CHEMICAL REVIEWS

REVIEW

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Chemical Modifications of Peptides and Their Impact on Food Properties

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CONTENTS

1. INTRODUCTION

During food storage and food processing, many different chemical reactions between the various ingredients proceed simultaneously. These reactions alter the characteristics of the foodstuff, such as nutritional, physical, toxicological, and sensory characteristics. Among others, amino acids, peptides, and proteins are subjected to modifications, because they contain reactive groups such as free amino, carbonyl, and sulfur-containing functional groups. Although modifications of amino acids and proteins have been studied and reviewed extensively, information on the alteration of peptides is very dispersed. However, peptides are very important in food, because they influence the functional properties, affect the product taste, and exhibit biological activity.[1] Peptides are widespread in nature and occur naturally in traditional foods.[2,3] In addition, peptides can also be added to foods to obtain the required properties. However, several authors already pointed out that possible adverse effects should be taken into account.[3−5] It is advisible to perform toxicity, cytotoxicity, and allergenicity studies before adding specific peptides to food products.

Naturally, modification of peptides during food storage or food processing alters their properties. In addition, peptide-specific degradation products can be formed that influence the characteristics of food products. To gain insight in the different reactions to which peptides are subjected, to highlight ambiguities and critical points, and to trigger additional research in this field, this review is presenting an overview of the chemical reactions that induce alteration of peptides. Although peptide modifications can be accomplished by chemical and/or enzymatic reactions, this review will only focus on the chemical reactions that can occur in food matrices. This implies that mainly studies in which the experimental setup was chosen to simulate food conditions will be handled. However, some studies dealing with modifications of peptides in physiological conditions (37 °C) will also be included, as these conditions can be relevant for food storage. The importance of peptides in food, the degradation of peptides without intervention of other reactive species, reactions between peptides and carbonyl compounds (sugars, lipids, degradation products, and other carbonyl compounds), reactions between peptides and inorganic additives, and the prevention of peptide modification will be addressed successively. Both degradation of starting products and formation of end products, as well as possible implications for the properties of food products, will be dealt with. Reactions of protein side chains have been studied extensively, and naturally, similar reactions can occur at peptide side chains. However, in this review, only reactions that have been described effectively for peptides will be treated, instead of giving an overview of hypothetical compound formation.

Peptides containing up to 100 amino acids will be handled in this review, as amino acid chains of more than 100 residues are commonly referred to as proteins.[2] By convention, the structure of a peptide is always drawn with the amino acid residue with the

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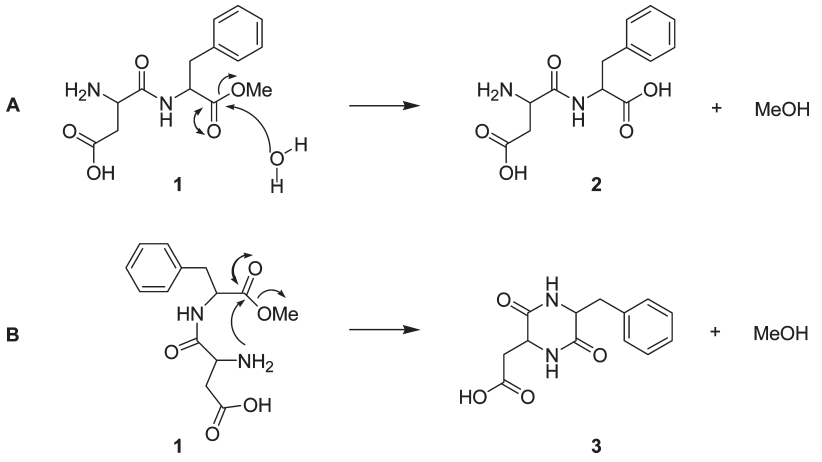
Scheme 1. Degradation of Aspartame 1 Due to (A) Hydrolysis of the Ester Linkage Resulting in the Formation of the Corresponding Carboxylic Acid 2 or (B) Cyclization to Produce the Corresponding Diketopiperazine 3



free amino group positioned on the left.[2] The amino acids of the chain ends are denoted as *N*-terminal and *C*-terminal amino acids. This notation will also be used throughout this review. In addition, chirality of the molecules is not represented in the schemes and figures because, first, in many cases the chirality of the starting products was not specified in the corresponding publications and, second, the chirality of the starting products might not be conserved due to high processing temperatures. Because of the rather innovativeness of the subject, this review mainly covers literature from the last two decades.

2. IMPORTANCE OF PEPTIDES IN FOOD

Initially, the discovery of oligopeptides as products of protein hydrolysis did not draw much attention. However, nowadays, peptides are highly valued in food because they influence the functional properties, affect the product taste, and exhibit biological activity.[1] Functional properties of peptides include solubilizing, emulsifying, gelling, and foaming properties.[5] Concerning taste properties, bitter-tasting peptides are described most extensively, while in fact, peptides may reveal all kinds of taste. Sweet, sour, salty, and umami tastes have also been reported.[1,5,6] Besides these five basic tastes, Japanese distinguish "kokumi", which has been described as continuity, mounthfulness, and thickness of taste. According to Ueda et al.,[7] the peptide glutathione increases the kokumi flavor of umami solutions and a model beef extract. In addition, interaction of these different tastes can result in the formation of typical food flavors. For instance, the dry-cured ham flavor is very characteristic and, besides volatile compounds and amino acids, also peptides have been shown to contribute to this specific flavor.[8−10]

The biological activity of peptides has been the subject of many studies, which have been compiled in multiple reviews. Biological activities include antimicrobial,[3,5,11,12] antioxidant,[3,12−15] antihypertensive (angiotensin converting enzyme [ACE] inhibitory),[3,12,13,16] antithrombotic,[3,13] hypocholesterolemic and hypotriglyceridemic,[3,13] antiobesity,[13] opioid agonist or antagonist,[3,5,12] immunomodulating,[3,5,12] cytomodulating,[3] mineral sequestering,[3,12] cytotoxic,[4] and allergenic[4,5] properties. Some peptides are multifunctional and can exert more than one of the effects mentioned.[3,13] Although most of the food peptides are degraded by the host during ingestion, digestion, and absorption, Sato et al.[17] demonstrated that some unmodified food-derived peptides are present in human blood.

3. DEGRADATION OF PEPTIDES WITHOUT THE INTERVENTION OF OTHER REACTIVE SPECIES

The degradation of peptides is, like any other chemical reaction, highly dependent on the reaction conditions. In 1997, a review concerning peptide stability in solids and solutions was published.[18] Temperature, buffer type and concentration, pH, and water activity were reported to influence the stability of peptides. In what follows, a short overview of these influential conditions will be given.

Temperature can exert an effect on the degradation of peptides. Aspartame **1** (Asp-Phe methyl ester), for example, is unstable at high temperatures. Under these conditions, the ester linkage of this intense sweetener may hydrolyze to the corresponding carboxylic acid **2** or cyclize to produce the corresponding diketopiperazine **3** (DKP) (Scheme 1), neither of which is sweet.[6] On the other hand, Beefy Meaty Peptide (BMP), an octapeptide (Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala) with desirable sensory properties identified in meat, was found to be relatively heat-stable.[19] Under pasteurization and sterilization conditions, respectively, 97.7% and 97.2% of the peptide remained unchanged. The authors suggested that the linearity of BMP and the peptide composition are responsible for this observation. As correctly pointed out by these authors, heat stability of peptides is also determined by the heat stability of the composing amino acids.

The stability of peptides is also influenced by the buffer type and concentration in which the peptide is stored. According to Bell,[18] this effect is largely due to peptides' susceptibility to acid−base-catalyzed reactions. It has been demonstrated by Bell and Wetzel[20] that, in a 0.1 M phosphate buffer at pH 3, catalysis by buffer components contributed to more than half of the observed aspartame degradation rate constant. In a 0.1 M phosphate buffer at pH 7, the contribution of buffer catalysis to the aspartame degradation rate constant increased to >98%. The rate of aspartame degradation also increased with increasing buffer concentration. Concerning the buffer type, Bell and Wetzel[20] also showed that phosphate buffers significantly enhanced the

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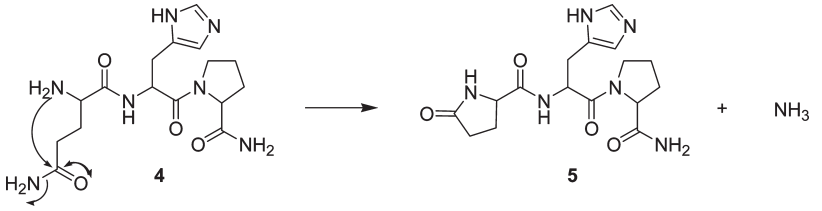
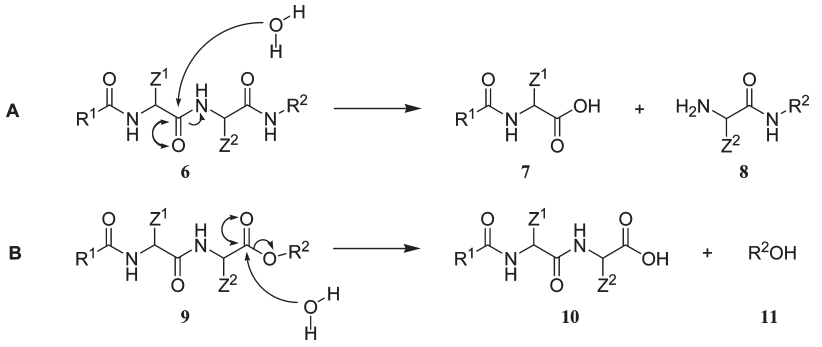
Scheme 2. Cyclization of Glutamine at the *N*-Terminus of Gln-His-Pro-NH_2 4, Yielding the Corresponding Tripeptide with Pyroglutamic Acid at the *N*-Terminus 5



Scheme 3. Hydrolysis of (A) a Peptide Bond and (B) an Ester Bond in a Peptide Ester



degradation rate of aspartame as compared to citrate buffers. The authors suggested that the differing ability of the buffers to accept and donate protons may explain the differences in reaction rate. Similar results were found for a hexapeptide (Val-Tyr-Pro-Asn-Gly-Ala) by Patel and Borchardt.[21]

In combination with the buffer type and buffer concentration, also the pH of the matrix influences the stability of peptides. According to Kato et al.,[6] aspartame is stable at pH 4 and unstable at pH 1 or 7−8. Wright[22] reported that the stability of Asn and Gln in peptides was the highest around pH 6. At this pH, the rate of ammonia loss from the side chains of Asn or Gln was the lowest. However, we would like to stress that comparison of the stability of peptides at different pH values is only possible if identical buffer types and concentrations are used.

Water activity also has a potential effect on the degradation of peptides. It has been shown that increasing residual moisture increased the degradation rate of the hexapeptide Val-Tyr-Pro-Asn-Gly-Ala.[23] Water activity has been pointed out to correlate with reaction rates.[24] In addition, water in food often acts as a plasticizer, leading to enhanced mobility and chemical reactivity as well.[25]

More recently, additional studies on the stability of peptides were performed and also high-pressure application, a promising technique in the preservation and processing of food, was pointed out to affect the stability of peptides. In milk (pH 6.8), already after 3 min of pressure treatment at 600 MPa and 60 °C about 50% of aspartame was lost, whereas thermal treatment at atmospheric pressure and 60 °C resulted in only 3% loss of aspartame.[26] In Tris buffer model systems at pH 7 and lower, the effect was smaller, but pressure-induced degradation of aspartame **1** was clearly present.[26] Similar as that due to thermal treatment, degradation of this intense sweetener under high-pressure application leads to the formation of linear **2** and cyclic **3** aspartylphenylalanine (Scheme 1). Both degradation products are not sweet, and therefore, high-pressure application of aspartame-containing food products will probably decrease their sweetness. Similar results were reported for leucylleucine methyl ester.[27] Decomposition of this dipeptide into Leu-Leu and the diketopiperazine of Leu-Leu increased with increasing pressure intensity at 60 °C.

Fernandez Garcia et al.[28] demonstrated that the application of heat alone, and of heat accompanied by high pressure, greatly accelerates the degradation of peptides such as Gln-Leu-Pro-NH_2 **4**, yielding the corresponding tripeptide with cyclized glutamine (pyroglutamic acid) at the *N*-terminus **5** (Scheme 2). According to these authors, the principle of Le Chatelier causes this cyclization under hyperbaric conditions, as a process associated with a decrease in volume will be favored by high pressure. The resulting peptides with pyroglutamic acid at the *N*-terminus usually have an endocrine and/or regulative function in the body and influence the synthesis pathways of important hormones.[28]

In addition, it has recently been shown that the molecular mass of peptides also influences their stability. According to Lan et al.,[29] no apparent degradation of soybean peptides below 1000 Da could be detected after thermal treatment at 130 °C for 2 h. In contrast, soybean peptides between 1000 and 5000 Da and mainly peptides above 5000 Da degraded during thermal treatment above 100 °C. It is not surprising that larger peptides are more susceptible to degradation, because they are more likely to contain a weak spot.

In general, low temperature, low pressure, low buffer concentrations, low water activity, and low molecular mass enhance the stability of peptides. Concerning the pH, it seems that the pH at which maximum stability is reached strongly depends on the nature of the peptide, which obviously determines the possible mechanisms of degradation. Different degradation mechanisms respond in another way to varying reaction

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conditions. In what follows, several degradation mechanisms reported for peptides will be discussed.

3.1. Peptide Chain Cleavage

Peptide chain cleavage results in the formation of free amino acids or smaller peptides. It has been reported that cleavage of long peptides and proteins enhances their solubility and increases the emulsification, foaming, and gelling properties of food products.[5] Several mechanisms have been described to cause cleavage of the peptide chain.

3.1.1. Peptide Hydrolysis. A first mechanism that induces peptide chain cleavage is acid−base-catalyzed hydrolysis. Hydrolysis takes place when water or a hydroxyl ion attacks the carbonyl group of an amide moiety of the peptide **6** (Scheme 3A), as opposed to other mechanisms of peptide cleavage (cf. below). It is important to assess the extent to which peptide hydrolysis occurs, as this allows one to discriminate true peptide reactivity as opposed to the reactivity of free amino acids after peptide hydrolysis. Hayase et al.[30] reported the hydrolysis of several tripeptides (Glu-Ala-Pro, Leu-Gly-Phe, and Pro-Phe-Lys) under dry roasting conditions (150−270 °C for 20 min), but yields of the resulting free amino acids and dipeptides were not mentioned. On the contrary, de Kok and Rosing[31] stated that peptides are rather stable compounds that do not hydrolyze in the absence of sugars during heating at 100 °C for 6 h. To the best of our knowledge, no other authors have reported the chemical hydrolysis of the peptide bond in the absence of other reactive species in food-simulating reaction conditions.

Because standard procedures for hydrolyzing peptide bonds in peptides and proteins concern extreme acidic treatments (6 N HCl) at high temperature for periods up to 24 h, we tend to believe that peptide hydrolysis, as such, is almost negligible in food matrices. We assume that intramolecular rearrangements, which will be discussed in section 3.1.2, are more important. Although it has been demonstrated that microwave heating leads to complete hydrolysis of peptides in only a few minutes,[32−34] more research is needed on the actual hydrolysis of peptides in food systems, because reported microwave hydrolysis methods also require strong acidic conditions (3 or 6 N HCl), which are mostly not present in food matrices.

In the case of peptide methyl esters **9** ($R^2 = CH_3$), such as aspartame **1**, hydrolysis can also take place at the ester bond (Scheme 3B). As already described earlier, the ester linkage of aspartame hydrolyses at high temperatures.[6] Upon application of high pressure, the degradation of aspartame[26] and leucylleucine methyl ester[27] also resulted, next to other degradation products, in the corresponding dipeptide.

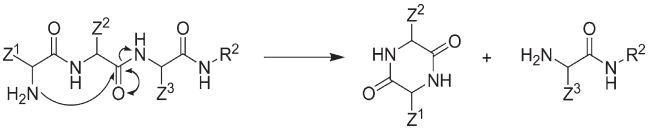
3.1.2. Intramolecular Cyclizations in the Peptide Chain. Because of an intramolecular cyclization of the *N*-terminal and neighboring amino acid, diketopiperazines **13** (DKPs) can be formed. Consequently, cleavage of the peptide chain takes place between the second and third amino acid (Scheme 4).

As mentioned in section 3, diketopiperazines have been reported to be produced during high-pressure treatment of aspartame[26] and leucylleucine methyl ester.[27] Also at ambient pressure, the cyclic Asp-Phe was formed from aspartame during heat treatment at 30 °C.[18] Rizzi[35] also described the formation of diketopiperazines during reflux of several di- and tripeptides in acetic acid (120 °C). Formation of diketopiperazines from dipeptides was much higher than from tripeptides.[35] This can be explained by the reduced steric hindrance in reactions with dipeptides. Obviously, formation of diketopiperazines from dipeptides does not lead to peptide chain cleavage. Hayase et al.[30] did not detect the formation of diketopiperazines from several tripeptides under dry roasting conditions, although cyclic Gly-Gly was formed from polyglycine under similar reaction conditions.[30] Diketopiperazine formation is relevant for food systems because these compounds possess a bitter taste due to their hydrophobicity. The formation of diketopiperazines has also been reported from the reactions of peptides with other reactive species, such as carbohydrates. This will be addressed in the corresponding section (section 4.1.3).

Intramolecular cyclization, which leads to peptide chain cleavage, can also occur at the *C*-terminus of an asparagine residue.[36,37] As can be seen in Scheme 5A, the side-chain amido NH_2-group of asparagine attacks its own main peptide chain carbonyl carbon, resulting in peptide cleavage and the formation of 3-(acylamino)pyrrolidine-2,5-dione **16** at the asparagine moiety.

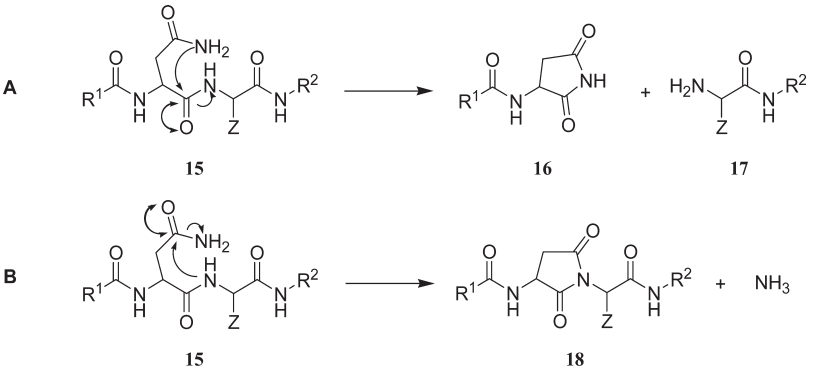
This intramolecular cyclization was observed when peptides containing Asn-Leu and Asn-Pro subunits were incubated at

Scheme 4. Proposed Formation Mechanism of Diketopiperazines 13 (DKPs) from a Peptide 12



12 13 14

Scheme 5. Mechanistic Pathway of Intramolecular Cyclization in Asparagine-Containing Peptides 15 Resulting in (A) Peptide Chain Cleavage or (B) Backbone Modification and Ammonia Release



15 16 17 (A)

15 18 (B)

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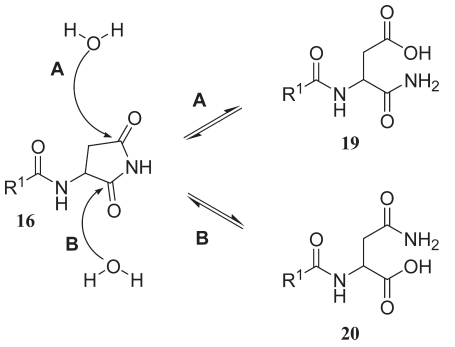
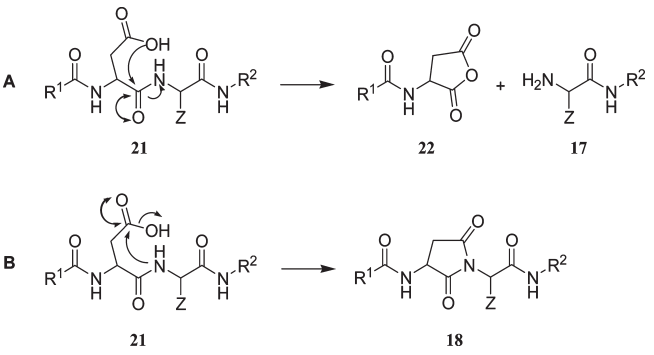
100 °C in a phosphate buffer at pH 7.4.[36] Similar peptide cleavage was not observed for peptides containing Asn-Gly subunits. According to the authors, in this specific case, the peptide-bound NH-group of glycine is more likely to attack the carbonyl carbon of the asparagine side chain due to less steric hindrance (Scheme 5B). This alternative pathway leads to a modification of the peptide backbone, but not to cleavage. However, the authors did not attempt to isolate this compound. In accordance with the results of Geiger and Clarke,[36] peptide cleavage following Scheme 5A was not observed for Asn-Gly-containing peptides, but all peptides tested were modified via pathway B in Scheme 5. These results suggest that, in Asn-Gly-containing peptides only pathway B takes place, whereas all other peptides containing asparagine can be modified via both pathways A and B. Patel and Borchardt[38] also reported peptide cleavage for Asn-Ser- and Asn-Val-containing peptides. Although the authors did not detect the 3-(acylamino)pyrrolidine-2,5-dione **16** but only its hydrolysis products **19** and **20** shown in Scheme 6, they also suggested pathway A in Scheme 5 as the actual reaction mechanism. Spontaneous cleavage of the peptide bond in α-Crystallin after the asparagine residue has also been demonstrated by Voorter et al.[37]

Scheme 6. Hydrolysis of the 3-(Acylamino)pyrrolidine-2, 5-dione



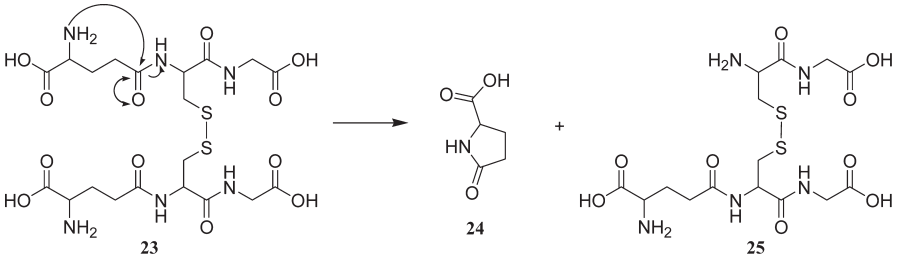
Similar intramolecular cyclizations, leading to peptide cleavage at the *C*-terminus of aspartic acid instead of asparagine residues, have been demonstrated under acidic conditions (Scheme 7). At 37 °C under acidic aqueous conditions, the major route of degradation of an Asp-Gly-containing hexapeptide was intramolecular cleavage of the Asp-Gly amide bond (Scheme 7A), whereas under basic conditions, the peptide-bound NH-group of glycine was more likely to attack the aspartic acid side chain, as in Scheme 7B.[39] According to the authors, the latter reaction involves nucleophilic attack by the deprotonated peptide amide nitrogen across the free carboxylic acid. Thus, as the pH of the solution is increased, the equilibrium concentration of the deprotonated amide increases, favoring the formation of the pyrrolidine-2,5-dione **18**. However, we tend to believe that deprotonation of the peptide amide nitrogen is not necessary for its nucleophilic properties and is rather slow. Deprotonation of the side-chain carboxylic acid will occur much faster. Therefore, we suggest that the difference between acidic and basic conditions is due to the higher reactivity of the nonprotonated peptide amide nitrogen under basic conditions as compared to the protonated peptide amide nitrogen under acidic conditions. In addition, it has also been shown that hydrolysis of the Asp-Gly peptide bond of this hexapeptide was suppressed in lyophilized samples.[23] Because dipeptides containing an aspartic acid residue exhibit a sour taste accompanied by bitterness,[5] this intramolecular cyclization can modify the flavor characteristics of food products.

Scheme 7. Proposed Mechanistic Pathway of Intramolecular Cyclization in Aspartic Acid-Containing Peptides 21 Resulting in (A) Peptide Chain Cleavage or (B) Backbone Modification



Although similar reactions can occur in glutamine and glutamic acid-containing peptides, this has not been reported yet to the best of our knowledge. Additional experiments should be performed to elucidate this, but it is possible that the greater distance between the main-chain and the side-chain amide for glutamine or acid for glutamic acid as compared to asparagine or aspartic acid, respectively, decreases the reaction rate. Then again, cyclization of the γ-linked glutamic acid at the *N*-terminus of oxidized glutathione **23** (GSSG) has been suggested, because both pyroglutamic acid **24** and oxidized glutathione, missing one of the *N*-terminal glutamic acid residues **25**, were detected from glutathione incubated in water for 5 days at 50 °C.[40] However, the reaction mechanism is somewhat different in this case, because the primary amino group of glutamic acid, instead of the side-chain amide, attacks the main chain of the peptide (Scheme 8).

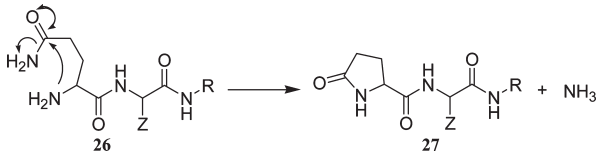
Scheme 8. Proposed Mechanism for the Formation of Pyroglutamic Acid 24 from Oxidized Glutathione 23



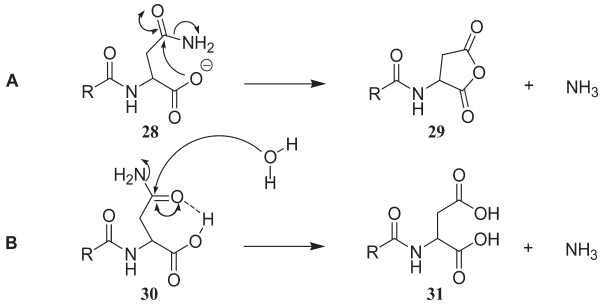
3.2. Peptide Backbone Modifications

Besides chain cleavage, alteration of the peptide structure can also arise from backbone modifications. Several mechanisms, in which ammonia is released from the side chains of peptide-bound asparagine or glutamine, lead to backbone modifications. In literature, these modifications are referred to as "deamidation". To provide a complete overview of the spontaneous chemical reactions affecting peptides in food systems, these deamidation mechanisms are briefly summarized here. For further information, the reader is referred to two extensive reviews on the subject.[22,41] The first two pathways of deamidation, leading to backbone modification, take place at the peptide termini. Amino-terminal glutamine **26** readily deamidates by cyclization with its own terminal amino group to form peptide-bound pyroglutamic acid **27** (Scheme 9). It has been shown that this cyclization of the *N*-terminal glutamine residue is accelerated by the application of elevated temperature and pressure.[28] As already mentioned in section 3, peptides with pyroglutamic acid at the *N*-terminus have been reported to possess hormone-like properties.[28]

Scheme 9. Mechanistic Pathway for the Cyclization of Amino-Terminal Glutamine 26 to Form Peptide-Bound Pyroglutamic Acid 27



Scheme 10. Mechanisms for the Deamidation of Carboxy-Terminal Asparagine



Cyclization does not occur at the amino-terminal asparagine because of the unfavorable size of the resulting ring structure (4-membered ring). On the contrary, at the carboxyl terminus, asparagine does undergo deamidation. Two possible mechanisms have been proposed for this reaction. A first hypothesis concerns a nucleophilic attack of the terminal carboxylate to form anhydride **29**[22] (Scheme 10A). Alternatively, it has been suggested that, under acidic conditions, the protonated terminal carboxylate group stabilizes the side chain of asparagine by hydrogen bonding in a conformation that is susceptible to the attack of a solvent nucleophile[22] (Scheme 10B). In fact, this mechanism leads to side-chain, instead of backbone, modification.

All these deamidation reactions at the termini of peptides are regulated by general acid−base catalysis. Another mechanism of deamidation, which alters the peptide backbone, is the so-called "β-aspartyl shift" mechanism. This mechanism has already been briefly introduced in section 3.1.2 (Scheme 5B). The peptide nitrogen of the residue at the *C*-terminus of asparagine (preferably glycine) functions as a nucleophile toward the side-chain amide group of asparagine. The resulting pyrrolidine-2,5-dione intermediate **18** can break down by two pathways, yielding an α-linked peptide **32** or a β-linked peptide **33** (Scheme 11). This β-form is referred to as an "isopeptide" and has been detected in several studies.[21,36−38]

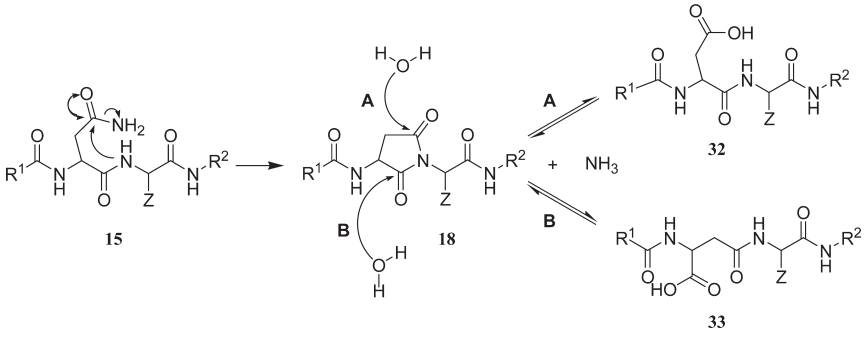
Recently, it has been shown that, for peptides containing the *N*-terminal X-Asn-Gly unit **34**, the pyrrolidine-2,5-dione intermediate **35** can also undergo an *N*-terminal cyclization reaction, resulting in the formation of a six-membered **36** or a seven-membered ring **37** (Scheme 12).[42] This peptide backbone modification was formed during purification of peptides originating from a tryptic bovine serum albumin (BSA) digest. Up to now, it has not been detected in food systems.

Obviously, during all these deamidation reactions ammonia is released. In combination with other food constituents or decomposition products, free ammonia can contribute to the formation of (desirable or undesirable) color and flavor.[41]

As already mentioned in section 3.1.2, similar reactions can take place with peptides containing aspartic acid (Scheme 7), but in this case no ammonia is released. For these peptides, formation of the backbone pyrrolidine-2,5-dione **18** (Scheme 7B) dominated cleavage of the peptide chain (Scheme 7A) under basic aqueous[39] or solid-state[23] reaction conditions.

Beside deamidation reactions, another pathway for backbone modification was postulated by Paulus et al.[43] According to these authors, intramolecular cyclization of cysteine,

Scheme 11. Mechanism for the Cyclization of Peptides Containing Asparagine 15 by the β-Aspartyl Shift Mechanism; The Pyrolidine-2,5-Dione Intermediate 18 Breaks Down to Form Either the α-Linked 32 or β-Linked 33 Peptide



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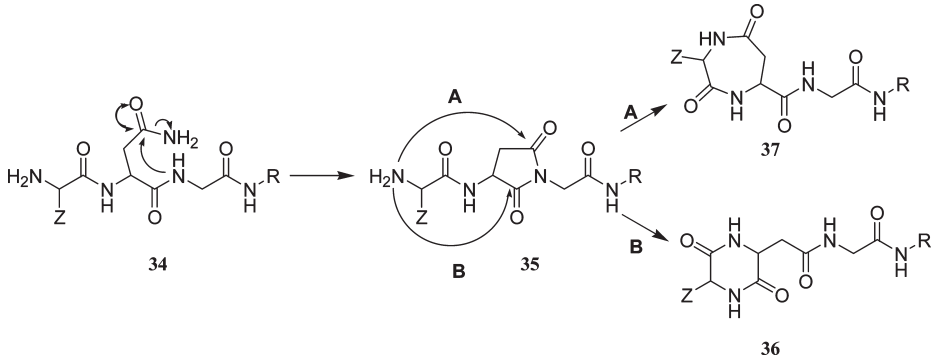
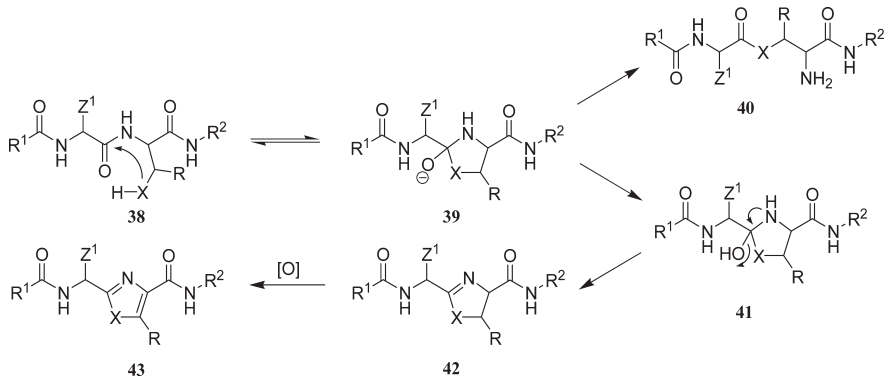
Scheme 12. Mechanism for the *N*-Terminal Cyclization in Peptides Containing an *N*-Terminal X-Asn-Gly Unit 34, after the Formation of the Pyrrolidine-2,5-dione Intermediate 35



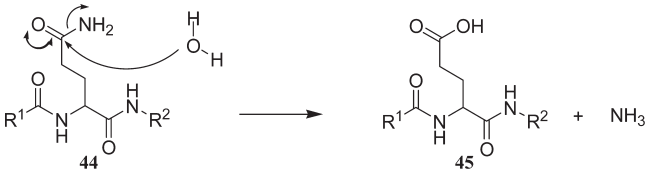
Scheme 13. Postulated Mechanism for the Formation of Thiazolines, Oxazolines, or Imidazolines (42) and Thiazoles, Oxazoles, or Imidazoles (43) As Protein Backbone Modifications; X = O, S, or NH; R = Either H (Serine, Cysteine, or β-Aminoalanine) or CH_3 (Threonine)



serine/threonine, or β-aminoalanine residues **38** results in the formation of thiazolines and thiazoles, oxazolines and oxazoles, or imidazolines and imidazoles, respectively, due to the attack of the side-chain heteroatom on the main-chain carbonyl group (Scheme 13). As visualized in Scheme 13, compound **39** is also an intermediate for the formation of an additional type of backbone modification **40**.

The formation of 2-methyl-4-carboxythiazoline from *N*-acetylcysteine at 60 °C has been demonstrated.[43] The highest concentrations were measured in strongly acidic media and at high water activity. However, under these conditions the obtained yields were <5%. On the basis of UV absorption measurements, similar substituted thiazolines were detected from Gly-Gly-Cys, Gly-Cys-Gly, and glutathione. Paulus et al.[43] also mentioned that oxazolines are rather labile compounds, which have only been found in a few natural compounds. Then again, the formation of imidazolines from N^{α}-acetyl-β-aminoalanine and longer peptides containing this amino acid has been demonstrated.[44] At 60 °C the highest conversion rate was measured at the highest pH (pH 9). With increasing temperature (80 and 100 °C), the maximum concentration was reached within a shorter reaction period and the formation of the imidazolines was favored at lower pH (conversion rates up to 30%). From these studies, it can be seen that only thiazolines, oxazolines, and imidazolines were detected

Scheme 14. General Mechanism of Deamidation of the Glutamine Side Chain; Similar Reactions Occur at Asparagine Side Chains



in model systems and not their oxidation products. However, according to Videnov et al.,[45] oxazoles have been found in many peptide-based bioactive compounds.

In general, only a few studies have reported the formation of thiazolines, thiazoles, oxazolines, oxazoles, imidazolines, or imidazoles. It is unclear whether these compounds were mostly not produced or not searched for.

3.3. Peptide Side-Chain Modifications

Next to peptide backbone cleavage and modifications, side-chain modifications can also alter the structure of a peptide. The previously mentioned reviews on deamidation of peptides[22,41] also discuss the modification of the asparagine and glutamine

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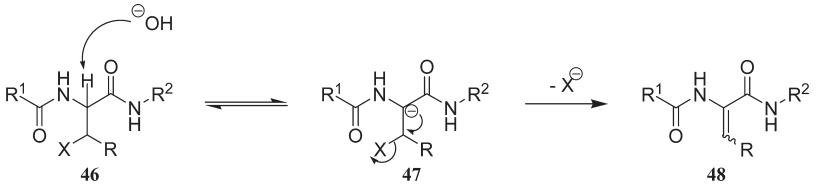
Scheme 15. Postulated Mechanism for the Formation of Dehydro Amino Acid Residues 48 in Peptides; X = OH, SH, or S−S−$CH_2CH(NH_2)COOH$; R = Either H (Serine, Cysteine, or Cystine) or CH_3 (Threonine)



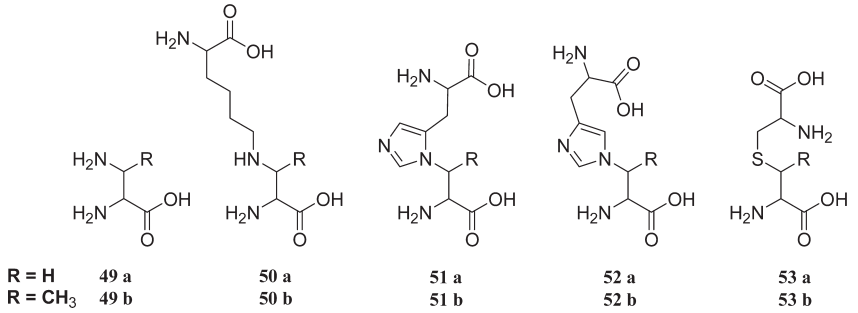
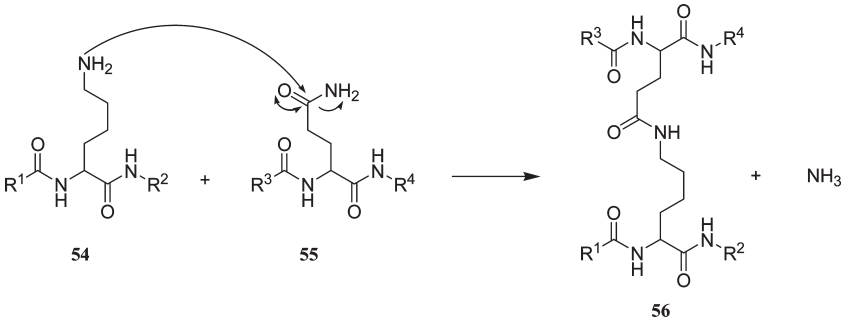


Figure 1. Chemical structures of β-aminoalanine **49a**, lysinoalanine **50a**, two isomers of histidinoalanine **51a** and **52a**, lanthionine **53a**, and their analogous compounds with a methyl substituent **49b**−**53b**.

Scheme 16. Mechanism for the Formation of the Isopeptide Bond ε-(γ-Glutamyl)lysine 56 from the Reaction Between the ε-Amino Group of Lysine 54 and the Side-Chain Amide Group of Glutamine 55



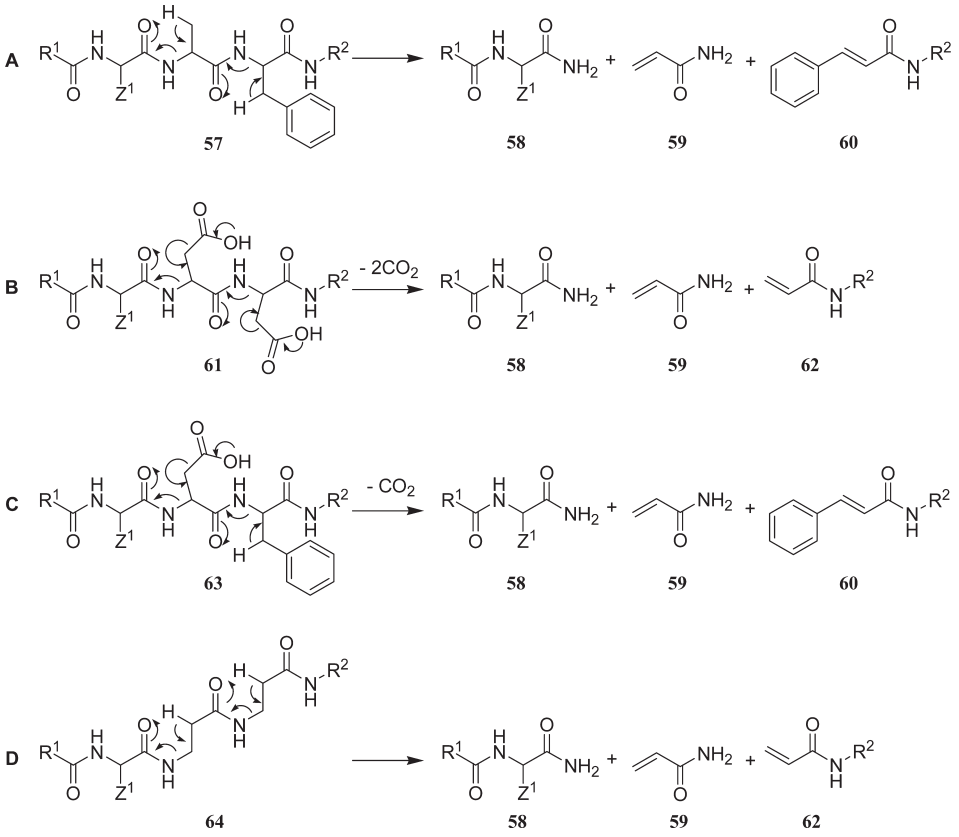
side chains into aspartic and glutamic acid, respectively. Because of general acid−base-catalyzed hydrolysis, the amide function of the side chain can be converted into an acid function (Scheme 14). Neighboring serine and threonine residues increase the rate of this deamidation reaction, because their side chains can provide a proton to the leaving group or stabilize the transition state.

Another pathway involving side-chain modifications in peptides is the formation of dehydro amino acids. This modification can occur during alkaline treatment of food.[46] As depicted in Scheme 15, abstraction of a proton at the α-position of a carbonyl group of the main peptide chain **46** results in the formation of a carbanion **47**, followed by an elimination reaction forming a dehydro amino acid **48**. According to Friedman,[46] it is not clear whether the reaction proceeds as it is depicted in Scheme 15 or via a one-step elimination reaction. However, in our opinion, second-order processes of elimination should be operative here. Anyway, both reaction pathways lead to the formation of dehydroalanine (R = H) from serine, cysteine, or cystine and of methyldehydroalanine (R = CH_3) from threonine. Although Friedman[46] mainly focused on the formation of dehydro amino acids in proteins, similar reactions can occur in peptides, because dehydro amino acids appear naturally in certain peptides.[46] Dehydro amino acids are very reactive and may lead to the formation of cross-links (section 3.5). In the case of cysteine, hydrogen sulfide is released during the formation of dehydroalanine. In combination with other food constituents or decomposition products, hydrogen sulfide contributes to the formation of specific *S*-containing volatiles.[47]

3.4. Peptide Cross-Linking

Like proteins, peptides are able to form cross-links. However, formation of cross-links between peptides as such is rare. Mostly, the intervention of other reactive species, such as degradation

Scheme 17. Hypothetical Formation Mechanism of Acrylamide 55 from Peptides Containing Subunits (A) Ala-Phe 53, (B) Asp-Asp 57, (C) Asp-Phe 59, and (D) β-Ala-β-Ala 60



products of carbohydrates or lipids, is required,[2] which is handled in the corresponding sections. Mainly dehydro amino acids, formed from serine, cysteine, cystine, or threonine via side-chain modifications (section 3.3), have been reported to form cross-links without the intervention of other reactive species.[46] Dehydroalanine can react with NH_3 to form β-aminoalanine **49a**, with the ε-NH_2-group of lysine to form lysinoalanine **50a**, with the NH-group of histidine to form histidinoalanine **51a** and **52a** and with the SH-group of cysteine to form lanthionine **53a** (Figure 1). Analogous compounds with a methyl substituent **49b**−**53b** are formed in the presence of methyldehydroalanine.[46]

Recently, the formation of the isopeptide bonds ε-(γ-glutamyl)lysine **56** and ε-(β-aspartyl)lysine, resulting from the reaction between the ε-amino group of lysine **54** with the side-chain amide group of glutamine **55** and asparagine, respectively (Scheme 16), has been detected for peptides. Rombouts et al.[48] found that heat treatment (130 °C for 24 h) of the mixture of the glutamine-containing peptide Gln-Gln-Pro-Gly-Gln-Gly and the lysine-containing peptide Val-Val-Pro-Pro-Lys-Gly-Gly, both representing amino acid sequences naturally occurring in glutenin, resulted in the formation of the isopeptide bond between lysine and the glutamine residue at position two.

As proteins, peptides have also been reported to cross-link through disulfide bridge formation. According to Jeric and Horvat,[40] incubation of glutathione (GSH) in water for 5 days at 50 °C resulted, among others, in the formation of oxidized glutathione **23** (GSSG).

3.5. Peptide Breakdown

Fragmentation of peptides, other than regular chain cleavage, followed by characterization of these fragments, has not been described much. To the best of our knowledge, only the formation of acrylamide, as a degradation product of peptides, has been characterized.

Acrylamide **59**, which was classified as "probably carcinogenic to humans" by the International Agency on Cancer Research (IARC),[49] is often found in heated food products. It is nowadays well-known that this compound is produced from the reaction of asparagine with reducing carbohydrates or its resulting cleavage products, during food processing at temperatures exceeding 100 °C.[2] However, this mechanism is inadequate to explain the formation of acrylamide in food products devoid of reducing sugars but rich in proteins, such as meat.[50,51] Peptides containing subunits as Ala-Phe **57**, Asp-Asp **61**, Asp-Phe **63**, β-Ala-β-Ala **64**, and Phe-Ala have been shown to produce acrylamide **59** during thermal treatment (roasting at 200 °C for 24 h).[52,53] The authors suggested a sequence of simultaneously occurring electrocyclic processes as the operating reaction mechanism (Scheme 17). The formation of *N*-substituted cinnamic amide **60** from peptides containing the sequences Ala-Phe and Asp-Phe at the *C*-terminus confirmed this formation pathway. For peptides containing Phe-Ala, additional reaction steps, which were not elucidated, are needed to yield acrylamide. It should be noted that the amounts of acrylamide produced in protein-rich foods are much lower than those produced in carbohydrate-rich foods.[50,51] Therefore, interaction of asparagine with

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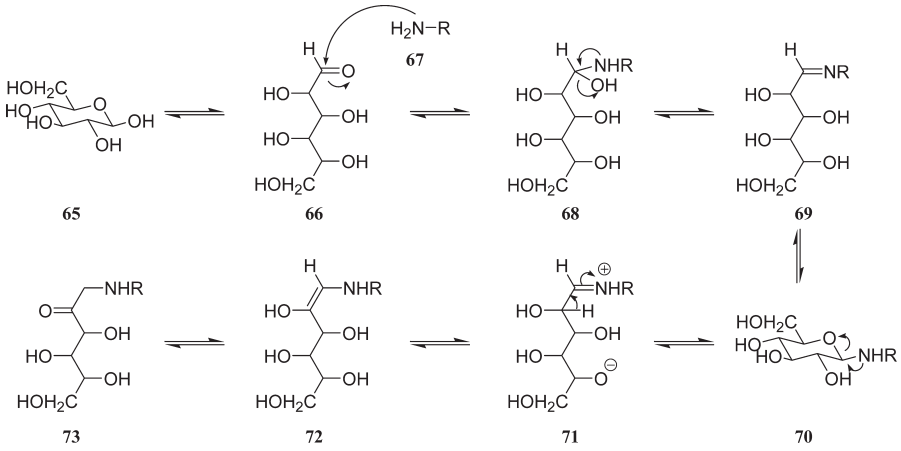
Scheme 18. Early Phase of the Maillard Reaction: Sugar−Amine Condensation to Form the *N*-Substituted Glycosylamine 70, Followed by the Amadori Rearrangement



sugars should still be considered as the main pathway for acrylamide formation in food products.

Although only acrylamide had been characterized as a degradation product of peptides, incubation of glutathione at 180 °C during 1 h led to the production of several *S*-containing volatiles, which require, in addition to hydrogen sulfide, fragmentation products of the peptide chain for their formation.[47] Elucidation of peptide fragments and the conditions of their formation remains a challenging task.

4. REACTIONS OF PEPTIDES WITH CARBONYL COMPOUNDS

Because of the presence of reactive nucleophilic groups in peptides, such as free amino, hydroxyl, and sulfhydryl groups, the reactions between peptides and carbonyl compounds are often observed in food systems. The reacting carbonyl compounds can be major food constituents, such as carbohydrates and lipids and their degradation products, or minor compounds, such as vitamins, additives, and carbonyl compounds derived from other reactions. Among these, the reactions between peptides and carbohydrates has been studied the most.

4.1. Reactions between Peptides and Carbohydrates

4.1.1. Introduction. The most well-known reaction between peptides and carbohydrates comprises the condensation reaction between a free amino group of a peptide and a carbonyl group of a reducing carbohydrate. This initial attack triggers a series of complex chemical reactions, known as the Maillard reaction or nonenzymatic browning reaction. As already mentioned in the introduction, the reactivity of proteins and free amino acids in the Maillard reaction has been studied and described much more than the reactivity of peptides and is outside the scope of this review.

In general, the Maillard reaction is initiated by the interaction of a carbohydrate **66** and a nitrogen source **67**, leading to the formation of an *N*-substituted glycosylamine **70** via aldimine **69**. Subsequently, this thermally labile glycosylamine undergoes what is called the Amadori rearrangement. The resulting 1-amino-1-deoxy-2-ketose **73** is referred to as the Amadori rearrangement product (ARP).[54] This early stage of the Maillard reaction is depicted in Scheme 18. Comparable to aldose sugars, ketose sugars undergo the Heyns rearrangement with the formation of 2-amino-2-deoxyaldoses.

In the advanced stages of the Maillard reaction, sugar fragmentation, condensation, elimination, dehydration, and cyclization reactions occur simultaneously, resulting in a huge diversity of reaction products.[54−56] Afterward, in the final stage, many of the functional groups generated in the advanced stage interact, yielding colored polycondensation products called melanoidins. Factors that influence the Maillard reaction, such as reactant concentration, thermal load, pH, buffer, and water activity, have been reviewed extensively, e.g., refs 57and 58.

4.1.2. Initial Stage of the Maillard Reaction. Several aspects on the reactivity of peptides and carbohydrates in the early phase of the Maillard reaction have been studied. For instance, monosaccharides have been reported to be more reactive than disaccharides.[59] In addition, glucose was found to be more reactive with peptides as compared to fructose in the formation of the Amadori or Heyns product, respectively.[60−62] Roscic and Horvat[63] reported that the reaction of leucine-enkephalin (Tyr-Gly-Gly-Phe-Leu) with glucose yielded more Amadori rearrangement product than the corresponding reaction with mannose or galactose.[63] On the contrary, Mennella et al.[59] stated that the reactivities of glucose and galactose toward peptides were similar. This difference could be due to the fact that in the latter study the model reactions were performed in anhydrous methanol instead of water. It is known that the solvent is an important criterion for the glycation of peptides.[63,64]

Besides the reacting carbohydrate, also the composition and sequence of the peptide influence the overall reactivity. Mori et al.[65] reported that the early stage of the Maillard reaction between di- or tripeptides and glyceraldehyde was influenced by the basicity of the second amino acid residue during heat treatment at 30 °C at pH 7. In the case of a positively charged basic amino acid in second position, such as histidine and arginine, the initial imine was converted to the ketoamine to a higher extent. In accordance to this, the presence of a negative charge at the second residue, as for aspartic acid, led to an extremely slow reaction with glyceraldehyde. It is possible that, because of the proximity of a protonated basic group, protonation of the imine **69** in Scheme 18 proceeds faster,

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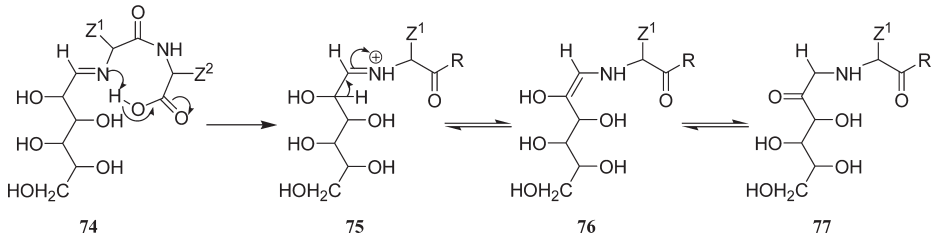
Scheme 19. Hypothetical Catalysis of the Amadori Rearrangement of the Dipeptide/Sugar Adduct 74, Due to a Proton Exchange between the Imino Nitrogen Atom and the *C*-Terminal COOH Group (R = NH−CH_2Z^2−COOH)



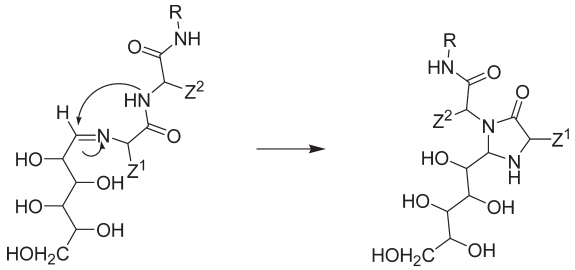
leading to an enhanced Amadori rearrangement. In contrast to these results, Venkatraman et al.[66] found that aspartic acid was an efficient catalyst of the Amadori rearrangement, when they studied the effect of neighboring amino acids on the reactivity of the ε-amino group of lysine.

Besides the basicity of neighboring amino acids, also the proximity of a carboxyl group to the imine has been reported to enhance the Amadori rearrangement. De Kok and Rosing[31] determined the overall reactivity of glycine, diglycine, and triglycine in the Maillard reaction at pH 5.6: the reactivity increased from glycine to diglycine, but when a third glycine residue was attached, the reverse effect was noticed. The authors proposed an intramolecular process to be responsible for the high reactivity if diglycine. It was suggested that the imino nitrogen is protonated by the COOH terminus of the dipeptide/glucose adduct **74**, leading to an enhanced Amadori rearrangement (Scheme 19). Substituting the dipeptide with glycine reduces the number of main-chain atoms, thereby prohibiting the formation of a structure in which the COOH group and the imine nitrogen are close. In addition, from a statistical point of view, the possibility of close interaction between the COOH group and the imino nitrogen will decrease with further increasing peptide chain length.

The hypothesis of intramolecular catalysis by a carboxyl group was confirmed by the observation that glutamic acid at the *C*-terminus in the peptides Gly-Glu and Met-Glu induced a higher reactivity as compared to other Gly-X and Met-X dipeptides, respectively.[31] Also for Gly-Asp, the catalytic effect of the side chain COOH group was detected, although less pronounced. As mentioned above, similar results were found by Venkatraman et al.,[66] who reported a catalytic effect of neighboring aspartic acid on the Amadori rearrangement at the ε-amino group of lysine. For Pro-X dipeptides this "Glu effect" was absent, because for this peptide an iminium cation is formed, which cannot be protonated.[31] In the Pro-X case, the highest reactivity was found for Pro-Lys, probably due to a base-induced deprotonation of the sugar C-2 atom. In contrast to the findings of de Kok and Rosing,[31] Mori et al.[65] did not detect the carboxylic residue as a moderator of any type of catalysis. It is unclear what causes these different catalytic effects, but as the reaction conditions and the determination of the rate constants were different, it is very difficult to compare both studies.

In addition, the hydrophobicity of neighboring amino acids has been reported to influence the reactivity of peptides. According to Mennella et al.,[59] hydrophobic amino acids at the *C*-terminus of lysine-containing dipeptides enhanced the reactivity of lysine toward different carbohydrates. The authors obtained

Scheme 20. Formation of Imidazolidin-4-one 79 from Imine 78 Derived from a Peptide and a Reducing Sugar



contrasting results for basic residues: Lys-Arg was among the most reactive whereas Lys-Lys showed a low reactivity.

A very nice and fast tool for comparing individual reactivities of amino acids in peptides in the early stage of the glycation reactions is the use of a peptide SPOT library, as Münch et al.[67] showed for dipeptides. All 400 possible dipeptides, composed of the 20 most common amino acids, attached to a membrane, were incubated with glucose and fructose to compare their reactivity. These authors found that the reactivities of the *N*-termini were quite similar, except for peptides with proline at the *C*-terminus and arginine at the *N*- or *C*-terminus, which were less reactive. Concerning side-chain reactivity, lysine, cysteine, and to a lesser extent histidine side chains revealed the highest reactivities.

Regarding the location within the peptide or protein where the Maillard reaction preferably takes place, it is known that the side chain of lysine is mostly modified in proteins.[54] This could be expected because, for proteins, the ratio of *N*-terminal to the easy accessible ε-amino group of lysine residues is very low. On the contrary, for peptides, the ratio of *N*-terminal to ε-amino groups of lysine increases with decreasing chain length, and therefore, the reactivity of the *N*-terminal amino group becomes more and more important. Amadori rearrangement products of *N*-terminal amino acids have been detected in hypoallergenic infant formulas.[68] Jakas et al.[62] demonstrated the formation of *N*-terminal Amadori or Heyns compounds from the reaction of glucose or fructose, respectively, with the lysine-containing peptide Leu-Ser-Lys-Leu, although the obtained results showed a preferential glycation of lysine side chains. Differences in reactivity of different lysine residues from one peptide have been reported by Tressl et al.[69] This can be due to neighboring amino acid residues, which influence the reactivity of the different amino acids, as already mentioned before.

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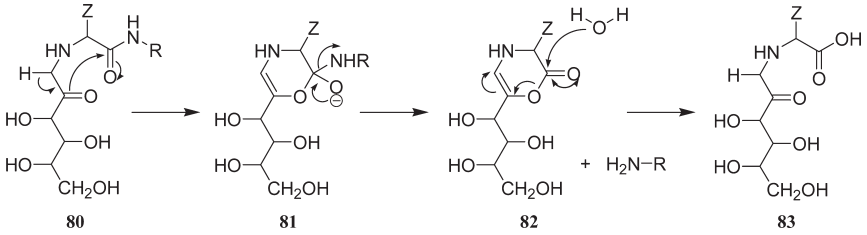
Scheme 21. Hypothetical Cleavage Mechanism of the Peptide Bond within the Amadori Product of a Peptide 80



Besides the formation of the Amadori product, the formation of imidazolidin-4-ones **79** from peptides and carbohydrates at relatively low temperatures (37 °C) has been reported.[61,63] Imidazolidin-4-ones **79** are formed when the imine at the *N*-terminal amino acid **78** undergoes a nucleophilic attack of the nitrogen of the second amino acid residue, instead of an Amadori rearrangement (Scheme 20).

The formation of these imidazolidin-4-ones depends on the reacting sugar. Jakas and Horvat[61] found that, in the case of glucose, the Amadori compound was primarily formed, whereas in the case of fructose, higher amounts of imidazolidin-4-one were produced. Roscic and Horvat[63] determined that the Amadori product was the major glycation pro duct from the reaction of leucine-enkephalin with glucose, whereas incubations with mannose and galactose resulted in a significant accumulation of the corresponding imidazolidin-4-ones. These authors also found that the equilibrium concentration ratio of the Amadori and the imidazolidin-4-one adduct depends upon the length, substitution, and amino acid sequence of the peptide. Accordingly, Jakas et al.[62] showed that no imidazolidin-4-ones were formed in the reaction of glucose or fructose with a lysine-containing tetrapeptide, since the ε-amino group of lysine was the primary glycation site and not the α-amino group.

In this context, it should be noted that it is not easy to evaluate the reactivity of peptides and sugars in the early phase of the Mailllard reaction. This is due to the fact that, under some conditions, such as very low pH or high temperature, the resulting ketoamine participates very fast in further rearrangement and condensation reactions of the Maillard reaction. Therefore, studies on the formation of the glycosylamine and the Amadori product are preferentially performed at neutral pH and moderate temperature. However, it is not certain whether the obtained order of reactivity will be similar at food processing conditions, involving higher temperatures.

4.1.3. Advanced Stages of the Maillard Reaction. During the later stages of the Maillard reaction, which are initiated by the degradation of the Amadori product, a wide variety of products are formed. The degradation of the Amadori product of peptides, similar to its formation, differs from the degradation of the Amadori compound of free amino acids and depends on the peptide sequence.

For instance, Hashiba[70] demonstrated that, at pH 5 and 50 °C, hydrolysis of the peptide bond within the Amadori product of diglycine and glucose was higher than hydrolysis of diglycine without the interference of sugars. An intramolecular rearrangement within the Amadori product was suggested by the author as visualized in Scheme 21. Hydrolysis of cyclic intermediate **86** forms the Amadori product of the liberated *N*-terminal amino acid.

This Amadori compound-induced peptide cleavage was not detected by Jakas and Horvat.[71] In their study, the degradation of the Amadori products of three similar peptides (Tyr-Gly-Gly-Phe-Leu, Tyr-Gly-Gly-Phe-Leu-OMe, and Tyr-Gly-Gly) was evaluated (phosphate buffer, pH 7.4). At 37 °C, the main degradation product was the original peptide, whereas at 70 °C, also other degradation products, mainly 4-hydroxybenzaldehyde and cyclic Tyr-Gly, were detected. 4-Hydroxybenzaldehyde was found to be a typical Maillard degradation product of tyrosine, and its formation was favored when tyrosine was linked with other amino acids at its *C*-terminus.

Besides 4-hydroxybenzaldehyde, also cyclic Tyr-Gly (diketopiperazine) was detected. As mentioned in section 3.1.2, diketopiperazines **13** can also be formed from peptides without the intervention of other reactive species (Scheme 4). In this context, Jakas and Horvat[71] showed that the yield of diketopiperazine formation from the parent peptide was more or less the same as the yield of diketopiperazine formation from the corresponding Amadori products. Diketopiperazines were also formed from the Maillard reaction of glucose with diglycine or triglycine (160 °C, 1 h).[72] The yield of formation of the diketopiperazine from triglycine was about 45−80% and increased as the water content increased, whereas diglycine yielded about 20% of diketopiperazine, independently of the water content. The impact of the Maillard reaction on this diketopiperazine formation is not known as a similar water content dependency is possible for the formation of diketopiperazines from the degradation of peptides without the intervention of other reactive species.

In addition to diketopiperazine formation, Lu et al.[72] found that the Maillard reaction between glucose and triglycine yielded higher amounts of glycine and diglycine with increasing water content, whereas only small amounts of glycine were formed in the glucose-diglycine model systems. This is as expected, since the formation of the diketopiperazine results in the liberation of glycine in the case of triglycine and not in the case of diglycine (Scheme 4).

As for amino acids and proteins, also peptide Maillard reaction products give rise to modifications in color, taste, aroma, biological activity, and nutritional value.[54] In what follows, several Maillard reaction products (MRPs) derived from the reactions of peptides with carbohydrates and their significance for foods will be discussed. Although MRPs can also be formed from the reaction between peptides and sugar fragmentation products, such as glyoxal and methylglyoxal, the reaction products derived from these two fragments will be discussed later (section 4.3), because they can also result from other degradation reactions such as the degradation of lipids.

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4.1.3.1. Formation of Color. Because the attractiveness of food depends on its appearance, it is obvious that the formation of colored compounds is a very important process in food chemistry. Kim and Lee[73] described that the development of brown color in a glucose-diglycine model system was the highest, followed by the corresponding glucose-triglycine and glucose-glycine model systems (100 °C, pH 7.8). The authors used this color measurement to evaluate the reactivity of the peptides. However, it is known that the amount of browning is not necessarily proportional to the conversion of peptides and/or sugars.[31] The degree of browning depends on the type of compounds formed, which in turn highly depend on the exact reaction parameters. Accordingly, the browning order in Maillard reaction mixtures with glucose reported by Buera et al.[74] (55 °C, pH 5) was triglycine > diglycine > glycine, and not diglycine > triglycine > glycine as in the study of Kim and Lee[73] (100 °C, pH 7.8). These differences illustrate that it is very difficult to explain or predict the degree of browning based on the composition, sequence, or length of a peptide.

To our knowledge, the isolation and identification of colored Maillard products from model systems containing peptides is not yet achieved. Possibly, similar compounds as in model systems containing free amino acids are formed, because according to Ledl and Schleicher,[54] for instance, deoxyaldoketoses and diketoses are important intermediates in the formation of colored compounds. These sugar degradation products can also be formed in the presence of peptides. Among others, a condensation product of 5-(hydroxymethyl)furfural with a pyranone **84**, a furanone **85**, and a pyrrolinone **86** (Figure 2) have been isolated and identified as colored Maillard reaction products, formed in model systems containing free amino acids.[54] Other colored Maillard reaction products are reviewed by Ledl and Schleicher.[54]

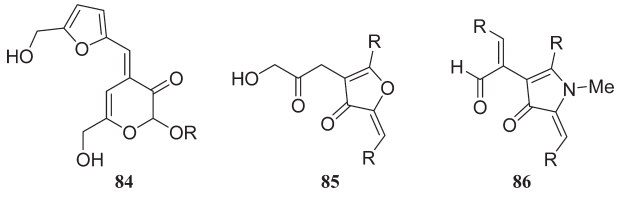


Figure 2. Chemical structures of colored Maillard reaction products identified in model systems containing free amino acids.

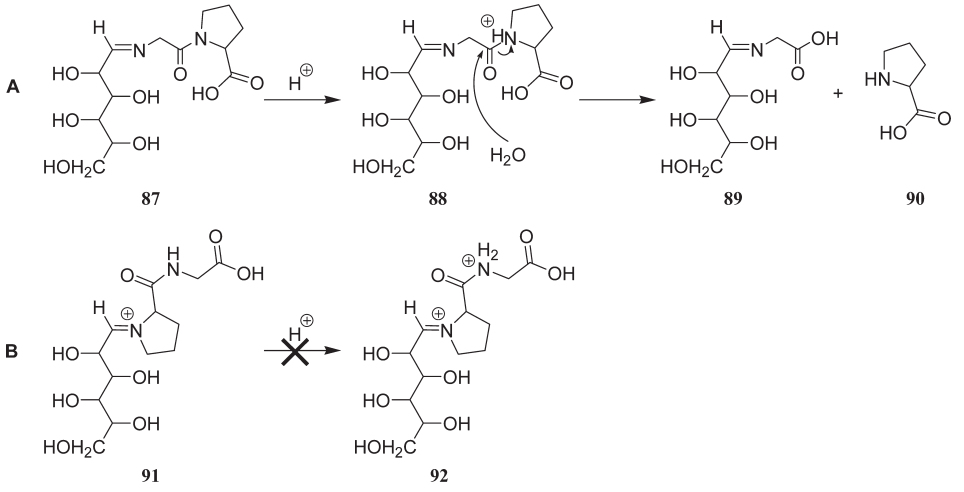
4.1.3.2. Formation of Taste. As mentioned in the Introduction, peptides as such reveal all kinds of taste, triggering the question whether Maillard reaction products of peptides also exhibit taste. Ogasawara et al.[75] studied the impact of the Maillard reaction between xylose and the 1000−5000 Da peptide fraction of soybean protein hydrolysate on the taste properties of the mixture. These authors found that this "Maillard reacted peptide" did not have a strong taste in aqueous solutions but significantly increased the intensity of mouthfulness and continuity in umami solutions and soup. Thus, the Maillard reacted peptide behaved as a taste enhancer.

4.1.3.3. Formation of Aroma. It is known that the Maillard reaction produces hundreds of volatile compounds, among which many contain various heterocyclic systems.[54] Multiple studies have focused on the differences in aroma formation during the Maillard reaction with peptides as compared to free amino acids. From these studies, it can be concluded, in general, that both systems generate similar aroma compounds but in different ratios and quantities. From the reactions with peptides, mainly the formation of pyrazines, furans, pyrrolizines, thiazoles, and thiophenes has been described.[76−80]

Comparison of the volatiles produced during the reactions of glucose with glycine, diglycine, triglycine, or tetraglycine (180 °C, 2 h, pH 4.2−4.4) revealed that both glycine and triglycine generated larger amounts of alkylpyrazines, whereas diglycine and tetraglycine produced greater quantities of furfural and 5-hydroxymethylfurfural.[76] The similarity between mono- and triglycine, on one hand, and di- and tetraglycine was explained by the fact that triglycine was degraded into glycine and diglycine via the formation of diketopiperazine, whereas tetraglycine was degraded primarily into diglycine.

As mentioned before, Lu et al.[72] confirmed that the Maillard reaction (160 °C, 1 h) between glucose and triglycine yielded higher amounts of glycine and diglycine, whereas only small amounts of glycine were formed in the model systems containing diglycine. These authors also determined the effect of the water content on the generation of volatiles during the Maillard

Scheme 22. Proposed Mechanism for (A) Acid Hydrolysis of the Imine from Gly-Pro 87 and (B) Hindered Acid Hydrolysis of the Imine from Pro-Gly 91 Due to the Close Proximity of Two Positively Charged van der Waals Radii



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reaction of glucose with glycine, diglycine, or triglycine. In general, substituted pyrazines, which are known to contribute significantly to the unique roasted aroma of many heated food products,[81] were the major volatiles. These aroma compounds were produced most in model systems containing glycine, followed by triglycine and diglycine. The formation of pyrazines increased as the water content decreased. A higher formation of furan derivatives from diglycine as compared to glycine or triglycine was also reported.

Similar results concerning the relative formation of pyrazines and furan derivatives were found by Oh et al.[77] in Maillard reaction systems of glucose and a 1:1 mixture of glycine and leucine, Gly-Leu or Leu-Gly (180 °C, 2 h, pH 5.3−5.5). In contrast to this, Van Lancker et al.[78] reported that pyrazines were produced more in case of model reactions between glucose and various dipeptides containing lysine at the *N*-terminus, as compared to similar reactions between glucose and the corresponding free amino acids (130 °C, 2 h, pH 8). This difference was mainly caused by the large amounts of 2,5(6)-dimethylpyrazine and trimethylpyrazine produced in the reactions with dipeptides. The contrasting results may be due to different reactions conditions, such as temperature and pH, but it is also possible that lysine-containing peptides behave in a different way than the previously studied glycine peptides.

As pointed out in section 3.2, deamidation reactions of peptides containing asparagine or glutamine result in the formation of free ammonia, which can also contribute to the formation of pyrazines.[41] Therefore, it can be expected that peptides containing high amounts of asparagine or glutamine produce relatively high amounts of pyrazines.

Pyrrolizines, which are proline-specific MRPs, were detected as major volatiles produced during the reactions of glucose with a 1:1 mixture of glycine and proline, Gly-Pro or Pro-Gly.[79] Comparison of volatile formation in Gly-Pro and Pro-Gly model systems revealed that Gly-Pro produced more volatiles, including pyrrolizines, than Pro-Gly. The authors suggested two reasons for this observation. First, the primary amino group of Gly-Pro is more reactive than the secondary amino group of Pro-Gly in catalyzing the transformation and fragmentation of glucose. In addition, the peptide bond in the Gly-Pro imine **87** is supposed to be more susceptible to acid hydrolysis than the peptide bond in the Pro-Gly imine **91**, because for Pro-Gly, the required protonation of the peptide bond nitrogen is energetically unfavorable due to the proximity of the positively charged imine, as illustrated in Scheme 22. However, the latter explanation is questionable since the amide nitrogen of structure **87** is much less basic than the imino nitrogen, which will be protonated and thus hydrolyzed much faster.

As compared to the volatiles produced from the mixture of glycine and proline, Gly-Pro produced more pyrrolizines. These results indicate that the Maillard reaction with amino acids bound in the peptide form may direct reactions toward specific types of aroma compounds.

In addition, also *S*-containing compounds, such as thiazoles and thiophenes, are formed during the Maillard reaction. It is known that, during heating, hydrogen sulfide is released from cysteine, which reacts with Maillard products and gives rise to a multitude of *S*-containing compounds.[54] Their important impact on food aroma is mainly due to their extremely low odor thresholds. It has been shown that also peptide-bound cysteine produces sulfur-containing heterocyclic compounds during the Maillard reaction. Comparison of the volatiles generated during glucose/cysteine reactions with glucose/glutathione reactions (180 °C, 1 h) showed that similar compounds were formed, but the glucose/cysteine model system produced about 3 times the amounts of thiazoles and thiophenes, 4 times the amount of pyrazines, and 30 times the amount of polysulfur-containing compounds than the corresponding glucose/glutathione model system.[80] The major difference in production of polysulfur compounds is suggested to be due to the lack of aldehydes formed in the reaction between glucose and glutathione.

Beside typical Maillard aroma compounds, also peptide-specific flavor compounds, such as pyrazinones, can be formed. Their formation requires the presence of glyoxal or methylglyoxal. As mentioned before, these sugar fragmentation products will be discussed separately (section 4.3).

4.1.3.4. Formation of Biological Activity. It is generally known that Maillard reaction products (MRPs) can exert several kinds of biological activities.[82] These findings result mainly from Maillard model reactions with free amino acids. Regarding peptide MRPs, some antioxidant and cytotoxic activities have been reported. Kim and Lee[83] determined the antioxidant activity of MRPs derived from glucose/glycine, glucose/diglycine, and glycose/triglycine model systems by means of several assays. As heating times increased, antioxidant activity also increased for all model systems. These authors found that the MRPs derived from the glucose/diglycine model system were the most effective antioxidants, followed by those from the glucose/triglycine and glucose/glycine model systems. It was suggested that the antioxidant activity of these MRPs is related to both the peptide chain length and the stability of the peptide bond toward heating, because it had been shown that diglycine is more stable than triglycine.[72]

In another study, the antioxidant activities of model reactions between xylose and a 1:1 mixture of glycine and histidine, Gly-His or His-Gly, were compared by means of oxidation of emulgated linoleic acid.[84] Glycine and histidine were chosen as the constituting amino acids, because the MRPs of these amino acids were shown to be poor and strong antioxidants, respectively. The results showed that Gly-His was equally effective as the mixture of the free amino acids, while His-Gly was much more effective. From this result, it can be concluded that the antioxidative effect depends not only on the constituting amino acids in the peptide but also on their sequence. The authors also studied the antioxidant activity of MRPs added to food. They showed that the addition of the initial reactants to food was more effective than adding preformed MRPs.

Regarding cytotoxicity, the influence of MRPs formed in model reactions of lysine, arginine, Lys-Arg, Arg-Lys, Lys-Lys, or Arg-Arg with ribose or glyceraldehyde (60 °C; 2 weeks) on the growth of cell cultures of murine RAW 264.7 macrophages was investigated.[85] The authors showed that none of the MRPs reduced the number of cells below the starting levels, indicating that the MRPs were rather cytostatic than cytotoxic. Interestingly, all Maillard-modified dipeptides, especially Lys-Lys, were more effective than the respective free amino acids.

Because no structural characterizations were performed to explain these antioxidant and cytotoxic activities, it is still not clear which of the many MRPs exert these properties. Such information

would be very valuable to optimize cooking and roasting procedures to minimize the formation of toxic and undesirable MRPs, while maximizing the formation of desired and beneficial MRPs in a food preparation.

4.1.3.5. Formation of Peptide Side-Chain Modifications and Cross-Links. Beside the MRPs described above, other peptide modifications have been described. These modifications mostly concern the side chains of lysine and arginine residues. Because lysine is an essential amino acid, modification of the side chain of lysine causes a decrease in nutritional value of the food product. Obviously, side-chain-bound MRPs of peptides are similar to those of proteins. However, for proteins, the specific tertiary structure may hamper or enhance the formation of some specific modifications, whereas for peptides, the probability of the formation of a tertiary structure decreases with decreasing peptide chain length. Therefore, it can be expected that all peptide side chains have equal chances of modification instead of only the most accessible protein side chains. In what follows, lysine side-chain modifications, arginine side-chain modification, and combined modifications of the lysine and arginine side chain will be addressed successively. The structures of two common lysine-bound peptide side-chain modifications are depicted in Figure 3.[86]

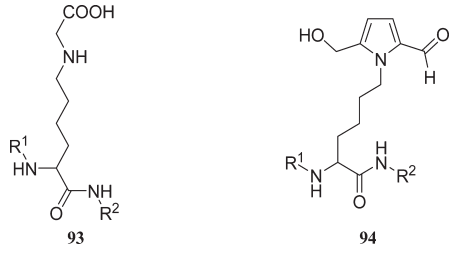

Figure 3. Chemical structures of carboxymethyllysine **93** and pyrraline **94**, two common lysine-bound peptide side-chain modifications.

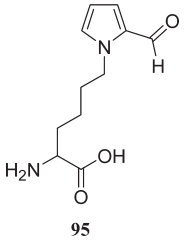

Figure 4. Chemical structure of formyline **95**.

Carboxymethyllysine **93** (CML) is a characteristic Maillard modification of the side chain of lysine, often used as a biomarker of protein damage. CML formation has been reported from the reaction between a heptapeptide (N^{α}-Ac-Lys-Lys-β-Ala-Lys-β-Ala-Lys-Gly) and glucose or lactose (110 °C, pH 6.7), together with furosine and formyllysine formation.[69] According to Cho et al.,[86] CML can be produced via several different pathways. Oxidative cleavage of the Amadori product has been proposed as the major pathway for CML formation.

Pyrraline **94** is another lysine derivative from the Maillard reaction and a potential indicator of heat treatment of food. In this context, Tressl et al.[69] found that the reaction between a heptapeptide (N^{α}-Ac-Lys-Lys-β-Ala-Lys-β-Ala-Lys-Gly) and glucose or lactose (110 °C, pH 6.7) did not yield pyrraline, whereas the reaction between 4-aminobutyric acid (representing bound lysine) and glucose or lactose under the same reaction conditions did. It was suggested that the vinylogous Amadori rearrangement, required for pyrraline formation, is not favored in the case of the heptapeptide. In contrast to these findings, Hayase et al.[87] described pyrraline formation from the reaction between the protein bovine serum albumin (BSA) and glucose or 3-deoxyglucosone. These results suggest that, although pyrraline was not detected from the reaction of the heptapeptide with glucose or lactose by Tressl et al.,[69] pyrraline formation from the Maillard reaction with peptides is possible. Recently, a pentose-derived lysine modification corresponding to the structure of pyrraline, namely, formyline **95** (Figure 4), has been described from the reaction of Boc-lysine and 3-deoxypentosone, pentoses, or disaccharides.[88]

Beside CML and pyrraline, other lysine modifications have been described for lysine-containing peptides or for N^{α}-protected lysine, used to simulate the behavior of the lysine side chain in peptides. Nakamura et al.[89] reported the formation of two epimeric fluorophores **96** during thermal treatment of N^{α}-acetyllysine and glucose during 6 weeks at 37 °C. The structure of these compounds is depicted in Figure 5. According to Hayase et al.,[90] the reaction between N^{α}-acetyllysine and 3-deoxyglucosone, a known degradation product of glucose, yields the formation of a pyrrolidinopyridinium compound **97**. As can be seen from its structure (Figure 5), this fluorescent compound is formed from the reaction of two molecules of N^{α}-acetyllysine and two molecules of 3-deoxyglucosone. From a statistical point of view, it is likely that, because of the required proximity of two lysine residues for both structures, the formation of similar compounds from peptides decreases with increasing chain length.

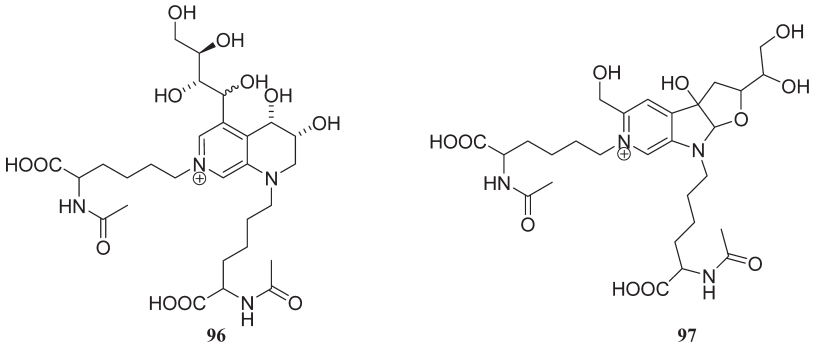

Figure 5. Chemical structures of MRPs isolated from the reaction mixture of N^{α}-acetyllysine with 3-deoxyglucosone (refs 89 and 90).

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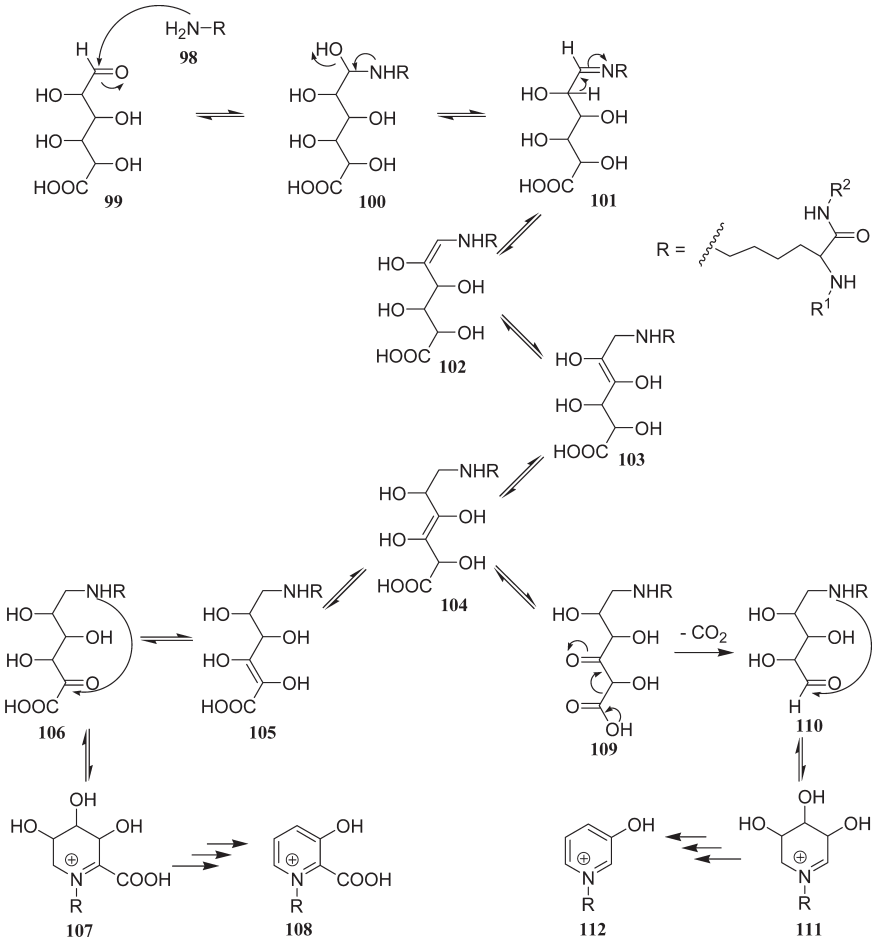
Scheme 23. Proposed Mechanism for the Formation of Pyridinium Derivatives 108 and 112 from the Reaction of Glucuronic Acid 99 and the Side Chain of Lysine 98



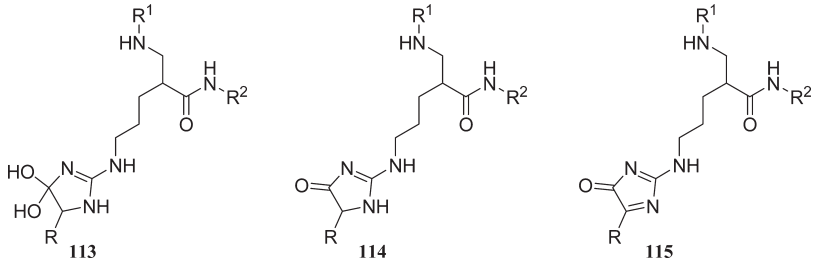


Figure 6. Chemical structure of identified arginine-bound peptide side-chain modifications: dihydroxyimidazolines **113**, imidazolinones **114**, and imidazolones **115**.

Recently, structurally more simple pyridinium compounds have been detected from the reaction between several lysine-containing pentapeptides and glucuronic acid **99** under dry heating conditions (50 °C; RH 75%; 5 days).[91] It was shown that the Amadori product was formed in the initial phase of the reaction and decreased later on, whereas the formation of the pyridinium derivatives increased linearly after an initial lag phase, suggesting the Amadori compound as their precursor. A mechanism for the formation of these pyridinium derivatives has been proposed[91] (Scheme 23).

It can be seen that, after the Amadori rearrangement, further keto−enol tautomerizations lead to the formation of an α-keto acid **106** and a β-keto acid **109** intermediate. The unstable β-keto acid eliminates CO_2 very fast, resulting in a 4-intermediate **110**. Intramolecular cyclization and subsequent dehydrations finally result in the formation of a 3-hydroxypyridinium salt **112** and a picolinic acid **108** derivative. Similar 3-hydroxypyridinium salt modifications have been reported at the *N*-terminus of opioid peptides.[92] In this study, picolonic acid derivatives, such as compound **108**, were not detected.

In addition to the lysine side chain, also the arginine side chain is often modified. Typical arginine modifications are dihydroxyimidazolines **113**, imidazolinones **114**, and

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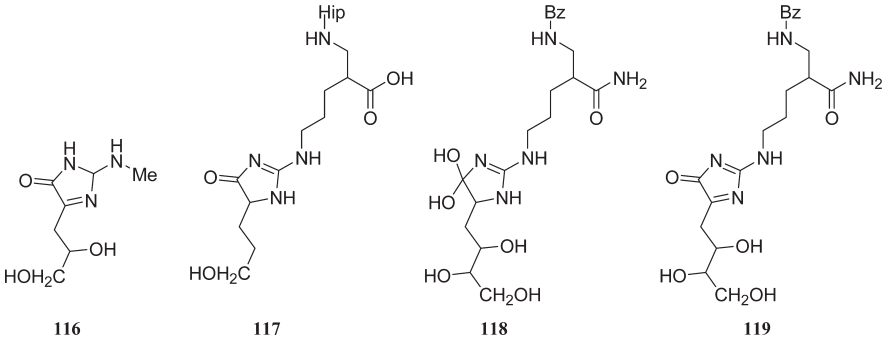
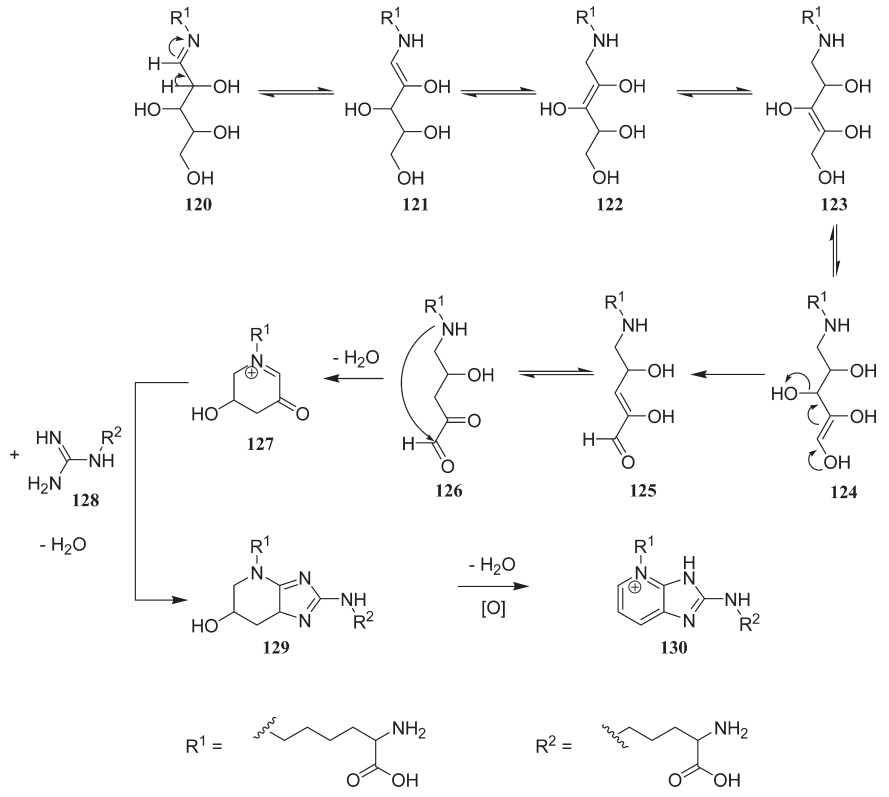


Figure 7. Chemical structures of some specific identified arginine-bound peptide side-chain modifications.

Scheme 24. Hypothetical Formation Pathway of Pentosidine 130 from Lysine, Arginine, and a Pentose



imidazolones **115** (Figure 6). These modifications are formed from the reaction of the arginine side chain with an α-dicarbonyl sugar degradation product via cyclization, dehydration, and oxidation reactions.[93−95]

Sopio and Lederer[93] found that the reaction between *N*-methylguanidine, representing the arginine side chain, and 3-deoxypentosone resulted in the formation of two diastereomeric imidazolinone structures **116** (Figure 7) in about 20% yield (reflux conditions, 3−4.5 h, pH 7). A similar imidazolinone compound **117** was detected from the reaction between N^{α}-protected arginine and disaccharides with a 1,4-glycosidic linkage after heating (100 °C, 8 h, pH 7).[94] Imidazolinone **117** was not detected in arginine model systems with monosaccharides and 1,6-linked disaccharides. The identified imidazolinone requires the intermediate 3,4-dideoxy-1,2-pentosone, an α-dicarbonyl degradation product of the sugar. From the results, it is assumed that this degradation product is characteristic for 1,4-linked disaccharides, whereas monosaccharides and 1,6-linked disaccharides preferably degrade into other α-dicarbonyl compounds. These other α-dicarbonyl compounds may eventually react with arginine side chains as well, resulting in other imidazolinones. Beside imidazolinones, Konishi et al.[96] and Hayase et al.[95] reported the formation of two diastereomeric dihydroxyimidazolines **118** and an imidazolone **119** after thermal treatment of a mixture containing N^{α}-protected arginine amide and 3-deoxyglucosone (50 °C, 4−7.5 days, pH 7.4) These authors also described the formation of bicyclic minor compounds derived from one molecule of N^{α}-protected arginine amide and two molecules of 3-deoxyglucosone during the same reaction.[97]

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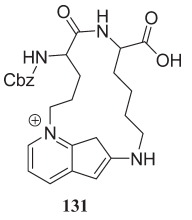
The formation of MRPs from carbohydrates and both lysine and arginine residues has also been described. In case both amino acids are constituents of different peptides, this reaction results in the formation of intermolecular cross-links. A common lysine- and arginine-containing cross-link is the pentosidine cross-link **130** (Scheme 24). This fluorescent compound can result from a plethora of pathways.[86] The major pathway for its formation presumably involves ring closure of a lysine-linked dideoxyosone **126** with arginine **128** (Scheme 24).

This dideoxyosone **126** with five carbon atoms results from the reaction between lysine and a pentose. Hexoses, such as glucose and fructose, however, have also been reported to degrade into fragmentation products with five carbon atoms during the Maillard reaction, resulting in the formation of pentosidine in the presence of lysine and arginine residues.[86]

Grandhee and Monnier[98] studied the formation of pentosidine in model systems containing N^{α}-Boc-lysine, N^{α}-Boc-arginine and ribose, glucose, fructose or 3-deoxypentosone, or N^{α}-Boc-arginine and the Amadori product of lysine and glucose or ribose. The yield of pentosidine from incubation mixtures containing 3-deoxypentosone was negligible, suggesting that 3-deoxypentosone is not an intermediate in pentosidine formation or that it is too reactive, leading to the preferential formation of other compounds. Comparison of the reactivity of the carbonyl compounds showed the following order: ribosylated lysine > ribose > glucosylated lysine > glucose. The yield of pentosidine from fructose was close to that of glucose. In ribose-containing systems, decreasing the reaction temperature from 80 to 60 °C led to a 2−2.5-fold increase in pentosidine yield. An alkaline pH also enhanced the production of pentosidine. In addition, it has been shown by Schwarzenbolz et al.[99] that the application of hydrostatic pressure up to 600 MPa on a solution of N^{α}-acetylarginine, N^{α}-acetyllysine, and ribose resulted in a pressure-dependent increase of the pentosidine content. The formation of an intramolecular pentosidine cross-link **131** has been reported from the reaction of an Arg-Lys dipeptide with either ribose, glucose, or fructose (Figure 8).[100]



131

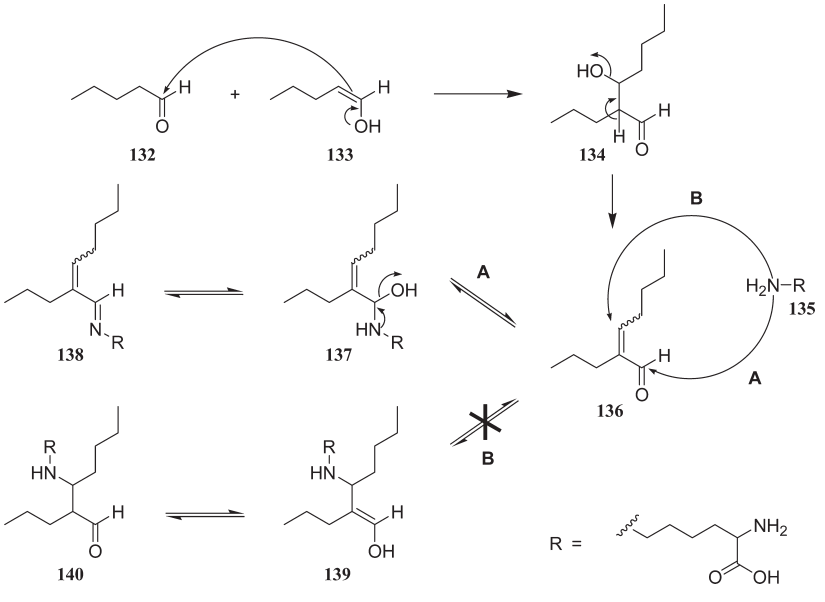
Figure 8. Chemical structure of an intramolecular pentosidine cross-link **131** produced in the reaction of N^{α}-protected Arg-Lys with either ribose, glucose, or fructose.

4.2. Reactions between Peptides and Lipids

It is known that unsaturated fatty acids are subject to lipid oxidation, which results in the formation of alkanes, alkenes, ketones, alkanals, alkenals, and hydroxyalkenals.[2] The carbonyl compounds formed are of major importance in the formation of off-flavor. Also, they undergo Maillard-type browning reactions.[101] As for the Maillard reaction, the interaction with amino groups starts with the formation of an imine, which results via rearrangements, cyclizations, and fragmentations in a wide variety of reaction products. Similar to the Maillard reaction, it is known that these reaction products influence color, taste, aroma, and nutritional value of food products. However, especially for peptides, little is known about the reaction with lipid oxidation products in food simulating conditions and about its implications. Reactions between lipid oxidation products and amino acids, peptides, or proteins under physiological conditions have been studied much more due to their potential contribution to tissue damage and diseases.[102,103]

A major drawback when studying the reaction between lipid oxidation products and amino acids, peptides, or proteins is the difficulty to find a suitable food model system because of the low water solubility of the lipid oxidation products. Therefore,

Scheme 25. Aldol Condensation of Pentanal 132, Followed by the Reaction with Lysine 135, Resulting in the Formation of (A) the Imine 138; Theoretically, Also the Formation of (B) the Michael Addition Product 140 Is Possible



132 133 134 135 136 137 138 139 140

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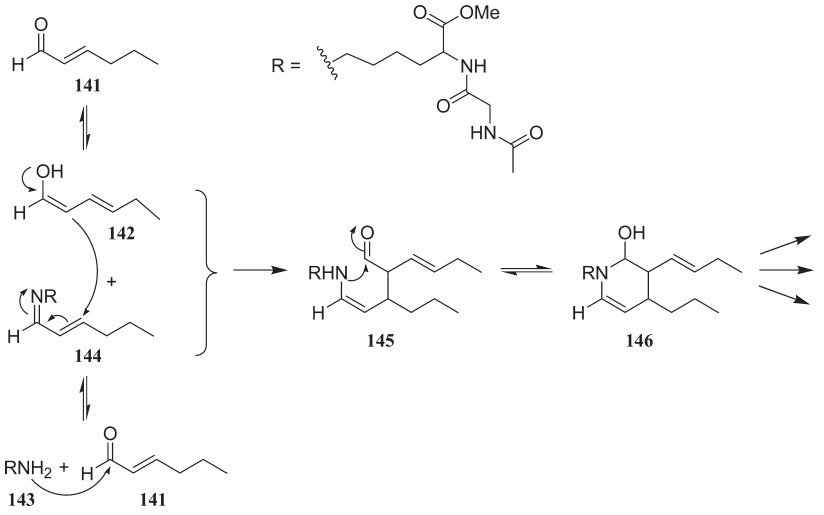
Scheme 26. Proposed Reaction Mechanism for the Formation of Quaternary Pyridinium Salts during the Reaction of *N*-Acetylglycinelysine Methyl Ester 143 and (*E*)-2-Hexenal 141



cosolvents such as methanol, ethanol, and acetonitrile are often used, but one should keep in mind that these solvents can influence the reaction and their use should be limited as much as possible.

The reaction between pentanal and two lysine-containing peptides at physiological conditions was studied by Dalsgaard et al.[104] The authors found that pentanal attached very quickly to the amino terminus of the peptides, whereas later, 1−3 additional pentanal molecules were attached to the lysine side chain as well. The adduct containing one molecule of pentanal at the lysine side chain was low in intensity at all times. In contrast, the adduct containing two molecules of pentanal at the lysine side chain was formed quite quickly, after the addition of the first pentanal molecule at the amino terminus, and later the adduct with three molecules of pentanal at the lysine side chain was formed. It was shown that pentanal **132** underwent a self-condensation reaction before it attached to the lysine side chain **135** to form the imine **138** (Scheme 25A). Although the side chain of lysine **135** could also react with the aldol condensation product to form the Michael addition product **140** (Scheme 25B), this reaction product was not observed.

The absence of the Michael addition in the initial steps of the reaction was also described in the reaction between (*E*)-2-hexenal and *N*-acetylglycyllysine methyl ester.[105] Seven variously substituted pyridinium salts were detected as the main reaction products. Four of them were composed out of one peptide molecule and two alkenals, two out of two peptide molecules and three alkenals, and one out of one peptide molecule and three alkenals. As suggested by the authors, the formation of these pyridinium salts starts with the formation of the imine **144** of the lysine side chain **143** and (*E*)-2-hexenal **141**, followed by the attachment of a second (*E*)-2-hexenal molecule via a Michael-type addition (Scheme 26). Afterward, dehydration, cyclization, and condensation reactions lead to the formation of the different pyridinium salts. The structure of the three main pyridinium salts is depicted in Figure 9.

Alaiz and Barragan[106] also reported the formation of pyridinium salts from the reaction of N^{α}-Cbz-lysine **152**

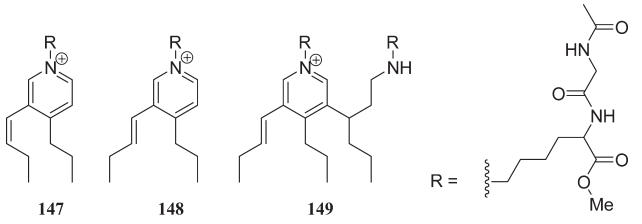


Figure 9. Chemical structures of the major compounds isolated from the reaction between *N*-acetylglycyllysine methyl ester **143** and (*E*)-2-hexenal **141**.

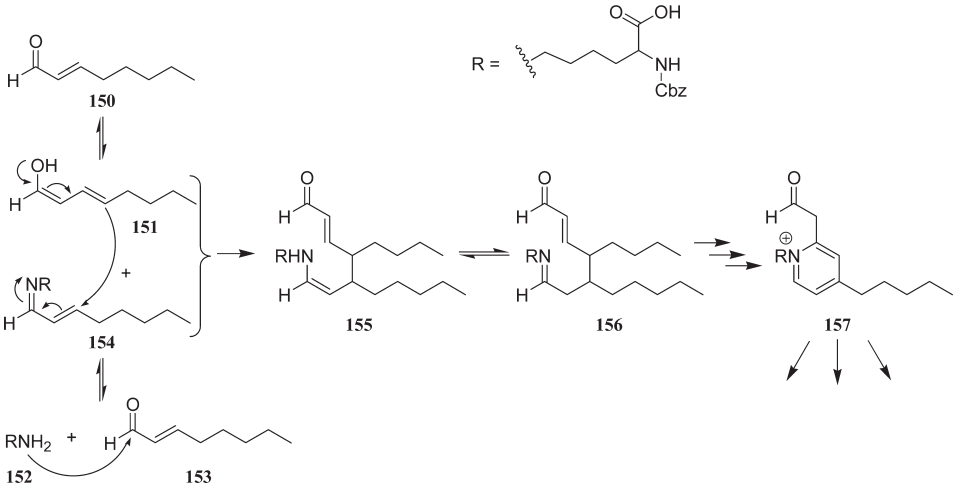
and (*E*)-2-octenal **150** under physiological conditions, beside aldol condensation products of (*E*)-2-octenal. In this study, the pyridinium salts were proposed to be formed from the reaction of imine **154** with the C-4 atom of (*E*)-2-octenal **150** (Scheme 27), instead of with the C-2 of (*E*)-2-hexenal in Scheme 26.[105] The resulting pyridinium salt **157** was not isolated but was suggested to be an intermediate in the formation of different pyridinium salts after additional oxidation and condensation reactions.

As can be seen from Scheme 27, loss of a butyl chain during the formation of the pyridinium core **157** was not explained. However, the pyridinium salts involved were positively identified in the reaction mixture, although the substitution pattern cannot be completely explained by means of Scheme 27.

Because lysine residues are often present at the *C*-terminus of biologically active peptides, for instance, ACE inhibitory peptides,[12,16] the formation of pyridinium salts can be expected in the presence of lipids, thereby modifying the activity of these peptides.

In addition to the side chain of lysine, also the side chain of histidine and arginine have been reported to react with lipid oxidation products. The reaction between N^{α}-Cbz-histidine and (*E*)-2-octenal under physiological conditions yielded mainly (*E*)-2-octenoic acid and two isomeric forms of the Michael-type addition product of the imidazole nitrogen of histidine to the C-3 of (*E*)-2-octenal.[107] The proposed

Scheme 27. Proposed Mechanism for the Formation of Quaternary Pyridinium Salts during the Reaction of N^{α}-Cbz-lysine 152 and (*E*)-2-Octenal 150 under Physiological Conditions



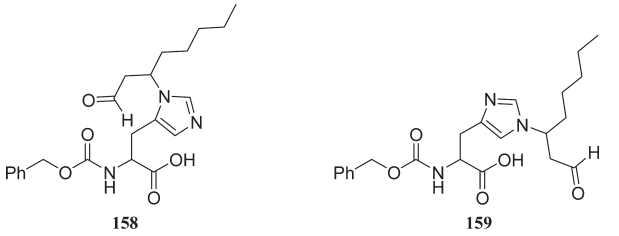


Figure 10. Proposed structures of N^{α}-Cbz-histidine/(*E*)-2-octenal adducts **158** and **159**.

structures of the N^{α}-Cbz-histidine/(*E*)-2-octenal adducts **158** and **159** are depicted in Figure 10.

Recently, the reaction between N^{α}-*t*Boc-histidine **160** and 4-hydroxy-(*E*)-2-nonenal (HNE) **161** has been described, albeit also under physiological reaction conditions. As proposed by the authors, the resulting cyclic hemiacetal **163** is formed via Michael addition of the imidazole NH-group to the double bond of HNE, followed by an intramolecular cyclization (Scheme 28).[108] As HNE is an important lipid oxidation product, this reaction, which decreases the concentration of HNE, reveals in fact some antioxidant activity. Possibly, this reaction is one of the operating mechanisms leading to the antioxidant activity of peptides, because it has been reported that those peptides typically contain a His-His unit in their sequence. Enhanced antioxidant activity has been reported when a leucine or proline residue is located at the *N*-terminus of the sequence.[3,12]

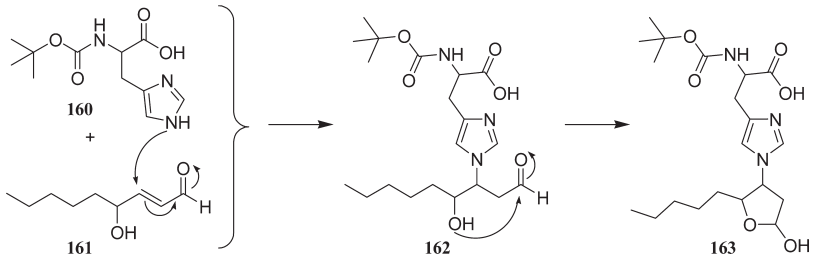
In addition, the side chain of arginine has been reported to react with a lipid oxidation product, namely, 4-oxo-(*E*)-2-nonenal. Under physiological conditions, the reaction between N^{α}-*t*Boc-arginine **164** and 4-oxo-(*E*)-2-nonenal **165** resulted in the formation of the substituted imidazole **168**.[108] According to the authors, the imidazole is formed through the intitial reaction of the guanidine group of arginine at the aldehyde moiety, followed by an intermolecular Michael addition and dehydration (Scheme 29).

To our knowledge, only one study has been performed so far on the reaction between peptides and lipid oxidation products in food-simulating conditions. A comparison of the volatiles produced during the reaction of 2,4-decadienal with cysteine and of 2,4-decadienal with glutathione (180 °C, 1 h, pH 7.5) was carried out by Zhang and Ho.[109] They found that both model systems produced about the same amounts of similar volatiles, but the ratio of these volatiles differed. In the model system containing cysteine, high amounts of hexanal were formed, whereas in the model system containing glutathione, higher amounts of 2-pentylpyridine were formed. As proposed by Josephson and Lindsay,[110] under aqueous conditions 2,4-decadienal undergoes hydration of the α,β-double-bond and retro-aldol condensation to afford hexanal and acetaldehyde as final aldehydes. 2-Pentylpyridine **176**, on the other hand, is formed via the unsaturated imine intermediate **172** from the reaction of 2,4-decadienal **169** and the amino group from an amino acid or peptide **170**, as visualized in Scheme 30.[109] After cyclization, the amino acid rest is split off in a radical way.

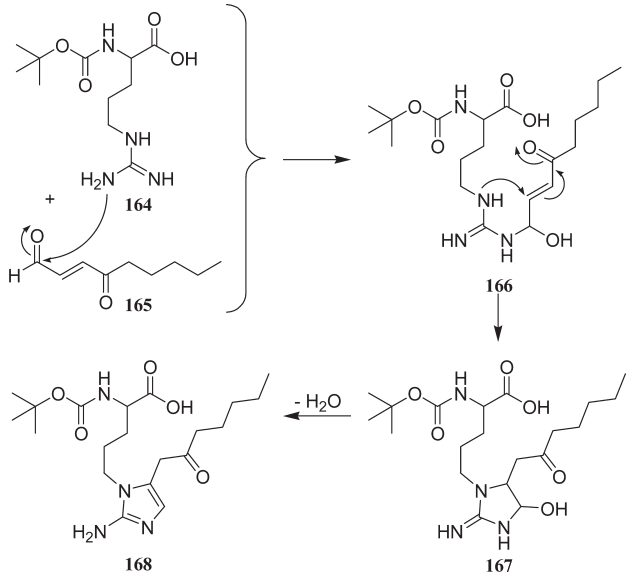
The differences between glutathione and cysteine model reactions indicate that, in the glutathione model system, 2,4-decadienal was involved in forming the imine **172** directly, and thus, less free 2,4-decadienal was available to undergo autoxidation and retro-aldolization. In addition, higher amounts of heterocyclic compounds containing both sulfur and nitrogen atoms were detected in the case of the cysteine model system, which is probably due to the faster elimination of hydrogen sulfide from cysteine as compared to glutathione.

It should be noted that, although only a few studies have focused on the reaction between peptides and lipid oxidation products under food-simulating conditions, probably similar compounds will be formed as from the corresponding reactions with amino acids and proteins, which have been studied much more.[101,102,111−113] However, it has been shown before that the amino acid sequence of peptides is of major importance in determining the final reaction products. Therefore, the compounds formed from the reactions of well-defined peptides with lipids need to be studied and characterized to evaluate their impact on food quality.

Scheme 28. Proposed Mechanism for the Formation of the Hemiacetal Adduct 163 of N^{α}-*t*Boc-histidine 160 and 4-Hydroxy-(*E*)-2-nonenal 161



Scheme 29. Proposed Mechanism for the Formation of the Substituted Imidazole Adduct 168 of N^{α}-*t*Boc-arginine 164 and 4-Oxo-(*E*)-2-nonenal 165



4.3. Reactions between Peptides and Glyoxal or Methylglyoxal

The reaction between peptides and these α-dicarbonyl compounds is discussed separately because glyoxal and methylglyoxal can both be formed from the degradation of sugars as well as from lipid oxidation.[101] Similar to the reaction between the side chain of arginine and 3-deoxyglucosone, the reaction between the side chain of arginine and methylglyoxal resulted in the formation of two tautomeric forms of an imidazolinone **177** (Figure 11).[114,115] It was expected that the reaction between N^{α}-acetylarginine and glyoxal[116] would lead to the formation of a similar imidazolinone, but this was not detected. Instead, imino imidazolidinone **178** was formed. This compound was formed very quickly under physiological conditions, but at higher temperatures, much lower amounts were formed.

Regarding the formation of cross-links, N^{α}-protected lysine-free peptides containing arginine did not cross-link with glyoxal or methylglyoxal under physiological conditions.[117] In the case of liberated *N*-termini, the peptide was cross-linked with glyoxal but not with methylglyoxal. In contrast, an arginine-free peptide containing lysine was cross-linked with methylglyoxal but not with glyoxal. In contrast to these findings, Wells-Knecht et al.[118] found that the incubation of N^{α}-hippuryllysine with glyoxal, under similar reaction conditions, yielded the formation of an imidazolium cross-link. This cross-link, known as GOLD **184** (glyoxal-lysine dimer), is a well-known advanced glycation end product (AGE) of the Maillard reaction with proteins.[86] A mechanism for the formation of GOLD was proposed by Wells-Knecht et al.[118] (Scheme 31). One molecule of glyoxal **179** reacts with two lysine side chains to form the α-diimine **181**, which then reacts with another molecule of glyoxal. The resulting intermediate **182** undergoes a Cannizaro-type rearrangement after nucleophilic attack of hydroxide, yielding a five-membered ring structure, which loses a hydroxyl group to form GOLD **184**.

Beside GOLD, Wells-Knecht et al.[118] also detected the formation of CML **93** from the reaction of N^{α}-hippuryllysine with glyoxal. The formation of CML **93** from the reaction of the N^{α}-Cbz-lysine and glyoxal was also reported by Al-Abed and Bucala.[119] A comparison of the reactivities of the arginine and lysine side chains toward glyoxal was investigated by Schwarzenbolz et al.[120] The authors found that a model system containing N^{α}-hippurylarginine, N^{α}-hippuryllysine, and glyoxal resulted in the modification of about 85% of the arginine side chains, while the lysine content remained almost unchanged (40 °C, 8 h, pH 7.4). Remarkably, addition of N^{α}-acetylcysteine to the reaction mixture led to the modification of the lysine side chains for more than 30%, while arginine derivatization was no longer visible. These results suggest that those three amino acids do not react independently with glyoxal but that these reactions are linked.

As already mentioned before, arginine and lysine residues are often present at the *C*-terminus of ACE inhibitory peptides,[12,16] and therefore, it should be kept in mind that these modifications could alter the activity of these peptides. In addition to such glyoxal and methylglyoxal modifications, which can also be formed from reactions with amino acids or proteins, the formation of pyrazinones **190** as peptide-specific modifications has been reported. A hypothetical reaction mechanism leading to their formation with glyoxal is depicted in Scheme 32. First, an imine **187** is formed between the *N*-terminal amino group of a peptide **185** and a glyoxal carbonyl group **179**. Cyclization, dehydration, and rearrangement produce the final substituted pyrazinone **190**.

In the case of methylglyoxal, two different pyrazinones can be formed that differ in the position of the methyl group (position 5 or 6 on the pyrazinone ring). It can be expected that the peptide reacts first with the aldehydic carbonyl group because it is less sterically hindered than the ketone carbonyl. The resulting

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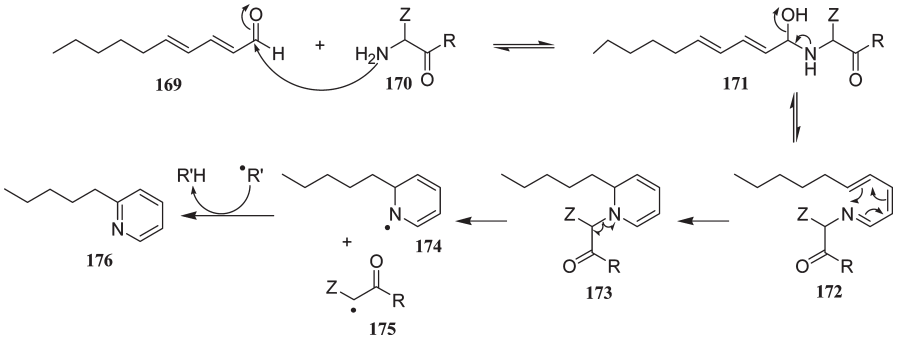
Scheme 30. Proposed Pathway for the Formation of 2-Pentylpyridine 176 from the Reaction of 2,4-Decadienal 169 and the Amino Group from Amino Acids or Peptides 170



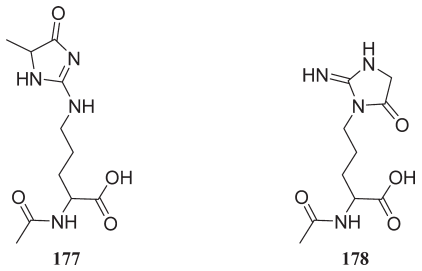


Figure 11. Chemical structures of two identified arginine side-chain modifications.

6-methylpyrazinones were indeed detected as the major pyrazinones in reaction systems containing methylglyoxal and Gly-Gly, Gly-Leu, or Leu-Gly (180 °C, 2 h, pH 5.3−5.5).[77] In contrast, Krause et al.[122] only detected the 5-methylpyrazinone after thermal treatment of methylglyoxal and Gly-Ala-Phe (37 °C, 14 days). According to these authors, nucleophilic attack is targeted on the ketone in the first step due to hydration of the aldehyde group in aqueous solution. As can be expected by the reaction mechanism, pyrazinones were not produced in reaction mixtures containing glucose and Pro-Gly or Gly-Pro (because of the secondary amino group in proline).[79]

Pyrazinones have been mainly described in reaction systems containing dipeptides.[77,121,123] It has been reported that, at temperatures of 180 °C, these pyrazinones **191** easily undergo decarboxylation,[77,121] as depicted in Scheme 33.

In addition, peculiar results were found for the reaction of Gly-Leu or Leu-Gly with glucose (180 °C, 2 h, pH 5.3−5.5),[77] as both reaction mixtures produced the same pyrazinones. Mostly pyrazinones expected from the dipeptide with leucine at the *N*-terminus were detected, also in the case of Gly-Leu reactions. The authors stated that the only possible explanation of this phenomenon is that both peptides, Gly-Leu and Leu-Gly, are interconverted through their cyclic intermediate. However, this has not been reported by any other study on the formation of pyrazinones, nor have other results suggested such equilibrium. Other experiments should be performed to elucidate this peculiar observation.

According to Izzo and Ho,[121] pyrazinones derived from the reaction of an α-dicarbonyl compound with a dipeptide possess a unique toasted aroma and are thus flavor-significant food constituents. As already mentioned before, besides pyrazinones from dipeptides, the formation of *N*-terminal pyrazinones has also been reported for longer peptides.[122]

4.4. Reactions between Peptides and Other Carbonyl Compounds

Peptides also react with other carbonyl compounds, present as minor constituents in food systems. Although these reactions might seem less important from a quantitative point of view, they can also have a substantial impact on the properties of food products.

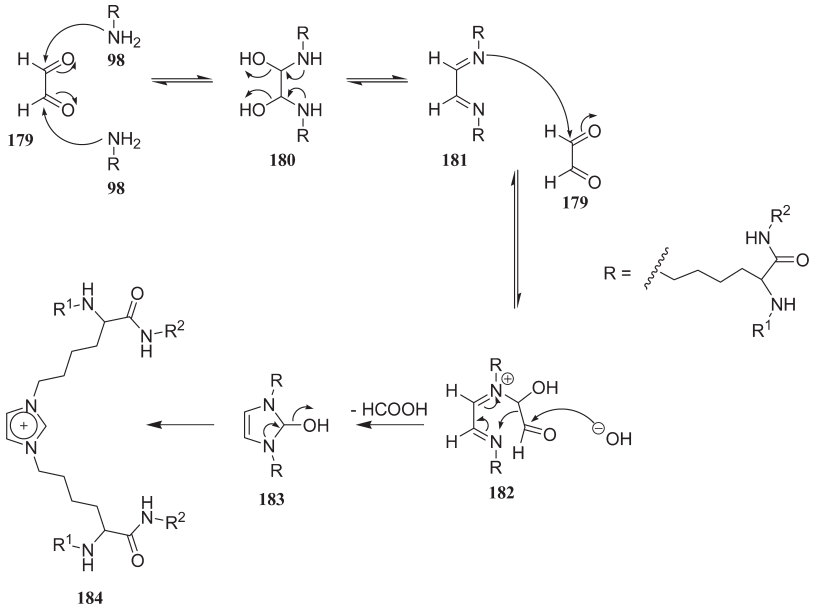
For instance, it is known that vitamin C, containing a reactive carbonyl group, undergoes Maillard-type browning reactions,[2] which results in a decrease of nutritional value. Regarding the reaction of vitamin C with peptides, Grandhee and Monnier[98] reported the formation of the fluorescent cross-link pentosidine **130** from the reaction of N^{α}-Boc-lysine and N^{α}-Boc-arginine with ascorbic acid or dehydroascorbic acid. As mentioned in section 4.1.3, pentosidine cross-links are also formed from the reaction of the side chains of lysine and arginine with pentoses or hexoses. The formation of other lysine-arginine cross-links in model systems containing N^{α}-Boc-lysine, N^{α}-Boc-arginine, and dehydroascorbic acid were also reported, in particular 4*H*-imidazol-4-ylidene-lysine derivatives.[124]

To our knowledge, no other studies have focused on the reactions of peptides with vitamin C, but the detection of the lysine-arginine cross-links mentioned above and the many reports on reactions between free amino acids and vitamin C[125−128] indicate that similar reaction products, as in the case of the Maillard reaction with carbohydrates, can be expected.

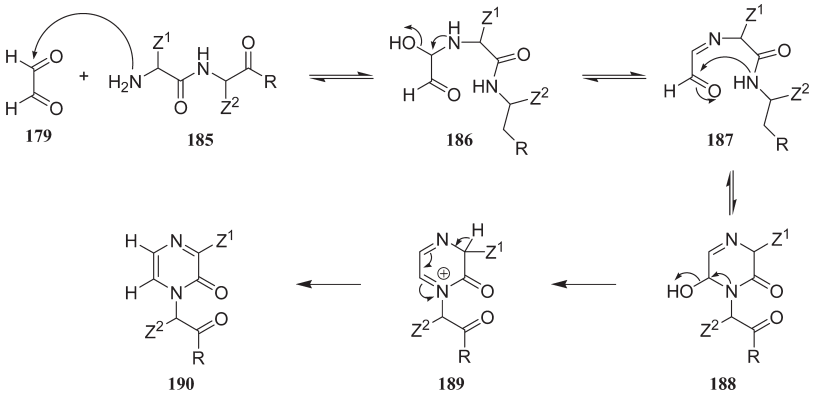
Chemical interactions between reactive flavor compounds and peptides in food can cause a decrease in aroma intensity. In this context, vanillin, the major compound in vanilla extract, has been reported to react with amino acids, peptides, and proteins.[129] Similar to the Maillard reaction, reaction rates were affected significantly by the type of amino acids or peptides involved, and by the reaction conditions. After 16 h at 55 °C, reduction of vanillin was lower than 11%, whereas at 75 °C, a much higher reduction (up to 85%) was observed. These results imply that the interaction between vanillin and peptides is probably not responsible for observed flavor losses in, for instance, ice cream, but can be important in heat-processed foods.

In addition, undesired reactive compounds, produced during food heating, can also undergo subsequent reactions with peptides. Such an example concerns acrylamide **59**, which is formed in heat-treated foods, among others, from the Maillard reaction between sugars and asparagine and from the degradation of peptides through

Scheme 31. Hypothesized Reaction Mechanism for the Formation of GOLD 184 (Glyoxal-Lysine Dimer) from Glyoxal 179 and the Side Chain of Lysine 98



Scheme 32. Hypothetical Reaction Mechanism for the Formation of a Pyrazinone 190 from the Reaction between Glyoxal 179 and a Peptide 185

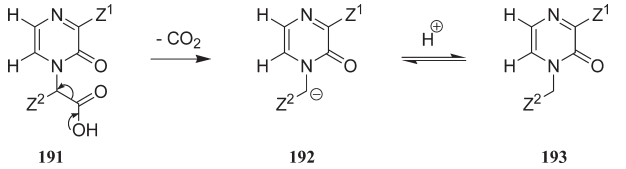


a sequence of electrocyclic processes (section 3.4). This carcinogenic compound has been reported to interact with various amino compounds, comprising peptides.[130] Acrylamide reacted very rapidly and easily with amino groups of peptides and other food constituents to produce the corresponding Michael adduct at low (37 °C), moderate (60 °C), and high temperatures (180 °C). However, this Michael adduct was not stable. During further heating, the Michael adduct decomposed again to form free acrylamide and may as such be an additional source of "hidden acrylamide" in food.

5. REACTIONS BETWEEN PEPTIDES AND INORGANIC ADDITIVES

To enhance the stability of food products, additives can be used. However, some of these additives can interact with food constituents, such as peptides.

Scheme 33. Decarboxylation of a Pyrazinone 191 Derived from Glyoxal and a Dipeptide



For instance, nitrite, which is added to meat to preserve its red color and as an antimicrobial agent,[2] has been reported to react with peptides even at room temperature. Especially, much attention has been paid to the formation of nitrosamines derived from peptides, compounds with strong carcinogenic activity. The formation of nitrosamines has been shown to take place at the *N*-terminal amino group[131] and at the side

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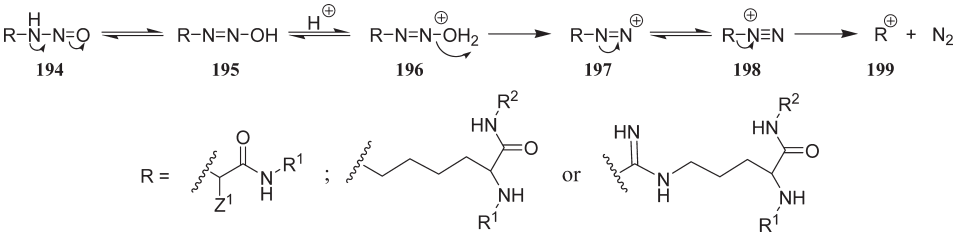
Scheme 34. Diazotation, Followed by Deamination of Nitrosated Lysine, Arginine, or *N*-Terminal Amino Groups



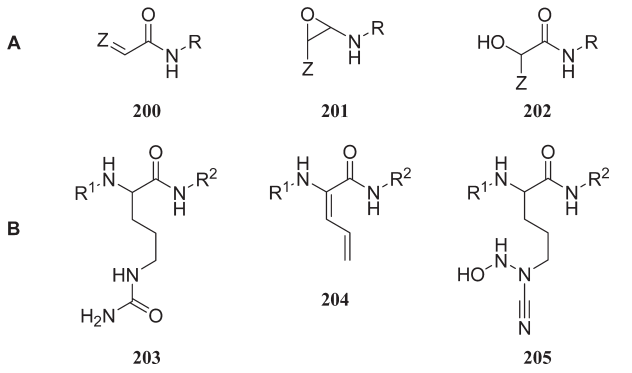


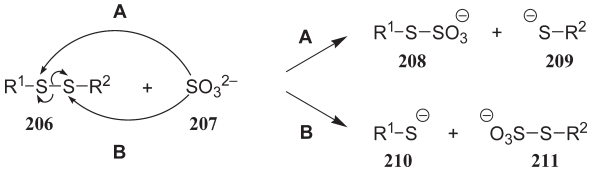
Figure 12. Chemical structures of the reaction products formed during the reaction of (A) nitrite and the *N*-terminal amino group of peptides or (B) nitrite and the side chain of arginine.

chains of tryptophan,[132−134] lysine,[131,135] and arginine[131,136] in peptides. The rate of the reaction increases with decreasing pH.[131,133] According to Bonnett and Holleyhead,[132] under acidic conditions, nitrite yields the electrophilic nitrosating species NO^+ or its equivalents $H_2NO_2^+$ or N_2O_3. However, according to Mellet et al., HNO_2 is the reactive species formed from nitrite. At the *N*-terminus and at the side chains of lysine and arginine, nitrosation is often followed by diazotation and deamination (Scheme 34), from which a carbenium ion **199** is formed. From this reactive carbenium ion, the formation of various additional peptide modifications and cross-links is possible.

As can be deduced from Scheme 34, this diazotation and deamination reaction cannot occur at the nitrosated side chain of tryptophan residues because of the presence of a secondary instead of a primary nitrosamine group. Diazotation, deamination, and subsequent reactions at the nitrosated *N*-terminus of a peptide can result in the formation of an alkene **200** (except for glycine), epoxide **201**, or alcohol **202**[131] (Figure 12A). The alkene derivative is formed by an elimination reaction, the epoxide from the reaction of the carbenium ion with the neighboring carbonyl group, and the alcohol from the addition of water. Side-chain nitrosated lysine also results in the formation of the corresponding alkene and alcohol, but as the side chain of lysine does not have a neighboring carbonyl group, the epoxide cannot be formed. For arginine, the formation of different reaction products has been reported.[136] The structures of the major reaction products of arginine and nitrite, identified by means of MS−MS and ^{1}H NMR, are depicted in Figure 12B.

Besides nitrosation at amino groups, nitrosation has also been reported at the thiol group of cysteine[134] and at the 3-position of the tyrosine side chain[134,136] in peptides. According to Zhang et al.,[134] nitrosation of the cysteine side chain of glutathione proceeds very fast under acidic conditions at room temperature. Already after 20−30 s, no thiol groups could be detected in an equimolar mixture of $NaNO_2$ and glutathione. In comparison to *N*- and *S*-nitrosation, nitrosation of tyrosine residues is less important and much slower.[136]

Scheme 35. Formation of *S*-Sulfonates 208 and 211 from the Reaction between Sulfite 207 and the Disulfide Bonds of Cystine, Peptides, or Proteins 206



Sulfur dioxide and sulfite are also commonly added to food products. Their principal preservative function is attributed to their antimicrobial activity, which allows prolongation of the shelf life of food products. In addition, these additives inhibit discoloration by blocking compounds with a reactive carbonyl group[2] and are alleged to prevent oxidative spoilage.[137] However, in food products, sulfites tend to bind to other food constituents even at relatively low temperatures, resulting in a nonactive form of sulfite. Differentiation must be made between reversibly and irreversibly bound sulfite. The formation of *S*-sulfonates **208** and **211**, from the reaction of sulfite **207** with the disulfide bonds of cystine, peptides, and proteins **206** (Scheme 35), is considered as irreversible.[137]

Although it is generally accepted that this reaction occurs with peptides, until now, the formation of *S*-sulfonates has only been studied in proteins. The formation of *S*-sulfonates has been shown in raw and cooked meat products,[138,139] shrimp,[140] and biscuits.[141] In some of these studies, a correlation was found between the levels of *S*-sulfonates and the concentration of total sulfite, whereas in other studies this was not the case. According to Garcia-Alonso et al.,[139] the limiting factor of the sulfonation reaction is not the total content of both reacting compounds but the accessibility of the disulfide groups. This could explain the different findings in these studies, and therefore, formation of *S*-sulfonates in peptides can be expected to be more important than the formation of *S*-sulfonates in proteins.

6. PREVENTING PEPTIDE MODIFICATION

Although modification of peptides in food can be desired for the development of a unique aroma and an agreeable brown

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color, it is undesired for many applications. Nowadays, peptides are often added to food systems because they influence functional properties, affect product taste, and exhibit biological activity.[1] Naturally, modification of peptides that alters these properties should be avoided as much as possible. It must be noted that complete inhibition of peptide modification is practically impossible. At some point, the reactive groups within peptides will always react with other reactive compounds present in the food matrix. In general, low temperature, low pressure, and low buffer concentrations contribute to the stability of peptides. These conditions are mostly achievable during food storage, but during food processing, less mild conditions are operative. Therefore, it is important to be aware of which part of the peptide is responsible for the desired properties. In this way, it is possible to search for specific modifications of the relevant amino acids residues (for which this review can serve as a guide) and avoid the conditions in which these modifications generally occur. For instance, if asparagine residues, which are known to undergo deamidation reactions, are important amino acids at the active site of a peptide, it is advisible to use a pH of 6, because at this pH the rate of ammonia loss is the lowest.[22]

7. SUMMARY AND OUTLOOK

Throughout this review, it was shown that the structure of peptides can be modified by a plethora of reaction pathways in food systems. Therefore, addition of peptides to food products to obtain desired properties might not be as easy as it seems, especially if a heat-treatment step is involved. In case one is interested in the properties of the peptide itself, modifications of this peptide are unwanted. However, specific modifications can also be desired if enhanced characteristics of the reaction products, such as color, taste, aroma, or biological activity, are sought for. Anyhow, in order to assess which modifications can take place, the exact structure of the peptide has to be known. Knowledge of the peptide structure thus is a first critical point in this research domain. Frequently, uncharacterized protein hydrolyzates are used or peptides for which it is not known which sequence is decisive for its activity.

Second, additional research on the possible modifications of peptides should be performed. In this review, an overview was presented of the chemical reactions that have been shown to induce alteration of the peptide structure in food systems. Regarding peptide degradation without the intervention of other reactive species, especially asparagine and glutamine residues represent the weakest spots in peptides. Naturally, peptides are not the only constituents of food products, and therefore, interactions between peptides and other reactive species are probably more important than peptide degradation as such. Mainly lysine and arginine residues are modified through various interactions with carbonyl compounds, such as carbohydrates and lipids. These modifications are, for instance, expected to take place in ACE inhibitory peptides, because they are typically very short peptides, rich in hydrophobic amino acids, from which the activity is enhanced if a proline, tryptophan, lysine, or arginine residue is located at the *C*-terminus.[12,16] Consequently, lysine or arginine modifications may alter the biological activity of these peptides. Besides lysine and arginine modifications, reactions at the *N*-terminus of peptides become more and more important with decreasing chain length. In addition, it should be stressed that not only the actual peptide modifications alter the food characteristics. The reactive groups in peptides also trigger degradation reactions of carbohydrates and lipids, resulting in the formation of color, taste, aroma, biological activity, etc. Regarding modifications of peptides due to the interaction with inorganic additives, nitrites are more important than sulfites. For sulfites, the formations of adducts is based on an equilibrium reaction, but for nitrites, nitrosation is often followed by diazotation and deamination, leading to additional peptide modifications. Although various modifications have been described, it can be expected that also other modifications take place, because many more modifications have been reported for free amino acids and proteins. In addition, little is known regarding the comparative reactivity of amino acids, peptides, and proteins. However, because free amino acids are rare in food systems and reactions with proteins are hindered due to their three-dimensional structure, it can be expected that modifications of peptides in foods are important. Moreover, conflicting results should be clarified. Different study results might be caused by different reaction conditions, which stresses the importance of choosing the right, food-relevant, reaction conditions. At first, simple model systems should be used, whereas in a later stage, more complex matrices should be studied to evaluate the actual modifications taking place when various reaction pathways occur simultaneously.

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BIOGRAPHIES

Fien Van Lancker obtained her Master's degree in Bioscience Engineering—Chemistry in 2007 from Ghent University (Ghent, Belgium). For her Master's thesis, she worked on the production of volatile fungal metabolites in relation with the sick building syndrome under the guidance of Prof. N. De Kimpe. Currently, she is working in the research team of Prof. N. De Kimpe as a Research Fellow of the Research Foundation Flanders (FWO-Vlaanderen) in a Ph.D. program at the Department of Sustainable Organic Chemistry and Technology at the Faculty of Bioscience Engineering of Ghent University. Her research interests are focused on thermal process contaminants and flavor chemistry, comprising the study of the Maillard

reaction and fungal metabolites. So far she has authored eight publications in international peer-reviewed journals.

An Adams obtained a Master's degree in Agricultural and Applied Biological Sciences (Chemistry) in 2001 at Ghent University (Belgium). In 2005, she was awarded the Ph.D. degree from the same institution (research team of Prof. N. De Kimpe), on the formation of valuable Maillard flavor compounds by model reactions and fermentation. She continues working as a Postdoctoral Fellow of the Research Foundation Flanders (FWO-Vlaanderen) at the Faculty of Bioscience Engineering of Ghent University, in the research team of Prof. N. De Kimpe. During her research career, she was a visiting research scientist at the University of Naples "Federico II" (Italy, 2003) and at Wageningen University (The Netherlands, 2006). Her main research interests are thermally generated flavors and contaminants, in particular resulting from the Maillard reaction and interactions with lipid oxidation, the analysis of natural products from medicinal plants or fungi, and general flavor research of food and essential oils. She authored 35 publications in international peer-reviewed journals.

Norbert De Kimpe obtained the diploma of chemical agricultural engineer in 1971, the Ph.D. degree in 1975, and the habilitation degree in 1985, all from Ghent University, Ghent (Belgium). He performed postdoctoral research work at the University of Massachusetts, Harbor Campus, at Boston (USA) (1979) and at the Centre National de Recherche Scientifique (CNRS) in Thiais, Paris (France) (1983), where he worked on unstable nitrogen-substituted sulfenyl derivatives and electron-deficient carbenium ions, respectively. He made his scientific career at the Belgian National Fund for Scientific Research, where he went through all stages up to the position of Research Director. During this career, he was affiliated with the Department of Organic Chemistry, Faculty of Agricultural and Applied Biological Sciences at Ghent University, where he took up teaching positions since 1987. He is now full professor in organic chemistry at the latter institution. He was a guest professor at the Centre Universitaire de Recherche sur la Pharmacopée et la Médecine Traditionelle in Butare (Rwanda, Central Africa) and at the Universities of Perpignan (France), Helsinki (Finland), Leuven (Belgium), Siena (Italy), Barcelona (Spain), Sofia (Bulgaria), Buenos Aires (Argentina), and Pretoria (South Africa). He was awarded the degree of Doctor honoris causa from the Russian Academy of Sciences in Novosibirsk (Russia) in 1998 and from the University of Szeged (Szeged, Hungary) in 2007. He obtained the Medal of Honour of Sofia University (Bulgaria) in 2006. He is Member of the Royal Flemish Academy of Belgium, Section Natural Sciences and the Academia Scientiarum et Artium Europea (European Academy of Sciences and Arts), Salzburg (Austria). He is Fellow of the Royal Society of Chemistry (U.K.). He is the author of 560 articles in international peer-reviewed journals. His research interests include (1) the synthesis of heterocyclic compounds, with focus on agrochemicals, pharmaceuticals, and natural products, (2) flavor and food chemistry, and (3) the bioassay-guided isolation of bioactive natural products from medicinal plants.

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