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FUNCTIONAL PROPERTIES AND ORGANOLEPTIC CHARACTERISTICS OF LEAF PROTEIN CONCENTRATES TREATED WITH SUPERCRITICAL CO₂

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ABSTRACT

Supercritical CO₂ was used to extract the pigments and lipid fraction from leaf protein concentrates. Extractions were performed using pressures of 10-70 MPa at 40°C and CO₂ flow rates of 5-6 L/min. Some functional properties and the organoleptic characteristics of the extracted meal were evaluated in comparison to those of LPC treated with acetone.

- Key words: functional properties, leaf protein concentrates, organoleptic characteristics, supercritical carbon dioxide. -

INTRODUCTION

Leaf protein concentrates (LPCs) have a high protein content and an appropriate amino acid composition that make them suitable in applications involving human nutrition. One of the major obstacles to the utilization of LPCs in the human diet is represented by their organoleptic characteristics. The color of LPC ranges from deep green to black, due to the presence of natural pigments (chlorophyll and carotenoids) and products formed by polyphenol oxidation and the Maillard reaction. Furthermore, LPCs have a strong grassy flavor, that can be ascribed to the lipid fraction and products originating from the oxidation of natural unsaturated fatty acids.

The solvent-properties of supercritical carbon dioxide (SC-CO₂) are well documented (PAUL and WISE, 1981; and McHUGH and KRUKONIS, 1986; STAHL et al., 1988) and among its potential applications are the extraction of lipids and the recovery of natural pigments (FRIEDRICH, 1984; MANABE et al., 1987; STAHL et al., 1988).

Furthermore STAHL et al. (1984) reported that treatment with SC-CO₂ at 40°C did not affect the nutritional value of various oil seed meals. The aim of this research was to extract the pigments and lipid fraction from LPC by using SC-CO₂ and to compare some properties of the residual LPC to those of decolorized LPC obtained via acetone extraction.

MATERIALS AND METHODS

LPC were prepared from alfalfa (*Medicago sativa* L.) harvested during the preflowering stage. After washing in a 0.1% sodium metabisulfite solution, the grass was chopped and squeezed according to a described pilot plant method (FIORENTINI and GALOPPINI, 1981), and the collected juice was rapidly centrifuged (4200 x g, retention time 15 sec) to separate any remaining fiber particles (LENCIONI et al., 1984). The juice was then heated to 85°-90°C by steam injection to obtain a protein coagulum that was recovered by centrifugation (2000 x g, retention time 5 min).

Acetone-extraction runs were conducted on portions of wet LPC according to FAVATI et al. (1988a), while SC-CO₂ extractions were performed on 45-50 g of freeze-dried LPC with the apparatus in Figure 1 and described in detail elsewhere (FAVATI et al., 1988b). In order to test the solubility power of SC-CO₂ at different densities, runs were conducted at 40°C and at pressures of 10, 30, 50 and 70 MPa. A standard amount of CO₂ (5.4 kg) was used in each run, with recorded flow rates of 5-6 L/min at ambient conditions.

The proximate composition of LPC samples before and after the extraction was determined according to standard AOAC (1984) methods. Nitrogen solubility over the pH range 3-10 was determined using a slight modification of the method of BETSCHART (1974). Five hundred milligrams of LPC were placed in a centrifuge tube with 45 ml of distilled water and the pH adjusted to the desired value with solutions of 1N or 0.1N NaOH and/or HCl. The sample was then stirred for 1 h at 25°C and the pH monitored throughout this period. Necessary pH readjustments were made with the above solutions, taking care not to exceed the total volume of 5 ml, including the distilled water utilized to wash the electrode. Afterwards the sample was centrifuged at 1610 x G for 30 min. The supernatant was then filtered (Whatman no.1 filter paper), the volume brought to 50 ml with distilled water, and the nitrogen content determined on a 5 ml aliquot by Kjeldahl procedure. The nitrogen solubility value at a given pH was calculated on the base of four determinations.

Water absorption was measured as follows: 10 ml of distilled water were added to 1 g of LPC and stirred for 30 min at 25°C. The mixture was then centrifuged at 1610 x G for 25 min and the free water recovered. Water absorption was expressed as g retained water/g LPC.

Fat absorption was determined by a modification of the procedure used by LIN and HUMBERT (1974). Five hundred milligrams of LPC were added to 3 ml of peanut oil and stirred at 25°C for 30 min. The mixture was then let rest for 25 min and subsequently centrifuged at 1610 x G for 30 min. The free oil was recovered and the fat absorption was expressed as g retained oil/g LPC.

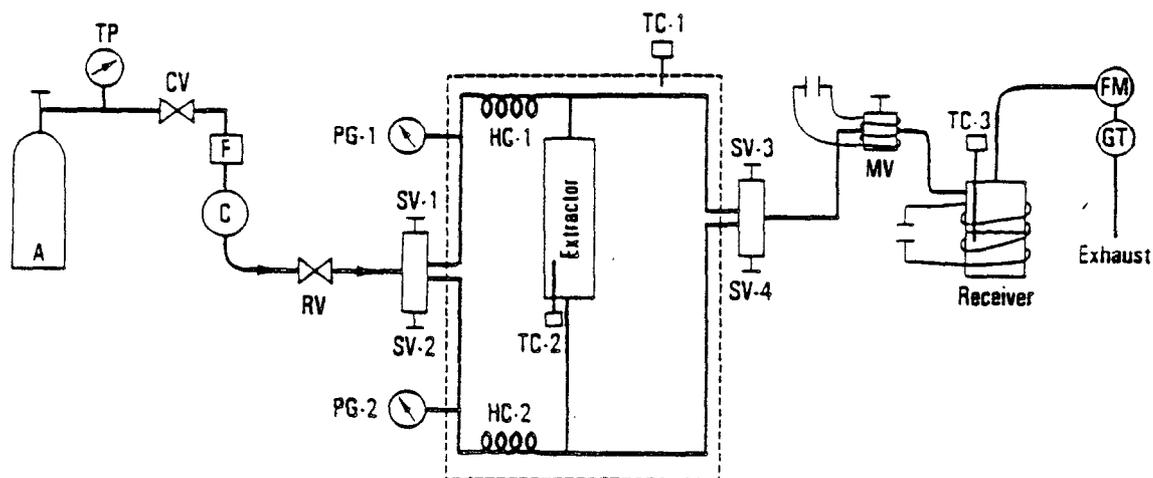


Fig. 1 - Supercritical fluid extraction system. Dashed lines indicate thermostated region. (A= CO₂ cylinder; TP= tank pressure gauge; CV= check valve; F= particulate filter; C= air-driven booster compressor; RV= relief valve; SV-1,2,3,4= valves; PG-1,2= pressure gauges; HC-1,2= thermal equilibrating coils; TC-1,2,3= thermocouples; MV= micrometering valve; FM= flow meter; GT= gas totalizer).

The color and the grassy flavor of the samples were assessed by sensory evaluation according to FAVATI et al. (1988a). Color was rated on the scale: deep green, green, pale green, pale green-gray, white-gray, pale white-gray, white. Grassy flavor was rated on the scale: very strong, strong, moderate, weak, absent.

RESULTS AND DISCUSSION

Complementary or alternative sources of proteins for human nutrition are in increasing demand. Aside from having an appropriate nutritional value, the successful use of LPCs mainly relies on their organoleptic characteristics and functional properties. The organoleptic characteristics are important for the acceptance of the proteins as a food by consumers, the functional properties are critical in predicting how these proteins will behave in a food system. In this work the effects of treatments with SC-CO₂ on LPC, have been studied taking into account both these parameters.

The proximate composition of untreated and residual LPC after extraction with acetone and SC-CO₂ at different pressures is reported in Table 1. Analytical data showed that the maximum removal of fat with SC-CO₂ was attained at the pressure of 70 MPa, where 58.5% of the theoretically available fat was extracted. Conversely, the extraction with acetone caused a reduction in the fat content equal to 98.5%. This difference should be partially ascribed to the fact that phospholipids show a negligible solubility in SC-CO₂. The removal of lipids affected the protein content of the residual product, raising crude protein from 46.2% to a maximum of 51.2% after extraction at 70 MPa. Protein content of the LPC after extraction with acetone was 56.1%.

Water absorption data were not significantly different for the untreated LPC and the extracted samples (Table 2). Results from fat absorption indicated that the actions performed with SC-CO₂ produced a definite reduction in the amount of oil absorbed, ranging from 18.9 to 22.3% over the pressure tested. This result was opposite from that obtained with acetone extraction, where no significant change in the fat absorption was recorded.

A comparison of the nitrogen solubility data for untreated and extracted LPC indicated substantial differences between the treatments (Fig. 2). While SC-CO₂ extractions did not produce major changes in the nitrogen solubility profile of LPC, extraction with acetone caused an appreciable reduction in this functional property over the entire range of pHs tested. This trend was substantiated by statistical analysis of the data ($p=0.05$).

Sensory evaluation allowed further characterization of the samples. The grassy flavor was reduced from "very strong" to "weak" by SC-CO₂ treatments at all pressures tested. The same assessment was given to the LPC extracted with acetone. LPC treated with SC-CO₂ did not show a significant reduction in color, being evaluated as "deep green", while extraction with acetone produced a "white-gray" residue. Though the removal of carotenoids from LPC by SC-CO₂ has been described (FAVATI et al., 1988), this minimal uptake of pigments should be ascribed to the chemical nature of chlorophylls and to the drying process. In fact the latter has been reported to reduce the extent of decolorization achievable also with traditional solvents (HUANG et al., 1971).

Table 1 - Proximate composition (dry basis) of untreated and residual LPC after extraction with acetone and SC-CO₂ at different pressures.

	Untreated LPC	Extracted LPC				
		Acetone	Extraction pressure (MPa)			
			10	30	50	70
Crude Protein (N x 6.25) (%)	46.2	56.1	48.0	50.6	51.1	51.2
Crude fat (%)	13.0	0.2	7.5	6.5	6.2	5.4
Ash (%)	12.8	15.6	13.2	14.1	14.2	13.9
Total N-free extract (%)	28.0	28.1	31.3	28.8	28.5	29.5

Table 2 - Comparison of some functional properties of untreated and residual LPC after extraction with acetone and SC-CO₂ at different pressures (mean of duplicate determinations).

	Untreated LPC	Extracted LPC				
		Acetone	Extraction pressure (MPa)			
			10	30	50	70
Water absorption (g H ₂ O/g LPC)	2.90	3.04	2.95	2.93	2.95	2.82
Fat absorption (g oil/g LPC)	2.33	2.31	1.81	1.84	1.89	1.87

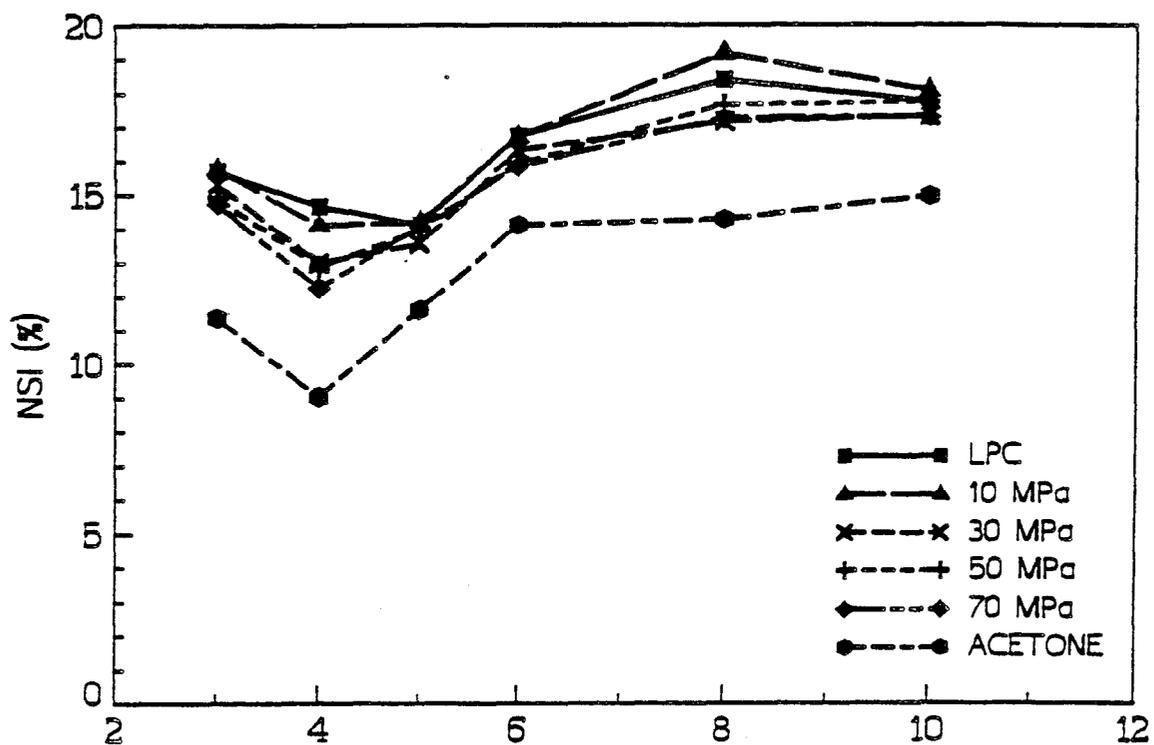


Fig. 2 - Nitrogen solubility profile of untreated and residual LPC after extraction with acetone and SC-CO₂ at different pressures.

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