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Effect of supercritical carbon dioxide treatment on the Maillard reaction in model food systems

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Abstract

The effect of supercritical carbon dioxide treatment on the development of the Maillard reaction in powdered model systems of lactose/ovine caseinmacropeptide and lactose/β-lactoglobulin at different pH values was studied. Supercritical carbon dioxide treatments in static conditions at 30 MPa and 50 °C for up to 5 h were applied to model systems. Control experiments at 50 °C were also performed. All assayed model systems treated with carbon dioxide under supercritical conditions showed lower extent of the Maillard reaction than control treated samples. Differences between supercritical carbon dioxide treated and control samples increased with pH. These results indicate that supercritical carbon dioxide treatment of food samples does not favour the Maillard reaction and thus can be applied in foods that may require special care to avoid excessive loss of available lysine.

Keywords: Supercritical carbon dioxide treatment; Caseinmacropeptide; β-Lactoglobulin; Maillard reaction

1. Introduction

Consumer demand for high quality fresh type foods has led to increasing development of minimally processed foods. Therefore, industry is interested in non-thermal food processing technology that has no detrimental influence on nutritional or sensory food quality.

Supercritical CO_2 technology offers a suitable gentle-action during processing of foods that may preserve their structural, nutritional and functional properties. Although the technique is largely used in extraction processes, a considerable number of applications of supercritical carbon dioxide in the manufacture of foods have been reported, including its use as spray medium for coating foods with additives (Sauer, Menjivar, & Burns, 1996), inactivation of microorganisms (Spilimbergo, Elvassore, & Bertucco, 2003), extrusion processing (Chen, Dogan, & Rizvi, 2002; Mulvaney & Rizvi, 1993), and enzymatic synthesis (Eckert, Knutson, & Debenedetti, 1996; Mishima, Matsuyama, Baba, & Chidori, 2003).

Studies on chemical reactions in supercritical carbon dioxide have shown that its use as a solvent allows the successful development of a variety of industrially available synthetic transformations, including the production of compounds which are difficult to obtain under commonly applied reaction conditions (Licence, Ke, Sokolova, Ross, & Poliakoff, 2003; Santrac & Skala, 2001; Wai, Hunt, Ji, & Chen, 1998). Yalpani (1993) showed that the treatment of mixtures of reducing carbohydrates with chitosan, a polysaccharide comprising copolymers of glucosamine and *N*-acetyl-glucosamine, in supercritical carbon dioxide afforded the corresponding imine-linked derivative with high degrees of conversion. These transformations were shown to be substantially more facile than in conventional media.

Reaction between free amino groups and reducing carbohydrates may lead to loss of viability of protein-

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bound L-lysine from foods, thereby reducing their nutritive value. It is therefore necessary to investigate whether supercritical fluid processing of food has a detrimental effect on the nutritional quality.

The Maillard reaction that occurs between sugars and compounds containing a free amino group is important for food processing because it leads to the development of substances which affect flavour, colour and nutritional quality. Maillard model systems consisting of mixtures of proteins, peptides or amino acids and reducing carbohydrates have been widely used to study the different aspects of this complex reaction (Broersen, Voragen, Hamer, & de Jongh, 2004; Faist, Muller, Drusch, & Erbersdobler, 2001; Moreno, Molina, Olano, & Lopez-Fandiño, 2003). The formation of Amadori compounds that takes place during the early stages is considered as the key step of the Maillard reaction. Evaluation of the early stages of this reaction can be achieved by the determination of furosine, formed during the acid hydrolysis of the Amadori compound, ε-deoxy-L-fructosyl-lysine (Erbesdobler, Dehn, Nangpal, & Reuter, 1987).

In this study, effect of supercritical carbon dioxide (SCF-CO_2) treatment on the development of Maillard reaction between caseinmacropeptide (CMP) or β-lactoglobulin (β-lg) and lactose was investigated under different reaction conditions.

2. Materials and methods

2.1. Generation of CMP

Ovine milk was provided by a local dairy farm from the central region of Spain. Whole casein was prepared by precipitation from skim milk by adjusting the pH to 4.6 with 1 M HCl, followed by centrifugation at 4500*g* and 5 °C for 15 min. The casein precipitate was washed three times with 1 M sodium acetate acetic acid buffer, pH 4.6, thoroughly dialyzed against water and lyophilized.

Commercial rennet powder containing 85% chymosin (EC 3.4.23.4) and 15% bovine pepsin (EC 3.4.23.1) was obtained from Chr. Hansen's Laboratorium (DK-1250 Copenhagen, Denmark). Rennet solution (1 ml, 4 mg/ml) was added to ovine casein solution (25 g/l) in 0.1 M sodium phosphate buffer, pH 6.5 (100 ml) and the mixture incubated at 35 °C for 20 min. To inactivate chymosin, 0.2 M NaOH was added to adjust the pH to 9.0–9.5, followed by heating at 60 °C for 15 min (Léonil & Mollé, 1991). The sample was adjusted to pH 4.6 with 1 M HCl and centrifuged at 4500*g* and 5 °C for 15 min. The supernatant was filtered through glass wool, subjected to exhaustive dialysis against water at 4 °C and finally lyophilized.

2.2. Preparation of powdered model systems

CMP powder systems were prepared by lyophilization of solutions of 2.0 mg/ml CMP and 2.5 mg/ml lactose (Scharlau Chemie) (lactose/CMP molar ratio = 24) in 0.1 M sodium phosphate buffer, pH 11.

β-Lactoglobulin powder systems were prepared by lyophilization of solutions of 1 mg/ml of β-lg 90% (Sigma) and 1.8 mg/ml of lactose (Scharlau Chemie) (lactose/β-lg molar ratio = 100) in 0.1 M sodium phosphate buffer, pH 6.5, or in 0.2 M sodium phosphate buffer, pH 7.5, or in 0.1 M sodium carbonate buffer, pH 9.5.

2.3. Water saturation device

Before treatment under supercritical conditions, model systems were treated with a water saturation device designed to ensure moisture absorption of the samples. The device was used under the conditions described by Page, Malik, Sumpter, and Lee (1993). The method used was as follows: a column (25 cm × 4.6 mm i.d.) was completely filled with Aluminium oxide 150 (Merck KgaA, Darmstadt, Germany) followed by addition of 10% (w/w) of HPLC-grade water (previously degassed by sonication). The precolumn was sealed and allowed to equilibrate overnight at 60 °C.

2.4. Treatment with supercritical CO_2

Fig. 1 shows a diagram of the system used to study the effect of supercritical carbon dioxide treatment on the development of Maillard reaction. The CO_2 is supplied into the system (at the selected pressure conditions) by a high pressure SFC 300 pump (Carlo Erba, Milan, Italy) when valve 1 is open. In order to maintain the water activity in all the samples up to the same level, a water saturation device was used, as described above. The device was placed between the valve 1 and the extraction cell inside the oven. Once the CO_2 passes through the alumina precolumn, some water is dissolved in the supercritical CO_2 (up to 0.2% depending on the conditions) and some is purged by the carbon dioxide, passing through the sample placed inside the extraction cell. The supercritical high pressure treatment (30 MPa and 50 °C) is maintained during the reaction time (for conditions of each experiment see Table 1) in static conditions. Once the reaction is completed, the system is depressurised by opening valve 2. The flow is directed towards a homemade device composed of a two piece chamber (Ibañez et al., 1999) containing a replaceable glass vial.

In control experiments, samples placed inside the extraction cell were previously treated with SCF-CO_2 at 8 MPa for 15 min to ensure moisture absorption and then kept at 50 °C under low pressure CO_2 during the reaction time.

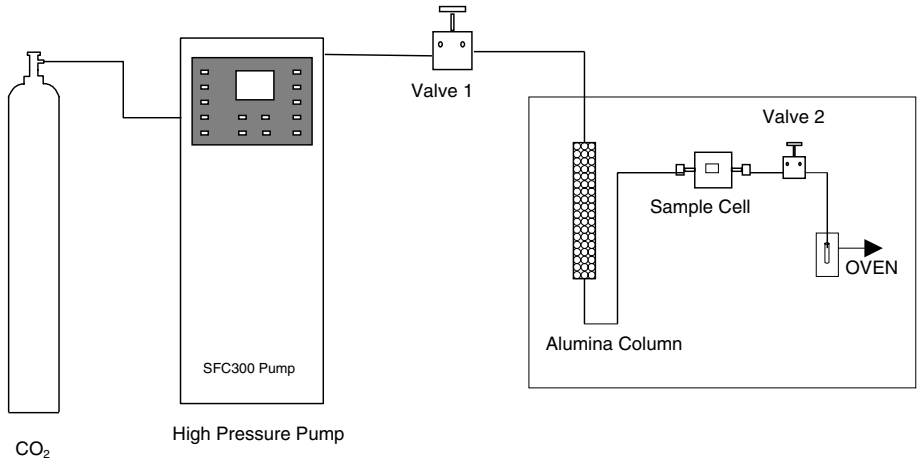

Fig. 1. Diagram of the instrumentation used for studying the effect of supercritical carbon dioxide treatment on the development of the Maillard reaction.

Table 1
Experimental conditions of supercritical fluid carbon dioxide (SCF-CO_2) and control treatments of model systems

Powdered model systems	SCF-CO_2 treatments		
	pH	*P* (MPa)	*t* (h)
CMP + lactose	11	Control[a]	1, 2, 4, 5
	11	30	1, 2, 4, 5
β-lg + lactose	7.5	Control[a]	1, 3, 5
	7.5	30	1, 3, 5
β-lg + lactose	6.5, 9.5	Control[a]	3
	6.5, 9.5	30	3

CMP, caseinmacropeptide; β-lg, β-lactoglobulin.
All treatments were carried out at 50 °C.
[a] Previously stabilised by SCF-CO_2 treatment at 8 MPa for 15 min.

Both, control and reaction treatments under SCF-CO_2 were performed in duplicate.

2.5. Furosine determination

Lactosylation of CMP and β-lg was measured through furosine determination by an ion-pair HPLC-RP method (Resmini, Pellegrino, & Batelli, 1990) after acid hydrolysis of the treated powders. Briefly, 400 μl of 8 N HCl was added to 2 mg of CMP or β-lg in hydrolysis tubes and heated at 110 °C for 23 h under inert conditions. After that 2 ml of 8 N HCl was added and the hydrolyzate was filtered through Whatman No. 40 filter paper. For determination of furosine, 500 μl of the filtered hydrolyzate was applied to a previously activated (with 5 ml of methanol and 10 ml of water) Sep-Pak C18 cartridge (Millipore). Furosine was eluted with 3 ml of 3 N HCl and 50 μl was injected.

HPLC analysis of furosine was performed using a chromatographic system composed of a 250 model pump (Perkin–Elmer), an oven (Kariba), a UV variable wavelength detector (λ = 280 nm) SM 4000 (LDC Analytical) and an 406 model interface (Beckman). Furosine separation was carried out using a C_8 (Alltech furosine-dedicated) column (250 × 4.6 mm i.d.), thermostated at 35 °C, and a linear binary gradient at a flow rate of 1.2 ml/min. Mobile phase was: solvent A: 0.4% HPLC grade acetic acid (Scharlau Chemie) in double-distilled water; and solvent B: 0.3% KCl (Merck) in solvent A.

Calibration was performed by the external standard method using solutions of known concentrations (from 0.52 to 5.2 mg/l) of commercial pure standard of furosine (Neosystem Laboratories).

3. Results and discussion

Conditions selected to perform the experiments were 50 °C and 30 MPa. As it has been previously described, relatively high temperatures favour the formation of the Amadori products (Guyomarc'h, Warin, Muir, & Leaver, 2000; Malec, Pereyra-Gonzales, Naranjo, & Vigo, 2002). Regarding the carbon dioxide pressure, only one reference could be found about the use of supercritical fluids as media to perform reactions involving an amino compound (chitosan) and reducing carbohydrates (glucose or malto-oligosaccharides) (Yalpani, 1993). In the study, the author suggested that high temperatures (up to 60 °C) and pressures ranging from 27 to 31 MPa favoured the Maillard reaction. Thus, in order to enhance the Maillard reaction in our model systems, similar conditions have been used.

Storage of lactose/β-lg mixture at 50 °C and pH 7.5 gave rise to the formation of lactosylated β-lg through the Maillard reaction. The extent of lactosylation increased with time during the period studied. Treatment of the mixture with SCF-CO_2 at 30 MPa during storage caused a decrease of β-lg lactosylation and no increase of lactosylated β-lg after 3 h storage was observed (see Fig. 2).

The effect of pH on the formation of lactosylated β-lg during storage is shown in Fig. 3. A slight lactosylation increase was observed with pH increase in the range 6.5–7.5, but a noticeable increase was detected at pH 9.5. It is generally accepted that glycation by Maillard reaction involves condensation of unprotonated amino groups with the reducing carbohydrate. The observed increase of lactosylation with pH may be due to the increase of unprotonated amino groups. As observed above, treatment under SCF-CO_2 at 30 MPa gave rise to a decrease in the formation of lactosylated β-lg. No changes in colour were observed after any of the conditions assayed. Moreover, no effect of initial pH of sample on lactosylation extent was observed.

Storage at 50 °C of lactose/CMP mixtures at pH 11.0 (Fig. 4) caused a noticeable lactosylation of CMP that increased with time. In the presence of SCF-CO_2, no lactosylation was observed after 1 h and a small formation of lactosylated CMP was detected during the storage period. The results obtained show generally good agreement with published data on the glycation of caseinmacropeptide and β-lg (Fenaille, Campos-Gimenez, Guy, Schmitt, & Morgan, 2003; Moreno, López-Fandiño, & Olano, 2002).

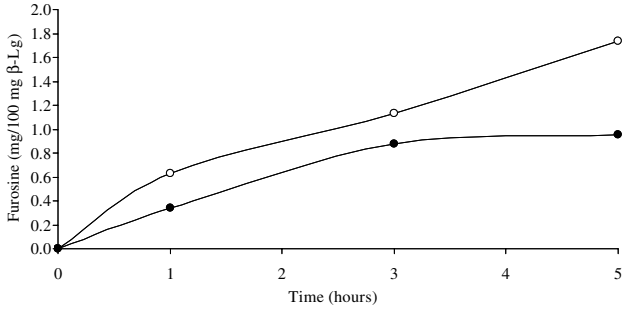

Fig. 2. Effect of supercritical carbon dioxide (SCF-CO_2) treatment at 50 °C on glycation of β-lactoglobulin (β-lg) during storage of powdered model systems of lactose/β-lg at pH 7.5: (•) SCF-CO_2 treatment (30 MPa); (○) control.

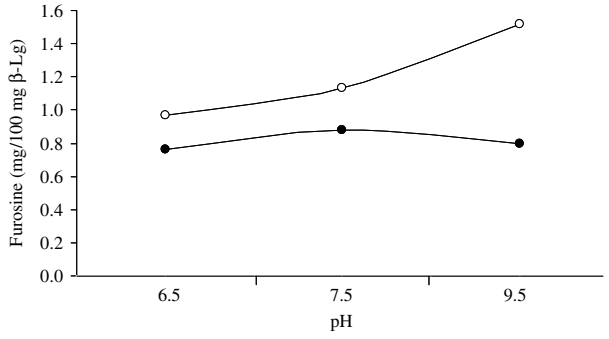

Fig. 3. Effect of pH on glycation of β-lactoglobulin (β-lg) during supercritical carbon dioxide (SCF-CO_2) treatment of powdered model systems of lactose/β-lg for 3 h at 50 °C: (•) SCF-CO_2 treatment (30 MPa); (○) control.

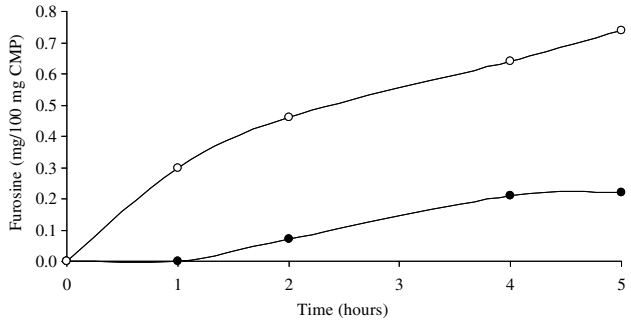

Fig. 4. Effect of supercritical carbon dioxide (SCF-CO_2) treatment at 50 °C, on glycation of CMP during storage of powdered model systems of lactose/CMP at pH 11: (•) SCF-CO_2 treatment (30 MPa); (○) control.

The observed slower lactosylation reaction under SCF-CO_2 conditions may be attributed to acidification of the sample by pressure and CO_2. Previous studies (Moreno et al., 2003) have shown that high pressure applied to sodium phosphate buffer may cause a drop of pH. Moreover, storage of milk under pressurized carbon dioxide gave rise to a considerable decrease of pH but after depressurisation under vacuum, the pH of CO_2-acidified samples returned to their initial state. Therefore, the treatment could be considered as being pH reversible (Gevaudan, Lagaude, Tarodo de la Fuente, & Cuq, 1996; Guillaume, Jiménez, Cuq, & Marchesseau, 2004). The presence of CO_2 may give rise to a decrease of pH causing a decrease of reactive unprotonated amino groups. Decrease in unprotonated amino groups is linked with a corresponding decrease in lactosylation rate. This pH-reversible acidification is more pronounced with increasing pH. This explains why the more marked differences between control and supercritical CO_2 treated samples were observed at the highest pH value.

These results indicate that SCF-CO_2 treatment of food samples does not favour the Maillard reaction and then can be applied in foods that may require special care to avoid excessive loss of available lysine.

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