SHORT COMMUNICATION

Supercritical CO_2 Extraction of Egg Yolk: Impact of Temperature and Entrainer on Residual Protein

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Differential scanning calorimetry (DSC) was used to monitor changes in protein conformation resulting from supercritical carbon dioxide (SC-CO_2) extraction of lipids from egg yolk. Extraction temperatures of 65°C and lower had no effect on protein conformation as indicated by similar denaturation temperatures and enthalpies of denaturation (ΔH). An extraction temperature of 75°C resulted in a reduction in the ΔH value for ovalbumin present in the egg yolk. The use of 3% methanol as an entrainer during extraction at 36 MPa and 40°C resulted in a 50% reduction in the ΔH value for ovalbumin. The use of high temperatures and/or entrainers during SC-CO_2 extraction can result in significant protein denaturation.

KEY WORDS: Differential scanning calorimetry, egg yolk, entrainer, protein conformation, supercritical CO_2.

Supercritical carbon dioxide (SC-CO_2) is becoming increasingly attractive as a solvent in food processing as it offers advantages in terms of low toxicity, low cost and lack of flammability, and it has a wide range of solvent properties. One food application that has received considerable attention is lipid extraction, for both oilseeds (1-4) and animal products, such as egg yolk (5,6).

In studies on lipid extraction and fractionation, little attention has been given to the impact of this processing on the conformation of the residual protein. Based on electrophoretic analysis, it has been shown that SC-CO_2 extractions at temperatures of 80°C and pressures of 300 bar (30 MPa) cause some protein fragmentation and oligomerization for both ribonuclease (7) and lysozyme (8). Because the temperature used in the study (80°C) is above the thermal denaturation temperature for lysozyme [approximately 75°C at neutral and alkaline pH values (9,10)] and ribonuclease [about 60°C (11)], these changes are not unexpected. In work with soy protein, extraction temperatures between 80 and 100°C and pressures between 10,600 and 12,400 psi (73-85.4 MPa) were shown to inactivate the enzyme lipoxygenase and alter the nitrogen solubility index (12). These changes were particularly noticeable when the moisture content of the soybean was elevated. Such results were predictable considering the high temperatures used for the extractions.

When lower temperatures have been used in supercritical extraction, the effect on protein conformation has not been as evident. For example, with soy protein there was little change in solubility and electrophoretic patterns resulting from SC-CO_2 extraction at 350 bar (35 MPa) and 40°C (13). The only evidence of protein denaturation due to SC-CO_2 extraction at lower temperatures was in the work of Christianson and coworkers (14). Using an extraction temperature of 50°C and pressures between 5000 and 8000 psi (34.5-55.2 MPa), peroxidase activity in corn germ was reduced tenfold, and there was also a slight reduction in nitrogen solubility. In this case, the decrease in peroxidase activity was considered to be beneficial in terms of shelf-life extension.

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Protein conformation is a key factor when the residual protein meal left after lipid extraction is used in food applications. As a result, protein conformational changes rather than just a loss in enzyme activity is a valuable parameter to consider. Relying on the loss of solubility as an indicator of conformation changes is open to criticism because changes in solubility are not always due to changes in conformation. A technique that has been shown to be of diagnostic value in this area of research is differential scanning calorimetry (DSC), where changes in the temperature and enthalpy of thermal protein denaturation can be related to protein conformation. Changes in these parameters reflect changes in protein conformation due to prior processing treatments.

In this investigation, DSC has been used to evaluate the impact of extraction temperatures up to 75°C during lipid extraction with SC-CO_2 on the conformation of residual ovalbumin protein found in egg yolk. In addition, the impact of using an entrainer (methanol) during the SC-CO_2 extraction has also been studied. Entrainers may be necessary to effectively remove polar lipids, and thus their impact on residual protein is of concern.

MATERIALS AND METHODS

Samples. Commercial egg yolk (Export Packers Co., Ltd., Winnipeg, Canada) was freeze-dried to a constant mass. The freeze-dried material was analyzed for moisture (15) and protein (16) content. All material was flushed with nitrogen, sealed and stored at −40°C, until required. Prior to SC-CO_2 extraction, the dried yolk was ground and sieved. The coarsely ground powder particles (passed a 1 mm sieve, retained on an 850 μm sieve) were used for SC-CO_2 extraction.

Supercritical extraction. The apparatus for SC-CO_2 extraction has been described previously (17). In this study, 40-50 g of freeze-dried egg yolk was placed into the 300-mL extraction vessel. Extraction pressure for all runs was 36 MPa and temperatures examined included 40, 55, 65 and 75°C. The CO_2 used in the extraction was commercial grade, supplied by Canadian Liquid Air Ltd. (Winnipeg, Canada). The entrained solvent (3% methanol in CO_2) was obtained pre-mixed from Matheson Gas Products (Whitby, Ontario). When entrained solvent was used, the temperature and pressure for the extraction were 40°C and 36 MPa, respectively. Methanol content was monitored during each entrained run to ensure proper entrainer concentration. Starting materials for the entrained SC-CO_2 extractions included the original egg yolk or the residue from a supercritical extraction at 40°C and 36 MPa with no entrainer. Upon completion of each supercritical extraction, the residue was removed from the vessel and analyzed for protein content (16) prior to DSC analysis.

Differential scanning calorimetry. The thermal properties of the original and SC-CO_2-treated egg yolk were determined in a Dupont 9900 thermal analyzer with a 910

differential scanning calorimeter cell base (Dupont, Boston, MA). Approximately 2 mg of material was accurately weighed into a DSC pan. Sufficient water was added so that a 20% dispersion was obtained. The pan was then sealed and loaded into the DSC. Thermal curves were obtained at a heating rate of 10°C/min with an empty pan as reference. Denaturation temperature (T_d), measured at the point of maximum heat flow, and enthalpy of denaturation (ΔH) were calculated by the General Analysis Utility Program (Version 2.2) available for the calorimeter. The basis for these calculations has been described previously (18). As the thermal curve obtained contained a prominent peak at 82°C, this was the endotherm used in this investigation. This denaturation temperature corresponded to that for ovalbumin (9). In calculating the ΔH value for this thermal transition, the total protein content has been used and results are expressed as J/g protein. As proteins other than ovalbumin are also present in egg yolk, the absolute ΔH value for ovalbumin is low compared to other references where the ΔH has been reported as J/g ovalbumin.

Statistical analysis. All samples were analyzed at least in duplicate. In some cases, such as the original yolk and at the 40°C, 36 MPa treatment, there were as many as four SC-CO_2 extractions and analyses. Average values and standard deviations are reported in the tables and figures. Statistical differences were determined with an analysis of variance in conjunction with Duncan's multiple range test (19).

RESULTS AND DISCUSSION

Composition of yolk. Analysis of the freeze-dried egg yolk used as the starting material for the supercritical extractions indicated a protein content of 32 ± 3% and a moisture content of 4.4 ± 0.2%. It is important to note that this moisture level is below that used by Eldridge and coworkers (12), who investigated the effect of moisture content on SC-CO_2 extraction of soy flakes. In that study, it was observed that changes in the nitrogen solubility index and the loss of lipoxygenase activity were minimal if the moisture content was 6.5% or less and the temperature was less than 90°C. Reduced moisture contents have been shown to increase the thermal stability for plant proteins because there is insufficient water in the vicinity of the protein system to bring about a transition in protein structure (20,21). As moisture content increases, the potential for thermal denaturation increases. In the present study, therefore, the moisture content is sufficiently low to preclude it as a major factor in evaluating the effect of SC-CO_2 on protein conformation.

Effect of temperature. Variations in temperature between 40 and 75°C for the supercritical extraction of egg yolks had no significant impact on the T_d values for the ovalbumin endotherm obtained during DSC analysis (Table 1). This indicated that the thermal stability of the protein was unaffected by the SC-CO_2 treatment at these treatment temperatures. The ΔH values, however, provide a better indicator of conformational change. Essentially, the energy required to unfold the protein during the thermal analysis is reflected in the ΔH value. If any prior processing has already partially unfolded the protein, the ΔH value measured during thermal analysis will be reduced.

Temperatures between 40 and 65°C had no significant effect on the ΔH values for ovalbumin; however, at 75°C, the ΔH value was significantly lower (Fig. 1). Despite the fact that the treatment temperature was below the denaturation temperature for ovalbumin (83.07°C, Table 1), the pressure/temperature combination was sufficient to cause significant conformational change. Note that heating the freeze-dried egg yolk at 75°C for 16 h in both air and CO_2 environments had no effect on the ΔH value. It would appear that elevated pressure during the SC-CO_2 extraction made the protein more susceptible to heat. The fact that an endotherm was obtained for ovalbumin indicates the protein has not been completely denatured. In some applications (*e.g.*, preparation of foams), partial protein unfolding can be an advantage. While this slight change in conformation does not jeopardize the use of this protein source, it clearly indicates that the pressure used in SC-CO_2 extractions made this storage protein more susceptible to thermal denaturation. High temperatures should be avoided if a native protein conformation is to be retained. Ideally, if protein conformation can be maintained, then denaturants can be added for those applications where some unfolding is an advantage.

TABLE 1

Effect of Extraction Temperature and Entrainer Use on the Denaturation Temperature (T_d) of Protein in Egg Yolk During Supercritical CO_2 Extraction (at 36 MPa)

Sample	Temperature during extraction (°C)	Entrainer used in extraction	T_d (°C)[a]
Yolk			83.07 ± 0.70[a]
Residue	40	None	82.53 ± 0.47[a]
Residue	55	None	83.18 ± 0.02[a]
Residue	65	None	83.08 ± 0.29[a]
Residue	75	None	82.34 ± 0.54[a,b]
Residue	40	3% Methanol	83.68 ± 6.92[a]
2nd Residue[b]	40	3% Methanol	81.07 ± 0.26[b]

[a]Column values followed by the same letter are not significantly different ($P < 0.05$).

[b]Residue resulting from re-extraction of a residue from a supercritical CO_2 extraction at 40°C and 36 MPa.

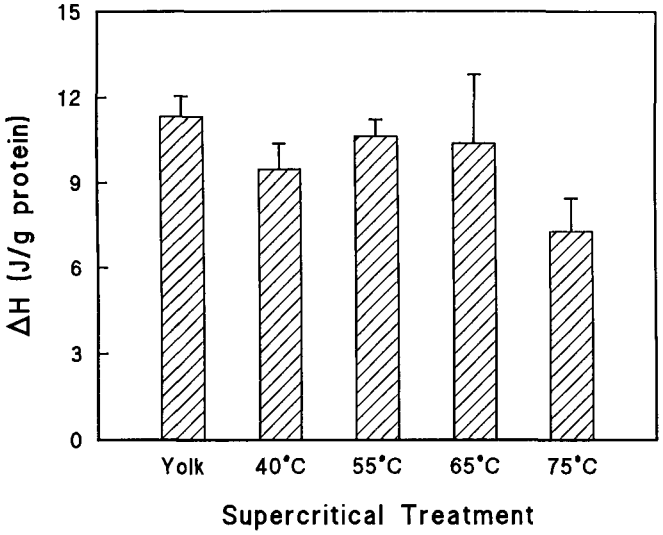

FIG. 1. Effect of temperature during SC-CO_2 extraction at 36 MPa on the enthalpy of denaturation (ΔH) of ovalbumin in egg yolk.

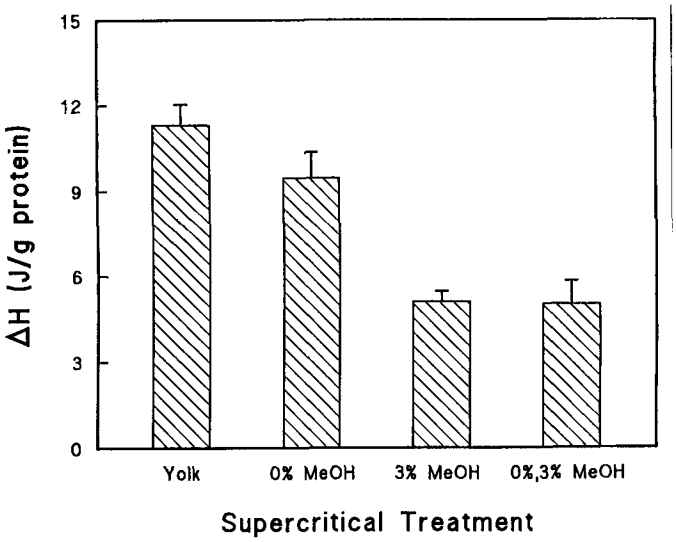

FIG. 2. Comparison of enthalpy of denaturation (ΔH) values for ovalbumin in egg yolk with and without 3% methanol entrainer during SC-CO_2 extraction at 40°C and 36 MPa. For 3% MeOH (methanol) sample, egg yolk was the starting material; for 0%, 3% MeOH, the residue from an unentrained extraction of egg yolk was re-extracted with SC-CO_2 containing the 3% methanol entrainer.

Effect of entrainer. The inclusion of 3% methanol as an entrainer during SC-CO_2 extraction of egg yolk was designed specifically to improve recovery of phospholipids (17). By including this entrainer in the initial extraction, phospholipids could be removed along with the nonpolar lipids, and by re-extracting a residue from an unentrained SC-CO_2 extraction, the phospholipids could be removed with a reduced level of nonpolar lipid.

While inclusion of the 3% methanol in the initial extraction had no impact on the T_d value for ovalbumin, the re-extraction procedure resulted in a small, albeit significant, decrease in T_d value. It is possible that the high lipid content in the yolk during the initial extraction made the protein less susceptible to the destabilizing effect of the entrainer. Alcohol (ethanol) has been used previously to reduce the T_d value for soybean globulins (22).

The slight reduction in T_d value, however, was minor in comparison to the effect of the 3% methanol on the ΔH values (Fig. 2). For both the original yolk and the residue from the unentrained SC-CO_2 extraction, ΔH values were approximately half what they were in the original yolk or unentrained residue. It has been reported previously that alcohols can affect protein conformation (23). For pure alcohols, the shorter-chain alcohols were more effective in denaturing fababean proteins, yet the maximum reduction in ΔH was 33%. In alcohol and water mixtures, high alcohol concentrations (2–5 M, depending on the alcohol) were required to reduce ΔH values and when denaturation was observed, the greatest decreases in ΔH values were observed with the longer-chain alcohols.

The 4.4% moisture in the egg yolk may have been sufficient to create a water and alcohol mixture during the SC-CO_2 extraction, thus explaining the 50% reduction in the ΔH values. If this is the case, then the use of longer-chain alcohols would be even more detrimental to protein structure. Clearly, this effect and the relationship between moisture content in the starting material and the use of entrainers during SC-CO_2 extraction requires further investigation. What is clear from this study is that, while CO_2 may be a mild solvent in terms of its impact on protein, the inclusion of an entrainer such as methanol during SC-CO_2 extractions can result in significant protein denaturation. Future research will focus on the impact of a more acceptable cosolvent from a food perspective, ethanol, and the role of sample moisture content on protein conformation during SC-CO_2 extraction.

ACKNOWLEDGMENTS

The support of the Natural Sciences and Engineering Research Council of Canada in the form of research grants is gratefully acknowledged. Excellent technical assistance was provided by A. Bernatsky, M. Szczygiel and L. Witty.

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[Received January 8, 1992; accepted May 24, 1992]