

## Progresses on processing methods of umami substances: A review

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### ABSTRACT

**Background:** Umami taste, which is one of the five basic tastes, refers to the taste expressed by specific chemicals such as glutamic and inosinic acids. Such a taste is known to make the overall taste of food softer, more harmoniously full-bodied; it also helps improve the overall sensory characteristics of food. Umami substances have therefore gained much attention during the past decade. So it is necessary to identify and/or explore effective umami processing technologies to meet the more pressing demands of the food industry.

**Scope and approach:** In this review, selected technologies that can be applied to produce or process umami substances, including fermentation, enzymatic hydrolysis, acid hydrolysis, Maillard reaction, water-based extraction, synthesis methods as well as microwave- and ultrasound-assisted processing are described. These methods all have their own advantages in processing umami substances from various raw materials. However, it has proven that composite processing methods in which selected methods are combined can be more effective for processing umami substances. Besides, major types of umami substances, umami raw materials, umami separation technologies and synergistic effect of umami substances are also discussed.

**Key findings and conclusions:** It is necessary to consider combined applications of the various reviewed technologies for the processing of umami substances. This can lead to significant improved production efficiency and economy of the whole production process. Some related issues, namely, environmental pollution, cost effectiveness ratio, production efficiency and convenience of process manipulation, should also be taken into consideration in any future studies.

### 1. Introduction

Taste is a characteristic that gives good (or bad) impression of a food consumer is having. Nowadays, it is well accepted that taste can be classified as sweet, bitter, sour, salty and umami and it is indeed our sensitive taste perception system that makes us feel the taste. The latter most taste, umami, not only increases the appetite of consumer, but is also an important source of nutrients for the human body (Jinap & Hajeb, 2010).

Until the discovery of the umami receptor, umami was not recognized as a basic taste and was mainly referred to as the taste of monosodium glutamate (MSG) (Chaudhari et al., 1996, 2009; Hoon et al., 1999; Montmayeur, Liberles, Matsunami, & Buck, 2001; Sami et al., 2003). However, there are also many other substances that exhibit this taste, including 20 kinds of natural amino acids constituting purine nucleotides such as 5'-inosinic acid (5'-IMP) and 5'-guanylic acid

(5'-GMP), which have a strengthening effect on the umami taste of glutamic acid (Yin, Venkitasamy, Chandrasekar, Pan, & Wei, 2013; Kong et al., 2017; Manninen, Rotola-Pukkila, Aisala, Hopia, & Laaksonen, 2018). As a result of many in-depth studies on umami, this taste has made a significant change to the way food can be produced. Through a combination of different umami substances, a series of new delicious tastes have been produced, thus improving our diet quality. This has indeed paved way for future food technologists and food industry to be able to prepare food that patients whose taste receptors are affected by certain diseases can appreciate.

Processing and identification of umami substances from different raw materials (Table 5) as well as study of the taste mechanisms of umami substances are among the topics that have received much attention (Chaudhari et al., 1996, 2009; Hoon et al., 1999; Montmayeur et al., 2001; Mouritsen, Duelund, Petersen, Hartmann, & Frøst, 2019; Sami et al., 2003). The major types of umami substances include amino

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acids, nucleotides, and umami peptides, while the raw materials of umami substances include animal materials (Dang, Gao, Ma, & Wu, 2015; Fu, Liu, Hansen, Bredie, & Lametsch, 2018; Kong et al., 2017; Song et al., 2016; Yamasaki & Maekawa, 1978), plant materials (Wu & Muir, 2010; Zhao, Sun-Waterhouse, Zhao, Qiu, & Su, 2018; Rhyu & Kim, 2011; Bagnasco et al., 2013; Mouritsen et al., 2017; Park & Seo, 2018; Zhang, Pan, Venkitasamy, Ma, & Li, 2015; Wei, Thakur, Liu, Zhang, & Wei, 2018; Istiqamah, Lioe, & Adawiyah, 2018; Wang, Zhang, & Mujumdar, 2010; Arai, Yamashita, & Fujimaki, 1972; Berends, Appel, Eisele, Rabe, & Fischer, 2014; Zhuang et al., 2016a; Lioe et al., 2018; Su, Cui, & Zheng, 2012; An, Zhang, Lu, & Zhang, 2006), aquatic products (Chen & Zhang, 2007; Cheung & Li-Chan, 2014; Duan et al., 2005, 2008; Laohakunjit, Selamassakul, & Kerdchoechuen, 2014; Lu et al., 2010; Mouritsen et al., 2019; Noguchi, Arai, Yamashita, Kato, & Fujimaki, 1975; Park et al., 2001, 2002; Sukkhown, Jangchud, Lorjaroenphon, & Pirak, 2017; Tao, Wu, Zhou, Gu, & Wu, 2014; Wang, Zhang, & Mujumdar, 2011; Wu, Zhang, Wang, Mothibe, & Chen, 2012; Zhang et al., 2012, 2017), fungi (Cho, Choi, & Kim, 2010; Lagnika, Zhang, & Mothibe, 2013; Li et al., 2014; Poojary, Orlien, Passamonti, & Olsen, 2017; Procopio, Brunner, & Becker, 2014; Sukkhown et al., 2017; Yang, Lin, & Mau, 2001; Zhang, Venkitasamy, Pan, & Wang, 2013) and even insects (Meyer-Rochow & Hakko, 2018; Meyer-Rochow & Hakko, 2018; Mouritsen, Duelund, Calleja, & Frøst, 2017; Yu et al., 2018; Zhou & Han, 2006). Among the possible raw materials, plant raw materials are the most abundant; these are followed by aquatic products and fungi.

A number of methods have been developed to process umami substances, including fermentation-based methods (Mouritsen et al., 2017), enzymatic hydrolysis (Gauthier & Pouliot, 2003), acid hydrolysis (Li et al., 2014), Maillard-reaction based methods (Muir, 2010), high-temperature and high-pressure processing (Dong et al., 2014), microwave-based methods (Chandrasekaran, Ramanathan, & Basak, 2013) and ultrasound-based methods (Shen, Gu, & Wang, 2013). It is important to note that since umami flavor is affected by many factors, such as pH and temperature as well as interactions among different umami substances, it is important to control these influencing factors in a desirable manner during processing by any of the above-mentioned methods.

With increasing demand for high-quality umami substances, processing methods of these substances should be innovated to meet the requirements. The objective of this article is to present an overview of the developments in technologies used for processing umami substances. The characteristics of these technologies and prospects of future research are also briefly discussed.

## 2. Umami substances

### 2.1. Amino acids

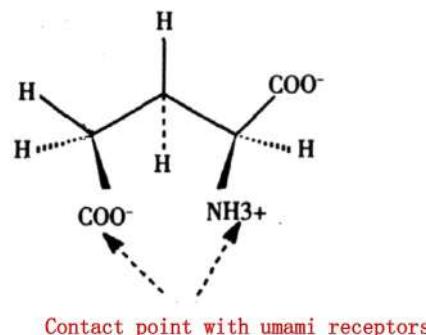
Umami amino acids include L-glutamic acid (L-Glu) and its sodium salt as well as L-aspartic acid (L-Asp) and its sodium salt (Manninen et al., 2018). These umami substances are widely available in nature, especially in fungi and aquatic products (Table 1) and constitute the main umami substances in processed foods. The threshold of L-glutamic acid and its sodium salt is 0.3 mg/mL, while that of L-aspartic acid and its sodium salt is 1.0 mg/mL (Wu & Kang, 2012). These amino acids can be classified as glutamate-type umami substances, which have a common basic skeleton structure of -O-(C<sub>n</sub>)-O-(n = 3–9); when n = 4–6, the umami taste is the strongest (Ardö, 2006). This is the reason why MSG can be used as a representative of umami amino acids. The taste mechanism of umami amino acids is the electrostatic interaction between the two groups of NH<sub>3</sub><sup>+</sup> and COO<sup>-</sup> to form a five-membered ring structure (Fig. 1) that can be sensed by the umami receptors (Ardö, 2006).

pH has a significant influence on the taste of umami amino acids due to the existence of NH<sub>3</sub><sup>+</sup> and COO<sup>-</sup>. Monosodium glutamate has

**Table 1**  
Umami taste amino acids in foods.

Food	L-Glu	L-Asp	Reference
<i>Flammulina velutipes</i>	29.98	2.59	Beluhan and Ranogajec (2011)
<i>Pleurotus ostreatus</i>	41.09	0.17	Beluhan and Ranogajec (2011)
<i>Boletus edulis</i>	39.09	0.33	Beluhan and Ranogajec (2011)
<i>Agaricus campestris</i>	34.78	0.21	Beluhan and Ranogajec (2011)
<i>Cantharellus cibarius</i>	29.99	0.06	Beluhan and Ranogajec (2011)
<i>Macrolepia procura</i>	33.65	0.12	Beluhan and Ranogajec (2011)
<i>Calocybe gambosa</i>	25.67	0.19	Beluhan and Ranogajec (2011)
<i>Agrocybe cylindracea</i>	2.18	0.94	Tsai, Tsai, and Mau (2008)
<i>Pleurotus ostreatus</i>	1.56	0.58	Tsai et al. (2009)
<i>Pleurotus ferulace</i>	1.40	0.36	Tsai et al. (2009)
<i>Boletus edulis</i>	0.59	0.65	Tsai et al. (2008)
<i>pleurotus cystidiosus</i>	1.16	0.05	Yang et al. (2001)
<i>Lentinus edodes</i>	1.30	0.41	Yang et al. (2001)
<i>Flammulina velutipes</i> (white)	1.54	0.03	Yang et al. (2001)
<i>Flammulina velutipes</i> (yellow)	6.82	0.24	Yang et al. (2001)
<i>Hericium erinaceus</i>	0.50	0.50	Mau, Lin, and Chen (2001)
<i>Dictyophora indusiata</i>	0.54	0.31	Mau, Lin, and Chen (2001)
<i>Tricholoma giganteum</i>	0.34	0.34	Mau, Lin, and Chen (2001)
<i>Coriolus Versicolor</i>	0.09	0.41	Mau Lin, & Chen (2001)
<i>Ganoderma tsugae</i>	0.06	0.22	Mau, Lin, and Chen (2001)
<i>Ganoderma lucidum</i>	0.11	0.06	Mau, Lin, and Chen (2001)
<i>Red tilmpa</i>	113.91	90.53	Huang et al. (2001)
<i>Prochlorodiscus scrofa</i>	130.60	76.02	Huang et al. (2001)
<i>Silurus asotus</i>	77.12	58.74	Huang et al. (2001)
<i>Silurus meridionalis</i>	73.11	55.52	Huang et al. (2001)
<i>P. fulvidraco</i> of Wuhan	133.12	91.14	Huang et al. (2001)
<i>Siniperca chuatsi</i>	147.51	93.04	Huang et al. (2001)
<i>I. cyprinellus</i>	113.00	76.71	Huang et al. (2001)
<i>Leiocassis longirostris</i>	96.82	61.91	Huang et al. (2001)
<i>Hemibagrus macropterus</i>	75.64	59.34	Huang et al. (2001)
<i>P. fulvidraco</i> of Guijiang	126.41	71.13	Huang et al. (2001)

Contents are listed as mg/g dry weight.



**Fig. 1.** Relationship between structure and taste properties of MSG (Ardö, 2006).

the lightest umami taste at pH less than 4.0, while the umami taste is the strongest at pH 5.5–8.0 (Wu, Gu, Tao, & Wang, 2014). When the pH is 8 or higher, such a compound is converted into disodium glutamate and the umami taste almost disappears (Wu et al., 2014).

### 2.2. Nucleotides

Nucleotides are widely used in food and pharmaceutical applications; they are often added to increase the taste of food (Kuninaka, 1960; Maruji et al., 2010). Not all 5'-nucleotides and their derivatives have an umami taste, however. Only the nucleotides of steroids exhibit umami taste, while the nucleotides of pyrimidines have no such taste. When the hydroxyl group on the phosphate ester is dissociated, the resulting nucleotides would exhibit umami taste. On the other hand, if the hydroxyl group is esterified or amidated, the umami taste disappears.

There are so far more than 30 kinds of nucleotides and their derivatives that have been noted to exhibit umami characteristics. These

**Table 2**

Umami 5'-nucleotides contents in different food species.

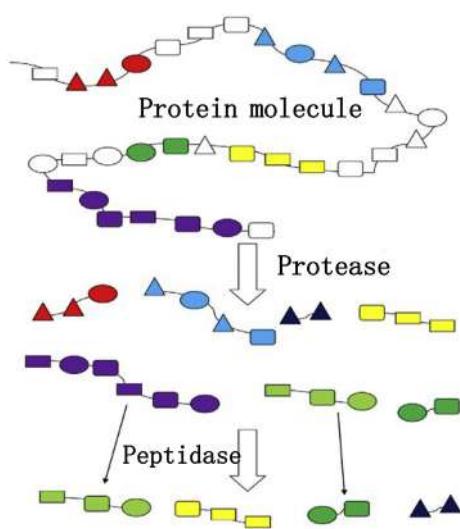
Species	5'-GMP	5'-IMP	5'-AMP	Reference
<i>Tricholoma giganteum</i>	0.10	0.29	0.26	Mau, Lin, and Chen (2001)
<i>Craterellus comucopoides</i>	2.88	3.97	0.35	Beluhan and Ranogajec (2011)
<i>Dictyophora indusiata</i>	2.97	0.02	0.21	Mau, Lin, and Chen (2001)
<i>Flammulina velutipes</i> (white)	1.16	0.17	0.53	Yang et al. (2001)
<i>Flammulina velutipes</i> (yellow)	0.22	0.13	0.42	Yang et al. (2001)
<i>Ganoderma lucidum</i>	1.11	0.47	0.91	Mau, Lin, and Chen (2001)
<i>Pleurotus cystidiosus</i>	1.38	0.05	1.56	Yang et al. (2001)
<i>Morchella elata</i>	1.19	1.77	6.57	Beluhan and Ranogajec (2011)
<i>Agaricus blazei</i>	0.06	0.07	0.15	Tsai et al. (2008)
<i>Macroleptiota procera</i>	0.12	0.16	1.03	Beluhan and Ranogajec (2011)
<i>Pleurotus ostreatus</i>	0.59	0.21	1.21	Beluhan and Ranogajec (2011)
<i>Pleurotus ferulae</i>	0.58	0.69	1.48	Tsai et al. (2009)
<i>Pleurotus ostreatus</i>	0.61	0.74	1.39	Tsai et al. (2009)
<i>Agrocybe cylindracea</i>	0.11	0.04	0.03	Tsai et al. (2008)
<i>Tremella fuciformis</i>	0.08	1.41	0.17	Mau, Lin, Chen, Wu, and Peng (1998)
<i>Boletus edulis</i>	0.64	0.28	0.09	Beluhan and Ranogajec (2011)
<i>Auricularia polytricha</i>	0.01	0.78	0.01	Mau et al. (1998)
<i>Agaricus campestris</i>	0.61	0.12	0.73	Beluhan and Ranogajec (2011)
<i>Flammulina velutipes</i>	0.45	0.28	1.48	Beluhan and Ranogajec (2011)
<i>Calocybe gambosa</i>	0.60	0.03	2.21	Beluhan and Ranogajec (2011)
<i>Auricularia fuscosuccinea</i> (white)	0.01	0.05	Not detected	Mau et al. (1998)
<i>Grifola frondosa</i>	0.56	0.08	0.6	Mau, Lin, and Chen (2001)
<i>Hericium erinaceus</i>	0.04	0.01	Not detected	Mau, Lin, and Chen (2001)
<i>Macroleptiota procera</i>	0.12	0.16	1.03	Beluhan and Ranogajec (2011)
<i>Auricularia mesenteria</i>	0.05	0.20	0.22	Mau et al. (1998)
<i>Cantharellus cibarius</i>	0.21	0.03	0.41	Beluhan and Ranogajec (2011)

Contents are listed as mg/g dry weight.

**Table 3**

Umami peptides in foods.

Umami peptide	Source	Reference
Glu-Glu	Fish protein hydrolysate; soybean protein; chicken enzymatic hydrolysate	Schindler et al. (2011); Noguchi et al. (1975); Arai et al. (1972); Maehashi et al. (1999)
Glu-Gly-Ser	Fish protein hydrolysate; soybean protein	Schindler et al. (2011); Noguchi et al. (1975); Arai et al. (1972)
Glu-Asp	Fish protein hydrolysate; soybean protein	Schindler et al. (2011); Noguchi et al. (1975); Arai et al. (1972)
Glu-Asp-Glu	Fish protein hydrolysate	Schindler et al. (2011)
Glu-Ser	Fish protein hydrolysate; soybean protein	Schindler et al. (2011); Noguchi et al. (1975); Arai et al. (1972)
Asp-Glu-Ser	Fish protein hydrolysate	Schindler et al. (2011); Noguchi et al. (1975)
Glu-Gln-Glu	Fish protein hydrolysate	Schindler et al. (2011); Noguchi et al. (1975)
Thr-Glu	Fish protein hydrolysate	Schindler et al. (2011); Noguchi et al. (1975)
Ser-Glu-Glu	Fish protein hydrolysate	Schindler et al. (2011); Noguchi et al. (1975)
Fru-Glu	Fish protein hydrolysate	Schindler et al. (2011)
Glu-Gly-Ser-Glu-Ala-Pro-Asp-Gly-Ser-Ser-Arg	Peanut hydrolysate	Su et al. (2012)
Ser-Ser-Arg-Asn-Glu-Gln-Ser-Arg	Peanut hydrolysate	Su et al. (2012)
Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala	Beef soup	Yamasaki and Maekawa (1978)
Ala-His-Ser-Val-Arg-Phe-Tyr	Parma hams	Dang et al. (2015)
Cys-Cys-Asn-Lys-Ser-Val	Jinhua hams	Dang et al. (2015)
Leu-Pro-Glu-Glu-Val	Soy sauce	Zhuang et al. (2016a)
Glu-Gln-Gln-Gln-Gln	Soy sauce	Zhuang et al. (2016a)
Ala-Gln-Ala-Leu-Gln-Ala-Gln-Ala	Soy sauce	Zhuang et al. (2016a)
pGlu-Pro-Ser	Wheat gluten	Schlachterle-cerny et al. (2002)
pGlu-Pro-Glu	Wheat gluten	Schlachterle-cerny et al. (2002)
pGlu-Pro-Glu	Wheat gluten	Schlachterle-cerny et al. (2002)
pGlu-Pro-Gln	Wheat gluten	Schlachterle-cerny et al. (2002)
Asp-Cys-Gly	Wheat gluten	Schlachterle-cerny et al. (2002)
pGlu-Pro	Wheat gluten	Schlachterle-cerny et al. (2002)
Glu-Glu-Glu	Chinese rice wine	Han and Xu (2012)
Glu-Val	Chicken enzymatic hydrolysate	Maehashi et al. (1999)
Ala-Glu-Ala	Synthesis peptide	Ohyama et al. (1988)
Gly-Asp	Synthesis peptide	Ohyama et al. (1988)
Val-Glu-Val	Synthesis peptide	Ohyama et al. (1988)
Val-Asp-Val	Synthesis peptide	Ohyama et al. (1988)
Gly-Asp-Gly	Synthesis peptide	Ohyama et al. (1988)
Glu-Leu	Synthesis peptide	Ohyama et al. (1988)
Asp-Leu	Synthesis peptide	Ohyama et al. (1988)



**Fig. 2.** Schematic diagram of peptides generation by enzymatic hydrolysis (Liu et al., 2016).

include (Table 2) the aforementioned 5'-IMP, 5'-GMP and 5'-adenylate (5'-AMP) (Yamaguchi, 1991). 5'-monophosphoric acid uridine disodium (5'-UMP) and 5'-monohydrocytidine disodium (5'-CMP) are, however, not good for their own umami taste, and can only cooperate with monosodium glutamate. These nucleotides thus play a role in helping to accentuate umami taste (Arai et al., 1972) (Table 3).

### 2.3. Umami peptides

In 1978, Japanese scientists first isolated and purified amino acids from papain-hydrolyzed beef hydrolysate and obtained octyl peptide with the amino acid sequence of Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala, which is then called an umami peptide (Yamasaki & Maekawa, 1978). This has opened up the research and utilization of umami peptides.

Umami peptides are small-molecule peptides (Fig. 2) with the molecular weights of 150 to 3,000 Da. These are important umami substances, which can supplement and enhance the overall taste of food, making it more harmonious, soft and full-bodied (Grigorov, Schlichtherle-Cerny, Affolter, & Kochhar, 2003; Smit et al., 2000). Compared with other peptides, umami peptide molecules usually contain glutamate residues or aspartic acid residues; these molecules also have their own umami taste receptors Rhyu & Kim, 2011; Toelstede, Dunkel, & Hofmann, 2009; Chaudhari et al., 2009; Shigemura et al., 2009. Umami peptides can be extracted from food or synthesized by enzymatic hydrolysis (Table 3) (Liu, Wang, Liu, & Ruan, 2016). The flavor of these peptides comes mainly from the intermediate products of protein synthesis and decomposition process (Zhang et al., 2012).

Umami peptides not only can directly enhance the delicious taste of food, but can also participate in the Maillard reaction as a precursor of volatile flavor substances, further enhancing the characteristic flavor of food (Lioe, Takara, & Yasuda, 2010; Wang, Yang, & Song, 2012).

**Table 4**  
Contents of succinic acid of some marine products.

Marine product	Succinic acid content (mg/100g)	Reference
Scallop	370	Weng and Sun (2007)
Corbicula meat	140	Weng and Sun (2007)
Clam	140	Weng and Sun (2007)
Conch meat	70	Weng and Sun (2007)
Oyster	50	Weng and Sun (2007)
Abalone	30	Weng and Sun (2007)

### 2.4. Organic acids

Organic acids which exhibit umami taste are mainly succinic acid and its salt; such umami substances are mostly from shellfish (Table 4; Anacleto, Maulvault, Barbosa, Nunes, & Marques, 2016; Weng, Yang, & Liu, 2016) and mushrooms (Hadar & Dosoretz, 1991). Currently, the approved organic acid flavoring agent for use in China is disodium succinate, which is the main umami ingredient in scallop (Christian et al., 2009). Such a flavoring agent is mainly used in wine, beverage, candy, soy sauce and other products (Chimirri, Bosco, Ceccarelli, Venturello, & Geobaldo, 2010). Studies have shown that the umami taste of succinic acid can be enhanced when combined with salt, sodium glutamate or other organic acids such as citric acid (Wang, Li, Wang, Zhang, & Liu, 2018; Yamaguchi, 1991) (Table 5).

## 3. Umami processing methods

### 3.1. Fermentation

Fermentation refers to the process that produces microbial cells or direct and/or secondary metabolites by the activities of microorganisms under aerobic or anaerobic condition (Barnett, 2010). *Bacillus subtilis* and yeast are commonly used in fermented umami foods since these microorganisms are easy to culture, require short culture period and exhibit high yield. Soy sauce, fish sauce, *Tianyou* (Fig. 3), oyster sauce and bean paste are among favorite fermented foods (Lioe et al., 2018; Lioe, Wada, Aoki, & Yasuda, 2007; Mouritsen et al., 2017). Fermentation is an economic and efficient method for producing umami substances; it is also a safe method (Zhao et al., 2018).

A number of materials from whom umami substances can be obtained through fermentation have been identified. These include fermented milk, fermented beans, fermented vegetables, fermented fishes, fermented meats as well as fermented grains and fermented tea (Mouritsen et al., 2017). The success of food fermentation is closely related to environmental conditions. High level of salt and low pH are the most important parameters suppressing the growth of undesired microorganisms and leading way to the degradation of proteins, carbohydrates and nucleic acids (Istiqamah et al., 2018).

Future research on fermentation should pay attention to the discovery and use of new food materials. For example, use of insects rich in high-quality protein should be explored (Mouritsen et al., 2017). Ways to improve fermentation efficiency and strain selection should also be explored.

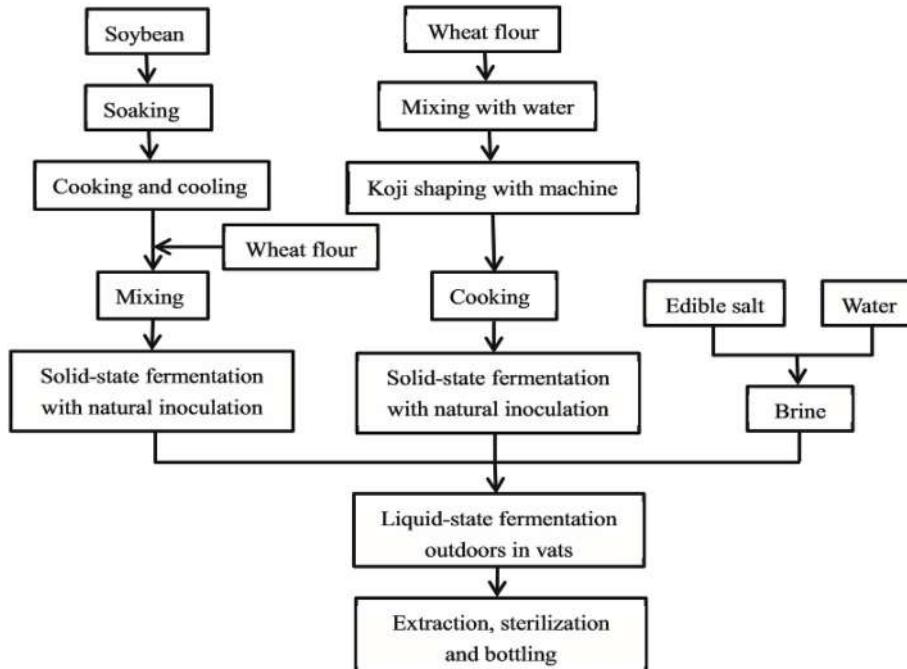
### 3.2. Enzymatic hydrolysis

Enzyme technology refers to the technology of converting corresponding raw materials into desirable substances via the use of a catalytic action of enzymes in a bioreactor. According to the specificity of enzyme (Yamasaki & Maekawa, 1978), suitable proteases or cellulases can be selected to enzymatically decompose cell walls of a raw material, so that the main components of the cell walls are decomposed, dissolved and can be extracted (Puri, Sharma, & Barrow, 2012). Besides cell wall components, some macromolecular proteins are also enzymatically decomposed into oligopeptides and free amino acids (Maehashi, Matsuzaki, Yamamoto, & Ueda, 1999).

Choice of enzymes is the key to producing umami substances. Enzymes that are currently used for such a purpose include acid proteases, neutral proteases, alkaline proteases, plant proteases and flavor proteases (Li & Lu, 2008; Ermis, 2017). When it is difficult to achieve the desired effect with only one enzyme, two or several enzymes can be combined to form a complex enzyme system (Ermis, 2017; Song et al., 2016). Suitable enzymes may also be engineered according to the structural characteristics of the desired taste substances (Gauthier & Pouliot, 2003). Although a complex enzyme system can be used, the mechanisms of interaction remains to be further explored.

**Table 5**  
Umami raw materials.

Category	Source
Plant materials	Defatted soybean meal (Zhao et al., 2018); soybean paste (Rhyu & Kim, 2011); rice bran (Bagnasco et al., 2013); pease (Mouritsen, 2017); ginseng (Park & Seo, 2018); tomato seeds (Zhang et al., 2015); linseed protein (Wei et al., 2018); asam sunti ( <i>Averrhoa bilimbi</i> L.) (Istiqamah et al., 2018); modified soybean protein (Arai et al., 1972); wheat protein (Berends et al., 2014); soybean sauce (Zhuang et al., 2016a); fermented soybean curd (Lioe et al., 2018); peanut (Su et al., 2012)
Animal materials	Cow bone (Song et al., 2016); chicken (Kong et al., 2017); Jinhua ham (Dang et al., 2015); beef (Yamasaki & Maekawa, 1978)
Aquatic products	Squid (Sukkhown et al., 2017); fish scrap (Park et al., 2002); shrimp (Cheung & Li-Chan, 2014); <i>Eriocheir sinensis</i> (Chen & Zhang, 2007); Puffer fish (Zhang et al., 2012); seaweed (Laohakunjit et al., 2014); fish protein (Noguchi et al., 1975); <i>Takifugu obscurus</i> (Tao et al., 2014)
Insect raw materials	Moth (Mouritsen et al., 2017); grasshopper (Meyer-Rochow & Hakko, 2018); Mealworm (Mouritsen et al., 2017); silkworm pupae (Meyer-Rochow & Hakko, 2018; Zhou & Han, 2006)
Fungi	Mushroom (Poojary et al., 2017); <i>Tricholoma</i> (Cho et al., 2010); <i>Boletus</i> (Zhang et al., 2013); <i>Flammulina velutipes</i> (Yang et al., 2001); <i>Pleurotus eryngii</i> (Li et al., 2014); brewery yeast (Procopio et al., 2014)



**Fig. 3.** Schematic diagram of *tianyou* (Chinese traditional fermented umami food) manufacturing process (Gao, Zhang, Regenstein, Yin, & Zhou, 2018).

Enzymatic hydrolysis possess unique characteristics in that the processing conditions are mild, the specificity is high, the side reaction is minimal and the resulting amino acids are not destroyed along the process. It is important to note, however, that although the hydrolysis process can be effectively regulated and controlled, undesirable flavor such as bitterness can be generated if the degree of hydrolysis is low. If the hydrolysis time is too lengthy, microorganisms would grow and multiply, causing product contamination. For these reasons, it is necessary to strictly control the hydrolysis conditions, e.g., hydrolysis time, temperature and pH, among others.

### 3.3. Acid hydrolysis

Acid hydrolysis is a simple process, low cost and requires low investment. It is a method that is widely used for protein hydrolysis (Aaslyng, Elmore, & Mottram, 1998a). There are still indeed many companies that use hydrochloric acid to hydrolyze plant proteins to produce umami substances in large quantity for food flavoring.

Acid hydrolysis, which usually employs soybean, wheat and corn as raw materials to obtain umami substances was first developed in Switzerland in 1866 (Aaslyng et al., 1998a,b). This method is characterized by rapid and thorough hydrolysis, with an L-type amino acid as a product and with no racemization. Note that in nature, amino acids are of L-type and are prone to racemization under alkaline conditions;

in such a case, L-type amino acids are converted to D-type amino acids and lose their physiological function. Racemization, however, is less likely to occur under acidic conditions. However, in the process of acid hydrolysis of vegetable proteins, chloropropanol, which is reported to have toxicity and carcinogenicity, is also produced along with umami substances. Many methods have been proposed to remove chloropropanol from acid-hydrolyzed protein solutions (Li et al., 2014). Most of these methods use the principle of forming chloropropanol through a reversible reaction. By adjusting the pH of the hydrolysate, chloropropanol is moved in the direction of glycerol production, thereby reducing the content of chloropropanol in the product. By using a high-protein and low-fat raw material along with a low-concentration solution of hydrochloric acid at a lower temperature, acid-hydrolyzed protein solution with low chloropropanol content can be produced via combined acid and enzyme hydrolyses (Li et al., 2014). Nevertheless, utilization of dilute HCl acid as an extraction solvent is not desirable in food processing; such a solvent may also lead to chemical residue and environmental pollution problem (Chanvorleak, Bokyoung, & Chan, 2016; Li et al., 2014; Tsai et al., 2009, 2007).

### 3.4. Maillard reaction

Maillard reaction, which involves non-enzymatic browning that typically occurs during thermal processing, is an interaction between

carbonyl compounds (reducing saccharides) and amino compounds (amino acids and proteins) (Ames, 1990). The reaction produces a variety of advanced compounds, intermediate and browning products, which exhibit aroma and taste-enhancing properties (Zhang et al., 2019; Zheng, 2005). These unique aromas, which can exhibit meat, bread and seafood flavors, among others, are known to cause appetite (Wang et al., 2012). In addition, the so-called Maillard peptides, which are peptide fractions with the molecular weights of 1,000 to 5,000 Da, are noted, for example, to have strong taste-enhancing properties in consommé soup (Ogasawara, Katsumata, & Egi, 2006).

Many factors are reported to affect the Maillard reaction, including types of carbonyl and amino compounds, temperature, pH, water activity, buffer system, ion species and even the entire food system (Ames, 1990; Qi, 2018). During the reaction, temperature is considered a major factor influencing the flavor properties of Maillard reaction products (Lan et al., 2010; Qi, 2018; Xu et al., 2010a, 2010b). Based on some previous studies, 110 °C might be the critical temperature that would reduce the bitter characteristics of Maillard reaction products due to the decomposition of bitter amino acids and bitter peptides at that temperature (Lan et al., 2010).

Cysteine has been recognized as a meaty flavor inducer during the formation of Maillard reaction products. Cysteine addition during the course of Maillard reaction has been noted to have a significant contribution to the color inhibition and mouthfulness and continuity enhancement (Mottram & And, 2002; Yu & Zhang, 2010; Zhang, Elfalleh, He, Tang, & Sun, 2018b). Some researchers (Lan et al., 2010; Qi et al., 2018) have also found that the combination of enzymatic and Maillard reactions can better improve the flavor of the products. For instance, Maillard reaction products from lipase- and papain-hydrolyzed beef bone hydrolysate was noted to exhibit good mouthful, umami and meaty characteristics (Song et al., 2016).

### 3.5. High-temperature and high-pressure processing

High-temperature and high-pressure processes are common methods used in the food industry to extract taste substances and nutrients from animal bones. Such processes are required due to the stable structure of the bones; it is difficult to extract proteins from the bones solely by enzymatic hydrolysis (Mahmoud, Malone, & Cordle, 1992). High-temperature and high-pressure processes can promote breakage of secondary bonds and partial peptide bonds of collagen triple helix, melting of collagen molecules as well as converting insoluble collagen into soluble proteins, peptides and amino acids. Heat-pressure extraction, for example, is a promising way to extract proteins from chicken

bone (Fig. 4); chicken bone extract and its hydrolysate (obtained via the use of flavourzyme), especially the small peptides, may have potential nutritional and flavor properties (Dong et al., 2014).

While many factors are noted to affect the high-temperature and high-pressure extraction process, including extraction temperature, extraction time, material-to-liquid ratio and extraction pressure, temperature is the most influential. The extraction temperature range is generally within 115–135 °C (Zeng et al., 2014); if the temperature is too high, color of the extract may turn dark brown and its flavor would be very poor.

Some investigator have reported that the sole application of ultra-high pressure processing could modify the content of 5'-IMP in fresh pork. The results showed that 5'-IMP would accumulate to its maximum level when a pressure of 300 MPa was applied (Zhang, Jian, Liang, & Xia, 2018a).

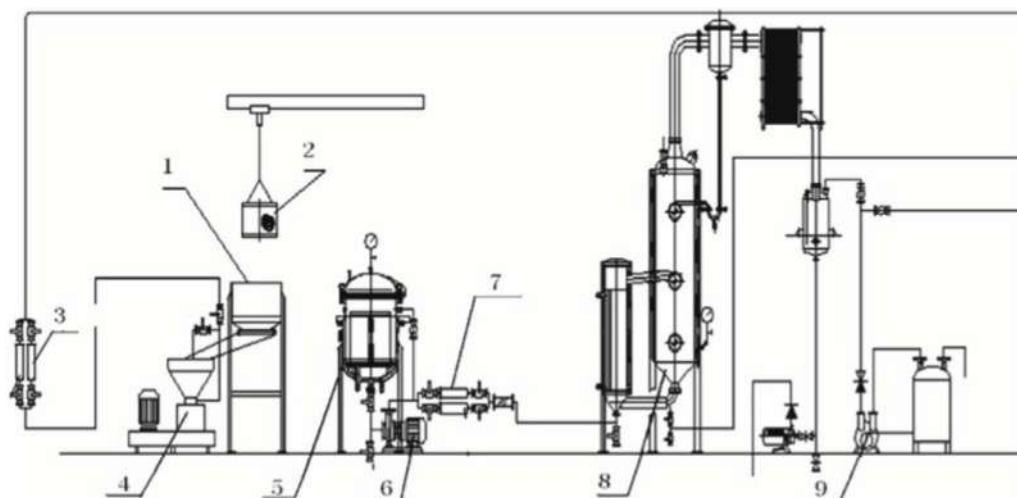
### 3.6. Water-based extraction

In recent years, consumers have been paying more attention to food safety and looking for foods that are more naturally produced. Food ingredients industry has thus been looking for more natural umami formula and processing methods to meet the need of health-conscious consumers (Huang, 1993; Radam, Yacob, Bee, & Selamat, 2010). Since umami substances such as umami amino acids and peptides are generally water-soluble, water can readily be used as a solvent to extract umami ingredients from umami raw materials. Some previous studies have indeed reported on water extraction and quantification of umami amino acids and nucleotides from Jinhua and Parma hams, mushrooms and doenjang (Dang et al., 2015; Poojary et al., 2017; Rhyu & Kim, 2011).

Water extraction is one of the most traditional extraction methods and can be applied to the extraction of various substances. As a classical extraction method, hot-water extraction has the advantages of being a simple operation, economical and environmentally friendly. However, it requires long extraction time and involves multi-step operation; high energy consumption is another drawback of this extraction method. In addition, raw materials for extraction should be rich in free umami amino acids and peptides; otherwise the extraction efficiency would be low and the quality of the umami substances obtained would also be low.

### 3.7. Synthesis methods

In terms of chemical synthesis, which is the most basic mode of



**Fig. 4.** Heat-pressure equipment for umami extraction (Dong et al., 2014). (1) Dump hopper; (2) crane cage; (3) and (4) colloid mills; (5) heat-pressure extraction pot; (6) circulating pump; (7) duplex strainer; (8) Inspissator and (9) vacuum pump.

**Table 6**

Comparison of different umami processing methods.

Umami processing method	Feature	Reference
Fermentation	The process is simple, inexpensive, easy to control and takes place at a milder condition. However, environmental hygiene requirements are high; process is prone to bacterial contamination.	Barnett (2010); Mouritsen et al. (2017); Lioe et al. (2018); Lioe et al. (2007); Zhao et al. (2018); Istiqamah et al. (2018)
Enzymatic hydrolysis	The reaction conditions are mild. The process is clean, safe and exhibits higher yield. However, enzymatic hydrolysis products are prone to bad taste such as bitter taste.	Puri et al. (2012); Yamasaki and Maekawa (1978); Maehashi et al. (1999); Gauthier and Pouliot (2003)
Acid hydrolysis	Acid hydrolysis is simple, inexpensive and allows for complete hydrolysis of substrates. However, hydrolysis products may be contaminated with solvent residues, which can be harmful.	(Aaslyng, Elmore, and Mottram 1998a); Li et al. (2014); Tsai, Wu, Huang, and Mau (2007, 2009); Chanvorleak et al. (2016)
Maillard reaction	The processing method is often used to assist other umami processing methods to improve product flavor and enhance umami taste.	Zheng (2005); Wang et al. (2012); Zhang, Elfalleh, He, Tang, & Sun (2018); Yu and Zhang (2010); Mottram & And (2002); Lan et al. (2010); Song et al. (2016); Corzo-Martínez et al. (2014)
High-temperature and high-pressure processing	This processing method is efficient, convenient and highly automated. However, processing equipment is expensive.	Mahmoud et al. (1992); Dong et al. (2014); Zhang, Jian, Liang & Xia (2018); Zeng et al. (2014)
Water-based extraction	The process is clean, simple and has low processing cost. However, the yield is low.	Radam et al. (2010); Huang (1993); Rhyu and Kim (2011); Dang et al. (2015); Poojary et al. (2017)
Synthesis methods	Chemical synthesis is relatively simple, gives higher yield. However, product purity is lower; product may also suffer chemical residues. Genetic engineering approaches have advantages of high specificity, wide sampling capability and provide considerably high yield. However, they are of high cost and difficult to scale up. More research efforts are needed for industrial applications of the approaches to realize.	Romero et al. (1997); Zhuang et al. (2016a); Yamasaki and Maekawa (1980); Zeng and Wang (2008); Ohyama, Ishibashi, Tamura, Nishizaki, & Okai (1988); Romero et al. (1997)
Microwave-assisted processing	This processing method has the advantages of fast heating and low solvent consumption; high recovery yield and less pollution can also be cited. However, the method involves high equipment cost and is prone to boiling, which may have an effect on heat-sensitive substances.	Yang et al. (2010); Li et al. (2018); Yang et al. (2009); Ochoa-Rivas et al. (2017); Phongthai et al. (2016); Song, Zhang, Mujumdar, and Fan (2009)
Ultrasound-assisted processing	This processing method has wide process adaptability, requires short processing time, low processing temperature, low processing cost and is easy to operate. However, due to limitation of ultrasonic attenuation, the processing efficiency of a large number of products is low.	Farid, Muhammed & Khan (2011); Shen et al. (2013); Wang et al. (2015); Siró et al. (2009); Jayasooryia, Bhandari, and Torley (2004); Mohibe, Zhang, Nsor-Atindana, and Wang (2011); Islam, Zhang, and Adhikari (2014); Wang et al. (2017)

synthesis, two options are generally available, namely, liquid-phase synthesis and solid-phase synthesis. The major difference between the two options is whether or not a solid phase carrier is used. In addition, liquid-phase synthesis is known to result in a product of higher purity; the process is nevertheless complicated and exhibits low synthesis yield. Solid-phase synthesis, on the other hand, is relatively simple, gives higher yield; product purity is nevertheless lower (Duhee, Neeraj, & Kent, 2004). Chemical synthesis methods have long been used to synthesize umami substances. Monosodium glutamate is a well-known umami substance obtained by chemical synthesis (Rudinger, 1954).

Synthesis of benzyloxycarbonyl-lysine-glycine methyl ester (CBZ-Lys-Gly-OMe) and benzyloxycarbonyl-serine-leucine methyl ester (CBZ-Ser-Leu-OMe) have been carried out in aqueous organic systems catalyzed by immobilized trypsin and thermolysin, respectively (Romero et al., 1997). Dipeptides obtained via the elimination of benzyloxycarbonyl and methoxy groups are two fragments of the delicious peptides, which are an octapeptide with umami/sour taste. At the optimum conditions, the synthetic yields were noted to be as high as 80% for CBZ-Lys-Gly-OMe and 100% for CBZ-Ser-Leu-OMe. Zhuang et al. (2016a) indeed found that synthetic umami peptides had a very good sensory taste and could significantly improve the umami taste of soy sauce; the peptides enhanced the taste better than monomeric amino acids.

With the trend towards natural ingredients and processing, however, use of chemical synthesis would soon be limited. In addition, the problem of racemization needs to be considered. Alternative synthesis method viz. genetic-engineering based synthesis has been proposed. In this method, DNA fragment containing umami target gene is introduced into a recipient cell via a vector or foreign gene, which is inserted into phage gene sequence to express. Processing and purification can then follow to obtain the target umami peptides (Yamasaki & Maekawa, 1980). Genetic engineering approaches have the advantages of high specificity, wide sampling capability and high yield. *Pichia pastoris* was, for example, used to efficiently express 16 copies of beef flavor peptides

(Zeng & Wang, 2008). Some synthesized peptides such as Gly-Asp, Ala-Glu, Gly-Asp-Gly, Val-Asp-Val, Asp-Leu and Val-Glu-Leu have also been described as eliciting a lingering umami taste (Ohyama, Ishibashi, Tamura, Nishizaki, & Okai, 1988). It is nevertheless necessary to well control the factors affecting the rate of synthesis and yield, including pH, temperature, substrate concentration, enzyme activity and enzyme type (Romero et al., 1997).

Although the genetic-engineering approaches can result in safe and hygienic products and can work with a wide range of raw materials and at the same time yield high-quality and naturally active peptides, the approaches have technical limitations, including difficulty related to separation, low yield, high cost and difficulty to scale up. More research efforts are needed for industrial application of the approaches to realize.

### 3.8. Microwave-assisted processing

The focus of microwave-assisted processing in this case is on microwave-assisted extraction. In one example study, shiitake mushroom was used as a raw material to extract tasty amino acids at a material-to-liquid ratio of 1:100 (g/mL), microwave power of 500 W and microwave irradiation time of 30 min (Yang, Shen, & Zhang, 2010); the results were compared with those of conventional extraction using water as a solvent. The ratio of amino acids yields by the two extraction methods was 131:100 (Yang et al., 2010). Microwave-assisted extraction of needle mushroom was also noted to help release the flavoring substances, free amino acids and 5'-nucleotides more efficiently from the mushroom when compared with traditional hot-water extraction (Li et al., 2018).

In another study, taste substances in pig bones were extracted by microwave at the following optimal conditions: material proportion of 1:11, microwave power of 500 W and extraction time of 1.5 h (Yang, Shen, & Zhang, 2009). Microwave extraction could significantly improve the extraction efficiency of odorous substances from the bones.

Compared with the traditional cooking method, the degree of hydrolysis and nitrogen yield of the microwave extract improved by 49.5% and 7.8%, respectively. Microwave can also be used to assist the extraction of free amino acids from peanut (Ochoa-Rivas, Nava-Valdez, Serna-Saldívar, & Chuck-Hernández, 2017) and rice bran (Phongthai, Lim, & Rawdkuen, 2016).

### 3.9. Ultrasound-assisted processing

Similar to that in the earlier section, the focus of ultrasound-assisted processing is on ultrasound-assisted extraction. Ultrasound-assisted extraction takes the advantage of acoustic cavitation to break the structure of an extracted raw material, so that taste components can transport to an extraction solvent more rapidly and extensively (Farid, Muhammed & Khan, 2011). Several extraction methods, namely, hot-water extraction, ultrasound-assisted and microwave-assisted extraction were, for example, comparatively used to extract umami substances from shiitake mushroom; the results showed that ultrasound-assisted extraction was superior to other tested methods (Shen et al., 2013). Umami amino acids could be well extracted from mushroom fruiting bodies by the aid of ultrasound at the following optimal condition: material-to-liquid ratio of 1:15, ultrasonic power of 500 W and time of 15 min (Wang, Chen, Wang, & Jing, 2015).

The above major processing methods have their own characteristics as well as advantages and disadvantages as summarized in Table 6. Appropriate processing methods should be selected according to the nature of raw materials, processing conditions and product types to achieve the best processing results.

## 4. Umami separation technologies

Umami substances, which are generally produced from microorganisms, animal and plant proteins, typically exist with other substances. Through the use of appropriate separation and purification

technologies, high-purity umami substances can be obtained, so that they can be better applied in food industry. Currently, popular separation methods include macroporous resin adsorption (Zhuang et al., 2016b), membrane separation (Lioe et al., 2018) and chromatography (Park, Seo, Lee, Na, & Son, 2018; Su et al., 2012).

Macroporous resins exhibit high recovery, high efficiency, excellent selectivity and require milder adsorption conditions due to their large surface area, unique pore structures, adsorption properties and surface functional groups (Fig. 5). Besides, macroporous resins also have the advantages of lower operating cost and easier regeneration capability (Zhuang et al., 2016b).

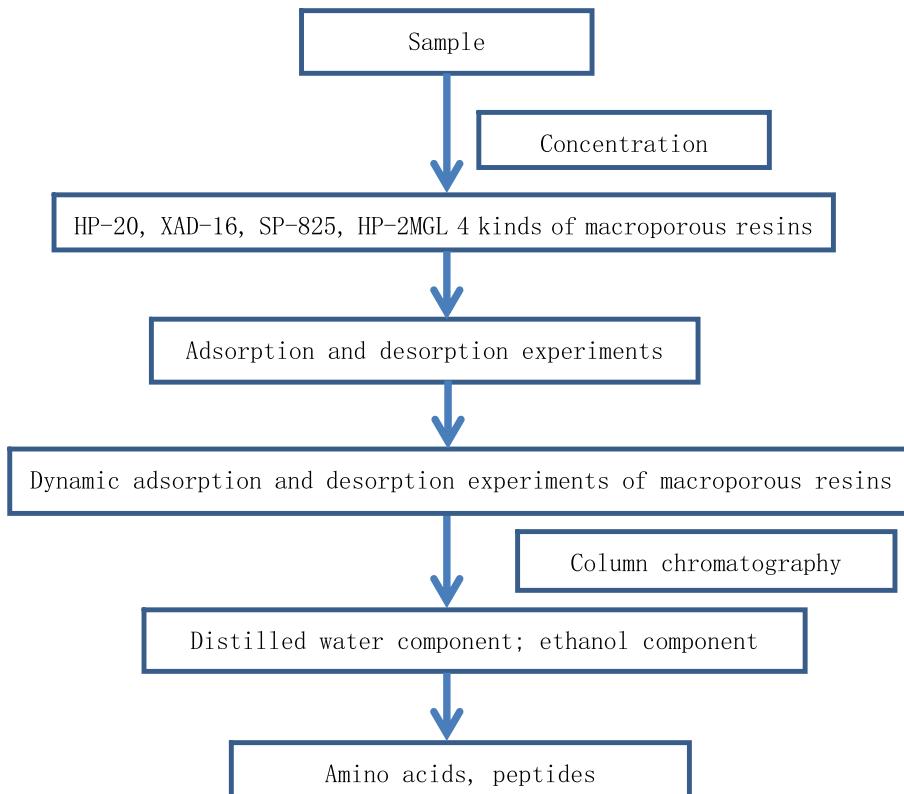
Membrane separation technology is another attractive technology for material separation, concentration and purification. Since membrane separation is generally conducted at room temperature, separated substances can maintain their original properties, aroma/flavor, color, nutrition and taste as well as many of their functional properties (Nazir et al., 2019). Membrane separation technology also generally boasts strong selectivity and can be applied over a wide range of conditions at relatively low energy consumption. Membrane separation is especially suitable for an operation in a continuous mode (Søtoft, Lizarazu, Parjikolaei, Karring, & Christensen, 2015; Yu et al., 2018).

Chromatography, which is also known as chromatographic separation, is among many effective methods for the separation of various compounds from complex mixtures and allows for further purification to obtain highly purified products with high recovery with no contamination (Cheng, Chen, & Xiong, 2010; Li et al., 2017; Xu et al., 2019).

Currently, no single method is used. Instead, various separation methods can be used in combination to separate umami substances from various protein hydrolysates.

## 5. Methods for enhancing umami

During food processing or storage, the umami taste may be



**Fig. 5.** Flow chart of separation and enrichment of tasty peptides by macroporous resins (Zhuang et al., 2016b).

weakened due to the influence of temperature, salt, pH, moisture and umami ingredients themselves (Liu, Wang, & Zhang, 2017; Wu et al., 2014). It is therefore necessary to enhance or stabilize umami. The taste intensity of monosodium glutamate, for example, varies with pH (Liu et al., 2017; Wu et al., 2014). When pH is 5.5–8.0, the umami taste is the strongest. On the other hand, when pH is lower than 4.0, the umami taste intensity decreases; the taste disappears when pH is higher than 8.0 (Liu et al., 2017; Wu et al., 2014). Under acidic conditions ( $\text{pH} < 5$ ), L-sodium glutamate undergoes intramolecular dehydration to form pyroglutamic acid if heated for an extended period of time, resulting in the disappearance of umami (Liu et al., 2017; Wu et al., 2014). 5'-nucleotide disodium salt has the strongest taste at pH 6.5–7.0; the compound has low stability under acidic conditions (Yamaguchi, 1998). Disodium succinate is weakly alkaline and exhibits the strongest umami taste at pH 7.5, while having good thermal stability at pH 5–8 (Li & Liu, 2013; Wang et al., 2018).

There is a synergistic effect among the umami components that helps enhance the umami taste (Yamaguchi, 1998); the enhanced intensity could be as high as 8 times of that of an individual component (Bellisle, 1999). There is a significant synergy between L-glutamic acid and 5'-nucleotide; 1:1 glutamate and inosinic acid produced 7-fold increase in umami compared with glutamate alone (Bellisle, 1999; Kuninaka, 1960; Maruji et al., 2010; Matsuo & Yamamoto, 1989). Interactions between umami nucleotides have also been reported and when used together the umami threshold can be significantly reduced. For example, 5'-inosine disodium alone has a savory threshold of 0.025 g/100 g, while when 5'-inosinate disodium is mixed with 5'-guanosine disodium at a ratio of 1:1, the threshold is lowered to 0.0063 g/100 g (Fuke & Ueda, 1996).

Salt can also enhance the umami taste.  $\text{Na}^+$  and  $\text{Cl}^-$  can significantly increase the overall umami intensity of a sample; the umami threshold of  $\text{Na}^+$  is 1.80 mg/mL (Nina, Andreas, & Thomas, 2006). Other substances, including umami peptides, can also enhance the umami taste during food processing (Yamasaki & Maekawa, 1978). Yeast extracts contain all the delicious precursor substances (nucleotides, peptides and amino acids) and the interaction of a variety of umami substances makes them give away a strong, long-lasting umami (Jin et al., 2017).

There are some substances that exhibit no umami taste or has high umami threshold. However, at lower concentrations, these compounds can significantly enhance the taste of umami substances or increase the fullness and taste durability of food. For example, six peptides isolated from chicken hydrolysate enzymatically prepared using bromelain (Maehashi et al., 1999) were noted to be slightly sour and tasteless, but could enhance the umami taste of IMP. Addition of reduced glutathione to an MSG solution or simulated beef soup solution was also noted to significantly increase the umami persistence, fullness and aftertaste of the solution (Ueda, Yonemitsu, Tsubuku, Sakaguchi, & Miyajima, 1997).

## 6. Conclusions

Umami is a very pleasant taste and hence has received much attention from both academia and industry. With further study on the umami science, new umami raw materials will continue to be discovered. At the same time, existing umami processing methods will be improved or otherwise abandoned, and new umami processing methods will be proposed and tested. According to the current research situation, future umami processing methods will be developed in the direction of composite processing technologies; some of which are reviewed in this article, namely, fermentation, enzymatic hydrolysis, Maillard reaction as well as microwave- and ultrasound-assisted technologies. Such composites as combined fermentation and Maillard reaction to produce umami substances or combined enzymatic hydrolysis with microwave-assisted extraction as well as the use of different enzymes in combination can be fully utilized to improve the production

and processing of umami substances. Bioengineering approaches, including fermentation, enzymatic hydrolysis and biosynthesis, warrant further research as potential alternatives to environmentally and efficiently produce umami substances. Genetic engineering for synthesizing umami substances will in particular have broad prospects for development and become more mature with further research. In addition, in order to make better use of umami substances, synergism among umami substances and separation and purification technologies of umami substances need further study.

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