

16 **Abstract:**

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

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

33

34 **1 Introduction**

35 Taste determines food selection, intake, absorption, and digestion, and thus contributes to
36 the nutritional status as well as to decisions on food purchase (Barylko-Pikielna & Kostyra,
37 2007; Beksan et al., 2003). Six basic tastes, salt, sweet, and umami, sour, bitter, and oleogustus,
38 are detected by taste receptors in taste buds on the tongue and palate epithelium (Running et al.,
39 2015). Sweet, umami and bitter tastes are particularly related to food acceptance or rejection
40 (Barylko-Pikielna & Kostyra, 2007). Sweet taste allows to identify carbohydrate-rich foods as a
41 source of energy (Behrens et al., 2011); sweet taste receptors are absent in carnivores (Jiang et
42 al., 2012). Umami molecules impart savory taste and increase other taste intensities (Jinap &
43 Hajeb, 2010). Umami taste is linked to meat intake and umami taste receptors are typically
44 absent in herbivores (Zhao et al., 2010). L-Glu and 5'-ribonucleotides elicit umami taste (Jinap
45 & Hajeb, 2010). Humans reject bitter tasting foods, however, a limited level of bitterness in food
46 may be desirable. Moreover, bitter taste reception in humans is highly variable (Meyerhof et al.,
47 2010). Saltiness often determines the sensory acceptance of savory foods, such as soups, sauces,
48 snacks, and bakery products (Schindler et al., 2011). However, salt intake in industrialized
49 nations exceeds by 80-100% the amount recommended by WHO. Sodium reduction, achieved
50 through partial replacement of sodium chloride with potassium chloride, a combination of
51 different taste enhancers, such as glutamate, peptides or modified physical properties of food, has
52 been investigated (Blesa et al., 2008; Schindler et al., 2011; Zhao et al., 2015). Kokumi-active
53 compounds are not taste active but enhance the taste intensity of other compounds by modulation
54 of the signal transduction from the taste receptors to the brain (Kuroda & Naohiro, 2015;

55 Maruyama et al., 2012). The kokumi taste activity imparts mouthfulness, complexity, and long
56 lasting taste (Ueda et al., 1997; Toelstede & Hofmann, 2008b; Toelstede et al., 2009).

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

68 **2 Generation of taste-active amino acids and peptides during food fermentation**

69 Taste compounds are generated through primary proteolysis of the raw material by proteases
70 from endogenous enzymes or microorganisms (Figure 1 and Table 1), followed by secondary
71 proteolysis, and enzymatic or chemical conversion of amino acids into derivatives. An overview
72 on enzymes with putative or known contribution to the formation of taste active peptides or
73 amino acids in fermented foods is provided in Table 2. Taste active peptides, amino acids, and
74 amino acid derivatives are the predominant tastants in many fermented foods and impart bitter,
75 umami, or kokumi taste (Toldra and Flores, 1998; Hillmann and Hofmann, 2016; see below).

76 Proteolysis in food fermentations has comprehensively been reviewed (Table 1; Gänzle et
77 al., 2008; Hughes et al., 2002; Savijoki et al., 2006; Toldra et al., 1993a; Toldra & Flores, 1998).
78 In cheese and soy sauce, microorganisms are the major or sole contributor of protease and
79 peptidase. During cheese ripening, casein is hydrolyzed by cell wall-bound proteinases from
80 LAB and peptides are subsequently hydrolyzed by intracellular peptidases of LAB (Broadbent et
81 al., 2002; Khalid & Marth, 1990). An imbalance of proteolysis and peptide hydrolysis, especially
82 proteolysis of β -casein, accumulates bitter peptides and imparts a bitter taste defect (Fallico et
83 al., 2005). In soy sauce, extracellular enzymes produced by koji starter cultures carry out primary
84 proteolysis. At the moromi stage, growth and metabolism of *Tetragenococcus halophilus* and
85 yeasts contribute to taste and flavor generation (Kaneko et al., 2011, Kaneko et al., 1994).

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

93 2.1 *Bitter taste*

94 The bitter taste of peptides depends on the amino acid composition and sequence (Kim &
95 Li-Chan, 2006). Bitter peptides were characterized by surface hydrophobicity as calculated by
96 the Q value (Arai et al., 1970; Ishibashi et al., 1987b; Ney, 1971). However, the Q rule does not
97 consider the effect of amino acid sequences and is thus inconsistent when determining the

98 bitterness of peptides (Toelstede & Hofmann, 2008b). Proline is a major contributor to bitter
99 taste of peptides (Ishibashi et al., 1988). The structure of peptides containing proline favours
100 binding to the bitter taste receptor (Tamura et al., 1990). The presence of Gly, Ala, Val, Leu,
101 Tyr, and Phe in peptides also imparts bitterness since these amino acids are also binding
102 determinants (Arai et al., 1970; Ishibashi et al., 1987a; Ishibashi et al., 1987b; Ishibashi et al.,
103 1988). In di- or tripeptides, bulky hydrophobic amino acids at any position determine bitterness,
104 whereas for larger peptides, a bitter taste is related to specific basic amino acids at the N-
105 terminus (Kim & Li-Chan, 2006). The composition of hydrophobic regions, the spatial
106 orientation of polar and hydrophobic regions, and the proximity between polar groups and
107 hydrophobic regions faced within the same plane were also suggested to be determinants for
108 bitterness (Kiw et al., 2008). Remarkably, the structural requirements for ACE-inhibitory activity
109 are related to the structural characteristics of bitter peptides and many bitter dipeptides show
110 ACE-inhibitory activity (Li et al., 2004; Pripp & Ardo, 2007).

111 2.2 *Umami taste*

112 Glutamate in fermented foods imparts umami taste if the concentration in the food product is
113 above the taste threshold of about 1 mM. Glutamate results from proteolysis or from conversion
114 of glutamine by glutaminase (Ito et al., 2013; Lioe et al., 2010). Glutaminase activity in
115 lactobacilli is strain- or species specific (Teixeira et al., 2014; Vermeulen et al., 2007; Zheng et
116 al., 2015). γ -Glutamyl transferase (GGT) also acts as a “glutaminase” if water is an acceptor and
117 GGT from *Bacillus* and *Aspergillus* convert glutamine to glutamate in soy sauce (Minami et al.,
118 2003a; Minami et al., 2003b).

119 Pyroglutamic acid (pGlu) and pyroglutamyl-Pro-X peptides impart umami taste with similar
120 activity to glutamate (Figure 1). Pyroglutamyl dipeptides are generated during heating by
121 cyclization of corresponding α -glutamyl- or α -glutaminyl dipeptides (Kasai et al., 1983).
122 Pyroglutamyl peptides are also produced by pGlu cyclase from pyroglutamic acid and free amino
123 acids (Altamura et al., 1970). *Lactobacillus helveticus*, *L. delbrueckii* subsp. *bulgaricus*, and
124 *Streptococcus thermophilus* were reported to have pGlu cyclase activity (Altamura et al., 1970;
125 Mucchetti et al., 2002). pGlu can be released from the N-terminus of proteins and peptides by the
126 action of a specific enzyme, such as pyrrolidone carboxyl peptidase (PCP) or l-pyro-glutamyl-
127 peptide hydrolase (PYRase) (Mucchetti et al., 2000).

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

134 Amadori products are intermediates of the Maillard reaction. Long fermentation times
135 support the Maillard reaction even at ambient temperature. Amadori products including Fru-Val,
136 Fru-Met, and Fru-pGlu were isolated from soy sauce and demonstrated astringency or bitterness
137 (Kaneko et al., 2011). Even though these compounds exist at subthreshold concentrations, they
138 provide the background taste of soy sauce and enhance the umami taste of glutamate (Kaneko et
139 al., 2011). Other products of the Maillard reaction such as alapyridaine, N-glycosides,

140 pyroglutamyl peptides, and N-acetylglycine also have umami taste (Figure 1) (Ottinger &
141 Hofmann, 2003; Shima et al., 1998; Winkel et al., 2008).

142 2.3 *Kokumi taste*

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

161 glutamyl dipeptides (Roudotalgaron et al., 1994). Other pathways or yet unknown enzymes may
162 also contribute to the formation of γ -glutamyl dipeptides (Toelstede & Hofmann, 2009).

163 **3 Contribution of taste active amino acids and peptides to food**

164 *3.1 Soy sauce*

165 Soy sauce is used as a condiment because of its umami and salty taste. The umami taste is
166 attributed to amino acids, particularly Glu, Ala, and Asp (Kaneko et al., 2011; Lioe et al., 2004;
167 Lioe et al., 2006). Although some small peptides including pGlu-Asp, pGlu-Val, and lac-Glu
168 exhibit umami taste, their direct contribution is negligible due to their low concentration in soy
169 sauce. However, these compounds provide the taste background and enhance other tastes
170 (Noguchi et al., 1975; Oka & Nagata, 1974a; Oka & Nagata, 1974b; Frerot & Chen, 2013).
171 Omission and reconstitution tests indicate that pyroglutamyl peptides and Amadori compounds
172 contribute to the umami taste when present at sub-threshold concentrations (Ferot & Chen,
173 2013). Shiga et al. (2014) identified Fru-Glu from 25 different soy sauces. Fru-Glu enhanced the
174 intensity of the umami taste at the subthreshold level due to a strong synergistic activity with
175 glutamate (Shiga et al., 2014).

176 *3.2 Cheese*

177 The taste and odour of cheese develops during ripening. Their composition of flavour active
178 compounds is strongly dependent on the technology used for cheese production and the
179 fermentation microbiota (McSweeney and Sousa, 2000). Free amino acids, particularly
180 glutamate, small peptides, and amino acid derivatives, such as lactoyl amino acids, and
181 pyroglutamyl amino acids, contribute to the taste of cheese (Drake, 2007; Andersen et al., 2010;

182 Sforza et al., 2009). Kokumi active peptides also contribute to the characteristic taste of cheese
183 (Table 3). Unbalanced bitterness is unpleasant to the consumer and thus constitutes a serious
184 economic concern for the cheese industry.

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

196 γ -Glutamyl peptides with kokumi taste, such as γ -Glu-Phe, γ -Glu-Tyr and γ -Glu-Leu, were
197 first isolated from Comte cheese (Roudotalgaron et al., 1994). In ripened Gouda and Parmesan
198 cheeses, γ -Glu-Glu and α -Glu-Glu were the most abundant dipeptides (Toelstede et al., 2009;
199 Hillmann and Hofmann, 2016). The concentration of the γ -glutamyl dipeptides increased 10-fold
200 to more than 100-fold over 44 weeks of ripening, whereas the concentration of α -glutamyl
201 dipeptides increased only 1-fold to 8-fold (Toelstede et al., 2009). The concentration of γ -
202 glutamyl-peptides in cheese exceeds the kokumi threshold concentrations. Omission and
203 reconstitution experiments confirmed that γ -glutamyl peptides contribute to the taste profile of

204 44 week matured Gouda cheese (Roudotalgaron et al., 1994; Toelstede & Hofmann, 2008b;
205 Toelstede et al., 2009; Toelstede & Hofmann, 2009). The formation of γ -glutamyl peptides in
206 Parmesan was attributed to the γ -glutamyltransferase activity from raw cow's milk rather than
207 microbial activity (Hillmann et al., 2016).

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

214 3.3 Fermented meat

215 The taste of dry cured hams results from enzymatic reactions, including proteolysis and
216 lipolysis, and chemical conversions, including lipid oxidation, and Strecker and Maillard
217 reactions throughout ripening. Microbial conversions additionally contribute to flavour
218 fermentation in production of dry cured sausages (Hammes and Hertel, 1998; Olesen et al., 2004;
219 Andrade et al., 2010). The contribution of microorganisms to proteolysis during meat
220 fermentations is limited, however, LAB, *Micrococcaceae* and surface moulds contribute to
221 flavor and taste due to amino acid metabolism (Table 1) (Olesen et al., 2004; Herranz et al.,
222 2006; Sinz et al., 2013). Free amino acids and peptides play a crucial role in producing taste and
223 flavor of ham and sausage (Herranz, Fernandez, de la Hoz, & Ordonez, 2006; Jurado, Garcia,
224 Timon, & Carrapiso, 2007; Sentandreu et al., 2003; Sforza et al., 2001; Sforza et al., 2006).

225 Glu and Asp are the most abundant free amino acids in fermented meats and their
226 concentration generally exceeds the taste threshold (Table 3) (Jurado et al., 2007). Pro, Ala, Val,
227 Ile, Leu, and Phe also increase to concentrations exceeding the taste threshold during ripening
228 (Kato et al., 1989). Lys, Tyr, Asp, Ala, and Glu were the most abundant free amino acids in the
229 ripening of Iberian ham and strongly influenced the taste of dry cured products (Careri et al.,
230 1993; Hughes et al., 2002; Toldra & Aristoy, 1993b).

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

240 3.4 Bread

241 Bread flavour is dependent on the activity of flour enzymes, and the metabolic activity of
242 yeasts and lactic acid bacteria (Gänzle, 2014). Sourdough fermented with lactic acid bacteria is
243 included in bread production to achieve dough leavening without baker's yeast, or as ingredient
244 to improve the texture, flavour, and storage life of yeast leavened bread (Brandt, 2007).
245 Fermentation microbiota in sourdoughs are process-specific; however, *L. sanfranciscensis*,
246 *L. plantarum*, and organisms of the *L. reuteri* group are key elements of sourdough microbiota

247 (Gänzle and Ripari, *in press*). In bread produced with yeasts as sole fermentation organisms
248 (straight dough processes), the concentration of amino acids including taste-active glutamate is
249 low because of the limited proteolysis at the dough stage, and the consumption of amino acids by
250 baker's yeast (Fujisawa & Yoshino, 1995; Gänzle et al., 2008; Johnson & Eldash, 1969, Zhao et
251 al., 2015). Sourdough fermentations which include lactic acid bacteria as fermentation
252 organisms, however provide suitable conditions for proteolytic conversion of cereal proteins, and
253 accumulate peptides and amino acids as flavour precursor compounds or taste-active compounds
254 (Gänzle et al., 2008; Thiele et al., 2002). Sourdough fermentations affect the concentrations of
255 the taste-active compounds glutamate and glutathione in a strain-specific manner. Glutamate
256 accumulated in sourdough fermentations to levels ranging from 27 to 120 mmol/kg; its
257 accumulation in sourdough depends on strain-specific glutamate decarboxylase and glutaminase
258 activities (Su et al., 2011; Stromeck et al., 2011; Vermeulen et al., 2007a). Glutamate generated
259 during sourdough fermentation significantly enhanced the umami taste of bread when glutamate
260 concentrations in bread exceeded the taste threshold of about 1 mmol / kg (Zhao et al., 2015).
261 The use of isogenic strains of *L. reuteri* expressing, or lacking, glutamate decarboxylase
262 minimized the influence of confounding factors on bread flavour (Su et al., 2011; Zhao et al.,
263 2015). Remarkably, the increased umami and sour taste intensities resulting from sourdough
264 addition were perceived as a higher intensity of saltiness by an untrained consumer panel (Zhao
265 et al., 2015). Taste active compounds produced during sourdough fermentation thus allow for
266 reduced salt levels in bread without compromising consumer acceptance (Zhao et al., 2015).

267 Wheat flour contains the kokumi active compound glutathione (Grosch & Wieser, 1999;
268 Ueda et al., 1997). At the dough stage, glutathione participates in thiol-disulfide exchange
269 reactions with gluten and integrates into the glutenin macropolymer (Grosch & Wieser., 1999;

270 Weegels et al.,1996). Glutathione levels in sourdough are influenced by glutathione reductase of
271 *L. sanfranciscensis* (Jänsch et al., 2007; Table 2). Glutathione concentrations in wheat dough,
272 however, are below the kokumi taste threshold of 300 $\mu\text{mol} / \text{kg}$ (Grosch & Wieser, 1999; Ueda
273 et al., 1997) and a contribution of glutathione to bread taste remains unclear.

274 **4 Interaction between taste active amino acids and peptides and salt**

275 NaCl tastes salty, masks metallic and bitter tastes, and enhances umami taste. Conversely,
276 amino acids and peptides may enhance the salty taste and thus allow reducing salt levels in food.
277 The salty taste of dry cured meat correlated to the concentrations of glutamate and aspartate
278 (Careri et al., 1993). Omission tests with cheese indicated that arginine at subthreshold
279 concentration significantly enhanced salty taste (Toelstede & Hofmann, 2008a; Toelstede et al.,
280 2009). Perception of salty and umami tastes is based on distinct receptors, however, the
281 simultaneous presence of umami and salty stimuli intensifies taste perception (Lioe et al., 2005).
282 Remarkably, the presence of bitter tasting aromatic amino acids at subthreshold levels also
283 enhanced the umami taste of soy sauce (Lioe et al., 2004). Subthreshold concentrations of taste
284 compounds can thus affect the intensity of other taste attributes (Lioe et al., 2005).

285 **5 Debitting in food fermentations**

286 Bitterness limits the acceptance and marketing of food and is of particular concern in cheese
287 production. Physical or chemical methods of debittering include the adsorption of bitter peptides
288 on suitable resins, their extraction, or rely on masking agents including cyclodextrin and poly- γ -
289 glutamate (Ley, 2008, Saha & Hayashi, 2001). Enzymatic debittering is achieved with
290 transglutaminase or peptidases (FitzGerald & O'Cuinn, 2006). Unbalanced proteolysis is

291 responsible for the formation of bitter peptides in food fermentations and reduction of bitter
292 peptides require enzymatic hydrolysis into products that lack bitter taste (Broadbent & Steele,
293 2007). Starter cultures combining a low propensity for the production of bitter peptides with a
294 high activity of debittering peptidases reduce bitterness in cheese (Broadbent et al., 2002). The
295 bitterness of casein hydrolysates was significantly reduced in the presence of amino peptidases
296 and post-proline dipeptidyl aminopeptidases (PPDA), which release amino acyl proline residues
297 from the N-terminus (Bouchier et al., 2001). Cell free extracts of different *Lc. lactis* were used
298 for debittering peptides due to the activity of PepXP, which can cleave the X-Pro-Y peptide bond
299 and liberate X-Pro from bitter peptides (Shimamura et al., 2009). In cheese, *L. helveticus* had a
300 significant debittering effect that was attributed to prolyl endopeptidases, such as PepO₂, PepO₃,
301 and PepF (Sridhar et al., 2005). The aminopeptidase PepN converts Pro-containing bitter
302 peptides into smaller peptides and amino acids (Broadbent & Steele, 2007).

303 An alternative strategy is to convert bitter amino acids into γ -glutamyl derivatives through
304 GGT. This approach not only reduces the concentration of bitter tasting amino acids Phe, Val,
305 Leu and His (Suzuki et al., 2002; Suzuki & Kumagai, 2004b; Suzuki et al., 2004a) but also
306 generates γ -glutamyl peptides with kokumi activity or umami taste that suppress the bitterness
307 and hence increase consumer preference (Son et al., 2015; Suzuki et al., 2002). Five
308 representative umami peptides suppressed salicin-induced intracellular calcium influx in a non-
309 competitive manner. α Glu-Glu at 1 mM was the most effective inhibitor of salicin-induced
310 intracellular Ca²⁺ response and hence inhibited the bitter taste sensation (Kim et al., 2015).
311 Glutamate with adenosine monophosphate or sodium salts of 5'-ribonucleotides as well as the
312 umami tasting peptides α Glu-Asp, α Glu-Glu, α Glu-Ser, and α Glu-Glu-Glu also suppressed the
313 bitter taste of peptides (Kemp & Beauchamp, 1994; Tokita & Boughter, 2012). In conclusion,

314 debittering in food fermentations is achieved by a combination of peptidase activity to reduce the
315 concentration of bitter peptides, and the accumulation of glutamate and related umami-tasting or
316 kokumi-active compounds that moderate the bitter taste impression.

317 **6 Conclusions and perspectives**

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

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

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674

675 **Figure legends.**

676 **Figure 1.** Overview on the generation of taste compounds from proteins during food
677 fermentation. Proteolysis generates taste active peptides and amino acids; glutathione reductase
678 generates the kokum-active glutathione. Further conversion of peptides or amino acids to taste
679 active compounds proceeds by enzymatic reactions. Enzymatic conversions were proposed to be
680 catalysed by lactoyl-transferase [2], succinyl transferase [3], pyroglutamyl cyclase [4], or by γ -
681 glutamyl-transferase [5]. Maillard / Amadori products [6] are formed by chemical conversion
682 during heating.

Table 1. Contributors to proteolysis in cheese, soy, meat, and sourdough fermentations.

	Primary proteolysis	Secondary proteolysis	Conversion of amino acids	Ref.
Cheese	Lactic acid bacteria (LAB) (surface microbiota)	LAB	<i>Penicillium</i> spp., LAB	(Khalid & Marth, 1990)
Soy sauce	<i>Aspergillus</i> spp.	LAB	<i>Aspergillus</i> , LAB	(Lioe et al., 2010)
Bread	Cereal enzymes (fungal or malt protease)	LAB	LAB	(Thiele et al., 2002; Gänzle et al., 2008; Gänzle et al., 2009)
Meat	Muscle enzymes; (surface microbiota)	LAB, staphylococci, (surface microbiota)	LAB, staphylococci, (surface microbiota)	(Benito et al., 2002; Benito et al., 2003; Freiding et al., 2012; Hughes et al., 2002; Sinz et al., 2013; Toldra et al., 1993a)

Table 2. Enzymes with putative or known contribution to the formation of taste active peptides or amino acids in fermented foods.

Enzyme	Microorganisms	Food	Reference
Glutaminase	<i>L. reuteri</i> , <i>Aspergillus sojae</i>	Sourdough, soy sauce	(Ito et al., 2013; Lioe et al., 2010; Teixeira et al., 2014)
Glutamate decarboxylase	<i>L. brevis</i> , <i>L. reuteri</i>	Cheese, sourdough	(Su et al., 2011; Teixeira et al., 2014)
γ -Glutamyl-transferase	<i>Aspergillus</i> spp., <i>Penicillium</i> spp., <i>Bacillus subtilis</i>	Cheese, soy sauce	(Minami et al., 2003a; Minami et al., 2003b)
Glutathione reductase	<i>L. sanfranciscensis</i>	Sourdough	(Jänsch et al., 2007)
Succinyl transferase	<i>Aspergillus</i> spp.	Soy sauce	(Frerot & Chen, 2013)
Pyroglutamyl cyclase	<i>L. helveticus</i> , <i>L. delbruechii</i> , <i>S. thermophilus</i>	Soy sauce, meat product	(Altamura, et al., 1970; Mucchetti et al., 2002)
Putative lactoyl transferase	<i>Lactobacillus</i> spp.	Soy sauce, cheese, meat product	(Sgarbi et al., 2013)

1 **Table 3.** Amino acids or peptides with sweet, umami taste or with kokumi taste activity that
 2 were isolated from fermented foods.

Compound ^{a)} , threshold (mM)		Source (ref)	Compound, threshold (mM)		Source
Sweet Taste					
Met	5	Cheese, soy sauce (3)	pGlu-Gly	2.2	Soy sauce (4)
Ala	6.7	Bread, soy, meat (1, 2)	Ile-Gln	<5.5	Soy sauce (4)
Gly	25	Cheese, soy sauce (3)	Leu-Gln	<5.5	Soy sauce (4)
Pro	25	Cheese, bread, meat (3)	Thr-Phe	<5.5	Soy sauce (4)
Ser	25	Cheese, soy sauce (3)	Ile-Glu	<5.5	Soy sauce (4)
pGlu-Gln	1.9	Soy sauce (4)	Pro-Lys	<5.5	Soy sauce (4)
Umami taste					
Glu	1.1, 0.3	Cheese, bread, soy sauce, meat (1, 3, 7, 8)	Asp-Glu-Ser	8.6	Fish (12)
Tyr	4	Tea (9-11)	Glu-Gln-Glu	7.4	Fish (12)
Asp	6.4	Soy sauce, cheese, bread, meat (5, 6)	Thr-Glu	12.1	Fish (12)
Lactoyl-glutamine	5	Cheese, soy sauce, meat (11)	Ser-Glu-Glu	5.5	Fish (12)
Glu-Glu	5.4	Fish (12)	Fru-Glu	0.8	Soy sauc (4) e
Glu-Gly-Ser	6.9	Fish (12)	N-glucosyl-Glu	1.6	Soy sauce (15)
Glu-Asp	7.6	Fish (12)	KGNEESLA	0.5	Meat (13, 14)
Glu-Asp-Glu	7.6	Fish (12)	γ -glutamyl-ethylamide	24	Tea (15)
Glu-Ser	8.5	Fish (12)	N-deoxyfructosyl-Glu	1.8	Soy sauce (15)
Kokumi activity					
γ -Glu-Glu ^{b)}	0.018	Cheese, bread (16, 17)	γ -Glu-Gln	0.008 ^a	Cheese (16, 17)
γ -Glu-Phe	2.5	Soy sauce 16, 18	γ -Glu-Val	0.003 ^a	Cheese (16, 17)
γ -Glu-Gly ^{b)}	0.018	Cheese, meat, soy sauce	γ -Glu-Tyr ^{b)}	2.5	Cheese, soy, meat ((16, 17, 19)
γ -Glu-His	0.01	Cheese (16, 17)	γ -Glu-Ala		(20)
γ -Glu-Leu	0.005	Cheese (16 – 18)	γ -Glu-Cys-Gly	0.3	Sourdough (21)
γ -Glu-Met	0.005	Cheese (16, 17)	γ -Glu-Val-Gly	0.3	Soy sauce (22)

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

5 ^{b)} The taste threshold was measured only in cheese.

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

13

14 **Table 4.** Bitter amino acids and peptides in fermented foods

Compound ^a	Threshold (mM)	Source (ref)	Compound	Threshold (mM)	Source
His	1.2	Cheese, sausage (1, 2)	Glu-Trp	5	Cheese (9, 10, 11)
Lys	3.4	Soy sauce, meat (4, 5)	Glu-Tyr	5	Cheese (9, 10, 11)
Val	3.4	Cheese, sausage (1, 2, 4)	YPFPGPIHNS	0.05 ^a	Cheese (12)
Try	4	Cheese (1)	SLVYPFPGPIHNS	0.06 ^a	Cheese (12)
Tyr	4	Cheese (1)	LVYPFPGPIHN	0.08 ^a	Cheese (12)
Phe	4, 5	Cheese, soy sauce, sausage (1, 2, 3, 6)	YPFPGPIHN	0.1 ^a	Cheese (12)
Ile	10	Cheese, sausage (1, 2, 3)	VYPFPGPIP	0.17 ^a	Cheese (12)
Leu	11	Cheese, sausage (1)	YQQPVLGPVRGPFPIIV	0.18 ^a	Cheese (12)
Met-Ile	0.42	Cheese (8)	YPFPGPIP	0.33 ^a	Cheese (12)
Fru-Met	1.6	Soy sauce (7)	YPFPGPIPNS	0.33 ^a	Cheese (12)
Fru-Val	1.8	Soy sauce (7)	VRGPF	0.42 ^a	Cheese (12)
Glu-Gly	2.5	Cheese (10, 11)	EIVPN	0.43 ^a	Cheese (12)
Glu-Thr	2.5	Cheese (9, 10, 11)	DIKQM	0.6 ^a	Cheese (1)
Fru-pGlu	2.6	Soy sauce (7)	LPQE	0.6 ^a	Cheese (1)
Glu-Val	5	Cheese (9, 10, 11)	GPVRGPF	1.18 ^a	Cheese (12)
Glu-Ala	10	Cheese (9, 10, 11)			

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

17 1, Toelstede & Hofmann, 2008a; 2, Henriksen & Stahnke, 1997; 3, Hughes et al., 2002; 4,
 18 Kato, Rhue, & Nishimura, 1989; 5, Toldra & Aristoy, 1993b; 6, Sgarbi et al., 2013; 7, Kaneko et
 19 al., 2011; 8, Toelstede & Hofmann, 2008b; 9, Roudotalgaron et al., 1994a; 10, Toelstede &
 20 Hofmann, 2009; 11, Toelstede et al., 2009; 12, Toelstede & Hofmann, 2008b.

21 .

22 **Figure 1.**

