SELECTIVE EXTRACTION OF PHOSPHOLIPIDS FROM EGG YOLK WITH SUPERCRITICAL CO_2.

K. Elst*, L. Van Ginneken and H. Weyten

Vito Process Technology, Boerentang 200, B-2400 Mol, Belgium
E-mail: Kathy.Elst@vito.be; Fax: +32 14 32 11 86

It is well known that supercritical CO_2 (SC-CO_2) is very efficient to remove neutral lipids from a variety of matrices, such as plant seeds [1]. The valuable, polar phospholipids (PL) remain behind, which is ascribed to their limited solubility in SC-CO_2 [2]. Typically, they are extracted by adding a polar modifier such as methanol or ethanol to the supercritical fluid (SC-fluid) [2-4].

In a recent study, PL could be extracted from egg yolk powder without using a modifier [5]. This was accomplished by 4 subsequent extractions at 517 bar and 40°C. The procedure is advantageous, because it is technically simpler and because no conventional solvents are needed.

In this study, the extraction of PL from egg yolk is explored in more detail. The extraction is performed with SC-CO_2, and the efficiency is examined as a function of time and process parameters such as temperature, pressure and pulsation. Furthermore, the effect of the polarity of the SC-fluid is investigated by adding ethanol as modifier.

Within the processing conditions studied, the PL cannot be extracted from egg yolk powder without adding at least 5 mole% ethanol as modifier. The extraction efficiency of the PL increases with the ethanol concentration and with the pressure. The processing temperature shows only a minor effect at a low ethanol concentration.

INTRODUCTION

Phospholipid mixtures are important natural emulsifiers, which are used in food, cosmetics and pharmaceuticals. They are traditionally obtained as a by-product from the oil processing industry, for instance from the refining of soybean oil [2].

Egg yolk is a rich source of phospholipids (PL). Dried egg yolk powder contains approximately 15% PL, which is mainly composed of roughly 70% phosphatidylcholine and 20% phosphatidylethanolamine [6]. PL-mixtures derived from egg yolk are commercially available and are used in some dedicated pharmaceutical and food applications.

SC-CO_2 efficiently removes neutral lipids from a variety of matrices, such as plant seeds. However, the more polar PL are not extracted, leaving a potentially valuable product behind.

In this study, the extraction of PL from egg yolk is explored in more detail. The efficiency is examined as a function of several process parameters, and the effect of a modifier is studied.

I - MATERIALS AND METHODS

Materials

The starting material consists of freeze dried egg yolk powder, and was kindly provided by BELOVO, SA (Belgium). The particles have a diameter of 60-100 µm. The moisture content of the egg yolk powder is specified to be less than 4%.

Apparatus
The extractions are performed in a laboratory scale apparatus (ISCO SFX^{TM} 220), designed for a maximum pressure of 510 bar and a maximum temperature of 150^{o}C. The supercritical fluid is led through an extraction cell and is depressurised over a heated capillary restrictor. The extract is collected in a sample vial of 20 ml.
In some experiments ethanol is added as modifier. This cosolvent is pumped independently with a GILSON 305-pump, and is mixed with the SC-fluid before entering the extraction cell. The cosolvent pump is operated at constant flow rate and is monitored gravimetrically.

Analysis
The extraction is monitored as follows.

- The weight loss of the sample is determined.
- The total amount of extracted PL is determined by phosphorus analysis. The extract is digested with aqua regia and H_2O_2 in a semi-open microwave destruction [7]. The P-content is subsequently analysed with ICP-AES. It is converted to the PL-mass by multiplying the P-mass by 24.5 to take into account the average molecular weight of the PL in the egg yolk.
- The PL are quantified on some extracts, using HPLC equipped with an UV-detector. A modified version of the procedure described by Heinze et al. is followed [8].

II – EXPERIMENTAL RESULTS & DISCUSSION

Extraction with supercritical CO_2
In the first experiments, the egg yolk powder is extracted with SC-CO_2. For these extractions a thimble of 2.5 ml is loaded with about 1.25 g egg yolk powder. The extraction is performed at 40^{o}C at an average CO_2 flow rate of 2.2 g/min. The extract is collected in a vial that is filled with hexane.

Figure 1 shows the extracted mass as a function of the CO_2-mass at 200 to 510 bar. All extraction curves have a similar shape. The extracted mass increases approximately linearly with the CO_2-mass, until 35 Wt% of the egg yolk is extracted. Thereafter, the extraction curve levels off to a plateau and no additional mass is collected.

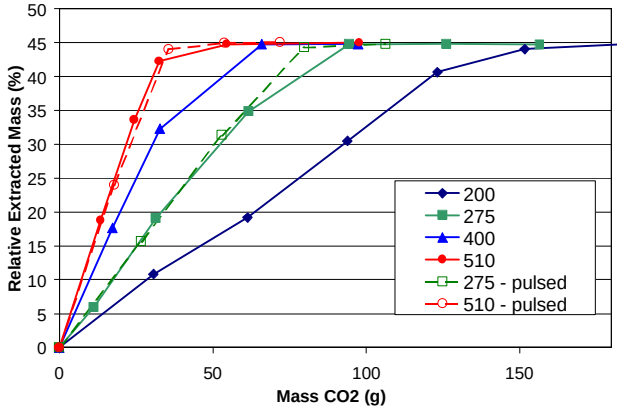

***Figure 1**: Extraction curves at several pressures between 200 and 510 bar. The extracted mass is taken relative to the original mass. The extraction curves that are obtained with 4 subsequent extractions are represented by open symbols.*

Figure 1 reveals that always 45 Wt% of the egg yolk powder is extracted at the end of the processing. This value is independent of the pressure that is applied.
Figure 1 also displays the curves for the pulsed extractions. These data are obtained by extracting the sample four times, and releasing the pressure after each extraction step. Similarly to the results of Boseli et al., the sample freezes during the pressure release when the extraction is performed at 510-bar [5]. Remark that the curves are identical to the uncycled experiments. This indicates that no additional egg yolk constituents are extracted by cycling the pressure (and by freezing the sample).
None of the extracts contains a detectable amount of P. The P-mass is lower than 0.05 mg / g egg yolk powder. This implies that less than 1% of the PL-content of the egg yolk is extracted and that less than 0.02 mg PL / g CO_2 is removed.

In literature, it has been suggested that the lack of PL-extraction may be due to the lipid-protein binding [5]. In fresh egg yolk, most of the PL are noncovalently bounded with proteins [6], forming lipoprotein particles which may hamper the extraction.
In order to ensure that no lipid-protein aggregates are present, which might deteriorate the extraction, the sample preparation is modified. Initially, all PL are removed from the protein matrix by dissolving them in an excess of ethanol (360 Wt% with respect to the egg yolk powder). The ethanol is evaporated and the precipitated PL are mixed with the remaining matrix. This mixture is extracted with 200 g SC-CO_2 at 510 bar and 40^{o}C. Table 2 summarizes the results. It shows that the pre-treatment with ethanol does not improve the extraction of the PL with SC-CO_2. The P-content of the extract remains below the detection limit, indicating that the solubility of the PL in SC-CO_2 is very low.

	Extracted mass (%)	P-content (mg/g powder)	Extracted PL (mg/g CO_2)
Extract	44.9	<0.05	< 0.02
Ethanol layer before evaporation	-	5.7	
PL-fraction of egg yolk [6]	-	5.7 – 7.9	
Lipids collected by solvent extraction [13]	-	7.0	

Table 2: *P-content of the extract. The value is compared with (1) the P-content in the ethanol before evaporation, (2) the expected value for the PL in the egg yolk sample, and (3) the P-content of the lipid fraction which is obtained by a solvent extraction (method of [13]).*

Within the present experimental conditions, no PL are extracted with SC-CO_2. The extracted egg yolk fraction is mainly composed of triglycerides containing fatty acids with sixteen to eighteen carbon atoms. Cholesterol is extracted as well, but represents less than 7% of the extracted mass [9].
Figure 4 shows a compilation of the extraction rate, which is expressed as g extract/ kg CO_2. These values are determined from the linear part of the extraction curves of Fig.1. The data obtained by Wu et al. at a different extraction temperature are added [9]. The data are compared with the solubility of triolein in SC-CO_2 [10,11] and with the modified Adachi-Lu curve [12]. The triglyceride triolein has a carbon number of 54 and has a slightly higher molecular mass than the most abundant triglycerides of the egg yolk [6]. The modified Adachi-Lu curve describes the solubility of vegetable oils, mainly composed of triglycerides with a carbon number of 53.4-53.6 [12].

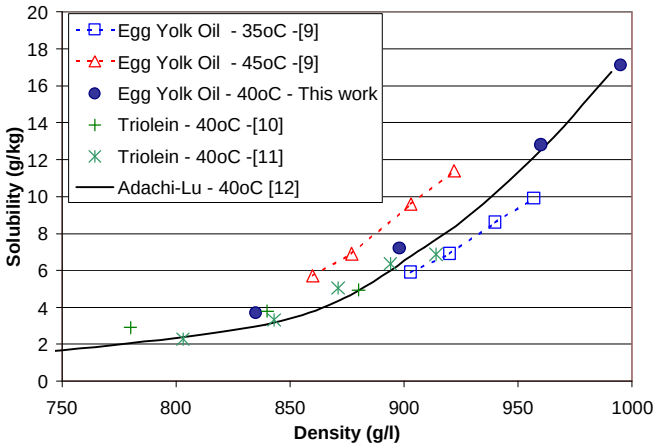

Figure 4: Comparison of the extraction rate with the solubility of representative triglycerides (triolein and some vegetable oils).

The extraction rates of the egg yolk oil are comparable to the solubility of triolein, and of vegetable oils which are described well with the Adachi-Lu curve [12]. The data also show a good agreement with the data of Wu et al. [9], which were derived at very different experimental conditions. Therefore, the results strongly indicate that the extraction of egg yolk powder with SC-CO_2 is limited by the solubility of the egg yolk oil, containing mostly triglycerides. In total 45 Wt% of the egg yolk is extracted, which corresponds well with the expected triglyceride and cholesterol content of the egg yolk [6].

Extraction with supercritical CO_2 and ethanol

In order to be able to extract the PL, a combined extraction is performed similarly as proposed for the extraction of phospholipids from soybeans [2].
The procedure consists of two stages:

- A first extraction with SC-CO_2, to remove the triglycerides and cholesterol. After the extraction, the thimble is removed from the apparatus and weighted. Glass beads are added to compensate for the loss in volume, so that the extraction cell is filled again.
- A second extraction with SC-CO_2 modified with ethanol, immediately followed by an extraction with 40-ml SC-CO_2 . The latter is done to remove the residual ethanol from the sample before weighing. The extract is collected in a collection vial filled with glass beads. The extract is washed from the glass beads with ethanol and collected for analysis. The collection efficiency of this procedure was checked with a spiked sample and found to be more than 90%.

To avoid cake formation during the extraction, due to the presence of the gummy PL, a different sample preparation is used [3]. The egg yolk powder is mixed with small ceramic particles (0.85 mm) in a weight ratio of 4:1. In this way the contact between the supercritical fluid and the sample is improved [3]. A stainless steel extraction thimble of 10 ml is filled with this mixture. The thimble contains about 2.7 g egg yolk powder and 10.8 g ceramic pearls. Glass beads are added at the end of the thimble to prevent clogging of the frit.

Figure 2 displays a typical curve, which is obtained with a combined extraction. In the first stage, the sample is processed with 180 g SC-CO_2. Afterwards it is extracted with 120 g SC-CO_2 modified with 15 mole% ethanol. The solid square represents the extracted mass as a

function the CO_2-mass. It shows that 45 Wt% of the egg yolk is removed at the end of the first stage. When the extraction is continued with modifier, additional mass is collected. At the end, approximately 60 Wt% of the egg yolk mass is removed, which corresponds well with the total lipid content of the egg yolk powder [6].

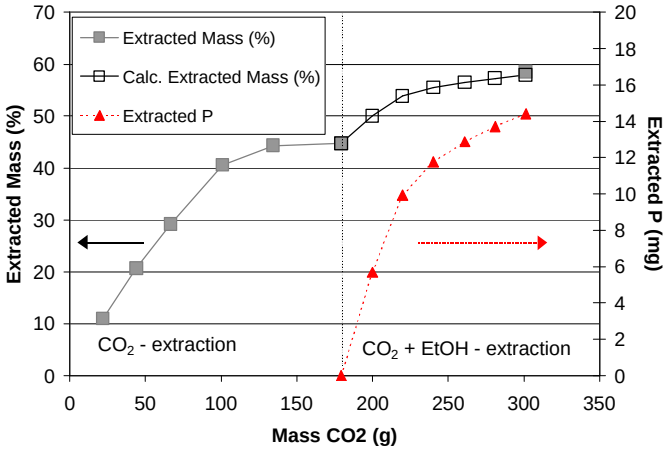

Figure 2: *Experimental data for an extraction containing two stages, (1) with SC-CO_2, and (2) with SC-CO_2 containing 15 mole% ethanol. The extracted mass is related to the original mass and is shown on the left axis. The P-content of the extract is displayed on the right axis.*

The dashed curve of Fig. 2 shows the P-content of the extract as a function of the CO_2-mass (right axis). From these data, the mass of the extracted PL is calculated by multiplying the P-mass with 24.5 to correct for the average weight of the PL. The value is added to the weight loss of the first extraction to obtain a "total" weight loss. The calculations are represented by the open squares. Remark that the calculated value at the end of the second extraction accounts well for the total mass loss which is determined gravimetrically. This indicates that the mass loss during the second part of the processing can mainly be attributed to the extraction of PL.

The extraction of the PL is studied by varying the processing conditions during the second stage of extraction. The first stage is kept constant and is not discussed in this section. It only serves to remove the triglycerides and the cholesterol from the sample.

In Figure 3a, the polarity of the SC-fluid is altered. The flow rate of the ethanol is altered and the CO_2-flow is adjusted to maintain a constant flow rate. Remark that the extraction rate of the PL strongly depends on the composition of the supercritical fluid. Notice also that approximately 16.5 mg P needs to be extracted before all PL are removed. Longer extraction times are required to achieve a total removal at the present experimental conditions.

Figure 3b shows the mass loss of the sample as a function of the extraction pressure. The composition of the SC-fluid is altered by changing the molar fraction of ethanol. At 100 bar, significantly less PL are extracted, which can be correlated with the reduced density of the supercritical fluid. In all cases, the extraction rate strongly increases with the ethanol content of the SC-fluid.

Finally, the influence of the extraction temperature is investigated at 500 bar, using SC-CO_2 with 7.5 mole% ethanol. The extraction is performed during 60 min at a total flow rate of 2 ml/min. Only a slight effect is seen. At 35^{o}C, approximately 3.3 +/- 0.4 mg P is extracted, whereas 2.3 mg P is extracted at 90^{o}C. Remark that the extraction efficiency is the lowest for the highest temperature. This is probably related to the lower density of the SC-fluid at 35 ^{o}C.

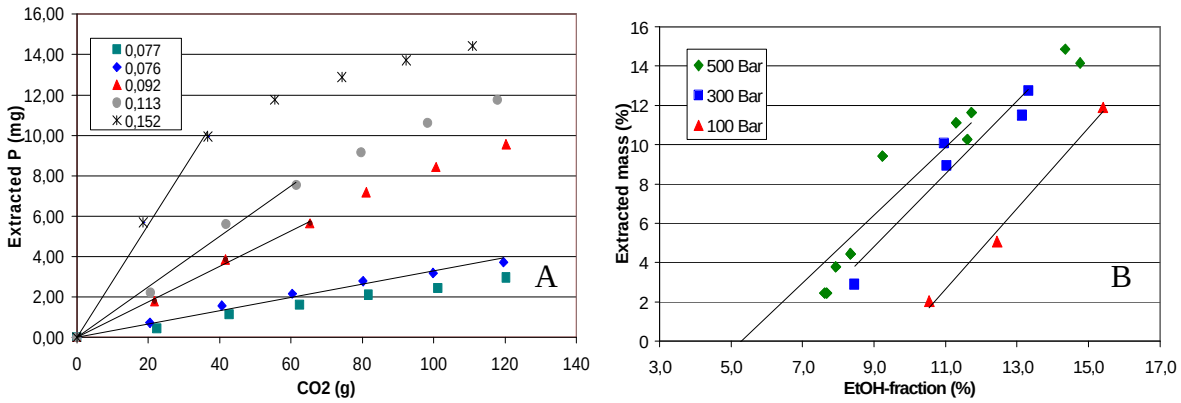

Figure 3 *(a) P-content of the extract as a function of the CO_2-mass. The composition of the SC-fluid is varied. The mole fraction of ethanol is shown in the legend. Figure 3 (b) shows the extracted mass for an extraction of 60 min. at a total flow rate of 2 ml/min. The composition of the SC-fluid and the pressure are altered.*

CONCLUSIONS

In this work, the extraction of PL from egg yolk powder is studied. Compared to previous studies, the extraction pressure is extended to higher pressures and the effect of the pulsation of the pressure is investigated. This work confirms that the PL cannot significantly be extracted with SC-CO_2. The polarity of the SC-fluid needs to be increased by adding ethanol as modifier. This work reveals that the extraction of PL strongly depends on the composition of the SC-fluid and the extraction pressure applied. The processing temperature shows only a minor effect at a low ethanol concentration.

REFERENCES

[1] Snyder J.M., Friedrich J.P., Christianson D.D., JAOCS, Vol. 61(12), **1984**, p. 1851.
[2] Montanari L., Fantozzi P., Snyder J., King J. J. of Supercritical Fluids 14, **1999**, p. 87.
[3] Dunford, N.T. Temelli, F., JAOCS, Vol. 72(9), **1995**, p. 1009.
[4] Ronyai, E., Simandi B., Tomoskozi, S. Deak, A., Vigh L., Weinbrenner Zs, J. of Supercritical Fluids, Vol 14, **1998**, p. 75.
[5] Boselli E., Carboni M.F., J. of Supercritical Fluids 19, **2000**, p. 45.
[6] Burley R.W., Vadehra D.V., The Avian Egg Chemistry and Biology, John Wiley & Sons, **1989**.
[7] NEN-EN 13657:2002
[8] Heinze T., Kynast G., Dudenhausen J.W., C. Schmitz, Saling E., Chromatographia, Vol. 25(6), 1988, p. 497.
[9] Wu W., Hou Y., J. of Supercritical Fluids 19, **2001**, p.149.
[10] Chastril, J., J. Phys. Chem. Vol. 86, **1982**, p. 3016.
[11] Nilson W.B., Gauglitz E.J., Hudson J.K., JAOCS 68, **1991**, p. 307.
[12] Sovova H., Zarevucka M, Vacek M, Stransky K, J.of Supercritical Fluids 20, **2001**, p.15
[13] Bligh E. G. and Dyer W.J., Can. J. Biochem. Physiol. 37, **1959**, p. 911.