Optimization of Supercritical Fluid Conditions for the Rapid Determination of Free Fatty Acids in Soy and Cottonseed Meals

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An alternative extraction method for the determination of fatty acids in cottonseed and soy meals employing supercritical carbon dioxide modified with methanol is described. Higher free fatty acid levels are an indicator of meals that have deteriorated in storage and may influence fermentation yields negatively. The extraction conditions were optimized using "design expert" software. Several optimum conditions for a quantitative extraction were determined. The addition of methanol increased the extraction yield considerably, which is demonstrated with the help of three-dimensional plots of recovery versus modifier and pressure while temperature was kept constant.

Keywords: *Supercritical fluid extraction; SFE; cottonseed meal; soy meal*

INTRODUCTION

Soy and cottonseed meals are frequently used as feeds for fermentation processes producing antibiotics, detergents, and vitamins. The analysis of the feed is an important factor for quality control and hence consistent yield. Furthermore, knowing the compositional makeup of the meals enables studies to be made on the effects that individual components may exert on the yield. Free fatty acids are one of the components that could influence the fermentation, and therefore a method for determining free fatty acid levels and composition could help to elucidate their importance on fermentation yields.

Supercritical fluid extraction (SFE) can offer a fast way of extracting free fatty acids from soy and cottonseed meals through its favorable physical and chemical properties. Viscosity and solute diffusivities of supercritical fluids are similar to those of gases; however, their solvating properties are comparable to those of liquids. These properties allow fast mass transfer and deep matrix penetration so that SFE can be faster than liquid extraction.

The efficiency of SFE for the extraction of seeds has already been demonstrated by several researchers (Friedrich et al., 1982; Stahl et al., 1980; Taylor et al., 1993; King et al., 1992). Taylor et al. (1993) investigated the accuracy and precision of SFE for the determination of oil content in oilseeds, such as soy flakes, canola flakes, and corn germ. The same group of researchers (King et al., 1992) also developed a rapid on-line SFE–supercritical fluid reaction–capillary gas chromatography method for the analysis of fatty acid compositions of triglycerides plus free fatty acids in oilseeds. However, this method did not independently measure the level of free fatty acids. The present paper describes the optimization of a method to determine free fatty acids in soy and cottonseed meals.

The selection of optimum operating parameters is often a laborious task when one parameter is optimized at a time. Several theoretical approaches for predicting the solubilities of compounds have been published (King, 1989; Mitra et al., 1991). These require physicochemical data which are often not readily available. Another approach for the estimation of solubility is the use of chromatographic means (Bartle et al., 1990b; Smith et al., 1987). However, these theories do not include the effect of using modifier with the supercritical fluid; hence, the modifier effect must still be determined by experiment.

Several statistical experimental design approaches have been used for the optimization of parameters involved in SFE. Mills et al. (1993) and Fisher (1989) used a simplex optimization scheme to find the optimum of two extraction parameters. Bicking et al. (1993) used a full factorial design approach to optimize temperature and pressure and plotted the results in three-dimensional plots which were beneficial to gain an overall understanding of the influence of these parameters on the extraction. The optimization scheme in this study used an expert design software which allowed the optimization of three factors and also provided three-dimensional plots, in which one of the parameters was kept constant.

The method reported here concentrates on free fatty acid profiles in cottonseed and soy meals, as the oil and hence the meal quality deteriorate when stored for a long time prior to being processed (Marshall et al., 1991). This is easily detected by the increase in free fatty acids in the oil. As there is still about 5% oil present in the meal, the decrease in meal quality can be monitored by determining the free fatty acid level in the meal and comparing it with that of superior meals which have not deteriorated in storage and hence contain fewer free fatty acids.

EXPERIMENTAL PROCEDURES

Instruments. All experiments were performed using a Jasco SFE system (Jasco U.K. Ltd., Great Dunmow, U.K.). An ethylene glycol/water mixture-filled cooler was used to maintain the head of the carbon dioxide pump Model 980-PU at −5 °C. The extraction vessel with an internal volume of 10 mL (Jasco, U.K.) was kept at a set temperature in a column oven Model 860-CO. A Model 880-81 backpressure regulator kept the entire system under a selected, constant backpressure. This backpressure is regulated by an electronic feedback regulator which is flow independent. A six-port Rheodyne valve was installed in place of an injection valve which enabled both dynamic and static extraction.

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All derivatized extracts were analyzed by high-performance liquid chromatography (HPLC) using a modular system consisting of an SP 8100 gradient pump (Spectra-Physics Analytical, Stone, U.K.) and a Model 3100 UV absorbance detector (LDC/Milton Roy, Stone, U.K.). Chromatographic data were collected using a HP 3395 integrator (Hewlett-Packard, Stockport, U.K.). The "design expert" system version 4.02 was purchased from Q D Consulting (Herts, U.K.) and the ISCO "SF-solver" version 2.5.1 from Jones Chromatography (Mid Glamorgan, U.K.).

Materials. Fatty acids were purchased from Aldrich (Gillingham, U.K.): myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids. Methanol, 2-propanol, and chloroform (all of HPLC grade) were obtained from Fisons (Loughborough, U.K.). α-Bromoacetophenone and triethylamine were purchased from Aldrich, and fibrous cellulose was obtained from Whatman Bio-Systems (Maidstone, U.K.).

Supercritical Fluid Extraction. Two grams of meal was mixed with about 6 g of cellulose and packed into the 10 mL extraction cell. This was topped up with more cellulose to minimize the void volume. Cellulose was used as filling material because it is porous and a weak adsorbent. The extraction cell was then tightened, connected to the SFE unit, and left for 15 min for temperature equilibration in the oven. The pump delivering the cooled carbon dioxide was then switched on and the system was pressurized. After pressurization, the modifier pump was switched on and the extraction was started. The fatty acids were collected in 3 mL of a 2-propanol/chloroform (1:1) mixture in a vial that was wrapped with aluminum foil to avoid exposure of the extracts to light. The vial was cooled in an ice−water bath. Following the extraction the contents in the receiver were transferred to a 5 mL volumetric flask and made up to volume.

Fatty Acid Derivatization. An aliquot of the extract was evaporated to dryness and derivatized with α-bromoacetophenone according to the method of Hanis et al. (1988) to form the phenacyl esters. After derivatization, the solvents were evaporated under a stream of dry nitrogen and the derivatization products were dissolved in methanol (1 mL). The fatty acid standards were dissolved in analytical grade acetone and derivatized using the same procedure as above. The derivatized fatty acids were separated on a 4.6 mm i.d. × 25 cm Hichrom ODS 5 μm column using a flow rate of 1.0 mL/min and a methanol/water gradient. The pump was programmed with a linear gradient from 70 to 85% methanol in 10 min, followed by an increase to 100% over the next 45 min. The values obtained for the individual free fatty acids were then added together to produce a value for total free fatty acids, making subsequent optimization calculations easier.

Liquid Extraction in Blender. Meal samples (5 g) were defatted with petroleum ether (60−80 °C fraction) by blending for 2 min in a conventional Waring Lab blender (Fisons, Loughborough, U.K.) with a meal/solvent ratio of 1:40 (Marshall et al., 1991).

Liquid Extraction Using an Ultrasonic Bath. Meal samples (2 g) were suspended in 10 mL of chloroform/methanol (2:1), sonicated for 15 min, and filtered using a vacuum unit (Jones Chromatography, U.K.). This was repeated with 9 mL of fresh solvent and sonication for another 30 min. Both extracts were combined and made up to 20 mL.

Time-Dependent Extractions. The cottonseed meal was treated and extracted in the same way as described under Supercritical Fluid Extraction; however, samples were taken after certain time intervals. This was done by stopping both pumps, quickly exchanging collection vials, and switching the pumps on again. Each extract was derivatized and analyzed. The sum of all free fatty acids present in the original sample was calculated using the equation (Bartle et al., 1990a)

$$m_0 = m_1 + m_2^2/(m_2 - m_3) \quad (1)$$

in which m_0 is the total amount of free fatty acids in the original sample, m_1 is the extracted amount after a time t_1, and m_2 and m_3 are amounts of fatty acids extracted in subsequent, equal time intervals t_2 and t_3. The extracted mass, m_1, should be taken from the nonexponential part of the plot $\ln(m/m_0)$ versus time, whereas m_2 and m_3 should be taken from the exponential part. This is important for the correct calculation of m_0 in eq 1. After m_0 is calculated, $\ln(m/m_0)$ versus time is plotted to ensure that the masses m_1, m_2, and m_3 are taken at the correct times. The value of m represents the mass of extractable fatty acids that remains in the matrix after a certain time.

Equation 1 is derived from the hot-ball model, which was used by Bartle et al. (1990a) to evaluate the effects of matrix shape, size variation, and solublity limitation on dynamic extraction. The model makes three assumptions, which, when fulfilled, result in complete conformity of the extraction behavior with the model. First, the particles of the matrix should be spherical, the size of the particles should have a narrow size distribution, and the analyte is assumed to be evenly distributed within the particles. Second, the flow rate is fast enough to ensure that the analyte concentration is zero at the particle's surface. Third, the analyte moves through the matrix by diffusion. The resulting plot of $\ln(m/m_0)$ versus time of a dynamic extraction is characterized by a steep initial decline, which is continued by an exponential decay whose slope is $1/t_c$ and dependent on particle size and the diffusion coefficient, and an intercept I of a theoretical value of −0.49977. When real samples are extracted, the three assumptions are often not fulfilled and hence influence the theoretically derived plot. If the particle has an irregular shape and hence a greater surface-to-volume ratio, the initial fall will be larger; the slope, however, remains the same. A smaller particle size, which can be attained by grinding, will allow faster extraction which can be observed by a larger slope. The grinding process presses the solute to the particle surface and thus yields a steeper initial fall and a longer time to establish a smooth concentration profile. The time, $0.5t_c$, is the time when the curve $\ln(m/m_0)$ versus time becomes linear and a smooth concentration profile is established. Another deviation of the theoretical curve can occur when the extraction is solubility limited. This reduces the initial rate of the extraction and therefore delays the establishment of the linear portion.

RESULTS AND DISCUSSION

Initially several extractions were performed on cottonseed meal 1 to compare recoveries using different modifiers, flow rates, and the combination of static and dynamic extraction. Cottonseed meal 1 was chosen as it contained the highest level of free fatty acids. The extracts were derivatized as described, and the sum of the fatty acids was used to estimate the overall recovery: myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids. The extraction conditions and recoveries are listed in Table 1. Methanol seemed to be a better modifier than 2-propanol; however, to judge the significance of the difference between the two modifiers, replicate extractions ($n = 6$) were performed to estimate the standard deviation. Condition D in Table 1 was chosen for this experiment, and the relative standard deviation was calculated to be ±6.4%. None of the conditions in Table 1 achieved any significant improvement compared to the rest as all of the recoveries lay within the limits of the standard deviation. One can conclude from pretrials that the extraction seems to be diffusion controlled rather than limited by solubility as no significant difference was determined when the dynamic extraction mode was compared with the static−dynamic combination. If the extraction had been solubility limited, then the amount of fluid used would have been important and hence the dynamic extraction would have shown a greater recovery. In the dynamic extraction 90 mL of CO_2/methanol fluid mixture was used, whereas in the static−dynamic combination only 40 mL was used. Condition E was then chosen for the optimization experiments.

Table 1. Conditions and Recoveries Used in the Pretrials[a]

conditions	flow rate (mL/min)	modifier (5 mol %)	recovery (%)
A, 30 min dynamic	3.0	methanol	77.6
B, 5 min dynamic, 15 min static, 10 min dynamic	3.0	methanol	81.9
C, 5 min dynamic, 15 min static, 10 min dynamic	3.0	2-propanol	75.7
D, 10 min dynamic, 10 min static, 10 min dynamic	3.0	methanol	81.2
E, 10 min dynamic, 10 min static, 10 min dynamic	2.0	methanol	77.6
F, 10 min dynamic, 10 min static, 10 min dynamic	4.0	methanol	77.6

[a] 50 °C, 200 kg/cm^2.

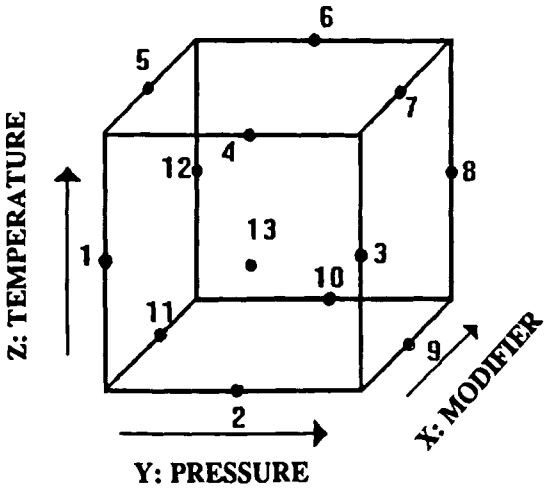

Figure 1. Schematic diagram showing the 13 experiments. Experiment 13 was carried out in triplicate.

Table 2. Extraction Conditions and Recoveries for the 15 Experiments Using the Statistical Design Approach

modifier[a] (mol %)	temp (°C)	pressure (kg/cm)	density[b] (g/mL)	solubility parameter[b]	recovery (%)
5	70	200	0.710	6.274	94.9
0	40	200	0.848	7.227	53.3
5	40	100	0.768	6.784	93.5
10	70	100	0.357	3.260	90.6
5	70	200	0.710	6.274	89.0
10	70	300	0.830	7.590	94.7
5	40	300	0.926	8.184	88.2
0	70	100	0.248	2.110	2.1
0	100	200	0.470	4.002	19.2
0	70	300	0.790	6.735	56.4
5	70	200	0.710	6.274	91.8
10	40	200	0.889	8.128	90.2
10	100	200	0.561	5.125	94.6
5	100	100	0.195	1.723	4.2
5	100	300	0.686	6.063	98.4

[a] Experiments listed in order of completion. [b] Density and solubility were calculated using the ISCO solvent solver.

Optimization. The expert system was used to design a series of experiments enabling the optimum conditions to be found by performing only 15 experiments. The schematic design visualizing the selected parameters is shown in Figure 1. This series of experiments includes one set of conditions with three replicates to determine the experimental uncertainty.

Cottonseed meal was used to conduct the experiments as spiked samples do not represent real samples. As shown in earlier experiments, the extraction seemed to be diffusion controlled, and hence, using spiked samples would only give an optimum for solubility-limited experiments and not for those limited by diffusion. Furthermore, spiked samples are not able to mimic active adsorption sites which influence the extraction, nor do they provide real distribution of the compound in the sample.

The SFE unit was rebuilt to avoid long transfer lines outside the temperature-controlled environment. The UV detector was not used to monitor the extraction as this may cause degradation of the fatty acids, so the outlet from the extraction cell was connected directly to the backpressure regulator. The extraction mode used condition E, Table 1, and the experimental conditions used for the extractions and their corresponding densities and solubility data are listed in Table 2. The extraction temperatures ranged from 40 to 100 °C, the pressures from 100 to 300 kg/cm^2, and the modifier concentrations from 0 to 10 mol %. The extraction recoveries for each of the 15 experiments are also listed in Table 2. The results were used to fit a quadratic model and the following equation was used for calculating the polynomial:

$$= x_0 + x_1A + x_2B + x_3C + x_{11}A^2 + x_{22}B^2 + x_{33}C^2 + x_{12}AB + x_{13}AC + x_{23}BC \quad (2)$$

The x values are the coefficients, and the letters $A-C$ represent the modifier, temperature, and pressure, respectively. The values were calculated by the expert system and show the influence of each parameter on extraction yield. The calculated values were then used to present the data in three-dimensional plots as seen in Figures 2A–4A. Additionally, the solubility parameter δ_1 of the pure and mixed fluid was also processed using the expert system software to present the data in the same form as the extraction recovery, enabling comparison between the solvation power of the fluid and the resulting recoveries. The solubility parameter δ_1 was calculated by the SF-solver software using the equation derived by Giddings et al. (1968). These data were then fitted to a quadratic model as this showed the best statistical correlation and is presented in Figures 2B–4B.

Figure 2A depicts the three-dimensional plot of the recoveries obtained with variations of pressure and modifier concentration at a constant temperature of 40 °C. Looking at the front of the plot and following the increase of modifier at constant pressure, a steep ascent indicates a rapid increase in percent recovery as the mole percent modifier rises to 8%. In the comparable solubility plot in Figure 2B, this steep ascent is not present, which suggests that the solubility data are not representing the real change in solvent strength achieved by the addition of modifier. This discrepency between solubility data and solvent strength has been noted previously (ISCO, 1991). Figure 2B depicts solvent strength in terms of density increase according to the solubility parameter theory; it therefore only shows the increase in solvent strength due to the increase in density caused by the addition of methanol and not the real increase in solvent strength. Therefore, Figure 2A shows the enhancing effect of methanol caused by the increased solvent strength, rather than Figure 2B. Deye et al. (1990) used the solvatochromic dye Nile Red

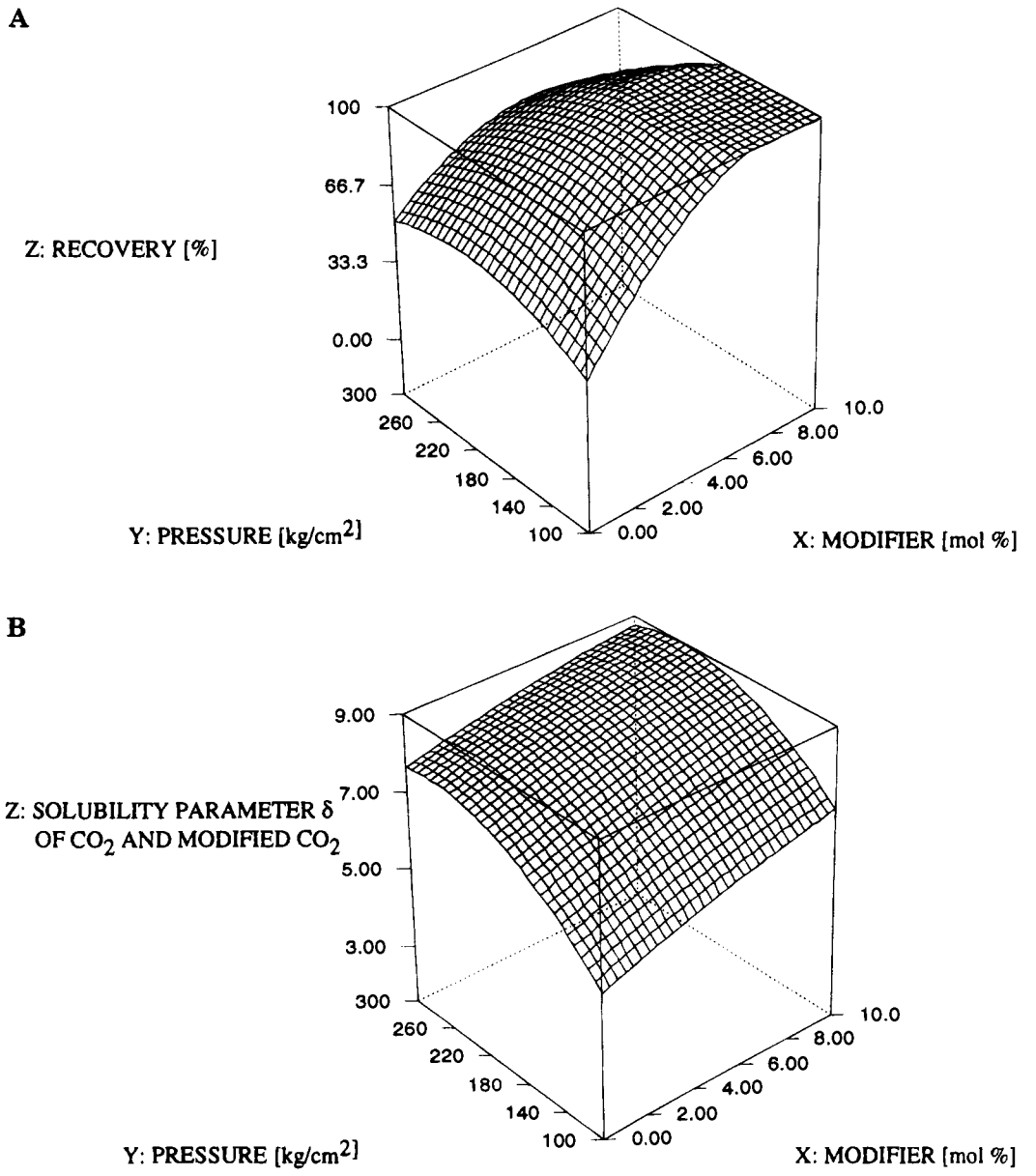

Figure 2. (A) Recovery of free fatty acids expressed in terms of the dependence upon modifier level and pressure at 40 °C. (B) Variation in solubility parameter of CO_2 and methanol in dependence of modifier level and pressure at 40 °C.

to correlate mobile phase solvent strength to chromatographic retention in supercritical fluid chromatography. They found that a small addition of methanol to carbon dioxide produced a large increase in solvent strength, causing nonlinear plots of chromatographic retention versus percent modifier. Better linearity was obtained when the retention data were plotted versus solvent strength determined with Nile Red. Rossi et al. (1990) further demonstrated the enhancing effect of ethanol on the extraction of egg lipids. The addition of 3% ethanol or more caused a considerable increase in extraction yield of cholesterol, cephalin, and lecithin.

Figure 2B further suggests that the recovery should be at its highest when pressure and modifier are at high levels. Comparing this to Figure 2A, however, shows that the recovery is actually lower in this region. This decline in recovery can be explained by assuming that once threshold pressure or maximum solubility is reached, extraction recovery is limited by diffusion of the analyte from the matrix to the bulk fluid. Therefore, a further increase in pressure beyond the threshold pressure or maximum solubility decreases the diffusion coefficient and increases viscosity of the fluid, resulting in the lower recovery. The decline in recovery is therefore caused by the change in viscosity and diffusity, and an increase of pressure beyond the pressure required for maximum solubility should therefore be avoided. The optimum conditions at 40 °C appear to be with a pressure of 100–200 kg/cm^2 and with a modifier concentration between 6 and 10%.

The temperature in Figure 3A is held constant at 70 °C. Again the steep increase due to the addition of modifier is not represented in the solublity plot in Figure 3B. Interestingly, the increase in recovery at 0% modifier with increasing pressure has almost the same ascent as in the solubility plot, proving that the solubilty parameter is a valid estimate using pure carbon dioxide. The highest recoveries were achieved at high pressure and high modifier levels. The high levels of these parameters are necessary to counteract the decrease in density compared to that at 40 °C.

Figure 4 shows the three-dimensional plot of the recoveries at 100 °C. Comparing Figures 2A–4A at low pressure and low modifier levels, it is obvious that the 100 °C plot shows the lowest recovery. The lower density and hence solvent strength of the fluid is

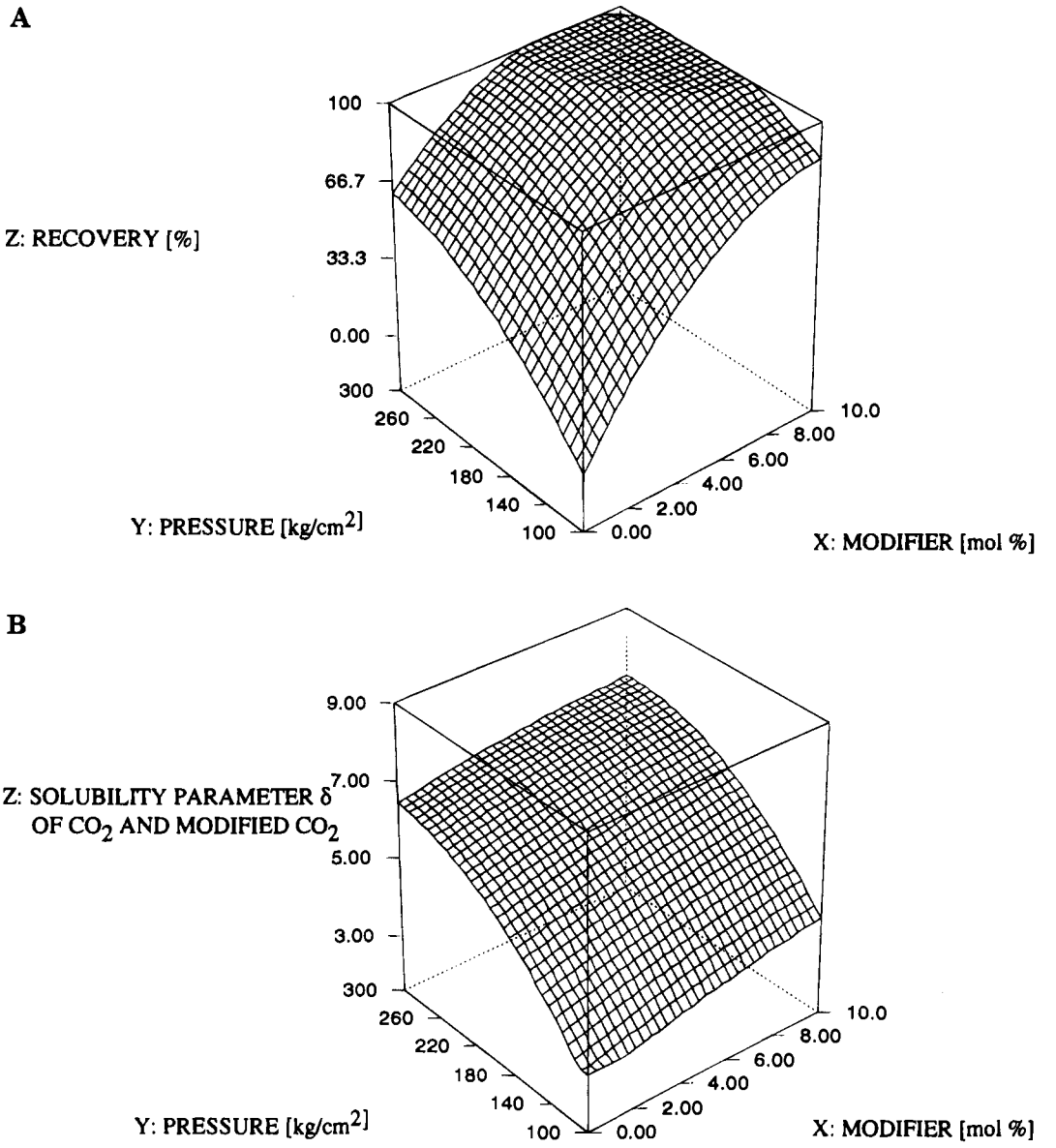

Figure 3. (A) Recovery of free fatty acids expressed in terms of the dependence upon modifier level and pressure at 70 °C. (B) Variation in solubility parameter of CO_2 and methanol in dependence of modifier level and pressure at 70 °C.

responsible for this behavior. Therefore, the temperature increase did not increase the vapor pressure of the free fatty acids to such an extent that it outweighed the decline in density.

The three-dimensional plots also demonstrate the possibility of analyte fractionation as they depict areas of low and high recoveries. This knowledge can be used to extract compounds other than fatty acids using conditions at which none of the free fatty acids are extracted. The expert system allows the calculation of conditions at which fractionation is possible. Brunner et al. (1982) demonstrated fractionation of free fatty acids from triglycerides in which the addition of ethanol had an enhancing effect on the fractionation. Fractionation was not of interest for the study reported here, as the triglycerides did not interfere with the analysis.

Validation of the Optimum Conditions. The expert program offers the ability to estimate optimum conditions for extractions at different temperatures. Two experiments from each optimized region in Figures 2A–4A were used to validate the optimum regions selected. The extraction conditions and recoveries are listed in Table 3, which shows that only one experiment (91.6%) had recovery outside the ±6.4% standard deviation of 100.0% recovery. This outlier could have been caused by several factors, the first being the low solubility of free fatty acids at these conditions. The density at which the low recovery was obtained is lower than that of the other experiments in Table 3. The density was obtained from experimental measurements published by Berger (1991). The SF-solver software, however, calculated a higher density at 100 kg/cm^2 than at 150 kg/cm^2. The SF-solver program did not calculate the density correctly because the temperature was below the critical temperature (T_c = 49.54 °C) and hence subcritical conditions existed, at which the program fails to calculate the correct density.

Second, Strubinger et al. (1991) measured the adsorption of methanol and carbon dioxide onto adsorptive material over a wide range of conditions. They found that a considerable amount of methanol is adsorbed in the near critical region, and this additional layer of adsorbed fluid possesses a high density. Assuming that the analyte has to diffuse through this additional layer, in which its diffusion coefficient is lower than in the bulk fluid, could explain the lower recovery of the outlier.

Additionally it has to be considered that the conditions lay on the edge of the cube representing the

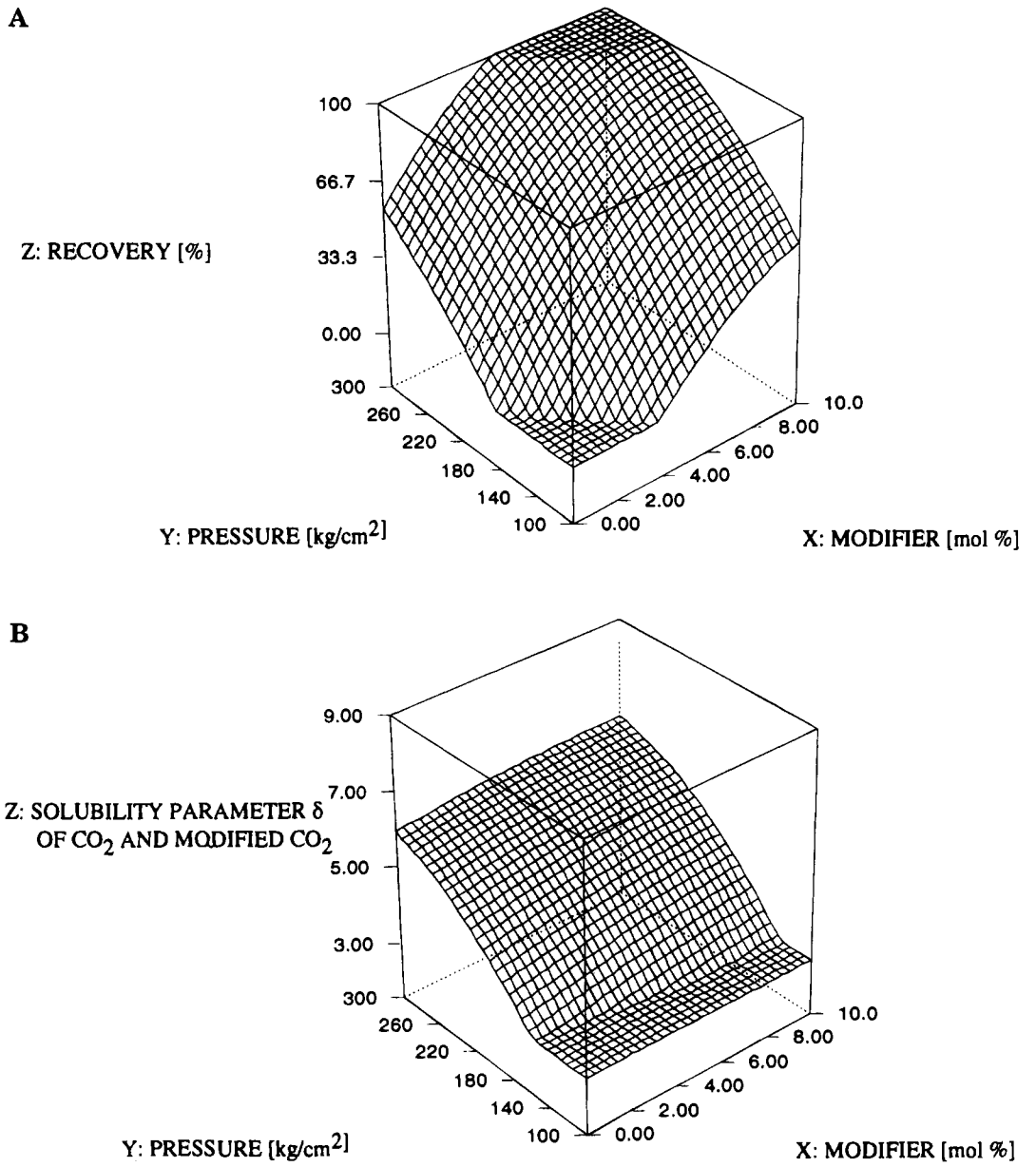

Figure 4. (A) Recovery of free fatty acids expressed in terms of the dependence upon modifier level and pressure at 100 °C. (B) Variation in solubility parameter of CO_2 and methanol in dependence of modifier level and pressure at 100 °C.

Table 3. Conditions Chosen for the Validation of the Optimum Conditions[a]

methanol (mol %)	temp (°C)	pressure (kg/cm^2)	recovery (%)
8	40	150	97.3
9	40	100	91.6
7	70	280	96.1
9	70	250	99.3
6	100	290	97.0
8	100	280	99.6

[a] Conditions E in Table 1.

experimental model (Figure 1), where statistical models often have the highest uncertainties. Therefore, it is best to choose the optimum conditions from the middle of the calculated optimum region. Overall, this proves that statistical experimental design is a valid technique of determining optimum conditions and provides useful information about the influence of each parameter.

Time-Dependent Extractions. To determine the original amount (m_0; eq 1) of free fatty acids in the meals, a time-dependent extraction experiment using

Table 4. Sum of Free Fatty Acids Extracted during the Time-Dependent Extraction and the Calculated Values of $\ln(m/m_0)$ for the Plot in Figure 5

time[a] (min)	extracted free fatty acids (FFA) (mg/g)	remaining amount m of FFA (mg/g)	$\ln(m/m_0)$
10	1.200	0.437	−1.321
15	1.507	0.130	−2.533
20	1.589	0.048	−3.529
30	1.606	0.031	−3.967
$t_1 = 60$	1.621	0.016	−4.628
$t_2 = 120$	1.629	0.008	−5.321
$t_3 = 180$	1.633	0.004	−6.014

[a] Condition E in Table 1.

cottonseed meal 1 was carried out using the following conditions: 9 mol % methanol, 70 °C, and 250 kg/cm^2. The time intervals and the sum parameter of the fatty acid recovery are listed in Table 4. The results were used to calculate the original amount (m_0) of the fatty acids in the meal according to the hot-ball model which Bartle et al. (1990a) used as a model for dynamic extraction. This original amount was calculated to be

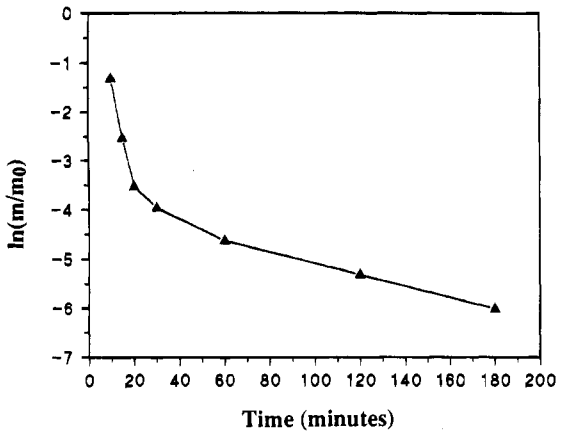

Figure 5. $\ln(m/m_0)$ vs time for the hot-ball model.

1.637 mg/g and was used for the previous calculation of recovery. Table 4 lists the sum of extracted fatty acids at a certain time and the remaining amount of fatty acids using 1.637 mg/g as the orignal amount. Figure 5 shows the corresponding graph in which $\ln(m/m_0)$ is plotted versus time. The evaluation of the plot $\ln(m/m_0)$ gave a value of -3.49 for I and 66 min for t_c. The high value of I suggests, according to the theory, uneven distribution of the free fatty acids in the matrix or irregular particle shape. The theory further predicts that the linear portion of the curve should start at $0.5t_c$, which was calculated to be 33 min. As shown in Figure 5 the linear portion of the plot starts at around 30 min. Additionally, the recovery at $0.5t_c$ is normally around 50–70%; however, about 98% was extracted at this time. The high recovery at $0.5t_c$ could be caused by the fact that the majority of the fatty acids are distributed in the outer part of the matrix and a higher percentage is extracted by the time a smooth concentration profile is achieved in the matrix. This demands maximum solubility and favorable diffusivity and viscosity at the beginning of the extraction to enable the fast mass transfer of the free fatty acids until equilibrium is reached, at which the concentration is zero at the surface and reaches its maximum in the middle of the particle. From this point onward the extraction begins to be limited by diffusion and an exponential decline can be observed in the plot $\ln(m/m_0)$ versus time.

This also explains the results obtained from the initial comparison between dynamic extraction (condition A, Table 1) and the dynamic–static combination (condition E). Once the majority of fatty acids were extracted, the static extraction mode allowed the interparticle diffusion to take place and, hence, no flow through the cell was required. The subsequent dynamic step swept the fatty acids then to the collection vial.

The time-dependent extraction could further determine the cause of the outlier in the validation experiment, since it distinguishes between extractions limited by diffusion or solubility.

Finally, the optimum SFE conditions of 9 mol % methanol, 70 °C, and 250 kg/cm^2 were applied to extract two different samples of both cottonseed and soy meals using condition E (Table 1). The results were compared with liquid extraction using either petroleum ether or a chloroform/methanol mixture as described under Experimental Procedures. The results are listed in Table 5 and show that cottonseed meals 1 and 2 have considerably different levels of free fatty acids. The petroleum ether extraction resulted in considerably lower recoveries, which is probably caused by the short extraction time of 2 min, which leaves no time for diffusion out of the matrix. The chloroform/methanol extractions achieved higher recoveries and are comparable to the SFE results. The longer extraction time and the fact that the meal was extracted twice helped achieve higher recoveries. The results show the advantage of SFE for the extraction of fatty acids in cottonseed and soy meals, as SFE achieved higher recoveries and the filtration step needed for the liquid extraction was eliminated.

Conclusions. The expert system provided great assistance in gaining an understanding of the dependence of the different parameters in SFE. It showed the increase in solubility due to the addition of methanol could not be predicted by the solubility parameter. The solubility parameter is, however, extremely useful when pure fluids are used, assuming that no excessive adsorption of the analyte on the matrix takes place. To improve the usage of the expert system, it is advisable to determine rough boundary extraction conditions so that optimization experiments need only to cover a small range of parameters and become more accurate.

Achieving maximum solubility was crucial for a fast extraction in this application as the analyte was present in a relatively high concentration compared to trace analysis, where solubility plays a minor role. Additionally, the fatty acids were mainly distributed in the outer layer, which required a high solvating power of the fluid in the beginning of the extraction. The comparison of SFE to liquid extraction showed that the SFE was faster, as no time-consuming filtration step was necessary, and higher recoveries were achieved. SFE for the determination of free fatty acids in cottonseed and soy meals minimizes the use of solvents and additionally produces reliable results as seen from the low standard deviation.

The method represented here is applicable to other matrices, e.g. food; however, a time-dependent extrac-

Table 5. Comparison of the Sum of Free Fatty Acid Levels in Different Samples Using Different Extraction Methods

		free fatty acid levels (mg/g)					
sample	extraction mode	myristic 14:0	palmitic 16:0	stearic 18:0	oleic 18:1	linoleic 18:2	total
cottonseed meal 1	SFE[a]	0.116	0.405	0.086	0.207	0.771	1.585
	petroleum ether	0.088	0.262	0.074	0.161	0.553	1.333
	$CHCl_3$[b]	0.109	0.336	0.081	0.189	0.683	1.400
cottonseed meal 2	SFE	0.014	0.088	0.018	0.032	0.101	0.253
	petroleum ether	0.010	0.039	0.020	0.019	0.063	0.151
	$CHCl_3$	0.014	0.051	0.016	0.022	0.087	0.190
soy meal 1	SFE	0.009	0.292	0.025	0.133	0.102	0.562
	petroleum ether	0.004	0.146	0.027	0.076	0.026	0.279
	$CHCl_3$	0.012	0.232	0.027	0.113	0.083	0.468
soy meal 2	SFE	0.010	0.297	0.039	0.139	0.115	0.600
	petroleum ether	0.005	0.116	0.022	0.065	0.024	0.232
	$CHCl_3$	0.012	0.271	0.027	0.138	0.107	0.556

[a] Condition E in Table 1. [b] Chloroform/methanol mixture (2:1).

tion should be conducted to estimate the required extraction time. This would then allow the calculation of the original amount of analyte in the matrix which can be compared with results obtained from conventional liquid extractions.

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