Food Chemistry 167 (2015) 78–83

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Isoflavone profile and protein quality during storage of sterilised soymilk treated by ultra high pressure homogenisation

N. Toro-Funes, J. Bosch-Fusté, M.L. Latorre-Moratalla, M.T. Veciana-Nogués, M.C. Vidal-Carou *

Department of Nutrition and Food Science-XaRTA, INSA, Faculty of Pharmacy, Campus de l'Alimentació de Torribera, University of Barcelona, Avda. Prat de la Riba 171, 08921 Santa Coloma de Gramenet, Barcelona, Spain

ARTICLE INFO

Article history:
Received 11 March 2014
Received in revised form 4 June 2014
Accepted 5 June 2014
Available online 20 June 2014

Keywords:
Isoflavones
Protein digestibility
Blocked lysine
Soymilk
UHPH
Storage

ABSTRACT

The application of ultra high pressure homogenisation (UHPH) treatments is useful to obtain fine and stable soymilk emulsions. Changes of isoflavones, protein digestibility and lysine availability during 4 months of storage at 20 ± 2 °C in soymilk treated by UHPH (300 MPa and 75 °C of inlet temperature) were studied in comparison to UHT-sterilised soymilk (142 °C, 6 s). Results indicated that although there was a significantly higher extractability of isoflavones in UHT (about 38%) than in UHPH-treated samples (about 15%), similar total contents were found at the end of storage. The interconversion of isoflavones into β-glucosides was faster in UHT than in UHPH-treated soymilk. Similar evolution of protein digestibility in both UHPH and UHT-treated soymilks was found, being slightly higher in the initial UHT (88.4%) than in UHPH-treated samples (83.3%). No great differences were observed in the % of blocked lysine among samples after treatments, neither in their evolution throughout storage.

1. Introduction

The consumption of soybean and soybean products is increasing because they are a good source of high-quality proteins and also of some bioactive compounds, like isoflavones. Accumulating evidence from dietary intervention studies support the health benefits of soybean proteins and isoflavones, such as reduction of serum levels of total cholesterol, low-density lipoprotein, and triglycerides (Liu, Ho, Chen, Liu, & Woo, 2014; Lobato et al., 2012; Torres, Torre-Villalvazo, & Tovar, 2006). The 60–70% of the changes observed in blood lipids has been attributed to the isoflavones (Anderson, Johnstone, & Cook-Newell, 1995). Soymilk is the highest soy-based product consumed over the world because it could be an alternative to cow's milk for lactose-intolerant and allergic to milk proteins individuals, or for those who avoid milk for other reasons (Reilly, Lanou, Barnard, Seidl, & Green, 2006). Soymilk manufacturing involves thermal processes, which provide longer shelf-life by reduction of microorganisms, improves the nutritional value by destroying antinutritional factors (Kunitz and Bowman–Birk trypsin inhibitors), and increases protein digestibility (Debruyne, 2006; Kwok & Niranjan, 1995). On the contrary, thermal processing favours the Maillard reaction, causing losses in nutritional quality and digestibility of proteins (Nursten, 2005; Seiquer, Díaz-Alguacil, Delgado-Andrade, López-Frías, et al., 2006). Furosine, which is an early Maillard reaction product, is generated by acid hydrolysis of the Amadori compound fructosyllysine, and used as an indicator of food quality and heat damage in lots of types of food, such as in milk products, cereals, pasta, honey and many other items to which moderate heat treatment is applied (Erbersdobler & Somoza, 2007). Since lysine is a precursor of fructosyllysine, the measure of lysine availability could be considered an index of protein quality (Feinberg, Dupont, Efstathiou, Louapre, & Guyonnet, 2006).

Alternative technologies to heat treatments have been extensively tested due to the increased interest in achieving high levels of food safety and nutritive quality. Ultra high pressure homogenisation (UHPH) is an emerging technology based on the dynamic application of high pressure (up to 400 MPa) allowing the continuous processing of fluid foods. Cavitation, turbulence, impact and shear forces are physical phenomena taking place during UHPH treatment (Floury, Legrand, & Desrumaux, 2004). These mechanical forces acting on the liquid food product cause fine and stable emulsions (Thiebaud, Dumay, Picart, Guiraud, & Cheftel, 2003) and microbial and enzymatic inactivation (Hayes, Fox, & Kelly, 2005). The application of UHPH treatments not only favours the food safety (Poliseli-Scopel, Hernández-Herrero, Guamis, & Ferragut, 2012; Valencia-Flores, Hernández-Herrero, Guamis, & Ferragut, 2013) but also allows an improvement of the extractability of potentially health-related compounds, such as phytosterols from the fat globules or isoflavones from proteins (Toro-Funes, Bosch-Fusté, Veciana-Nogués, & Vidal-Carou, 2014a). Isoflavone

* Corresponding author. Tel.: +34 934033785; fax: +34 934035931.
E-mail address: mcvidal@ub.edu (M.C. Vidal-Carou).

http://dx.doi.org/10.1016/j.foodchem.2014.06.023
0308-8146/

interactions with proteins involve hydrogen bonding and hydrophobic interactions, which are believed to be a function of the protein denaturation degree (Malaypally & Ismail, 2010). Denaturation of proteins by high pressure differs from heat-induced denaturation. Protein denaturation by heat is mainly irreversible because of the breakage of covalent bonds and aggregation of the unfolded protein (Tedford, Smith, & Schaschke, 1999). On the contrary, protein under high pressure at levels of <400 MPa in isostatic high pressure processing tend to unfold till their secondary or terciary state, and the hydrogen bonds are not affected by pressure while the electrostatic and hydrophobic interactions between proteins are affected causing reversible dissociations and aggregations (Floury, Desrumaux, & Legrand, 2002). In a previous work, it has been studied the changes of isoflavones and nutritive quality of proteins, in terms of digestibility and available lysine, in pasteurised UHPH-treated (200 MPa/55 °C and 200 MPa/75 °C of inlet temperature) soymilk during refrigerated storage at 4 °C (Toro-Funes, Bosch-Fusté, Veciana-Nogués, & Vidal-Carou, 2014b). Therefore, the aim of this work is to monitor the changes of the isoflavones, the "*in vitro*" protein digestibility and the blocked lysine throughout 4 months of storage at 20 ± 2 °C in sterilised UHPH-treated soymilk compared to conventional UHT-treated samples in order to observe if there are differences with the pasteurised UHPH-treated samples.

2. Materials and methods

2.1. Methods

2.1.1. Isoflavones

Isoflavone determination was performed by UHPLC (Waters Acquity System, Milford, MA, USA) with a diode array detector (Waters 2996, Milford, MA) following the method described by Toro-Funes et al. (2012).

The non-treated soymilk (the base product) showed 125.24 ± 4.14 mg/L of total isoflavone contents.

2.1.2. "In vitro" protein digestibility

The *in vitro* digestibility was carried out following the 3-enzyme method described by Hsu, Vavak, Satterlee, and Miller (1977) that involves the use of pancreatic trypsin (Type IX), bovine pancreatic chymotrypsin (Type II), and porcine intestinal peptidase (Type III). The digestibility of each sample was calculated using the following regression equation:

$$Y = 210.464 - 18.103(X),$$

where Y = % protein digestibility and X = pH of the protein suspension after 10 min of digestion with the enzyme solution.

The base product showed 76.22 ± 0.81% of protein digestibility.

2.1.3. Blocked lysine

To calculate the % of blocked lysine, furosine was quantified by ion-pair liquid chromatographic procedure using UV detection (Delgado, Corzo, Santa-María, Jimeno, & Olano, 1992) and total lysine content was determined by cation exchange chromatography and post-column derivatization with ninhydrin, and UV detection using a Biochrom 30 (Biochrom Ltd., Cambridge, UK) amino acid analyser following the method of Moore, Spackman, and Stein (1958). Results were expressed as the percentage of blockage of lysine, as reported by Bosch, Alegría, Farré, and Clemente (2008), according to the formula:

$$\%\ \text{Blocked lysine} = (3.1 \times \text{furosine} \times 100) / (\text{total lysine} + 1.86\ \text{furosine})$$

The base product showed 29.77 ± 1.34 mg/L of furosine and 5.21 ± 0.10% of blocked lysine.

2.2. Samples

Soymilk samples were provided by the pilot plant of the Autonomous University of Barcelona (Centre Especial de Recerca Planta de Tecnologia dels Aliments (CERPTA). The elaboration process and the technological conditions applied for their production are described in the work reported by Poliseli-Scopel et al. (2012).

Three productions of soymilk were performed. For each production, whole soybeans (*Glycine max* var Majesta) were hydrated (1:3 water:soybean) during 15 h at room temperature and then grinded for 20 min at 80 °C. The pulp obtained was filtrated to obtain the soymilk base product (2.61 ± 0.04 g protein/100 mL; 1.78 ± 0.08 g fat/100 mL; 1.29 ± 0.08 g carbohydrates/100 mL; 5.56 ± 0.12 g dry matter/100 mL; 0.28 ± 0.02 g ash/100 mL; pH value was 6.72 ± 0.02) for further processing by UHPH, or UHT.

For each soymilk production, two treatments were applied on the base product: one by UHPH and the other by UHT. UHPH treatments were conducted with a high pressure homogeniser (FPG11300, Stansted Fluid Powder Ltd., Essex. UK). The UHPH conditions were 300 MPa at 75 °C of inlet temperature (UHPH). And, the UHT treatment was done in a two step homogeniser at 18 MPa and 4 MPa (X68IE+X68, Niro Soavi, Italy) previous to a heat treatment at 142 °C for 6 s with an indirect system equipment (6500/010, GEA Finnah GmbH Ahaus, Germany).

During the pressurisation operation, soymilk experienced an adiabatic heating in the high pressure valve during approximately 0.7 s reaching a temperature of 135.7 ± 1.5 °C. And, the temperature after cooling the liquid was 26.2 ± 2.2 °C. Soymilk was packaged in a coater paperboard cartons (200 mL Tetra Brick containers) by using a Tetra Pak (TBA9 slim line, Switzerland) aseptic technology. Both UHPH and UHT-treated soymilks did not show microbiological growth (total mesophilic aerobic bacteria, aerobic spores, Bacillus cereus, and enterobacteria) during 6 months of storage at room temperature (Poliseli-Scopel, Hernández-Herrero, Guamis, & Ferragut, 2014).

UHT and UHPH samples were stored at 20 ± 2 °C in a dry and dark place up to 4 months. Samples were taken monthly (time 0, 1, 2, 3 and 4) and stored at −30 °C until analysis.

2.3. Statistical analysis

All analyses were made in triplicate. All statistical tests were performed by means of the Statistical Software Package for Windows PASW Statistic 18.0 (SPSS, Chicago, IL, USA).

3. Results and discussion

3.1. Isoflavones

The initial isoflavone contents (mg/L) in the UHT and UHPH-treated soymilks are shown in Table 1. Total isoflavones were 154.67 and 186.20 mg/L in UHPH and UHT-treated samples, respectively. The UHT soymilks showed higher isoflavone amounts than the UHPH-treated ones ($p < 0.05$), both samples obtained from the same base product. The isoflavone amounts in the UHT-treated samples were also significantly higher than the ones in the pasteurised UHPH and heat-treated soymilk reported in a previous work (Toro-Funes et al., 2014b). It has been reported an increase in the extractability of isoflavones in UHPH-treated soymilks compared to their base product due to the combined levels of homogenisation pressure and temperature, and probably to the fluid temperature reached in the high pressure valve (Toro-Funes et al., 2014a). However, the UHT treatment increased the isoflavone contents more than the different UHPH treatments.