belacan*), 1.5–2.5% for *kangkong goreng belacan* (stir fried water convolous with belacan), and 2% for *nasi goreng belacan*.

Introduction

Umami taste is one of the basic taste categories, alongside sweet, sour, bitter and salty (Fuke & Shimizu, 1993; Imafidou & Spanier, 1994; Nagodawithana, 1994). The receptors for different tastes, including umami taste have also been identified by molecular biology (Lindemann, 2001). Umami taste is triggered by monosodium glutamate (MSG), and 5′-ribonucleotides, namely disodium 5′-innosinate (IMP) and disodium 5′-guanylate (GMP), which are used as flavour enhancers throughout the world (Bellisle, 1999; Ikeda, 1909). The intensity of umami taste in food is usually produced throughout synergistic interactions of free glutamate and 5′-ribonucleotides. The umami taste of L-glutamate can be significantly enhanced by 5′-ribonucleotides and the synergy is a property of this taste quality (Zhang et al., 2008). Most food materials contain naturally occurring umami substances whether at suprathreshold or at subthreshold amounts (Maga, 1983). Yamaguchi and Komata (1987) and others (Marcus, 2005; Peralta et al., 2005) established that most savoury food contain umami substances released through ripening, drying, curing, aging and/or fermentation and that their umami properties can be further developed by a small amount of added glutamate.

Malaysian cuisine reflects the multi-ethnic shape of the country and the culinary culture of each ethnic group contributes to the characteristics of Malaysian cuisine (Hutton, 2005). *Belacan* (shrimp paste) is one of the most popular traditional condiments used in countless local dishes such as chilli *belacan* (*sambal belacan*), spicy noodle soup (*laksa*), fried rice with *belacan* (*nasi goreng belacan*), stir fried chilli paste (*sambal tumis*), sour and spicy fish stew with *belacan* (*asam pedas*), stir fried water convolvulus with *belacan* (*kangkung goreng belacan*) and Indian fried noodles (Hutton, 2003, 2005; Lee, 2001). *Belacan* is also used in other Southeast Asian countries and it is known as *kapi* in Thailand, *ngapi* in Burma, *trasi* in Indonesia, and *andaramang* in Philippine. In Malaysia, it is made from fermented tiny shrimp of a species known as *geragau or geragok*. *Belacan* is salty in taste and has strong shrimp odor (Md Adnan, 1984). High content of salt in *belacan* make it inconsumable in a raw form, but it is often used as a flavour enhancer in dishes.

Kuznesof, Tregear, and Moxey (1997) stated that consumers' acceptance, choice and preference towards foods are motivated by a number of factors. Flavour is an important attribute of the eating quality of foods (Flores, Armero, Aristoy, & Toldra, 1999; Imafidou & Spanier, 1994). One of the taste properties resulting from the natural occurrence or intentional addition of compounds such as monosodium glutamate and certain 5′-nucleotides is the umami

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taste that has also been described as a savoury and brothy sensation (Maga, 1994). Studies showed that the taste quality associated with glutamate in foods, known as umami, is recognized and coded as a unique quality by the gustatory system (Bayliss & Rolls, 1991; Faurion, 1991; Ninomiya, Tanimukai, Yoshida, & Funakoshi, 1991; Prescott, 2004; Rolls, 2000; Rolls, Critchley, Wakeman, & Mason, 1996). Prawn and shrimp are popular seafood in many countries because of their appetizing flavour (Morita, Kubota, & Aishima, 2001). The exploration of Malaysian consumer perception on *belacan* has been studied recently (Leong et al., 2009). However, so far the study on the potential of *belacan* as a umami enhancer has not been carried out. This study was conducted to determine the concentrations of free glutamate and 5′-ribonucleotides in *belacan* and Malaysian cuisines and the sensory attributes of the products.

Materials and methods

Food samples

Samples of 11 types of *belacan* used in the study were purchased from local grocery stores and markets in the state of Selangor, Penang and Melaka, while homemade *belacan* was purchased at its originality places being processed. Four local dishes namely *Sambal Belacan* (SB), *Kangkung Goreng Belacan* (KGB), *Nasi Goreng Belacan* (NGB) and *Asam Pedas* (AP) were cooked by experienced chef using the same *belacan*. The dishes were cooked according to the standard recipe and through discussion an hour before the sensory evaluation of the products.

Chemicals and materials

Standard for L-glutamate (minimum 99%-TLC, Sigma Adrich, Steinhelm, Germany) and dansyl chloride (Assay $\geq$ 99.0%, Fluka, Buchs, Switzerland) were used. All the reagents used were of HPLC analytical grade: acetone, methanol and 5N sodium hydroxide solution (Merck, Darmstadt, Germany), glacial acetic acid and sodium hydrogen bicarbonate (Scharlau, Barcelona, Spain). De-ionized water was obtained through a Millipore-Q50 Ultrapure water system (Model 63310-3; Sartorius, Goettingen, Germany).

Preparation of chemicals and solutions

Dansyl chloride solution was prepared in the concentration of 1.5 mg ml^{-1} in dried acetone. Fresh solution was prepared each time, kept at 4 °C in the refrigerator and wrapped in the aluminum foil until being used. The buffer was a pH 10.5 solution of 4 g/l sodium hydrogen carbonate. The solution was adjusted by using 5N sodium hydroxide. The stock solutions of L-glutamic acid, disodium IMP and disodium GMP were further diluted in de-ionized water to five working standards. Mobile phase used for L-glutamic acid analysis was 1% (v/v) glacial acetic acid in 45% methanol, filtered through a 0.45 μm Milipore filter. Concentratetion of 0.5 M potassium dihydrogen phosphate was prepared fresh daily in de-ionized water, adjusted pH 4.0 with phosphoric acid using a digital pH Meter (CyberScan 1100, Eutech Instrument, Singapore), degassed and filtered through Millipore paper (0.45 μm) and used as mobile phase for disodium IMP and disodium GMP determination by HPLC.

Glutamate extraction and derivatization

The samples of *belacan* in the paste form were diluted by adding 5 ml de-ionized water to 5 g sample and mixed for 15 min using the stirrer (MR3001, Heidolph, Germany). Then, 1 g of homogenized sample and 0.5 g sample (in powder form) was transferred into 25 ml volumetric flask, buffer was added and the mixture was stirred for at least 15 min before being filtered through a Whatman No.1 filter paper. The food samples were blended homogenously using a blender (model Autovortex, Stuart, England) and 5 g of the homogenized sample was transferred into 25 ml volumetric flask before it was added with buffer. The solution was mixed for 15 min using magnetic bar and stirrer plate, and filtered through a Whatman No.1 filter paper. The samples were prepared in duplicates.

Aliquots of 10 μl of the standard, sample or blank (de-ionised water) were transferred into a 1 ml Pierce Reacti-Vial before 50 μl of the buffer solution and 100 μl of the dansyl chloride solution were added. The mix reagents were then mixed vigorously using vortex. Then, the vials were heated in the boiling water bath at 100 °C for 10 min. The vial contents changed from a pale yellow (before reaction) to colourless (after reaction). The vials were cooled under running water then 300 μl of methanol was added. Methanol was added to minimize any errors that might occur owing to loss of the reaction mixture through evaporation in the heating process. Sample was filtered through 0.45 μm pore size (13 mm) before injection onto HPLC.

Glutamate determination

The method used for the determination of glutamate was adapted from Williams and Winfield (1982) with some modifications made previously by Populin, Moret, Truant, and Conte (2007). The methodology was based on the HPLC method using pre-column dansylation and fluorescence detection. The dansyl chloride (5-dimethylaminonaphthalene-1-sulphonyl chloride or Dns-Cl) derivatives were readily separated using liquid chromatography (JASCO PU-2080 Plus, with JASCO DG-2080-54 (4-line degasser), manual injector (Model Rheodyne 7725i) and JASCO FP-2020 Plus fluorescence detector, Easton, MD, USA). The C18 Waters BondaPak column (4.6 mm × 300 mm) (Milford, Massachusetts, USA) was used. The flow rate was set at 1.2 ml/min and the sample loop size was 20 μl via Rheodyne injector. Isocratic elution was performed using the solvent which was held at 1% (v/v) glacial acetic acid in 45% methanol. The fluorescence detector was set at the excitation wavelength of 328 nm and emission wavelength of 530 nm. The peaks were detected and analyzed with Borwin Chromatography Software.

5′-Nucleotides extraction

The procedure by Liu, Vujayakumar, Hall, Hardley, & Wolf-Hall (2005) were used to determine the content of 5′-ribonucleotides which are 5′-inosine monophosphate (5′-IMP) and 5′-guanosine monophosphate (5′-GMP). Briefly, ground freeze dried of *belacan* and local dishes with and without *belacan* (500 mg) were boiled in de-ionized water (25 ml) for 1 min, cooled to room temperature and then centrifuged at 22,200 × *g* for 15 min at 10 °C in high speed centrifuge (Beckman, Model Avanti J25, Golden Valley, MN, USA). After removal of water, the final volume was measured and the extraction was repeated once using de-ionized water (20 ml) and then filtered through polytetrafluoroethylene (PTFE, 0.20 mm) filter before HPLC analysis.

5′-Nucleotides determination

The 5′-nucleotides were separated using a HPLC system (Model LC-20AT, Shimadzu, Kyoto, Japan) attached with a UV-spectrophotometric detector (Model CTO-6A, Shimadzu, Kyoto, Japan), LC-10AT VP pump, a Rheodyne 7725i injector and a 20 μl sample loop, and using a mobile phase 0.5 M KH2PO4 (pH 4.0) at a flow rate of 1 ml/min, a Bondapak column (3.9 mm × 300 mm) (Milford, MA, USA) and UV detection was 254 nm (run time 15.0 min). Each