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DSC STUDY OF THE EFFECTS OF HIGH PRESSURE AND SPRAY-DRYING TREATMENT ON PORCINE PLASMA

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Abstract

One use of blood generated in abattoirs is to obtain dehydrated plasma which can be used as a functional ingredient in the preparation of foods. High hydrostatic pressure is a new technique for improvement of the sanitary quality of food products or their ingredients. The changes caused in the proteins by treatment can affect their functional properties, and differential scanning calorimetry DSC was therefore applied to detect possible conformational changes in the plasma proteins. The DSC results in the present study show that spray-drying does not appreciably affect the protein structure, but high-pressure treatment seems to have a denaturing effect.

Keywords: DSC, high hydrostatic pressure, plasma proteins, spray-drying

Introduction

One way of upgrading porcine blood generated in industrial abattoirs consists in making use of the functional and nutritional properties of the protein fraction of this by-product [1–3]. The blood or its fractions can be used as functional ingredients in the preparation of certain foods, since their properties are comparable with those of some commercial products, e.g. egg albumen [4, 5]. One important property of both plasma and egg proteins is their capacity to form gels on heating. The changes caused by thermal treatment in these proteins often results in unfolding of the native structure. Under appropriate conditions, the polypeptides may form a coagulum or a three-dimensional network (a gel). The capacity to produce such structures is interesting in foods because the gels give texture, consistency and a medium which permits the retention of water, nutrients and aromas [6].

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One of the main products of the blood collected hygienically in abattoirs in some industrialized countries is spray-dried plasma. However, even if the blood is collected hygienically, microbial contamination is still a serious problem, which needs to be solved in order for this plasma powder to be used as an ingredient in human food.

The interest in maintaining functional properties supposes a limitation in the use of conventional systems to improve sanitary quality. While the thermal treatment of food effectively controls microorganisms, it can alter the taste of the food, destroy vitamins and provoke structural modifications in the proteins, which can lead to a decrease in or loss of some of these functional properties.

The application of high hydrostatic pressure (HHP) is a new technique which may be used to make spoilage microorganisms inactive in certain foods and food ingredients. HHP induces a number of changes is the morphology, biochemical reactions, genetic mechanisms, and cell membrane and wall of microorganisms [7]. As compared with classical heat treatment, HHP seems not to disrupt convalent bonding and acts without dependence on product size and geometry. As a consequence, HHP may produce food products with sufficient microbial stability and with the original nutritional and organoleptic qualities maintained [8]. Since the pressure is uniform throughout the food, the food is preserved evenly throughout, without any particle escaping preservation. Unlike thermal processing, isopressing is not time/mass-dependent, which reduces the processing time [9].

Differential scanning calorimetry (DSC) has been applied to detect possible conformational changes in plasma proteins after the application of HHP and dehydration through spray-drying.

Thermal denaturation is a cooperative phenomenon which can be detected as an endothermic peak in DSC thermal curves. The values of enthalpy changes (ΔH), calculated from the areas of the transition peaks, can permit an estimate of the thermal energy required to denature the protein [10, 11]. In many cases, the enthalpy may be used as indicators of the native state of the protein, and the technique can therefore be very useful as a method of monitoring denaturation during processing [12, 13].

The aim of the present study was to evaluate, by means of calorimetric analysis, the effects of technological treatment, spray-drying and HHP, on the structure of the protein fraction of porcine plasma.

Material and methods

Refrigerated porcine blood, hygienically collected in an industrial abattoir and containing sodium citrate as an anticoagulant, was used. The plasma fraction was obtained by centrifuging the blood for 15 min at 2520 g at a temperature of 4–5°C and by separating it later through decanting.

Spray-drying

The dehydrated plasma was obtained by spray-drying liquid plasma with Lab-Plant Spray Dryer SD-05 laboratory equipment. The process parameters used were as follows: compressor pressure: 1.8 bar; input flow of the product: 800 mL h^{-1}; input flow of hot air: 64 m^3 h^{-1}; air inlet temperature: 180°C; air outlet temperature: 80°C. These processing conditions allowed us to eliminate about 90% of the water content of the plasma and to obtain a product with the composition described in Table 1.

Table 1 Chemical composition (in g/100 g of powder) of spray-dried blood plasma[a]

Component	Content
water	11.83±0.52
ash	14.13±0.75
protein	66.45±1.92
fat	3.88±0.75

[a] Means of at least triplicate determination (means ± confidence interval α=0.05)

Samples of liquid plasma and solutions of dehydrated plasma in distilled water with a final dry matter content of 10% (corresponding to the original plasma solid content) were analysed. The study was undertaken with samples at pH 7.3 (the pH of the plasma immediately after separation by centrifuging) and with samples acidified with 4 N HCl to pH 5.5, 4.5 or 4 in order to compare the influence of different pH conditions on the thermal properties of both products.

High hydrostatic pressure (HHP)

HHP experiments up to 500 MPa were performed on a cold isostatic press of ACB Gec Alsthom (Nantes, France).

Flexible polyvinylidene chloride bags were filled with 250 mL of plasma and sealed. Water was used to apply pressure around the bag inside the HHP vessel. Operating pressures of 400, 450 or 500 MPa were applied during total compression times of 15 or 30 min. The temperature of the samples before and during HHP processing was maintained at 5°C. Control (non-pressurized plasma) was maintained at the same temperature.

All HHP processing conditions were repeated at least twice, and processed samples were analysed within 3–6 h (5°C) after pressure release.

Differential scanning calorimetry (DSC)

The heat capacity measurements were carried out with a Mettler DSC30 differential scanning calorimeter. The instrument was calibrated by standard proce-

dures with indium, lead and zinc. Aliquots of samples measuring 100 μL were weighed into aluminium pans and hermetically sealed. An appropriate quantity of distilled water was used as the reference material to obtain a flat baseline. Each sample was analysed at least twice. The temperature was raised from 30 to 100°C at a heating rate of 3°C min^{-1}.

Both the thermal denaturation point (T_d), measured at the point of minimum heat flow, and the enthalpy of denaturation (ΔH) were calculated. The calorimetric enthalpy was calculated as the peak area, which was integrated using a straight baseline. The starting and the end limits of the endotherm were determined as the visual departure from linear traces.

Results and discussion

Spray-drying

The protein structure undergoes important modifications, given that heat induces gelation in plasma proteins during thermal analysis. In the early stages, denaturation or unfolding of the native structure occurs. This is an endothermic process which involves the breaking of non-covalent bonds. Later, but before unfolding is completed, the aggregation of proteins occurs and a three-dimensional network structure is formed. This part of the process is largely exothermic. The variation in global enthalpy calculated from the endothermic peak observed in the DSC curves is a result of all these exothermic and endothermic reactions induced by heating [14, 15].

The differences between the values of enthalpy calculated for the two types of sample, liquid and spray-dried plasma (Table 2), were not significant (P>0.05) and it can therefore be deduced that spray-drying does not denature plasma proteins. However, a slight movement of the peak temperature towards higher values can be observed for all the samples of dehydrated plasma with respect to the values for liquid plasma. This might indicate the existence of structural modifications of the proteins which affect their thermal stability but do not produce vari-

Table 2 Thermal properties of porcine blood plasma[a]

pH	Blood plasma		Spray-dried blood plasma	
	ΔH[b]/J g^{-1}	T_d[c]/°C	ΔH/J g^{-1}	T_d/°C
7.3	10.1±0.8	75.7±0.6	10.5±1.3	76.4±1.0
5.5	6.5±0.6	75.6±0.2	56.±1.0	76.3±1.7
4.5	3.5±1.0	64.5±2.3	2.5±1.0	67.2±1.8
4.0	0	–	0	–

[a] Means of at least triplicate determinations (means ± confidence interval α=0.05).
[b] Enthalpy of transition.
[c] Denaturation peak temperature.

ations in the total energy involved in the process of denaturation and later gelation.

There are some factors, such as pH or ionic strength, that affect the capacity for gelation and the characteristics of the gel. In this study, the effects of pH on DSC parameters have been evaluated because these functional ingredients are mainly used in a number of formulated food products whose pH varies from neutrality (cooked meat products) to acidity (fermented products).

Figure 1 shows thermal curves obtained in the analysis of samples of liquid plasma and spray-dried plasma for two of the pH values studied (7.3 and 5.5). Table 2 reports the means of the analyses at all pH levels. It is possible to observe that variations exist in the calorimetric parameters ΔH and T_d, depending on pH. These variations are significant ($P<0.05$) and indicate that the different levels of acid denaturation of the samples affect the thermal stabilities of the plasmatic proteins and facilitate the process of denaturation during calorimetric analysis. These observations coincide with those described for egg-white proteins in similar studies [16]. The variations determined in the DSC curves are correlated with an appreciable weakening of the gel consistency when pH decreases, up to the point where protein solutions at pH≤4 have completely lost their capacity for gelation.

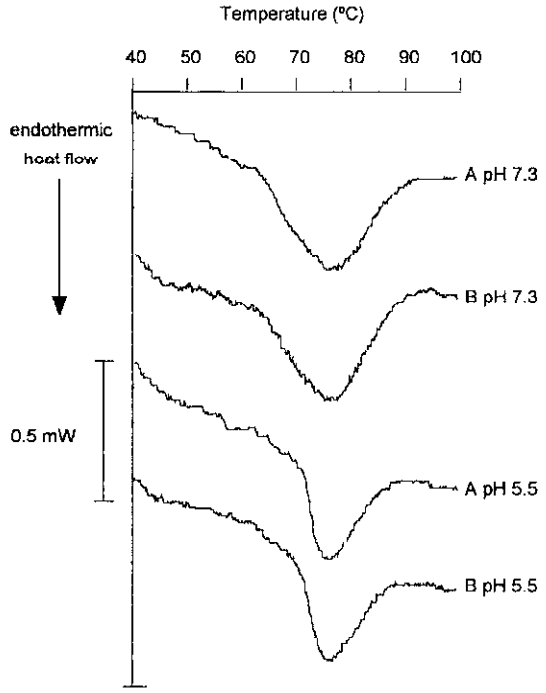

Fig. 1 Heating DSC curves for 100 µL samples of blood plasma (A) and 10% (w/v) spray-dried plasma solution in distilled water (B) at different pHs. Heating rate: 3°C min^{-1}

Enthalpy values calculated from the DSC curves for both kinds of samples (spray-dried and liquid plasma) are always identical in practice when the pH conditions are the same. These results confirm, as expected, that the spray-drying treatment does not induce appreciable denaturation of plasma proteins.

HHP treatment

HHP treatment affects proteins at the level of non-covalent interactions, which are involved in secondary and tertiary structure stabilization [17]. Suzuky (1960) [18] found that denaturation is induced at high pressure at different temperatures, which suggested that different mechanisms of denaturation operate when specified combinations of pressure and temperature are applied.

Figure 2 shows thermal curves obtained in the analysis of high-pressurized liquid plasma. The thermograms of the samples treated at the same pressure are practically identical for both compression time values. As can be observed in Table 3, where the means of the analyses for all HHP treatments are shown, when the pressure applied to the samples was increased, the ΔH values of the transition

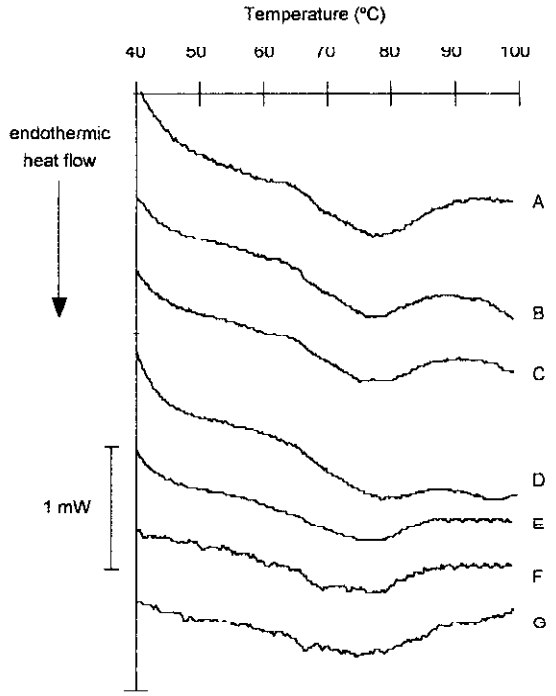

Fig. 2 Heating DSC curves for 100 μL samples of blood plasma without treatment (A) and high-pressurized liquid plasma under different operating conditions: 400 MPa/15 min (B), 400 MPa/30 min (C), 450 MPa/15 min (D), 450 MPa/30 min (E), 500 MPa/15 min (F) and 500 MPa/30 min (G). Heating rate: 3°C min^{-1}

Table 3 Thermal properties of pressurized porcine blood plasma[a]

Pressure	15 min		30 min	
	ΔH^b/J g^{-1}	T_d^c/°C	ΔH/J g^{-1}	T_d/°C
Control	10.1±0.8	75.74±0.6	10.5±1.3	76.4±1.0
400 MPa	7.5±0.5	77.20±1.0	8.0±1.0	77.4±2.4
450 MPa	6.5±1.5	76.90±0.2	7.5±0.5	75.3±0.6
500 MPa	4.5±0.5	76.55±0.4	4.0±0.0	75.1±0.5

[a] Means of at least duplicate determinations (means ± confidence interval α=0.05).
[b] Enthalpy of transition.
[c] Denaturation peak temperature.

peaks decreased. It can therefore be concluded that all the HHP treatments applied have a denaturing effect on the plasma proteins. The degree of denaturation increases as the applied pressure increases. However, the duration of the treatment, defined as the time during which the sample is effectively subjected to the desired operating pressure, does not contribute significantly to the degree of denaturation.

It was also found that the texture of the liquid plasma was affected by the HHP treatment. This might suggest that a slight protein modification occurred, allowing a certain degree of protein unfolding, and a limited aggregation may have taken place, although not sufficient for network formation to occur during treatment. However, when pressures above 450 MPa were applied, HHP induced plasma gelation at room temperature just a few hours after pressurization. This suggests that the modifications of protein structure are enough to destablize the protein.

These observations do not coincide with the findings of Hayakama *et al.* (1992) [14], who reported that the main protein component of bovine serum albumin remains fairly stable up to pressures beyond 400 MPa in phosphate buffer at pH 7 and at a protein concentration of 0.4 g L^{-1}. As the concentration of the samples studied in our experiment was 100 g L^{-1}, it may be deduced that the protein concentration affects the HHP-induced gelation process, depending on the heat-induced gelation behaviour of the proteins.

Despite the fact that gelation occurs after a few hours, this will not be a problem in the atomization and spray-drying system if spray-drying is applied to pressurized plasma immediately after pressurization. Therefore, it is possible to obtain dehydrated plasma with acceptable functional characteristics and improved microbiological quality.

Additional research is needed to determine the relationship between the processing conditions and the HHP-induced gelation of protein concentrates.

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