The novel umami peptides identified from *Takifugu obscurus* and *Takifugu rubripes*

YUAN LIU and Bei Li
Department of Food Science and Technology, Shanghai Jiao Tong University, 800 DongChuan Road, Shanghai

Abstract

Consuming Takifugu is very popular in China because of its delicious taste. In this study, Takifugu obscurus (*T. obscurus*) and Takifugu rubripes (*T. rubripes*), edible puffer fishes, were used as the raw materials to isolate and identify the umami peptides. Different fractions were separated and purified using some isolation technologies such as membrane separation. Then sensory evaluation was performed of theses fractions to distinguish the peptides with the umami taste. The separated umami peptides were synthesized to verify their umami taste using the sensory evaluation. Finally, 12 novel umami peptides were identified and validated. This study could provide theoretical basis and technical support to understand the flavour of Takifugu and develop the new flavouring using the umami peptides.

Introduction

Flavour perception involves in the olfactory and gustatory interaction, which results from the volatile and non-volatile compounds releasing from the food. It also has a decisive influence on the eating quality, preference and acceptability of foods, especially for meat products [1]. And this is the reason why the number of research on understanding the chemistry of meat flavour, and determining the dominant influencing factors on flavour quality during the production and processing of meat has increased over the years. Inorganic salts, peptides, amino acids, organic acids, ATP breakdown products (ATP derivatives) and sugars contribute to generating the taste of meat and meat products in their water soluble substances [2]. Among these contributors, flavour peptides possess unique taste properties owing to their primary structure and amino acid sequence, which participates in the formation of the flavour and improve the overall flavour of food [3].

The pioneer study of the flavour peptide can be traced back to 1987. Japanese scientist firstly isolated the beefy meaty peptide (BMP) from beef meat, which was identified as a savoury taste similar to monosodium glutamate (MSG) [4]. After that, many researchers are gradually focusing on isolating the novel flavour peptides using gel filtration chromatography, reverse phase high performance liquid chromatography, tandem mass spectrometry, sensory evaluation and the electronic tongue.

Puffer fish, known as blowfish, bubble fish, fugu and porcupine fish, is a popular and edible fish species in both China and eastern Asian countries, owing to its unique umami taste. Peptides and free amino acids are the key components to release the delicious taste of puffer fish [5]. The relationship between the formation of flavour and free amino acids, ATP in puffer fish has been well studied. However, the isolation and identification of the novel umami peptides in puffer fish is a neglected topic.

Therefore, our present study is to identify the novel umami peptides from *Takifugu obscurus* and *Takifugu rubripes.* Different treatments including water soluble, heating, and enzymatic hydrolysate were used on *Takifugu* to extract and separate the umami peptides. The meaning of the study is not only to identify the novel umami peptides, but also to provide information on developing the new umami flavours or seasonings.

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Experimental

Materials

Takifugu obscurus and *Takifugu rubripes* fish were both purchased from Jiangsu Zhongyang Group Co., Ltd (Nantong City, Jiangsu Province, China). And they were killed by a puffer fish licensed chef. Muscle filets were removed and three different treatments, including water soluble, heating, and enzymatic hydrolysate were used to extract the fractions from fresh cultured muscle filets. The supernatants were collected freeze-dried and stored at -80℃.

Isolation and purification [6]

The T. *obscurus* and T. *rubripes* extractions with a molecular weight of less than 3KDa were fractionated by ultra-filtration using membranes with a MW cut off size of 3KDa. The condition of ultrafiltration was at 5℃ under 2.5-3.0 Psi N_2 pressure. All the ultrafiltration fractions were collected for freeze-drying and stored at -80℃. Sephadex G-15 gel filtration chromatography (column 1.6*60cm) was used to elute the extracted fractions with deionized water as the eluting solvent and the flow rating being 0.75ml/min. The UV absorbance of the eluent was monitored at 220nm. Each filtration fraction from successive runs were pooled and lyophilized for the sensory evaluation (described in the following).

The most intense umami taste fraction, obtained from the gel filtration chromatography, was separated using a Waters 2695 Allicance® HPLC system under the condition of a Kromasil C18 column at 30℃ to get several sub-fractions. The elution condition was an isocratic elution with eluting solvent 90% A (Milli-Q water) and 10% B (ACN HPLC grade) for 20 minutes at a flow rate of 1ml/min. And the elution peaks were monitored at 215nm.

Identification of the umami peptides by MALDI-TOF/TOF MS/MS [6]

Freeze-dried RP-HPLC fraction with the most intense umami taste was first re-dissolved in 50% ACN, 0.1% TFA containing 4mg/ml a-cyano-4-hydroxycinnamic acid (HCCA) and filtered. Myoglobin digested with trypsin was used to calibrate the mass instrument with internal calibration mode. The MALDI-TOF/TOF MS/MS (mass range scanning from 450 to 2000Da) was run in the positive refractor mode. MS/MS spectra were acquired from 2000 shots by adjusting the laser intensity above the threshold for generation of molecular ions for each umami peptide.

Synthesis of the umami peptides

The sequenced umami peptides by *MALDI-TOF/TOF MS/MS* were synthesised using the Solid phase peptide synthesis technique. The purity of synthetic peptides was higher than 97%.

Sensory evaluation [6]

The sensory panel consisted of 3 males and 5 females, who were screened by recognising the basic tastes. The sensory evaluation was conducted in the sensory lab. The sensory attributes, including sweetness, sourness, bitterness, umami, and kokumi, were evaluated to describe the taste perception of sample fractions, including the ultrafiltration, Sephadex G-15 gel filtration, RP-HPLC fractions, and the synthetic umami peptides.

Results and discussion

After ultrafiltration and gel chromatography, the fractions of *T. obscurus* and *T. rubripes* were separated and subjected to the sensory evaluation in order to screen out the most intense fraction with the umami taste. RP-HPLC was conducted on this fraction to get several individual sub-fractions. The descriptive analysis was used to describe and assess the sensory properties of the sub-fractions. The sub-fractions with the most intense umami taste were considered as the umami peptides and their sequences were identified. In order to validate the umami taste with these identified peptides, the synthetic peptides were subjected to the sensory evaluation. The umami taste was also found and described by the synthetic peptides, which indicates the validation of these identified peptides as the umami peptides.

Table 1 lists the sensory description of the synthetic umami peptides. The result shows that totally, 12 novel umami peptides were identified and validated from *T. obscurus* and *T. rubripes* using the different treating methods. Additionally, regardless of the different treatments, another attribute kokumi, is one of the main sensory properties of these umami peptides, which is more likely to contributing the delicious taste of puffer fish.

Table 1: The sequences, sources and sensory description of the synthetic umami peptides [7]

The sequences of identified umami peptides	*Sources*	*Sensory Properties*
Leu-Tyr-Glu-Arg	Takifugu Obscurus enzymatic hydrolysate	Sweetness, Umami, Kokumi
Val-Arg-Ser-Tyr	Takifugu Obscurus enzymatic hydrolysate	Sweetness, Umami, Kokumi
Cys-Ala-Leu-Thr-Pro	Takifugu Obscurus (100℃)	Umami, Kokumi
Arg-Pro-Leu-Gly-Asn-Cys	Takifugu Obscurus (100℃)	Umami, Kokumi
Glu-His-Ala-Met-Leu-Asn	Takifugu Rubripes (4℃)	Umami, Kokumi
Lys-Gly-Arg-Tyr-Glu-Arg	Takifugu Obscurus enzymatic hydrolysate	Sweetness, Umami, Kokumi
Thr-Leu-Arg-Arg-Cys-Met*	Takifugu Obscurus (4℃)	Umami, Kokumi
Pro-Gly-Gly-Val-Arg-Asn-Gly	Takifugu Rubripes (4℃)	Umami, Kokumi,Sourness
Pro-Val-Ala-Arg-Met*-Cys-Arg	Takifugu Obscurus (4℃)	Umami, Kokumi
Tyr-Gly-Gly-Thr-Pro-Pro-Phe-Val	Takifugu Obscurus (100℃)	Umami, Sweetness
Tyr-Lys-Cys-Lys-Asp-Gly-Asp-Leu-Arg	Takifugu Obscurus enzymatic hydrolysate	Umami, Kokumi, Fish flavour
Glu-Phe-Lys-Glu-Tyr-Asn	Takifugu Rubripes (4℃)	Umami, Kokumi, Sourness

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