TITLE: EMULSIFYING PROPERTIES OF α -LACTALBUMIN AFTER HIGH PRESSURE TREATMENT AND SUBSEQUENT LACTOSYLATION

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Running title: High pressured and lactosylated α -lactalbumin functionality.

ABSTRACT

The effect of high pressure (HP) (300 MPa 25 °C, 1 h) and subsequent lactosylation of α -lactalbumin (α -La) (50°C, 44% RH, 168 h) was investigated regarding to its functional properties. The degree of lactosylation was evaluated by means of furosine determination, and expressed as the number of blocked lysines, being very similar to the native form. Taking into account these values and the number of free amino acids, there was evidence of higher aggregation in the pressurised samples than in the native ones. In general, emulsifying properties improved by the use of high pressure prior to lactosylation but there was no correlation between emulsifying activity index (EAI) and surface hydrophobicity. Solubility was over 80 % in all cases.

Keywords: High-pressure, α -lactalbumin, lactosylation, functional properties, whey proteins, Maillard reaction

INTRODUCTION

Maillard reaction between polysaccharides and proteins, carried out under controlled conditions, is an efficient procedure to improve the functional properties of proteins without using chemical compounds that could constitute a problem in foods [1]. Different studies have demonstrated the improvement of solubility, thermal stability and emulsifying and foaming properties of proteins bounded with oligosaccharides [2, 3].

On the other hand, HP offers a technique by which the functional characteristics of proteins can be modified in a consumer friendly, ecological way to lead to products of added value. There are several papers dealing with the effect of high-pressure on emulsifying properties of β -lactoglobulin (β -Lg) [4, 5, 6, among others] and recently a study of the effect of heat-treatment and high-pressure on the subsequent lactosylation of β -Lg [7]. However, to our knowledge there are no studies about the effect of the denaturation of α -La by HP, subsequent lactosylation and functional properties of the produced conjugates.

The aim of this work was to ascertain if lactosylation of α -La denatured by HP at 300 MPa improved its functional properties to be used as ingredient in different foodstuffs. The degree of lactosylation of native and HP treated α -lactalbumin was determined and compared by means of furosine. Solubility, hydrophobicity and emulsifying activity were evaluated.

MATERIALS AND METHODS

Sample Preparation, High Pressure Treatment and Glycosylation Experiments.

α -La (3mg/mL) was submitted to 300 MPa in a 900 HP equipment (Eurotherm Automation, Lyon, France). Pressure was raised at a rate of 2.5 MPa s^{-1} , maintained for 1 h at 25°C , and released at the same rate. After treatment, samples were immediately cooled in an ice-water bath and lactose was then added at 0.55 molar ratio, (α -La:lactose). The resulting solutions were freeze-dried and kept at 50°C and 44% relative humidity (RH). Samples were taken for analysis after 0, 8, 24, 48, 72, 96, 120, 144 and 168 h storage. As control, solutions of native α -La and lactose were treated in a similar way. All glycosylation experiments were performed in duplicate. Aliquots of the samples were stored for functional properties at 0, 24, 72 and 120 hours and delactosylated using Centricon devices.

Determination of Furosine (2-furoyl-methyl-lysine) and Colour Development

The extent of α -La lactosylation was evaluated by the furosine assay (generated by acid hydrolysis of the Amadori compound, first stable compound of Maillard reaction) [8]. Data were expressed as lactosylated lysines [9]. Absorbance was measured at room temperature at 294 nm as an indication of the formation of intermediate products of non-enzymatic browning.

Determination of Free Amino Groups

The content of free amino groups was determined using trinitrobenzenesulfonic acid (Sigma Chemical Co., St. Louis, MO). The absorbance at 420 nm was transformed into μmol of leucine/mL, using a calibration curve within the range 0.25-2.1 μM .

Emulsifying Activity and Stability

Emulsifying activity index (EAI) and emulsifying stability index (ESI) were measured following Pearce and Kinsella method [10]. Different concentrations (0.025, 0.05, 0.075 and 0.1%) of an aqueous protein solution and an oil volumetric fraction (ϕ) of 0.25 were homogenised using an Ultra-turrax (3 minutes at its maximum capacity). After the homogenisation 20 μL of the emulsions were put into 10 mL of 0.1% SDS, being 1/500 the final protein dilution. The absorbance was measured at 500 nm.

Hydrophobicity

Surface hydrophobicity was determined by the SDS-binding method according to Kato et al. [11]. Hydrophobicity was represented as μg of SDS bound to 500 μg of protein.

Solubility

The protein solubility was expressed as the ratio of the protein content in the supernatant to the protein content in the original suspension.

RESULTS AND DISCUSSION

The extent of lactosylation of native and high-pressure treated α -La, during storage at 50 °C and 44 % of relative humidity, is shown in Fig. 1A. There were no differences in the reactivity towards lactose between the native and HP samples. Colour development (Figure 1B) was also similar to HP treated samples at all the storage times tested, compared to the native α -La. The decrease in free amino groups of both native and pressurized α -La correlates well with the results obtained for the number of blocked lysines (figure 1a), although the number of blocked lysines in high-pressurised α -La was slightly lower than in the native stage, suggesting that aggregation phenomena took also place during the storage.

FIGURE 1

Emulsifying activity at concentrations ranging between 0.025 to 0.1 % of total protein content in the emulsion of native and pressurised α -La, and native and pressurised α -La with added lactose was tested (Figure 2).

FIGURE 2

All the samples showed similar patterns of the emulsifying activity indexes (EAI). In all cases, the lower the concentration, the higher the EAI. The best EAI was obtained for lactosylated native α -La at 24 h of storage and in general it seems that lactosylation slightly improves the EAI of α -La, as it occurs for β -Lg [12], in the native or in the HP denatured form. But in general, there was an improvement of the EAI in all pressurised samples (with or without lactose) with the exception mentioned above (Figure 2). It is probable that denaturation by high-pressure unfolds the protein, and although the levels of glycosylation are very similar to those obtained from native α -La, the flexibility acquired under high-pressure was enough to improve its emulsifying properties.

Solubility of native α -La at pH 7 was over 90 % after storage, whereas pressurised α -La maintained the solubility around 80 % (data not shown). Solubility is an important parameter in determining the EAI of proteins [13] as the protein needs to remain soluble to exhibit its improved emulsifying properties. Although solubility is not improved after high-pressure treatment, the value of 80 % is still quite acceptable for industrial applications and the EAI of the pressurised samples is not affected by this decrease in solubility.

Surface hydrophobicity is not correlated to the EAI as shown in Figure 3. Protein before lactosylation is more hydrophobic than their lactosylated counterparts, although pressurised samples (with or without lactose) exhibits more hydrophobicity. It is also noteworthy that the lactosylated forms decrease its hydrophobicity during the storage.

FIGURE 3

The present results show the effect of denaturation by high-pressure of proteins before lactosylation under dry-heating conditions. More research is currently underway to understand the changes in the protein structure during the storage probably related to the EAI, solubility and hydrophobicity.

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Figure captions:

Figure 1. Number of blocked lysines (estimated from the furosine concentration) (A); colour development (B) and number of free amino groups (C) during 120 h of dry-heating of α -La and lactose. Native α -La (●) and pressurised α -La at 300 MPa, 25 °C, 60 min (▲).

Figure 2: Emulsifying activity index of native and pressurised at 300 MPa, 25 °C, 60 min α -La without lactose (A) and with lactose (B). Solid line is for pressurised samples and dot line is for non-pressurised samples. Times of storage: (◆) 0 h, (●) 24 h, (▲) 72 h and (■) 120 h.

Figure 3: Surface hydrophobicity of native and pressurised at 300 MPa, 25°C, 60 min α -La with and without lactose. Native α -La (●) and pressurised α -La at 300 MPa, 25 °C, 60 min (▲). Solid line is for lactosylated samples and dot line is for non-lactosylated samples.

Figure 1:

Figure 2:

Figure 3: