Influence of High Hydrostatic Pressure on Aspartame: Instability at Neutral pH

Keywords: *High pressure; aspartame; diketopiperazine; peptides; acceptable daily intake*

INTRODUCTION

High-pressure application is a promising technique for the preservation and processing of food. Advantages such as better retention of color, taste, and nutrients compared to thermal processing are explained by the failure of pressure to break covalent bonds. However, pressure as a thermodynamic factor similar to temperature is known to influence chemical equilibria and reaction rates according to the Le Chatelier principle, allowing covalent bond ruptures as a consequence of chemical reactions which are associated with reductions in volume (Tauscher, 1995). This may be exemplified by the rate of nonenzymatic browning in a glucose−lysine system at 50 °C, which has been shown to be either retarded or accelerated by pressure of 600 MPa, depending on pH (Hill et al., 1996; Tamaoka et al., 1991). As incubation times of some hours or even days were required in these experiments to recognize pressure effects, the results are of little relevance for commercial high-pressure food processing, during which treatments should not exceed a few minutes. In experiments on the pressure stability of the synthetic dipeptide sweetener aspartame (aspartylphenylalanyl methyl ester) we have found drastic pressure effects within minutes under conditions of commercial pressure application.

MATERIALS AND METHODS

Materials. Aspartame and diketopiperazine were gifts from Nutrasweet AG, Zug, Switzerland. L-Phenylalanyl-L-aspartic acid, L-aspartyl-L-phenylalanine, and phenylalanine methyl ester were from Bachem AG, Switzerland.

High-Pressure Treatment. Experiments were conducted in a high-pressure device consisting of a series of thermostated microautoclaves (i.d. 16 mm, ca. 10 mL) connected by valves (Butz et al., 1994). Pressure was generated manually by a hand pump in combination with a pressure intensifier. The pressure-transmitting medium was water. Due to adiabatic heating there was an initial temperature rise of not more than 4 °C on pressure buildup; the initial temperature was usually restored after 1−2 min. Aspartame was dissolved in 0.05 mol/L Tris/HCl buffer, pH 7 (Merck 8382), water, and pasteurized full-cream milk to yield a concentration of about 500 μg/mL. Samples were pressure treated in Teflon tubes (inner/outer diameter 6/8 mm; 1−2 mL) with silicon stoppers. Temperature controls were identically packaged samples in pressureless microautoclaves of the same device. Process parameters are shown in the legends to figures.

HPLC. Aspartame and its degradation products were analyzed according to the method of Langguth et al. (1991) with minor modifications. A high-performance liquid chromatograph SPD-10AD/LC-10AD (Shimadzu, Düsseldorf, Germany) with UV detection was used with a reversed-phase HD-Sil-18-5s-80 column (Orpegen, Heidelberg, Germany). Milk-containing samples were acidified, centrifuged, and filtrated for HPLC according to the procedure of Romero et al. (1996). Detection was at 200 nm; flow rate was 1.0 mL/min. The mobile phase was an 80:20 mixture of 0.5 mol/L NaH_2PO_4 (Merck 106346) adjusted to pH 2.1 by H_3PO_4 and methanol (Merck 106007), the injection volume was 20 μL, and the pressure was 26−28 MPa. Peak areas were measured by integration software (PC Integration Pack, Kontron).

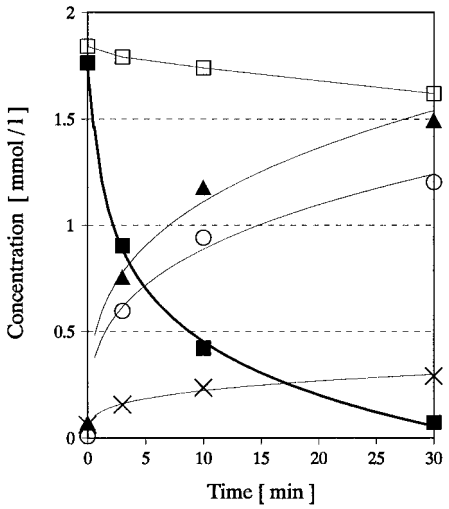

Figure 1. Pressure treatment of aspartame solutions of 0.5 g/L (ca. 1.7 mmol/L) in full-cream milk at 600 MPa and 60 °C: (■) aspartame; (□) aspartame control at 60 °C; (▲) molar sum of diketopiperazine and aspartylphenylalanine; (○) diketopiperazine; (×) aspartylphenylalanine. Data points are means of two replications.

RESULTS AND DISCUSSION

An important future application of high-pressure pasteurization will be light food and diets for diabetics in which artificial sweeteners substitute for sugar. In the pressure tests, aspartame solutions of ca. 0.5 g/L corresponding to 1.7 mmol of active substance/L were used (pH 7). This corresponds to the concentration in commercial diet cola and chocolate milk (Prodolliet and Bruelhart, 1993). In milk (pH 6.8) as soon as 3 min after pressure treatment at 600 MPa and 60 °C (Figure 1) ca. 50% of active substance is lost while the nonsweet compounds aspartylphenylalanine and diketopiperazine are forming (verified by t_R value and cochromatography of the authentic substances). The curve of the molar sum of these two compounds is a symmetrical reflection of the aspartame degradation curve. Results obtained for aspartame solutions in Tris buffer of pH 7 (Figure 2) resemble those in milk, but effects are smaller, suggesting additional matrix effects in milk. Aqueous aspartame solutions show even smaller pressure effects (Figure 3). This may be explained by self-ionization of water, which is promoted by pressure (Tauscher, 1995), resulting in a lowering of pH and stabilization of aspartame. In contrast to water, the pH of Tris buffer, frequently used in high-pressure experiments, is nearly pressure independent because of its very small ionization volume. Nonpressurized controls (Figures 1−3) show heat degradation of only a few percent in the three different media, with the strongest effect again in milk (ca. 3% loss after 3 min at 60 °C). Similar heat stability was reported for tea sweetened by aspartame (pH 3.5), in which after 7 h at 80 °C only 10% of sweetener was lost (Scherz et al., 1983). Hence, the drastic degradation reaction is caused mainly by pressure. The loss in substance within a few minutes under pressure is comparable to that recorded when diet cola was stored at 20 °C for >200 days. Comparably large pressure

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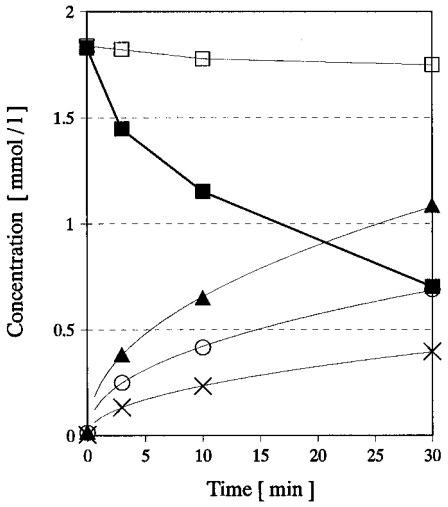

Figure 2. Pressure treatment of aspartame solutions of 0.5 g/L (ca. 1.7 mmol/L) in 0.05 mol/L Tris/HCl buffer, pH 7, at 600 MPa and 60 °C: (■) aspartame; (□) aspartame control at 60 °C; (▲) molar sum of diketopiperazine and aspartylphenylalanine; (○) diketopiperazine; (×) aspartylphenylalanine. Data points are means of two replications.

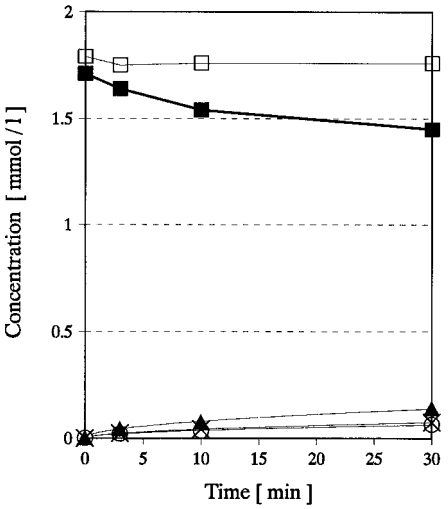

Figure 3. Pressure treatment of aspartame solutions of 0.5 g/L (ca. 1.7 mmol/L) in water at 600 MPa and 60 °C: (■) aspartame; (□) aspartame control at 60 °C; (▲) molar sum of diketopiperazine and aspartylphenylalanine; (○) diketopiperazine; (×) aspartylphenylalanine. Data points are means of two replications.

effects have not been recorded in any food or food ingredient so far. In studies at lower pH [pH 2 (acetic acid); and ca. pH 3 in grape juice; Butz et al., unpublished results] the effects were less drastic and comparable to the results in aqueous aspartame solutions (see Figure 3). Hence, sour food containing aspartame may be high pressure treated without greater loss in active substance. High-pressure treatment of dairy products of neutral pH such as chocolate milk and ice cream may present problems (possibly including also toxicological ones). In Germany, aspartame in dairy products is allowed at up to 1.2 g/kg, equivalent to ca. 4 mmol/L (EC regulations, 1000 mg/kg; U.S. regulations, no limits but demand for accordance to GMP). During pressure treatment at 60 °C, 600 MPa, and pH 7, according to Figure 1, 1.15 mmol of diketopiperazine/L (corresonding to 300 mg/L) could be present after only 5 min. A human individual of 40 kg consuming 1 L of pressurized chocolate milk would ingest the upper limit of diketopiperazine (acceptable daily intake, ADI) of 7.5 mg/kg of body weight. Compensation of the pressure-related aspartame loss by higher aspartame doses admixed before pressure treatment would result in even higher diketopiperazine concentrations in the end product.

The example of aspartame shows that high-pressure pasteurization of food may lead to unexpected chemical reactions of considerable extent. It underscores the need for studies of undesirable (possibly also desirable) chemical effects involved. Studies to explore the mechanism of pressure-induced aspartame degradation are underway. The behavior of other smaller peptide structures under pressure is investigated as well.

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