Denaturation of Egg Yolk Proteins during Processing with near-Critical Dimethylether

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Abstract

Near-critical dimethylether (DME) is an effective solvent for extraction of polar and neutral lipids from natural compounds. In some cases processing using DME may cause denaturation of residual proteins, particularly in the presence of water, and this may be undesirable where the enriched protein fraction is also a product of value. This work describes measurements of denaturation of egg proteins when DME is used to extract the lipid fraction from egg yolks. Both fresh and diluted egg yolks were processed using DME as an antisolvent to precipitate water and protein from the feed stream. Protein denaturation, based on changes in water solubility, was measured for different processing temperatures, and water to DME flow ratios. Results show that the amount of water present in the system has some effect on changes in protein solubility.

Introduction

Near-critical dimethylether (DME) is an effective solvent for extraction of polar and neutral lipids from natural compounds leaving a protein enriched residual fraction [1]. The extraction can be carried out by first drying the product and then passing DME through to extract lipids and leave a residual protein enriched residue. Lipids can also be extracted directly from an aqueous feed stream by contacting it directly with the near-critical solvent and then separating the DME phase containing dissolved lipids from DME-insoluble solids and excess water that is in the system.

During processing the protein fraction may experience some denaturation depending on the processing conditions and the extent of water present in the system. Denaturation of the proteins may be undesirable if the protein is required in a functional form in the product, of if good solubility of the product in water is required. Denaturation of proteins may however be desirable for some applications such as production of food products where denatured proteins can impart favourable textural properties. This paper describes measurements of the effect of processing parameters on protein denaturation.

Experimental

Experiments were conducted using fresh egg yolks, diluted 5 fold in distilled water, giving a solution approximately 10% solids by mass with a pH of 6.0. This feed was mixed with DME at 40 bar pressure and passed into a 500 mL phase separation (extraction) vessel. DME and extracted water and lipids were taken off the top of the vessel and depressurised into a separator to vaporise the DME and recover water and lipid. Excess water and non DME soluble solids were allowed to accumulate in the extraction vessel and were recovered at the end of the run. A schematic of the process is shown in Figure 1. Experimental runs took between 30 minutes and 60 minutes to complete.

The feed flow rate to DME flow rate ratio was adjusted to give one run where the total amount of water in the feed is completely soluble in the DME, and two runs where there was

an excess of water in the system. Experiments were also conducted at higher and lower temperatures.

Further experiments were also conducted using a freeze dried sample of fresh egg yolks to compare the effects of DME processing of a dry bed of powder.

The degree of denaturation is determined here by measuring changes in the water solubility of egg yolk proteins exposed to DME during antisolvent extraction of the lipid phase. Measurement of changes in the solubility does not give a complete or absolute measure of protein denaturation but is a common result of significant changes in the protein structure. Protein solubility is also a factor that is often more directly of interest in development of many powdered food products.

Results and Discussion

The fresh egg yolk solution was measured to contain 58% soluble protein. Freeze dried egg yolk powder was measured to have a protein solubility of only 22%. Commercially available egg yolk powder produced by spray drying was found to be almost completely insoluble.

Figure 2 shows results for the protein solubility before and after processing. The solubility of water in DME at 40°C [2] is also shown for convenience. The results show a noticeable effect of processing temperature, as can be expected. After processing at 30°C the protein solubility had dropped to 21%, whereas processing at 60°C resulted in a protein solubility of only 6%. The results also give some indication of a dependence on the water loading in the system. The largest reduction in protein solubility occurred for the run closest to

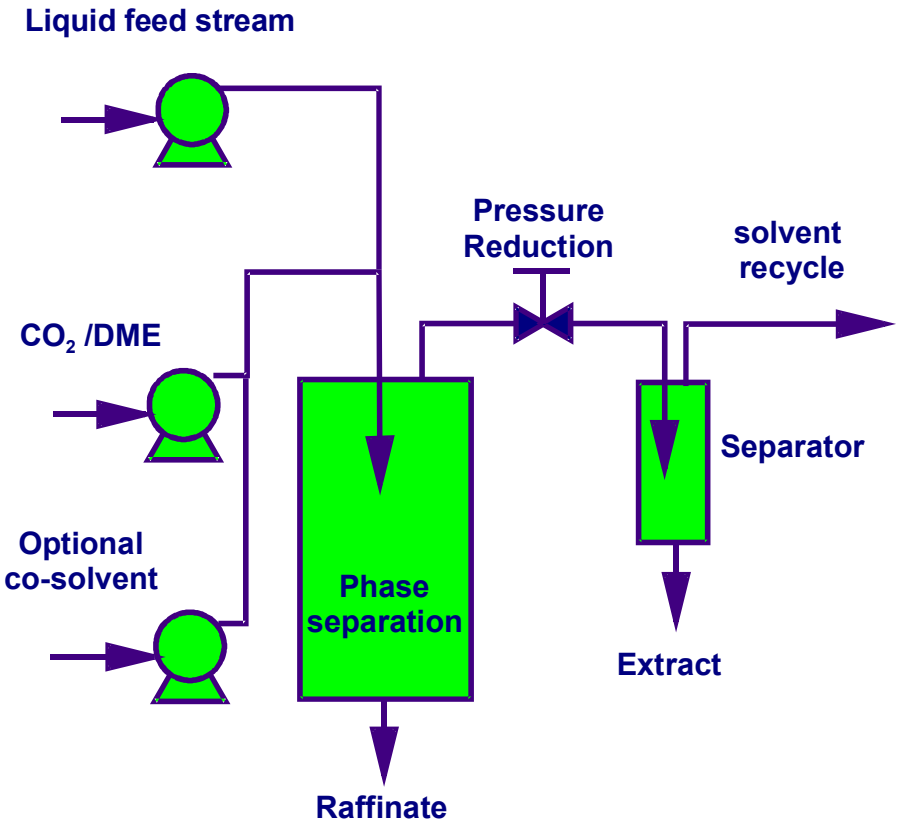

Figure 1 – Schematic of an antisolvent process.

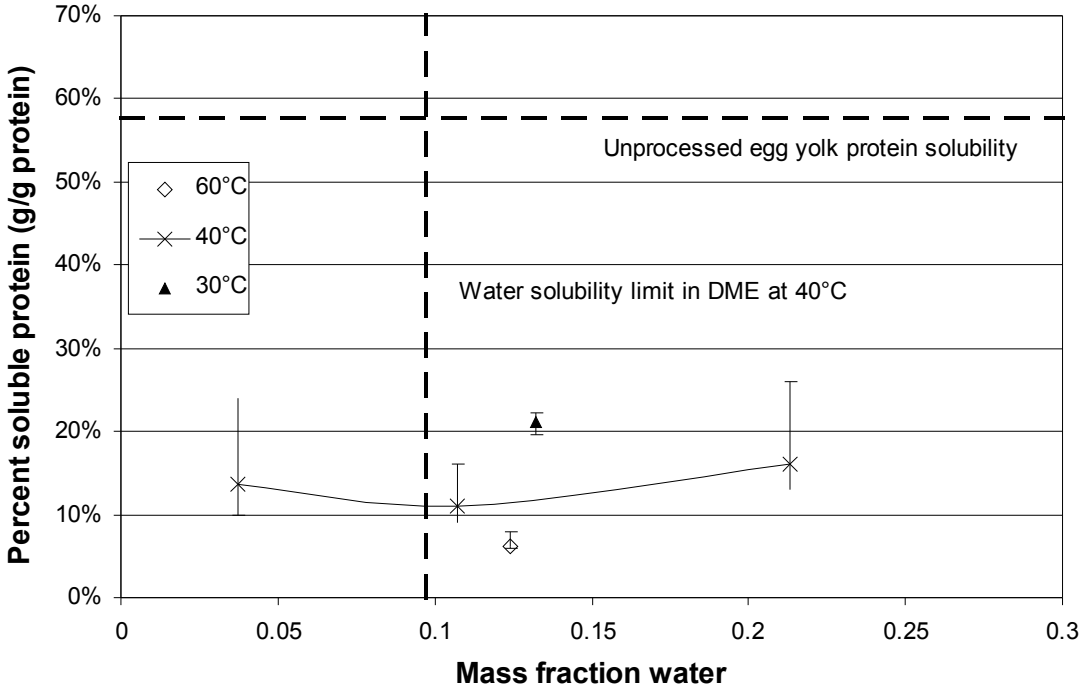

Figure 2 – Change in egg yolk protein solubility after processing with DME

the water saturation limit. Processing with either limited water, or an excess of water, resulted in a lower reduction in solubility.

Extraction of lipids from the freeze dried egg powder, by comparison, showed very little change in the protein solubility. The freeze dried powder was measured to have a protein solubility of 22.1%. This dropped to 21.1% after DME extraction at 40°C, and to 17.9% after processing at 60°C.

SDS Page analysis results are shown in Figure 3. They are used to give an indication of any significant changes in the profile of the non-soluble proteins after processing. After processing there is a band of insoluble protein that emerges with a molecular mass above 116 kDa. For the un-saturated run a noticeable band around 6 kDa also emerges.

There are a number of factors that may be contributing to the protein denaturation. These include exposure time, temperature, the presence of phase boundaries, and changes in the dielectric constant of the solvent as the ratio of DME to water changes. During processing in a system undersaturated in water, the water will be rapidly drawn into the DME phase during the initial contacting period. The protein fraction is insoluble in this DME/water phase and may not be significantly exposed to structural change after the initial transient contacting time. The smaller drop in solubility seen in Figure 2 for the undersaturated process may be a reflection of this.

During processing with higher water content in the system, the protein will be exposed to the excess water phase containing dissolved DME. The dissolved DME is expected to lower the dielectric constant and may make unfolding of the protein easier. The degree to which this

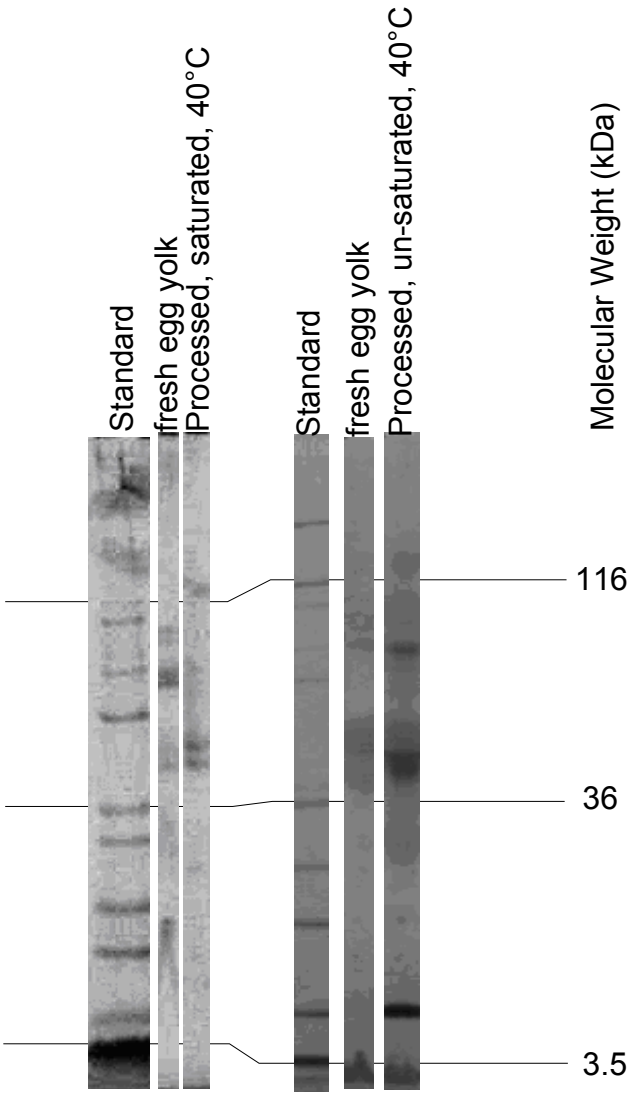

Figure 3 - SDS Page gel plates of the insoluble egg yolk protein fraction before and after antisolvent processing.

unfolding becomes permanent may be affected by the rate at which the DME is removed from this water phase after the processing run. Rapid removal of the DME would result in sudden exposure of the hydrophobic groups of the protein to water which may prevent refolding of the molecule. In these experiments the DME was removed gradually over a period of 10-20 minutes, but in a continuous antisolvent process it is expected that this water phase would be removed through a rapid depressurisation as it is removed from the extraction vessel. This may result in higher rates of denaturation than observed in this work.

Conclusions

Measurements of the change in solubility of egg yolk proteins after processing showed that higher temperatures encourage a greater degree of protein denaturation. The amount of water present in the process also affected the degree of solubility reduction. Solubility reduction was greatest for a water content just in excess of the solubility limit of water in DME. Dry powder bed extraction with pure DME (i.e. no water), in comparison, showed very little reduction in solubility after processing.

References

1. Yano et al, “Decholesterolized and defatted egg powder and method for producing same”, US patent 4234619, (1980)
2. Stephen Tallon, Owen Catchpole, “Measurement and modeling of the solubility of phosphatidylcholine in dimethylether and water”, conference proceedings of the 2004 AIChE annual meeting, Austin, Texas, 7th to 12th November, (2004)