Strecker Degradation Products of Aspartic and Glutamic Acids and their Amides

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Abstract


Aspartic and glutamic acids, asparagine and glutamine were oxidised with either potassium peroxydisulphate or glyoxal. Non-volatile products were derivatised and analysed by GC/FID and GC/MS. Volatile reaction products were isolated and analysed by the same methods. It was found that the degradation reactions of amino acids are complex. Amino acids are principally degraded via the corresponding α-keto acids to Strecker aldehydes (aspartic acid to oxalacetic and 3-oxopropionic acids and glutamic acid to α-ketoglutaric and 4-oxobutyric acids), which are unstable and decomposed by decarboxylation to the corresponding aldehydes. Aspartic acid also eliminates ammonia and yields fumaric acid whereas glutamic acid gives rise to an imine, pyroglutamic acid. A recombination of free radicals leads to dicarboxylic acids (succinic acid from aspartic acid, succinic, glutaric and adipic acids from glutamic acid). The major volatile products (besides the aldehydes) are lower carboxylic acids (acetic acid from aspartic acid and propionic acid acid from glutamic acid). The major volatile products (besides the aldehydes) are lower carboxylic acids (acetic acid from aspartic acid and propionic acid acid from glutamic acid) that can at least partly arise by radical reactions. In both quality and quantity terms, a higher amount of degradation products arises by oxidation of amino acids by peroxydisulphate.

Keywords: Strecker degradation; Strecker aldehydes; amino acids; glyoxal; sodium peroxydisulphate; aspartic acid; glutamic acid; asparagine; glutamine; radicals

The reaction of an α-amino acid with an oxidation reagent to give carbon dioxide and an aldehyde containing one carbon atom less is known as Strecker degradation (SCHÖNBERG et al. 1948). A number of chemical reagents have been recognised as having the power to cause such oxidative decarboxylation of amino acids. The reactions between amino acids and α-dicarbonyl compounds are of special importance. They involve transaminations and yield carbon dioxide, aldehyde and aminocarboxyls. The aldehydes formed, often called Strecker aldehydes, can act as food odourants per se. The aminocarboxyls formed can yield pyrazine derivatives known as important flavour-active constituents of many processed foods. Mechanisms of these reactions have been recently described (BELITZ & GROSCH 1999; ADAMIEC et al. 2001a, b).

When amino acids with functional groups in the side chain are involved in the Strecker degradation, even more complex reactions are possible. Such amino acids are aspartic (Asp) and glutamic (Glu) acids and their amides asparagine (Asn) and glutamine (Gln). The Strecker degradation of Asp theoretically leads to 3-oxopropionic acid (malonic semialdehyde). This Strecker aldehyde has been identified as a product of Asp oxidation with N-bromoacetamide (BISHNOI & BANERJI 1985; REDDY et al. 1990), chloramine T (GOWDA & RAO 1987) and potassium peroxydisulphate (SRIVASTAVA & MATHUR 1982). On the other hand, the action of methylglyoxal on Asp resulted in the formation of acetaldehyde, which arises by decarboxylation of 3-oxopropionic acid (SCHÖNBERG & MOUBACHER 1952). Analogously, the Strecker degradation of Glu leads to 4-oxobutyric acid (succinic semialdehyde), which was found to be a product of Glu oxidation by isatin (indole-2,3-dione) (SCHÖNBERG & MOUBACHER 1952), sodium hypochlorite (FRIEDMAN & MORGULIS 1936) or sodium hypobromite (FOX & BULLOCK 1951), N-bromoacetamide (REDDY et al. 1990) and potassium peroxydisulphate (SRIVASTAVA & MATHUR 1982).

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It is well known that Asp also behaves as a β-amino acid. It can eliminate ammonia and yield fumaric acid (Belitz & Grosch 1999). Similarly, Glu behaves as a γ-amino acid as it readily forms 3-oxopyrrolidone carboxylic (pyroglutamic) acid upon heating. Under roasting conditions where radical reaction mechanisms can predominate, Asp and Asn yield various pyrrole-2,5-diones (Wilken & Baltes 1990). Asp gives rise to maleimide (pyrrole-2,5-dione) as the major component. A pathway for the formation of this compound may be maleic acid formed from Asp by the loss of ammonia, which subsequently reacts with carboxyl groups of dicarboxylic acid, yielding the imide. Substituted pyrrole-2,5-diones (3-methyl and 3,4-dimethyl) were minor products. Asn yields predominantly 3-methyl- and 3,4-dimethyl-pyrrole-2,5-diones, whereas the concentration of succinimide (pyrrolidine-2,5-dione) is low. It is suggested that pyrrole-2,5-diones arise by a radical methyl group transfer to either maleimide or succinimide.

This study was undertaken as a part of the investigation of beer changes due to oxidation during pasteurization and storage which is followed by a decomposition of amino acids and other compounds. Glutamin is a very labile amino acid. It was even used as an indicator of beer staling (Hill et al. 1998). The aim was to identify the compounds arising during Strecker degradation of Asp and Glu, their amides Asn and Gln, and to show the importance of radical reactions in the pathways leading to these products.

**MATERIAL AND METHODS**

**Chemicals:** Aspartic acid (L-isomer), glyoxalhydrate trimer and 4-oxobutanoic acid (Sigma Chemical Company, St. Louis, USA), L-asparagine monohydrate, L-glutamine (Aldrich, Steinheim, Germany), L-glutamic, acetic, propionic, butanoic, 2-methylbutanoic, pentanoic (Lachema, Brno, Czech Republic), 2-oxobutyric acid and oxalacetic acid (Merck, Darmstadt, Germany) were commercial products. Potassium peroxydisulphate (K$_2$S$_2$O$_8$), hydroxylamine.HCl and 1 ml of dry pyridine were added and the mixture was let to stand for 10 min at room temperature. Then 0.1 ml of hexamethyldisilazane and 0.1 ml of trimethylchlorosilane were added and 1 µl of the solution was analysed by GC/FID and GC/MS.

**Oxoaacids:** Using another aliquot of the evaporated aqueous phase, 10 mg of hydroxylamine.HCl and 1 ml of dry pyridine were added and the mixture was let to stand for 10 min at room temperature. Then 0.1 ml of hexamethyldisilazane and 0.1 ml of trimethylchlorosilane were added and 1 µl of the solution was analysed by GC/FID and GC/MS after 5 min of standing at room temperature.

**Gas Chromatographic (GC/FID) and Gas Chromatographic/Mass Spectrometric (GC/MS) Analysis:** A Hewlett-Packard (H/P) Model 4890A gas chromatograph equipped with a flame ionization detector and a fused capillary column (HP-Innowax, 30 m × 0.25 mm i.d., film thickness: 0.25 µm) was used in this study. The GC oven was temperature programmed from 60 to 220°C at a rate of 5°C/min, the injector and detector temperatures were held at 220 and 250°C, respectively. The carrier gas (N$_2$) flow rate was 1 ml/min. The sample (1 µl) was injected using a split ratio of 1:10. Duplicate analyses of samples were done.

GC retention indices (relative retention index, R.I.) were determined internally with a series of n-alkanes (Van den Dool & Kratz 1963). The GC conditions were the same as described above.

A H/P Model G1800A apparatus equipped with the same column operating under conditions described above was used for GC/MS analysis. Carrier gas (He) flow rate was 0.7 ml/min. Mass spectra were obtained by EI ionization at 70 eV. The ion source temperature was maintained at 250°C. The NIST/EPA/NIST 75k Mass Spectral Database (Hewlett-Packard) enabled the tentative identification of analysed compounds.

**RESULTS AND DISCUSSION**

The oxidative decarboxylation of amino acids in this study was induced by a free radical initiator, potassium peroxydisulphate, or achieved by glyoxal, a representative of α-dicarbonyl compounds (Adamiec et al. 2001a, b). Under the reaction conditions employed, the decomposed amount of Asp ranged from 53 to 64% and that of Asn from 40 to 46%. Similar results were achieved with Glu (52–67%) and its amide Gln (37–62%) (Table 1).

Major pathways of Asp transformation are outlined in Fig. 1. The intermediate oxalacetic acid can yield either pyruvic or 3-oxopropanoic acid (the Strecker aldehyde) by decarboxylation and fumaric acid by the elimination of ammonia. Decarboxylation of pyruvic acid gives acetaldehyde. Acetaldehyde was identified as a product of
Asp, oxalacetic and pyruvic acids but not quantified. Analogous reactions catalysed by enzymes occur during transamination of Asp. Analysis of the keto acids arising by the oxidation of Asp with peroxodisulphate revealed the presence of only 0.006 mg of pyruvic acid and 0.010 mg of 3-oxopropanoic acid (malonic semialdehyde) and no oxalacetic acid. The absence of oxalacetic acid in the reaction mixture is not surprising as it was found in another product identified but the intermediate $\alpha$-keto-glutaric acid was not identified.

Dicarboxylic and other nonvolatile acids were analysed as methyl esters and the results obtained are summarized in Table 2. As can be seen, fumaric acid arises as the major product from Asp in both systems studied. This acid was accompanied by two other dicarboxylic acids, i.e. maleic acid (an isomer of fumaric acid) and succinic acid. The occurrence of succinic acid suggests a homolytic cleavage of Asp (or intermediates) and subsequent recombination of so formed free radicals, as is schematically shown in Fig. 3. Only traces of fumaric acid were detected in systems comprising Asn. Other nonvolatile products are probably formed from Asn as this compound is in equilibrium with two other forms, i.e. isoasparagine and 3-aminosuccinimide (SHU & LAWRENCE 1995). As expected, Glu yielded its lactame, pyroglutamic acid as the major product and the same compound also arose from Gln. Succinic and adipic acids were found in relatively high levels. Again, this finding suggests a radical

![Fig. 1. Major pathways of Asp transformation](image1)

![Fig. 2. Major pathways of Glu transformation](image2)
Propionic acid was the major volatile mixture and in systems comprising Asn. The same volatile product found in the Glu/glyoxal and Gln/glyoxal reaction mixtures were formed from 5 mmol of Asp. Acetic acid was the only volatile product from the Asp/K₂S₂O₈ system (from 5 mmol of Asp, 665 mg) as valeric acid. Acetic acid was the only volatile acid arising by oxidation of Asp by K₂S₂O₈; however, only 0.50 mg (0.008 mmol) were formed from 5 mmol of Asp. Acetic acid was the only volatile product found in the Asp/glyoxal reaction mixture and in systems comprising Asn. The same volatile acids (acetic, propionic and butyric acids) were isolated from Glu/K₂S₂O₈. Propionic acid was the major volatile acid. Most of it arose from Gln oxidised by K₂S₂O₈. Only 1.34 mg (0.018 mmol) of this acid arose from 5 mmol of Gln. Valeric and 2-methyl/butyric acids were the newly identified compounds. The latter acid was only formed by reactions of Glu and Gln with K₂S₂O₈ at about the same concentrations as valeric acid. Acetic acid was the only volatile product found in the Glu/glyoxal and Gln/glyoxal reaction mixtures. In addition to the compounds listed in Table 3, pyrazin, 3-furancarbaldehyde and 5-methyl-2-furancarbaldehyde, which undoubtedly arose from glyoxal (ADAMIEC et al. 2001a, b), were the minor constituents of the volatile fraction.

In all cases, the major methyl esters of N-methyl and N,N-dimethyl amino acids were the major constituents of the derivatised aqueous phase that arose by methylation of the residual amino acids with diazomethane (LIEBICH & FÖRST 1985).

The volatile fraction obtained by simultaneous steam distillation/solvent extraction of Asp/K₂S₂O₈ reaction mixture contained acetic acid as the major volatile product and smaller amounts of propionic and butyric acid (Table 3). The major portion of acetic acid arose by oxidation of Asp by K₂S₂O₈, nevertheless only 0.50 mg (0.008 mmol) were formed from 5 mmol of Asp. Acetic acid was the only volatile product found in the Asp/glyoxal reaction mixture and in systems comprising Asn. Acetic acid, propionic acid and butyric acid were the major constituents of the volatile fraction.

However, the amount of acids arising as products of decomposition of the amino acids studied was relatively low. For example, the amount of volatile acids arising in the Asp/K₂S₂O₈ system (from 5 mmol of Asp, 665 mg) totals only 0.5 mg and the amount of the nonvolatile acids 4.3 mg, which corresponds to 0.7 % of Asp decomposed. Similarly, the total amount of volatile acids arising in the Glu/K₂S₂O₈ system (from 5 mmol of Glu, 735 mg) is 0.6 mg and the total amount of the nonvolatile acids is 16.6 mg, which corresponds to 2.3 % of Asp decomposed.

**CONCLUSIONS**

It was found that dicarboxylic amino acids Asp, Glu and their amides decompose by reactions with peroxodisulphate or glyoxal. They are not only decomposed by Strecker degradation but also by other pathways. The major reaction products are keto acids including Strecker aldehydes, dicarboxylic acids and lower fatty acids. Some of these products may arise by reactions involving recombination of free radicals.

**Abbreviations**

<table>
<thead>
<tr>
<th>Acid</th>
<th>Asp</th>
<th>Glu</th>
<th>Asn</th>
<th>Gln</th>
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<tbody>
<tr>
<td>l-aspartic acid</td>
<td>l-glutamic acid</td>
<td>l-asparagine</td>
<td>l-glutamine</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 3. Products arising from Asp by radical reactions**

- Acetic acid
- Propionic acid
- Butyric acid
- Succinic acid
References


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Souhrn


Asparagová a glutamová kyselina, asparagín a glutamin byly oxidovány peroxidosulfátem nebo glyoxalem. Netěkavé reakční produkty po derivatizaci a těkavé produkty po zlacení byly analyzovány metodou GC/FID a GC/MS. Bylo prokázáno, že degradovaná reakce studovaných aminokyselin jsou znázorněny kompletně. V zásadě se aminokyseliny rozkládají přes odpovídající α-ketokyseliny na příslušné Streckerovy aldehydy (asparagová kyselina přes oxaloctovou na 3-oxoproprionovou kyselinu, glutamová kyselina přes α-ketoglutarovou na 4-oxomáslenu kyselinu), které jsou nestálé a dekarboxylovány na příslušné aldehydy. Asparagová kyselina rovněž eliminuje amoníak a poskytuje fumarovou kyselinu, zatímco glutamová kyselina cykлизuje na imin, pyroglutamovou kyselinu. Rekombinace při reakci vzniklých volných radikálů vede k dikarboxylovým kyselinám (ke kyselině jantarové z asparagové a ke kyselině jantarové, glutarové a adipové z kyseliny glutamové). Hlavními těkavými produkty rozkladu aminokyselin jsou vedle aldehydů nízší karboxylové kyseliny (octová z asparagové a proprionová z glutamové kyseliny), které mohou částečně vznikat rovněž radikálovými reakcemi. Kvalitativně i kvantitativně poskytují více reakčních produktů oxidace aminokyselin peroxidosulfátem.

Klíčová slova: Streckerova degradace; Streckerovy aldehydy; aminokyseliny; glyoxal; peroxidosulfát sodný; asparagová kyselina; glutamová kyselina; asparagín; glutamin; radikály

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