Formation of taste-active amino acids, amino acid derivatives and peptides in food fermentations – a review

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Abstract:

Fermented foods are valued for their rich and complex odour and taste. The metabolic activity of food-fermenting microorganisms determines food quality and generates odour and taste compounds. This communication reviews the formation of taste-active amino acids, amino acid derivatives and peptides in food fermentations. Pathways of the generation of taste compounds are presented for soy sauce, cheese, fermented meats, and bread. Proteolysis or autolysis during food fermentations generates taste-active amino acids and peptides; peptides derived from proteolysis particularly impart umami taste (e.g. α-glutamyl peptides) or bitter taste (e.g. hydrophobic peptides containing proline). Taste active peptide derivatives include pyroglutamyl peptides, γ-glutamyl peptides, and succinyl- or lactoyl amino acids. The influence of fermentation microbiota on proteolysis, and peptide hydrolysis, and the metabolism of glutamate and arginine is well understood, however, the understanding of microbial metabolic activities related to the formation of taste-active peptide derivatives is incomplete. Improved knowledge of the interactions between taste-active compounds will enable the development of novel fermentation strategies to develop tastier, less bitter, and low-salt food products, and may provide novel and “clean label” ingredients to improve the taste of other food products.

Keywords: food fermentation, sourdough, cheese, soy sauce, kokumi, taste, proteolysis
Introduction

Taste determines food selection, intake, absorption, and digestion, and thus contributes to the nutritional status as well as to decisions on food purchase (Barylko-Pikielna & Kostyra, 2007; Beksen et al., 2003). Six basic tastes, salt, sweet, and umami, sour, bitter, and oleogustus, are detected by taste receptors in taste buds on the tongue and palate epithelium (Running et al., 2015). Sweet, umami and bitter tastes are particularly related to food acceptance or rejection (Barylko-Pikielna & Kostyra, 2007). Sweet taste allows to identify carbohydrate-rich foods as a source of energy (Behrens et al., 2011); sweet taste receptors are absent in carnivores (Jiang et al., 2012). Umami molecules impart savory taste and increase other taste intensities (Jinap & Hajeb, 2010). Umami taste is linked to meat intake and umami taste receptors are typically absent in herbivores (Zhao et al., 2010). L-Glu and 5’-ribonucleotides elicit umami taste (Jinap & Hajeb, 2010). Humans reject bitter tasting foods, however, a limited level of bitterness in food may be desirable. Moreover, bitter taste reception in humans is highly variable (Meyerhof et al., 2010). Saltiness often determines the sensory acceptance of savory foods, such as soups, sauces, snacks, and bakery products (Schindler et al., 2011). However, salt intake in industrialized nations exceeds by 80-100% the amount recommended by WHO. Sodium reduction, achieved through partial replacement of sodium chloride with potassium chloride, a combination of different taste enhancers, such as glutamate, peptides or modified physical properties of food, has been investigated (Blesa et al., 2008; Schindler et al., 2011; Zhao et al., 2015). Kokumi-active compounds are not taste active but enhance the taste intensity of other compounds by modulation of the signal transduction from the taste receptors to the brain (Kuroda & Naohiro, 2015;
Maruyama et al., 2012). The kokumi taste activity imparts mouthfulness, complexity, and long-lasting taste (Ueda et al., 1997; Toelstede & Hofmann, 2008b; Toelstede et al., 2009).

Food fermentation is one of the oldest methods for food processing and traditional fermented foods are highly valued for their rich and complex taste and odour (Hutkins, 2006). The metabolic activity of food-fermenting microorganisms determines food quality, generates flavour, and enhances palatability. This communication aims to review the current knowledge related to taste active compounds in fermented foods, focussing on taste-active amino acids, amino acid derivatives, and peptides. The established or putative pathways of the generation of taste-active compounds are discussed for soy sauce, cheese, fermented meats, and bread. While these foods do not represent the diversity of fermented foods, they provide a cross-section of the different fermentation procedures, raw materials, and fermentation organisms that are employed in food fermentations (Hutkins, 2006, Gänzle, 2015). The conversion of sugars to organic acids is common to all food fermentations with lactic acid bacteria and is therefore not considered.

2 Generation of taste-active amino acids and peptides during food fermentation

Taste compounds are generated through primary proteolysis of the raw material by proteases from endogenous enzymes or microorganisms (Figure 1 and Table 1), followed by secondary proteolysis, and enzymatic or chemical conversion of amino acids into derivatives. An overview on enzymes with putative or known contribution to the formation of taste active peptides or amino acids in fermented foods is provided in Table 2. Taste active peptides, amino acids, and amino acid derivatives are the predominant tastants in many fermented foods and impart bitter, umami, or kokumi taste (Toldra and Flores, 1998; Hillmann and Hofmann, 2016; see below).
Proteolysis in food fermentations has comprehensively been reviewed (Table 1; Gänzle et al., 2008; Hughes et al., 2002; Savijoki et al., 2006; Toldra et al., 1993a; Toldra & Flores, 1998). In cheese and soy sauce, microorganisms are the major or sole contributor of protease and peptidase. During cheese ripening, casein is hydrolyzed by cell wall-bound proteinases from LAB and peptides are subsequently hydrolyzed by intracellular peptidases of LAB (Broadbent et al., 2002; Khalid & Marth, 1990). An imbalance of proteolysis and peptide hydrolysis, especially proteolysis of $\beta$-casein, accumulates bitter peptides and imparts a bitter taste defect (Fallico et al., 2005). In soy sauce, extracellular enzymes produced by koji starter cultures carry out primary proteolysis. At the moromi stage, growth and metabolism of *Tetragenococcus halophilus* and yeasts contribute to taste and flavor generation (Kaneko et al., 2011, Kaneko et al., 1994).

In sourdough and meat fermentations, enzymes from cereals and meat, respectively, determine proteolysis (Gänzle et al., 2008; Hammes and Hertel, 1998; Ordonez et al., 1999; Toldra et al., 1993a). Meat endogenous enzymes including dipeptidyl peptidases (DPP) and cathepsin B are main contributors to proteolysis during sausage and ham production (Sentandreu et al., 2003; Ordonez et al., 1999; Molina & Toldra, 1992; Toldra & Flores, 1998). Sourdough lactobacilli are generally non-proteolytic (Zheng et al., 2015); however, peptidase activity of sourdough LAB contributes significantly to the hydrolysis of peptides (Gänzle et al., 2008).

2.1 Bitter taste

The bitter taste of peptides depends on the amino acid composition and sequence (Kim & Li-Chan, 2006). Bitter peptides were characterized by surface hydrophobicity as calculated by the Q value (Arai et al., 1970; Ishibashi et al., 1987b; Ney, 1971). However, the Q rule does not consider the effect of amino acid sequences and is thus inconsistent when determining the
bitterness of peptides (Toelstede & Hofmann, 2008b). Proline is a major contributor to bitter taste of peptides (Ishibashi et al., 1988). The structure of peptides containing proline favours binding to the bitter taste receptor (Tamura et al., 1990). The presence of Gly, Ala, Val, Leu, Tyr, and Phe in peptides also imparts bitterness since these amino acids are also binding determinants (Arai et al., 1970; Ishibashi et al., 1987a; Ishibashi et al., 1987b; Ishibashi et al., 1988). In di- or tripeptides, bulky hydrophobic amino acids at any position determine bitterness, whereas for larger peptides, a bitter taste is related to specific basic amino acids at the N-terminus (Kim & Li-Chan, 2006). The composition of hydrophobic regions, the spatial orientation of polar and hydrophobic regions, and the proximity between polar groups and hydrophobic regions faced within the same plane were also suggested to be determinants for bitterness (Kiw et al., 2008). Remarkably, the structural requirements for ACE-inhibitory activity are related to the structural characteristics of bitter peptides and many bitter dipeptides show ACE-inhibitory activity (Li et al., 2004; Pripp & Ardo, 2007).

2.2 Umami taste

Glutamate in fermented foods imparts umami taste if the concentration in the food product is above the taste threshold of about 1 mM. Glutamate results from proteolysis or from conversion of glutamine by glutaminase (Ito et al., 2013; Lioe et al., 2010). Glutaminase activity in lactobacilli is strain- or species specific (Teixeira et al., 2014; Vermeulen et al., 2007; Zheng et al., 2015). γ-Glutamyl transferase (GGT) also acts as a “glutaminase” if water is an acceptor and GGT from Bacillus and Aspergillus convert glutamine to glutamate in soy sauce (Minami et al., 2003a; Minami et al., 2003b).
Pyroglutamic acid (pGlu) and pyroglutamyl-Pro-X peptides impart umami taste with similar activity to glutamate (Figure 1). Pyroglutamyl dipeptides are generated during heating by cyclization of corresponding α-glutamyl- or α-glutaminyl dipeptides (Kasai et al., 1983). Pyroglutamyl peptides are also produced by pGlu cyclase from pyroglutamic acid and free amino acids (Altamura et al., 1970). *Lactobacillus helveticus*, *L. delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus* were reported to have pGlu cyclase activity (Altamura et al., 1970; Mucchetti et al., 2002). pGlu can be released from the N-terminus of proteins and peptides by the action of a specific enzyme, such as pyrrolidone carboxyl peptidase (PCP) or 1-pyro-glutamyl-peptide hydrolase (PYRase) (Mucchetti et al., 2000).

Succinyl amino acids, especially suc-Arg and suc-Glu, impart umami taste (Table 3) (Frerot & Chen, 2013). They may arise from arginine catabolism or from fungal succinyl transferase activity (Frerot & Chen, 2013). Lactoyl amino acids were first isolated from cheese (Frerot & Escher, 1998) and (Figure 1) are produced from lactic acid and free amino acids in the presence of live or lysed *L. rhamnosus* and *L. helveticus* (Table 2). The enzymes involved in their formation remain unknown (Sgarbi et al., 2013).

Amadori products are intermediates of the Maillard reaction. Long fermentation times support the Maillard reaction even at ambient temperature. Amadori products including Fru-Val, Fru-Met, and Fru-pGlu were isolated from soy sauce and demonstrated astringency or bitterness (Kaneko et al., 2011). Even though these compounds exist at subthreshold concentrations, they provide the background taste of soy sauce and enhance the umami taste of glutamate (Kaneko et al., 2011). Other products of the Maillard reaction such as alapyridaine, N-glycosides,
pyroglutamyl peptides, and N-acetylglucine also have umami taste (Figure 1) (Ottinger & Hofmann, 2003; Shima et al., 1998; Winkel et al., 2008).

2.3 *Kokumi* taste

Glutathione (GSH) was the first kokumi active compound that was identified. It enhances continuity, mouthfulness and thickness when added to a solution containing glutamate and inosine-5’-monophosphate (Ueda et al., 1997). Several γ-glutamyl dipeptides also have kokumi taste activity (Toelstede & Hofmann, 2009); they have a higher solubility in water than α-Glutamyl peptides and resist hydrolysis by peptidases (Suzuki & Kumagai, 2004b). Glutathione is present in yeast extract and in cereals (Ueda et al., 1997) and thus may be generated by autolysis of yeasts. In cereal fermentations, GSH is produced from oxidized glutathione by glutathione reductase of lactobacilli (Jänsch et al., 2007). Microbial synthesis of γ-glutamyl peptides may be related to the activity of γ-glutamyl transferase, γ-glutamyl transpeptidase, or γ-Glu-Cys synthetase (Roudotalgaron et al., 1994). γ-Glutamyl transferase (GGT) catalyzes the hydrolytic cleavage of the isopeptide bond and the transfer of the γ-glutamyl unit to amino acids or peptides (Toelstede & Hofmann, 2009). In GGT reaction, acidic and basic amino acids are poor acceptors, whereas neutral amino acids are preferred (Toelstede & Hofmann, 2009). GGT is found in *Penicillium* spp. in cheese, and in *Aspergillus* or *Bacillus* from soy sauce. Recent studies indicated endogenous enzymes from milk and some *Lactobacillus* species also show GGT activity, but the contribution of GGT to the taste of fermented foods remains unclear (Arai et al., 1973; Sgarbi et al., 2013; Toelstede & Hofmann, 2009). γ-Glutamyl dipeptides and GSH are substrates of γ-glutamyl transpeptidase and generate a large variety of γ-
glutamyl dipeptides (Roudotalgaron et al., 1994). Other pathways or yet unknown enzymes may also contribute to the formation of γ-glutamyl dipeptides (Toelstede & Hofmann, 2009).

3 Contribution of taste active amino acids and peptides to food

3.1 Soy sauce

Soy sauce is used as a condiment because of its umami and salty taste. The umami taste is attributed to amino acids, particularly Glu, Ala, and Asp (Kaneko et al., 2011; Lioe et al., 2004; Lioe et al., 2006). Although some small peptides including pGlu-Asp, pGlu-Val, and lac-Glu exhibit umami taste, their direct contribution is negligible due to their low concentration in soy sauce. However, these compounds provide the taste background and enhance other tastes (Noguchi et al., 1975; Oka & Nagata, 1974a; Oka & Nagata, 1974b; Frerot & Chen, 2013). Omission and reconstitution tests indicate that pyroglutamyl peptides and Amadori compounds contribute to the umami taste when present at sub-threshold concentrations (Frerot & Chen, 2013). Shiga et al. (2014) identified Fru-Glu from 25 different soy sauces. Fru-Glu enhanced the intensity of the umami taste at the subthreshold level due to a strong synergistic activity with glutamate (Shiga et al., 2014).

3.2 Cheese

The taste and odour of cheese develops during ripening. Their composition of flavour active compounds is strongly dependent on the technology used for cheese production and the fermentation microbiota (McSweeney and Sousa, 2000). Free amino acids, particularly glutamate, small peptides, and amino acid derivatives, such as lactoyl amino acids, and pyroglutamyl amino acids, contribute to the taste of cheese (Drake, 2007; Andersen et al., 2010;
Sforza et al., 2009). Kokumi active peptides also contribute to the characteristic taste of cheese (Table 3). Unbalanced bitterness is unpleasant to the consumer and thus constitutes a serious economic concern for the cheese industry.

$\alpha$-Glutamyl di- and tripeptides, especially Asp-, Thr- and Ser-containing peptides, have umami taste (Table 3). The taste of $\alpha$-Glu-X is highly determined by the hydrophobicity of the second amino acid. For example, Glu-Asp, Glu-Thr, Glu-Ser, Glu-Glu and Glu-Gly-Ser have umami taste, Glu-Gly, Glu-Ala, Glu-Pro and Glu-Val have a flat or no taste; and Glu-Ile, Glu-Leu, Glu-Tyr and Glu-Phe possess bitter taste (Table 3 and Table 4) (Arai et al., 1973). Other dipeptides in cheese, including Arg-Pro, Asp-Asp, Arg-Asp, pGlu-Gln, pGlu-Gly, Asp-Glu, Glu-Glu, also impart umami taste. Glu-enriched hydrophilic oligopeptides, especially with a Glu residue at the N- or C-terminal position, possess umami taste (Kim et al., 2015). The taste of $\alpha$-Glu-X or their formation is unrelated to $\gamma$-Glu-X. $\alpha$-Glutamyl dipeptides are formed by proteolysis of casein, whereas $\gamma$-glutamyl dipeptides are generated by $\gamma$-glutamyl transpeptidase or $\gamma$-glutamyl transferase from amino acids (Toelstede et al., 2009; Toelstede & Hofmann, 2009).

$\gamma$-Glutamyl peptides with kokumi taste, such as $\gamma$-Glu-Phe, $\gamma$-Glu-Tyr and $\gamma$-Glu-Leu, were first isolated from Comte cheese (Roudotalgaron et al., 1994). In ripened Gouda and Parmesan cheeses, $\gamma$-Glu-Glu and $\alpha$-Glu-Glu were the most abundant dipeptides (Toelstede et al., 2009; Hillmann and Hofmann, 2016). The concentration of the $\gamma$-glutamyl dipeptides increased 10-fold to more than 100-fold over 44 weeks of ripening, whereas the concentration of $\alpha$-glutamyl dipeptides increased only 1-fold to 8-fold (Toelstede et al., 2009). The concentration of $\gamma$-glutamyl-peptides in cheese exceeds the kokumi threshold concentrations. Omission and reconstitution experiments confirmed that $\gamma$-glutamyl peptides contribute to the taste profile of
44 week matured Gouda cheese (Roudotalgaron et al., 1994; Toelstede & Hofmann, 2008b; Toelstede et al., 2009; Toelstede & Hofmann, 2009). The formation of γ-glutamyl peptides in Parmesan was attributed to the γ-glutamyltransferase activity from raw cow’s milk rather than microbial activity (Hillmann et al., 2016).

Development of bitter taste in cheese results from unbalanced levels of proteolysis and peptide hydrolysis. Peptides are major contributors to bitter taste as proven by an omission test (Engel et al., 2001a; Engel et al., 2001b). Cheese production with isogenic cultures of Lactococcus lactis demonstrated that the accumulation of bitter peptides depends on the substrate specificity of the extracellular protease lactocepin (Broadbend et al., 2002). Salt and acidity additionally influence the perception of bitterness (Engel et al., 2001b; Engel et al., 2001c).

3.3 Fermented meat

The taste of dry cured hams results from enzymatic reactions, including proteolysis and lipolysis, and chemical conversions, including lipid oxidation, and Strecker and Maillard reactions throughout ripening. Microbial conversions additionally contribute to flavour fermentation in production of dry cured sausages (Hammes and Hertel, 1998; Olesen et al., 2004; Andrade et al., 2010). The contribution of microorganisms to proteolysis during meat fermentations is limited, however, LAB, Micrococccaceae and surface moulds contribute to flavor and taste due to amino acid metabolism (Table 1) (Olesen et al., 2004; Herranz et al., 2006; Sinz et al., 2013). Free amino acids and peptides play a crucial role in producing taste and flavor of ham and sausage (Herranz, Fernandez, de la Hoz, & Ordonez, 2006; Jurado, Garcia, Timon, & Carrapiso, 2007; Sentandreu et al., 2003; Sforza et al., 2001; Sforza et al., 2006).
Glu and Asp are the most abundant free amino acids in fermented meats and their concentration generally exceeds the taste threshold (Table 3) (Jurado et al., 2007). Pro, Ala, Val, Ile, Leu, and Phe also increase to concentrations exceeding the taste threshold during ripening (Kato et al., 1989). Lys, Tyr, Asp, Ala, and Glu were the most abundant free amino acids in the ripening of Iberian ham and strongly influenced the taste of dry cured products (Careri et al., 1993; Hughes et al., 2002; Toldra & Aristoy, 1993b).

Peptides in meat also contribute to taste (Reina et al., 2014). Bitter tasting dipeptides such as Ile-Val, Leu-Gly, Ile-Asp and Pro-Leu were isolated from ripened ham (Table 4) (Sentandreu et al., 2003). The bitter peptides in meat result from a low activity of aminopeptidase, which cleaves peptides into free amino acids with reduced bitterness (Reina et al., 2014). High endopeptidase activity leads to a high content of Met, Asn and Ile, which impart bitterness in aged hams (Sforza et al., 2001; Sforza et al., 2006). The release of bitter peptides and amino acids from muscle protein is less pronounced compared to casein; therefore, bitterness is not a major issue in meat products (Henriksen & Stahnke, 1997). Di- and tri-peptides with umami taste or kokumi activity were also identified in fermented meats (Suzuki et al., 2002).

3.4 Bread

Bread flavour is dependent on the activity of flour enzymes, and the metabolic activity of yeasts and lactic acid bacteria (Gänzle, 2014). Sourdough fermented with lactic acid bacteria is included in bread production to achieve dough leavening without baker’s yeast, or as ingredient to improve the texture, flavour, and storage life of yeast leavened bread (Brandt, 2007). Fermentation microbiota in sourdoughs are process-specific; however, *L. sanfranciscensis*, *L. plantarum*, and organisms of the *L. reuteri* group are key elements of sourdough microbiota.
(Gänzle and Ripari, *in press*). In bread produced with yeasts as sole fermentation organisms (straight dough processes), the concentration of amino acids including taste-active glutamate is low because of the limited proteolysis at the dough stage, and the consumption of amino acids by baker’s yeast (Fujisawa & Yoshino, 1995; Gänzle et al., 2008; Johnson & Eldash, 1969, Zhao et al., 2015). Sourdough fermentations which include lactic acid bacteria as fermentation organisms, however provide suitable conditions for proteolytic conversion of cereal proteins, and accumulate peptides and amino acids as flavour precursor compounds or taste-active compounds (Gänzle et al., 2008; Thiele et al., 2002). Sourdough fermentations affect the concentrations of the taste-active compounds glutamate and glutathione in a strain-specific manner. Glutamate accumulated in sourdough fermentations to levels ranging from 27 to 120 mmol/kg; its accumulation in sourdough depends on strain-specific glutamate decarboxylase and glutaminase activities (Su et al., 2011; Stromeck et al., 2011; Vermeulen et al., 2007a). Glutamate generated during sourdough fermentation significantly enhanced the umami taste of bread when glutamate concentrations in bread exceeded the taste threshold of about 1 mmol / kg (Zhao et al., 2015). The use of isogenic strains of *L. reuteri* expressing, or lacking, glutamate decarboxylase minimized the influence of confounding factors on bread flavour (Su et al., 2011; Zhao et al., 2015). Remarkably, the increased umami and sour taste intensities resulting from sourdough addition were perceived as a higher intensity of saltiness by an untrained consumer panel (Zhao et al., 2015). Taste active compounds produced during sourdough fermentation thus allow for reduced salt levels in bread without compromising consumer acceptance (Zhao et al., 2015).

Wheat flour contains the kokumi active compound glutathione (Grosch & Wieser, 1999; Ueda et al., 1997). At the dough stage, glutathione participates in thiol-disulfide exchange reactions with gluten and integrates into the glutenin macropolymer (Grosch & Wieser, 1999;
Weegels et al., 1996). Glutathione levels in sourdough are influenced by glutathione reductase of *L. sanfranciscensis* (Jänsch et al., 2007; Table 2). Glutathione concentrations in wheat dough, however, are below the kokumi taste threshold of 300 µmol / kg (Grosch & Wieser, 1999; Ueda et al., 1997) and a contribution of glutathione to bread taste remains unclear.

### 4 Interaction between taste active amino acids and peptides and salt

NaCl tastes salty, masks metallic and bitter tastes, and enhances umami taste. Conversely, amino acids and peptides may enhance the salty taste and thus allow reducing salt levels in food. The salty taste of dry cured meat correlated to the concentrations of glutamate and aspartate (Careri et al., 1993). Omission tests with cheese indicated that arginine at subthreshold concentration significantly enhanced salty taste (Toelstede & Hofmann, 2008a; Toelstede et al., 2009). Perception of salty and umami tastes is based on distinct receptors, however, the simultaneous presence of umami and salty stimuli intensifies taste perception (Lioe et al., 2005). Remarkably, the presence of bitter tasting aromatic amino acids at subthreshold levels also enhanced the umami taste of soy sauce (Lioe et al., 2004). Subthreshold concentrations of taste compounds can thus affect the intensity of other taste attributes (Lioe et al., 2005).

### 5 Debittering in food fermentations

Bitterness limits the acceptance and marketing of food and is of particular concern in cheese production. Physical or chemical methods of debittering include the adsorption of bitter peptides on suitable resins, their extraction, or rely on masking agents including cyclodextrin and poly-γ-glutamate (Ley, 2008, Saha & Hayashi, 2001). Enzymatic debittering is achieved with transglutaminase or peptidases (FitzGerald & O'Cuinn, 2006). Unbalanced proteolysis is
responsible for the formation of bitter peptides in food fermentations and reduction of bitter peptides require enzymatic hydrolysis into products that lack bitter taste (Broadbent & Steele, 2007). Starter cultures combining a low propensity for the production of bitter peptides with a high activity of debittering peptidases reduce bitterness in cheese (Broadbent et al., 2002). The bitterness of casein hydrolysates was significantly reduced in the presence of amino peptidases and post-proline dipeptidyl aminopeptidases (PPDA), which release amino acyl proline residues from the N-terminus (Bouchier et al., 2001). Cell free extracts of different *Lc. lactis* were used for debittering peptides due to the activity of PepXP, which can cleave the X-Pro-Y peptide bond and liberate X-Pro from bitter peptides (Shimamura et al., 2009). In cheese, *L. helveticus* had a significant debittering effect that was attributed to prolyl endopeptidases, such as PepO2, PepO3, and PepF (Sridhar et al., 2005). The aminopeptidase PepN converts Pro-containing bitter peptides into smaller peptides and amino acids (Broadbent & Steele, 2007).

An alternative strategy is to convert bitter amino acids into γ-glutamyl derivatives through GGT. This approach not only reduces the concentration of bitter tasting amino acids Phe, Val, Leu and His (Suzuki et al., 2002; Suzuki & Kumagai, 2004b; Suzuki et al., 2004a) but also generates γ-glutamyl peptides with kokumi activity or umami taste that suppress the bitterness and hence increase consumer preference (Son et al., 2015; Suzuki et al., 2002). Five representative umami peptides suppressed sialicin-induced intracellular calcium influx in a non-competitive manner. αGlu-Glu at 1 mM was the most effective inhibitor of salicin-induced intracellular Ca\(^{2+}\) response and hence inhibited the bitter taste sensation (Kim et al., 2015). Glutamate with adenosine monophosphate or sodium salts of 5’-ribonucleotides as well as the umami tasting peptides αGlu-Asp, αGlu-Glu, αGlu-Ser, and αGlu-Glu-Glu also suppressed the bitter taste of peptides (Kemp & Beauchamp, 1994; Tokita & Boughter, 2012). In conclusion,
debittering in food fermentations is achieved by a combination of peptidase activity to reduce the concentration of bitter peptides, and the accumulation of glutamate and related umami-tasting or kokumi-active compounds that moderate the bitter taste impression.

6 Conclusions and perspectives

The generation of taste active compounds in food fermentation results from proteolysis and peptide hydrolysis in combination with amino acid conversion and formation of taste-active amino acid derivatives. Proteolysis in food fermentations is well understood, however, the elucidation of enzymes and metabolic pathways converting peptides or amino acids to taste-active derivatives is still in its infancy. The use of isogenic mutant strains in food fermentations in combination with chemical and sensory analyses of the products provides a powerful tool to further elucidate the influence of specific enzymes and metabolic pathways of food fermenting lactic acid bacteria on the taste of fermented foods. These studies will facilitate the selection of specific starter cultures to achieve improved sensory properties of foods.

A multitude of taste-active compounds is produced during food fermentations, highlighting the role of taste-taste interactions as well as the contribution of compounds that are present at subthreshold levels. The appreciation of fermented foods thus relates to a large diversity of tastants providing a complex taste impression. Improved knowledge of the interactions between taste-active compounds will enable the development of novel fermentation strategies to develop tastier, less bitter, and low-salt food products, and may provide novel and “clean label” ingredients to improve the taste of other food products.

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Figure legends.

Figure 1. Overview on the generation of taste compounds from proteins during food fermentation. Proteolysis generates taste active peptides and amino acids; glutathione reductase generates the kokum-active glutathione. Further conversion of peptides or amino acids to taste active compounds proceeds by enzymatic reactions. Enzymatic conversions were proposed to be catalysed by lactoyl-transferase [2], succinyl transferase [3], pyroglutamyl cyclase [4], or by $\gamma$-glutamyl-transferase [5]. Maillard / Amadori products [6] are formed by chemical conversion during heating.
**Table 1.** Contributors to proteolysis in cheese, soy, meat, and sourdough fermentations.

<table>
<thead>
<tr>
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<th><strong>Primary proteolysis</strong></th>
<th><strong>Secondary proteolysis</strong></th>
<th><strong>Conversion of amino acids</strong></th>
<th><strong>Ref.</strong></th>
</tr>
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<tbody>
<tr>
<td>Cheese</td>
<td>Lactic acid bacteria (LAB) (surface microbiota)</td>
<td>LAB</td>
<td>Penicillium spp., LAB</td>
<td>(Khalid &amp; Marth, 1990)</td>
</tr>
<tr>
<td>Soy sauce</td>
<td>Aspergillus spp.</td>
<td>LAB</td>
<td>Aspergillus, LAB</td>
<td>(Lioe et al., 2010)</td>
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<tr>
<td>Bread</td>
<td>Cereal enzymes (fungal or malt protease)</td>
<td>LAB</td>
<td>LAB</td>
<td>(Thiele et al., 2002; Gänzle et al., 2008; Gänzle et al., 2009)</td>
</tr>
<tr>
<td>Meat</td>
<td>Muscle enzymes; (surface microbiota)</td>
<td>LAB, staphylococci, (surface microbiota)</td>
<td>LAB, staphylococci, (surface microbiota)</td>
<td>(Benito et al., 2002; Benito et al., 2003; Freiding et al., 2012; Hughes et al., 2002; Sinz et al., 2013; Toldra et al., 1993a)</td>
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Table 2. Enzymes with putative or known contribution to the formation of taste active peptides or amino acids in fermented foods.

<table>
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<tr>
<th>Enzyme</th>
<th>Microorganisms</th>
<th>Food</th>
<th>Reference</th>
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<tr>
<td>Glutaminase</td>
<td><em>L. reuteri, Aspergillus sojae</em></td>
<td>Sourdough, soy sauce</td>
<td>(Ito et al., 2013; Lioe et al., 2010; Teixeira et al., 2014)</td>
</tr>
<tr>
<td>Glutamate decarboxylase</td>
<td><em>L. brevis, L. reuteri</em></td>
<td>Cheese, sourdough</td>
<td>(Su et al., 2011; Teixeira et al., 2014)</td>
</tr>
<tr>
<td>γ-Glutamyl-transferase</td>
<td><em>Aspergillus spp., Penicillium spp., Bacillus subtilis</em></td>
<td>Cheese, soy sauce</td>
<td>(Minami et al., 2003a; Minami et al., 2003b)</td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td><em>L. sanfranciscensis</em></td>
<td>Sourdough</td>
<td>(Jänsch et al., 2007)</td>
</tr>
<tr>
<td>Succinyl transferase</td>
<td><em>Aspergillus spp.</em></td>
<td>Soy sauce</td>
<td>(Frerot &amp; Chen, 2013)</td>
</tr>
<tr>
<td>Pyroglutamyl cyclase</td>
<td><em>L. helveticus, L. delbruechii, S. thermophilus</em></td>
<td>Soy sauce, meat product</td>
<td>(Altamura, et al., 1970; Mucchetti et al., 2002)</td>
</tr>
<tr>
<td>Putative lactoyl transferase</td>
<td><em>Lactobacillus spp.</em></td>
<td>Soy sauce, cheese, meat product</td>
<td>(Sgarbi et al., 2013)</td>
</tr>
</tbody>
</table>
Table 3. Amino acids or peptides with sweet, umami taste or with kokumi taste activity that were isolated from fermented foods.

<table>
<thead>
<tr>
<th>Compound, threshold (mM)</th>
<th>Source (ref)</th>
<th>Compound, threshold (mM)</th>
<th>Sweet Taste</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met</td>
<td>5</td>
<td>pGlu-Gly</td>
<td>2.2</td>
<td>Soy sauce (4)</td>
</tr>
<tr>
<td>Ala</td>
<td>6.7</td>
<td>Ile-Gln</td>
<td>&lt;5.5</td>
<td>Soy sauce (4)</td>
</tr>
<tr>
<td>Gly</td>
<td>25</td>
<td>Leu-Gln</td>
<td>&lt;5.5</td>
<td>Soy sauce (4)</td>
</tr>
<tr>
<td>Pro</td>
<td>25</td>
<td>Thr-Phe</td>
<td>&lt;5.5</td>
<td>Soy sauce (4)</td>
</tr>
<tr>
<td>Ser</td>
<td>25</td>
<td>Ile-Glu</td>
<td>&lt;5.5</td>
<td>Soy sauce (4)</td>
</tr>
<tr>
<td>pGlu-Gln</td>
<td>1.9</td>
<td>Pro-Lys</td>
<td>&lt;5.5</td>
<td>Soy sauce (4)</td>
</tr>
<tr>
<td>Glu</td>
<td>1.1, 0.3</td>
<td>Asp-Glu-Ser</td>
<td>8.6</td>
<td>Fish (12)</td>
</tr>
<tr>
<td>Tyr</td>
<td>4</td>
<td>Glu-Gln-Glu</td>
<td>7.4</td>
<td>Fish (12)</td>
</tr>
<tr>
<td>Asp</td>
<td>6.4</td>
<td>Thr-Glu</td>
<td>12.1</td>
<td>Fish (12)</td>
</tr>
<tr>
<td>Lactoyl-glutamine</td>
<td>5</td>
<td>Ser-Glu-Glu</td>
<td>5.5</td>
<td>Fish (12)</td>
</tr>
<tr>
<td>Glu-Glu</td>
<td>5.4</td>
<td>Fru-Glu</td>
<td>0.8</td>
<td>Soy sauce (4)</td>
</tr>
<tr>
<td>Glu-Gly-Ser</td>
<td>6.9</td>
<td>N-glucosyl-Glu</td>
<td>1.6</td>
<td>Soy sauce (15)</td>
</tr>
<tr>
<td>Glu-Asp</td>
<td>7.6</td>
<td>KGNEESLA</td>
<td>0.5</td>
<td>Meat (13, 14)</td>
</tr>
<tr>
<td>Glu-Asp-Glu</td>
<td>7.6</td>
<td>γ-glutamyl-ethylamide</td>
<td>24</td>
<td>Tea (15)</td>
</tr>
<tr>
<td>Glu-Ser</td>
<td>8.5</td>
<td>N-deoxyfructosyl-Glu</td>
<td>1.8</td>
<td>Soy sauce (15)</td>
</tr>
<tr>
<td>γ-Glu-Glu&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.018</td>
<td>Cheese, bread (16, 17)</td>
<td>γ-Glu-Gln</td>
<td>Cheese (16, 17)</td>
</tr>
<tr>
<td>γ-Glu-Phe</td>
<td>2.5</td>
<td>γ-Glu-Val</td>
<td>0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cheese (16, 17)</td>
</tr>
<tr>
<td>γ-Glu-Gly&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.018</td>
<td>Cheese, meat, soy sauce</td>
<td>γ-Glu-Tyr&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(16, 17, 19)</td>
</tr>
<tr>
<td>γ-Glu-His</td>
<td>0.01</td>
<td>Cheese (16, 17)</td>
<td>γ-Glu-Ala</td>
<td>(20)</td>
</tr>
<tr>
<td>γ-Glu-Leu</td>
<td>0.005</td>
<td>Cheese (16 – 18)</td>
<td>γ-Glu-Cys-Gly</td>
<td>0.3</td>
</tr>
<tr>
<td>γ-Glu-Met</td>
<td>0.005</td>
<td>Cheese (16, 17)</td>
<td>γ-Glu-Val-Gly</td>
<td>0.3</td>
</tr>
</tbody>
</table>

<sup>b</sup>a  All amino acids and peptides are L-type. The free amino acids, di- and tripeptides are written in three letter code, longer peptides are written in one letter code.

<sup>b</sup>b The taste threshold was measured only in cheese.

References: (1), Henriksen & Stahnke, 1997; (2), Lioe et al., 2006; (3), Toelstede & Hofmann, 2008a; (4), Yamamoto et al., 2014; (5), Kaneko et al., 2011; (6); Khalid & Marth, 1990; (7), Drake et al., 2007; (8), Zhao et al., 2015; (9), Careri et al., 1993; (10), Sforza et al., 2001; (11), Sgarbi et al., 2013; (12), Schindler et al., 2011; (13), Tamura et al., 1989; (14), Wang et al., 1996; (15), Kaneko et al., 2006; (16), Beksan et al., 2003; (16), Toelstede et al., 2009; (17), Toelstede & Hofmann, 2009; (18), Sforza et al., 2006; (19), Roudotalgaron et al., 1994; (20), Hillmann and Hoffmann, 2016; (21), Ueda et al., 1997; (22), Kuroda et al., 2013.
### Table 4. Bitter amino acids and peptides in fermented foods

<table>
<thead>
<tr>
<th>Compound</th>
<th>Threshold (mM)</th>
<th>Source (ref)</th>
<th>Compound</th>
<th>Threshold (mM)</th>
<th>Source (ref)</th>
</tr>
</thead>
<tbody>
<tr>
<td>His</td>
<td>1.2</td>
<td>Cheese, sausage (1, 2)</td>
<td>Glu-Trp</td>
<td>5</td>
<td>Cheese (9, 10, 11)</td>
</tr>
<tr>
<td>Lys</td>
<td>3.4</td>
<td>Soy sauce, meat (4, 5)</td>
<td>Glu-Tyr</td>
<td>5</td>
<td>Cheese (9, 10, 11)</td>
</tr>
<tr>
<td>Val</td>
<td>3.4</td>
<td>Cheese, sausage (1, 2, 4)</td>
<td>YPFPGPHNS</td>
<td>0.05$^a$</td>
<td>Cheese (12)</td>
</tr>
<tr>
<td>Try</td>
<td>4</td>
<td>Cheese (1)</td>
<td>SLVYPFPAGPHNS</td>
<td>0.06$^a$</td>
<td>Cheese (12)</td>
</tr>
<tr>
<td>Tyr</td>
<td>4</td>
<td>Cheese (1)</td>
<td>LVYPFPAGPHN</td>
<td>0.08$^a$</td>
<td>Cheese (12)</td>
</tr>
<tr>
<td>Phe</td>
<td>4, 5</td>
<td>Cheese, soy sauce, sausage (1, 2, 3, 6)</td>
<td>YPFPGPHN</td>
<td>0.1$^a$</td>
<td>Cheese (12)</td>
</tr>
<tr>
<td>Ile</td>
<td>10</td>
<td>Cheese, sausage (1, 2, 3)</td>
<td>VYPFPAGPHN</td>
<td>0.17$^a$</td>
<td>Cheese (12)</td>
</tr>
<tr>
<td>Leu</td>
<td>11</td>
<td>Cheese, sausage (1)</td>
<td>YQPVLGPVREGPFPFIV</td>
<td>0.18$^a$</td>
<td>Cheese (12)</td>
</tr>
<tr>
<td>Met-Ile</td>
<td>0.42</td>
<td>Cheese (8)</td>
<td>YPFPGPHN</td>
<td>0.33$^a$</td>
<td>Cheese (12)</td>
</tr>
<tr>
<td>Fru-Met</td>
<td>1.6</td>
<td>Soy sauce (7)</td>
<td>YPFPGPHN</td>
<td>0.33$^a$</td>
<td>Cheese (12)</td>
</tr>
<tr>
<td>Fru-Val</td>
<td>1.8</td>
<td>Soy sauce (7)</td>
<td>VRGPFP</td>
<td>0.42$^a$</td>
<td>Cheese (12)</td>
</tr>
<tr>
<td>Glu-Gly</td>
<td>2.5</td>
<td>Cheese (10, 11)</td>
<td>EIVPN</td>
<td>0.43$^a$</td>
<td>Cheese (12)</td>
</tr>
<tr>
<td>Glu-Thr</td>
<td>2.5</td>
<td>Cheese (9, 10, 11)</td>
<td>DIKQM</td>
<td>0.6$^a$</td>
<td>Cheese (1)</td>
</tr>
<tr>
<td>Fru-pGlu</td>
<td>2.6</td>
<td>Soy sauce (7)</td>
<td>LPQE</td>
<td>0.6$^a$</td>
<td>Cheese (1)</td>
</tr>
<tr>
<td>Glu-Val</td>
<td>5</td>
<td>Cheese (9, 10, 11)</td>
<td>GPVRGFPF</td>
<td>1.18$^a$</td>
<td>Cheese (12)</td>
</tr>
<tr>
<td>Glu-Ala</td>
<td>10</td>
<td>Cheese (9, 10, 11)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ All amino acids and peptides are L-type. The free amino acids, di- and tripeptides are written in three letter code, longer peptides are written in one letter code.

Figure 1.

[primary proteolysis]

proteins

bitter peptides

peptides

oxidized or protein-bound glutathione

[1]

[secondary proteolysis]

amino acids

lactoyl amino acids

[2]

succinyl amino acids

[3]

pyroglutamate peptides

[4]

γ-glutamyl peptides

[5]

Maillard products/Amadori compounds

[6]