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# Influence of L-pyroglutamic acid on the color formation process of non-enzymatic browning reactions

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ABSTRACT

Heating aqueous D-glucose model reactions with L-glutamine and L-alanine yielded similar colored solutions. However, size-exclusion chromatography (SEC) revealed that both non-enzymatic browning reactions proceeded differently. Due to a fast occurring cyclization of L-glutamine to pyroglutamic acid, the typical amino-carbonyl reaction was slowed down. However, L-glutamine and L-alanine model reactions showed the same browning index. Closer investigations could prove that L-pyroglutamic acid was able to influence non-enzymatic browning reactions. SEC analyses of D-glucose model reactions with and without L-pyroglutamic acid revealed an increase of low molecular colored compounds in the presence of L-pyroglutamic acid. Polarimetric measurements showed a doubling of D-glucose mutarotation velocity and HPLC analyses of D-fructose formation during thermal treatment indicated a tripling of aldose-ketose transformation in the presence of L-pyroglutamic acid, which are signs of a faster proceeding non-enzymatic browning process. 2-Pyrrolidone showed no such behavior, thus the additional carboxylic group should be responsible for the observed effects.



## 1. Introduction

The Maillard reaction is one of the most important reaction cascades in the field of food chemistry. Many food properties, such as taste, flavor and color, are contributed to by Maillard reaction products and thus the control of such non-enzymatic browning reactions is an essential technological task to ensure food quality. However, there are certain fields of application like milk or juice production that suffer from adverse effects induced by the Maillard reaction (Yeom, Streaker, Zhang, & Min, 2000). Another important technological branch is the sugar producing industry. During sucrose production sugar cane or sugar beets are extracted in a thermal process. Besides sucrose, sugar beets are rich in different amino compounds and, despite the well-established technological extraction and purification processes, small portions of monosaccharides (Andrews, Godshall, & Moore, 2002), nitrogen-containing substances and reaction intermediates, such as D-glucose, D-fructose and L-arabinose, L-glutamine, L-glutamic acid, L-asparagine and L-aspartic acid, 4-aminobutanoic acid and pyroglutamic acid (5-oxopyrrolidine-2-carboxylic acid), pass into the final product (Godshall, Buchler, Boyle, & Clarke, 1998). After that, the Maillard reaction can take place during the post-production process and product storage leading to a decline in quality by colored reaction products (Shore, Broughton, Dutton, & Sissons, 1984).

Pyroglutamic acid is a cyclization product of L-glutamic acid and especially L-glutamine and is supposed to be a precursor of 4-aminobutanoic acid (Reinefeld, Bliesener, & Schulze, 1982). This amino acid has already been subject of many investigations and is known to be very reactive and thus mainly responsible for browning reactions in this field of application (Reinefeld, Bliesener, Reinefeld, & Rexilius, 1975). Furthermore, pyroglutamic acid was found to be chemically stable at food relevant pH and temperature conditions (Airaudo, Gayte-Sorbier, & Armand, 1987), which is why there has been little focus on its influence on non-enzymatic browning processes so far. Therefore, a detailed study about the effect of L-glutamine – an amino acid with polar side chain – on the Maillard reaction with a special focus on the cyclic intermediate L-pyroglutamic acid is presented here. In order to assess the non-enzymatic browning reactivity of L-glutamine and the effect of L-pyroglutamic acid on the course of non-

*Abbreviations:* Ala, L-alanine; Asn, L-asparagine; Asp, L-aspartic acid; BuLa, 2-pyrrolidone; DAD, diode array detector; Fru, D-fructose; GC-FID, gas chromatography with flame ionization detector; Glc, D-glucose; Gln, L-glutamine; Glu, L-glutamic acid; Pyr, L-pyroglutamic acid; SEC, size-exclusion chromatography; HPLC, high performance liquid chromatography.

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enzymatic browning reactions, unbuffered aqueous model systems with D-glucose at different starting pH values were analyzed with size-exclusion chromatography and GC-FID. HPLC-PAD analyses and polarimetric measurements of D-glucose solutions with and without L-pyroglutamic acid and the structurally related compound 2-pyrrolidone were conducted as well and should reveal which structural feature is responsible for the observed catalytic effects. In addition, a comparison to L-alanine with its hydrophobic side chain was supposed to show reactivity differences in the reaction course of the amino-carbonyl reaction pathway and the carboxylic group catalysis of non-enzymatic browning reactions.

## 2. Materials and methods

### 2.1. Chemicals

The following compounds were obtained commercially: D-Glucose (PubChem CID: 5793) (Roth, Germany); D-fructose (PubChem CID: 5984), sodium hydroxide solution (PubChem CID: 5984) (Merck, Germany); L-alanine (PubChem CID: 5950), L-glutamine (PubChem CID: 5961) (Acros Organics, Belgium); L-rhamnose (PubChem CID: 25310), L-pyroglutamic acid (PubChem CID: 7405), 2-pyrrolidone (PubChem CID: 12025) (Sigma-Aldrich, Germany); hydrochloric acid solution (PubChem CID: 313) (Bernd Kraft GmbH, Germany); EZ:Faast™ GC-FID Physiological Amino Acid Analysis Kit (Phenomenex, Germany).

### 2.2. Model reactions

#### 2.2.1. D-Glucose alone and in mixture with L-glutamine, L-alanine, L-pyroglutamic acid and 2-pyrrolidone

To assess the different amino compounds' non-enzymatic reaction behavior, each amino compound (0.1 m) was dissolved in purified water and the pH value was adjusted to 5 or 8 by adding diluted hydrochloric acid or sodium hydroxide solution. The employed D-glucose solutions (1 m) were prepared the same way; 2 mL of the carbohydrate solution alone or 1-mL aliquots of the carbohydrate solution mixed with 1 mL of the amino compound model solutions in glass ampoules were incubated in a heating block at 130 °C for different periods of time. After 30, 60, 120 and 180 min the reaction was stopped by cooling down the samples in a water bath. The amino-acid-containing model reactions were analyzed by spectrophotometry, GC-FID, HPLC-PAD and SEC-DAD. The mixtures with L-pyroglutamic acid were subjected to spectrophotometry, HPLC-PAD, HPLC-DAD, SEC-DAD and polarimetric analyses. The reactions in the presence of 2-pyrrolidone were analyzed by HPLC-PAD and polarimetric measurements and the models with pure D-glucose were analyzed by spectrophotometry, HPLC-PAD, SEC-DAD and polarimetry as described below.

In general, the application of buffer substances is avoided, since buffers are known to cause unintended side reactions and influence color formation (Bell, White, & Chen, 1998). The employed 10 to 1 ratio between carbohydrate and amino compounds is chosen to simulate non-enzymatic browning based on the glucose/glutamine ratio found in sugar beets (Reinefeld et al., 1975; Schiweck & Büsching, 1974).

### 2.3. Qualification and quantification

#### 2.3.1. UV/Vis spectrophotometry

To investigate the general formation of colored reaction products, aliquots of the model reactions were diluted 1:10 with purified water (and undiluted in the case of pure D-glucose) and the browning was measured as the absorption at 420 nm (Specord® 200 Plus, Analytik Jena, Jena, Germany; WinAspect® Plus, Software Version 4.1.0.0). The results were specified as browning index (absorption @ 420 nm × dilution factor).

#### 2.3.2. pH measurement

To make sure that no reaction compound is acting as a buffer during the browning process changing the reaction course, pH values were measured after tempering the samples to room temperature with a pH-electrode (SenTix Mic, WTW, Weilheim, Germany; pH-meter CG 820, Schott, Mainz, Germany) (results not shown).

#### 2.3.3. Size-exclusion chromatography

The prepared non-enzymatic browning reaction models were diluted (1:5) with purified water and analyzed by size-exclusion chromatography-DAD (degasser, Degasys DG 1310; pump, Shimadzu LC-10AD$_{VP}$; autosampler, Merck AS-4000; guard column, Agilent PL Aquagel OH, 3 μm; 1st column, Agilent PL Aquagel OH Mixed-H, 8 μm; 2nd column, Agilent PL Aquagel Mixed, 8 μm; detector, Shimadzu SPD 10A$_{VP}$; software, Shimadzu LabSolutions v5.71 SP1) to compare the molecular size of colored reaction intermediates and end-products dependent on the utilized amino compound. The analyses were carried out at room temperature (22 °C), the injection volume was 40 μL and purified water with a flow rate of 1.0 mL/min was used as eluent. The effluent was monitored at a wavelength of 420 nm. Size calibration was achieved with five pullulan reference substances of different molecular weights (1, 5, 20, 100 and 400 kDa) (results not shown).

#### 2.3.4. HPLC-PAD

D-Glucose degradation and D-fructose formation was monitored by HPLC analysis with pulsed amperometric detection (pump, Dionex GP 40; autosampler, Perkin Elmer Series 200 Autosampler; guard column, Dionex CarboPac PA1 BioLC, 4 × 50 mm; 1st column, Dionex CarboPac PA100, 250 × 4 mm; 2ndcolumn, Dionex CarboPac PA100, 250 × 4 mm; detector, Dionex PAD; software, Shimadzu LabSolutions v5.71 SP1). For quantification the thermally treated D-glucose solutions (0.5 m) alone and in mixture with L-pyroglutamic acid (0.05 m) and 2-pyrrolidone (0.05 m) were spiked with L-rhamnose solution as internal standard and diluted 1:1000 in total with purified water, to achieve maximum concentrations of 90 μg/mL for D-glucose and D-fructose and of 50 μg/mL for L-rhamnose. The analyses were carried out at room temperature (22 °C), the injection volume was 20 μL and 0.15 m sodium hydroxide solution with a flow rate of 0.5 mL/min was used as eluent. Identification of the compounds was tentatively accomplished by comparing their retention times with those of authentic known commercially available references.pH measurements, UV/Vis spectrophotometrical measurements and HPLC-PAD analyses were carried out in duplicate.

#### 2.3.5. HPLC-DAD

In order to monitor the L-pyroglutamic acid formation process, D-glucose and L-glutamine model reactions were analyzed by HPLC-DAD at a wavelength of 210 nm after dilution with purified water (1:20) (pump, Shimadzu LC-9A; degasser, Shimadzu DGU-4; autosampler, Jasco AS-950; column oven, Shimadzu CTO-10AS VP; guard column, Macherey-Nagel EC 4/3 Nucleosil 120-5 C18; column, Macherey-Nagel EC 120-5 C18; detector, Gynkotek UVD 340 s; software, Dionex Chromeleon Version 6.00 Build 435). The analyses were carried out at 35 °C with a flow of 0.5 mL/min and with an eluent gradient starting at 99/1 (*v*/*v*) water/methanol. Within the first 15 min methanol is increased to 20 percent and kept constant for another 5 min. From 20 to 25 min the methanol level is increased to 99% with a holding time of 10 min. Then the eluent composition was adjusted to the original (99/1, *v*/*v*)