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Aqueous processing of sunflower kernels with enzymatic technology

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An enzymatic treatment with cell-wall-degrading activities was carried out during the aqueous processing of sunflower kernels into oil and protein. The oil extraction yield was improved up to 30% of the total oil, and a meal of light colour and free from antinutritional compounds was obtained. More than 87% of phenolic compounds, as chlorogenic acid, were successfully removed during aqueous processing. When specific phenolics removal was incorporated in the aqueous extraction process, the *in vitro* protein digestibility increased up to 7·5% over that of the standard process.

INTRODUCTION

Conventional processes to extract oil from seeds (sources of vegetable protein), although giving high oil extraction yields, require expensive capital investment and operational costs, and cause undesirable effects on the quality of end-products at high temperatures. The new tendency to avoid the use of toxic organic solvents in large installations has renewed interest in alternative extraction processes (involving the use of water, alcohol aqueous solutions and supercritical fluids) and has led during the last two decades to continuous research on biorenewable solvents (Hron *et al.*, 1982; Johnson & Lusas, 1983).

Although water is not a specific oil solvent, aqueous processes represent an innovation in extraction technology, for any processed oilseed (Rhee *et al.*, 1972; Hagenmaier, 1974; Staron & Guillaumin, 1979; Lawhon *et al.*, 1981; Cruz Madueño, 1990). Aqueous extraction is advantageous because it presents no risks of fires and explosions, the solvent is not toxic, and the mild processing ensures high quality in the products of the process, oil and protein. The operation is more flexible since start-up and shut-down are safer in the absence of flammable solvents, favouring batch operation with less initial investment and operation costs. The main disadvantages, as compared to conventional technologies, are the lower efficiency of oil extraction, the reduction of the product stability which contains more residual oil and the easier microbial contamination.

Permeability of the cells wall to oil passage can be increased either by mechanical or thermal conditioning or by enzymatic digestion of the cell walls. Hydrolytic enzymes were reported to enhance the oil yield from aqueous extracted seeds since cellulose, hemicellulose and pectic substances constitute 80–90% of the cell wall polysaccharide (Fullbrook, 1983, 1984; Bhatnagar & Johari, 1987; Marek *et al.*, 1990). The enzymatic treatment can be incorporated during the mixing step of the ground seeds using water at a suitable pH and temperature.

The acceptance of dehulled sunflower meal as a food ingredient largely depends on the colour which phenolic compounds and reducing sugars impart to processed food products. Phenolics interact with proteins via oxidation and the products formed are responsible for the dark colour, and they can bind to the amino, thiol and methylene groups of the proteins, reducing the nutritive quality, since the new compounds cannot be metabolised (Sabir *et al.*, 1974; Hurrell & Finot, 1985; Shamanthaka & Sastry, 1990).

The content of phenolic compounds in sunflower meals ranges from 3–4% (w/w), of which 79% are soluble and 21% protein-bound (Bau *et al.*, 1983). Chlorogenic acid (an ester of caffeic and quinic acids), with an astringent taste (Nagel *et al.*, 1987), and caffeic acid constitute 70% of all phenolics (chlorogenic, caffeic, *p*-hydroxybenzoic, *p*-coumaric, cinnamic, *m*-hydroxybenzoic, vanillic, syringic, transcinnamic, isoferulic and sinapic) (Sosulski, 1979; Leung *et al.*, 1981).

To avoid oxidative processes involving phenolic compounds and subsequent irreversible bonding with proteins, their extraction is required. Among the several methods proposed to remove chlorogenic acid, worth mentioning is the use of acidified solvents, reaching the maximum solubility of the phenolics without protein

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losses; this yields sunflower products with mild taste and light colour (Fan & Sosulski, 1976; Sodini & Canella, 1977).

The diffusion-extraction from kernels proposed by Sosulski *et al.* (1973) selectively removes low-molecular-weight substances (polyphenolics — located in the outer part of the kernel — single sugars, minerals and non-proteinic nitrogen compounds) which diffuse through the cell walls. Temperature causes a remarkable effect, reflected in 90% removal at 80°C in 60 min, while at 60°C, 120 min are required to remove 87%. However, protein solubility is affected by high temperatures unless short treatment periods are employed (Lanzani *et al.*, 1979).

In this work, the aqueous extraction of oil from dehulled sunflower seeds is studied, and the efficiency of the enzymatic treatment during the aqueous extraction process is evaluated. Additional stages of phenolics removal were carried out and the process (oil yield and quality of products) was compared with the aqueous extraction process in order to evaluate the efficiency of the enzymatic action in both the presence and absence of phenolic compounds. The removal of these latter are refered to as chlorogenic acid removal.

MATERIALS AND METHODS

Dehulled sunflower seeds (obtained from commercial suppliers), wrapped in plastic bags and stored at 4°C until use, were ground in a coffee grinder and screened to a particle size < 2 mm.

A mixture of a cellulase Celluclast 1·5 L and a pectinase, Pectinex ULTRA SP both kindly supplied by Novo Nordisk A/S, and a multiactivity complex (hemicellulase, cellulase, and other side cell wall degrading activities) Multifect, a gift from Finnish Sugars Co. Ltd, were used.

Analytical methods

Moisture, ash and fat content were analysed by AOAC (1990) procedures (934·01, 942·05 and 920·39, respectively). NDF (neutral detergent fibre) was determined according to Goering and Van Soest (1970). Protein was calculated as Kjeldahl N × 6·25. The chlorogenic acid content in the defatted meal was determined extracting a 1 : 80 (w/v) meal suspension with 50% ethanol, shaking at 25°C for 30 min, filtering and twice re-extracting the residue with the ethanol solution. The absorbance in the supernatant resulting from the three extractions was read at 330 nm with 50% ethanol as blank. Available lysine was determined by the TNBS (trinitrobenzenesulphonic acid) method (James & Ryley, 1986). Amino acid content was analysed after acid hydrolysis with 6 M HCl, followed by derivatization with phenylisothiocyanate and analysed by reverse-phase HPLC, in a Waters Pico-Tag Station. *In vitro* protein digestibility was measured by an enzymatic assay proposed by Hsu *et al.* (1977), involving digestion with trypsin, chymotrypsin and peptidase. C-PER (computed protein efficiency ratio) was calculated as indicated by Hsu *et al.* (1978). The colour of the protein samples was visually determined with the procedure of Flemming and Sosulski (1977), based on complexing the proteins with phenolics at basic pH to measure the degree of extraction of chlorogenic acid. The colour of the protein product in a 1 : 20 (w/v) meal suspension at pH 10, kept at room temperature for 1 h was examined. The protein solubility was determined (in a 1% suspension) in a 0·2 M NaCl solution at pH 9, stirring at room temperature, centrifuging and determining the fraction of initial protein that is recovered in the supernatant. The reducing sugars content in the whey was determined by the Somogyi (1952) method with glucose as standard.

The oil was recovered after the oil–water emulsion was broken following the method of Lawhon *et al.* (1981). The emulsion was progressively enriched in oil, by adding the oil recovered by centrifugation of small amounts of emulsion. Optimal conditions for de-emulsification are: moisture (the most critical variable) under 20%, pH in the range 4–6, shearing agitation for 1–3 min, and centrifugation at 1000 rpm (147 × *g*) or higher for 1–3 min at 40°C. Free fatty acids as oleic, and phosphorus as phospholipids in the oil were measured by the colorimetric methods of Lowry and Tinsley (1976) and Raheja *et al.* (1973), respectively. Peroxide value was measured as the iodine liberated from the cadmium-iodide complex, by the peroxides in the oil (AOCS, 1985).

Experimental

Aqueous extraction process

Ground sunflower kernels were resuspended in water, adjusting pH at 4·8–5·0, since at acidic pH sunflower proteins can be recovered as concentrate in the solid phase, removing oil and phenolic compounds in the liquid one. In order to keep a good contact between solid and liquid phases, a mixing step shaking at 200 rpm was performed. Other experimental conditions (solvent : kernels ratio, particle size) were fixed as a function of the oil recovery. The separation of the solid and liquid phases was achieved by mechanical means, with a Beckman J2-21 centrifuge at 10 000 rpm (14784 × *g*) for 20 min. The solid residue from the first centrifugation was redissolved in water at a 1 : 5 (w/v) ratio (this reduction in the water volumes facilitates the de-emulsification of the oil), adjusted to acidic pH, and the aqueous slurry was centrifuged under the former conditions. The solid was air-dried from 60–70% moisture after centrifugation to 7–8%, and the liquid phases were pooled and submitted to a de-emulsification step to separate the oil.

Diffusion–extraction of phenolic compounds

During this study two specific procedures were used for phenolics removal during the aqueous processing. To discover whether the phenolics present in the media (concentrations around 1·2 g/litre, which was found to reduce the enzyme activity to 85% of the initial filter

paper activity) affected the enzymatic efficiency, a process involving the removal of phenolics before aqueous extraction kernels was tried (a). The second method was done on semi-defatted meal, and was tried with the aim of comparing the effect of the complete removal of the free phenolic compounds (as chlorogenic acid) on the meal quality (b).

(a) Continuous diffusion from transversally cut in halves kernels (Sosulski *et al.*, 1973). Operational conditions were maintained during 2 h at 60°C and pH 4·5 by addition of 0·01 M HCl and stirring to maintain the particles suspended in the solvent. Solvent : kernel ratio was 120 : 1 (v : w). The use of cut kernels facilitated the elimination of the testa which, once separated from the kernels, could be removed from the surface by flotation. Once dried and ground, the kernels were processed to extract oil and protein.

(b) Diffusion from ground meal from the aqueous processing (Lanzani *et al.*, 1979). Samples were contacted with water at 100°C for 10 s, with a volume of boiling water 25 times the initial weight of kernels before aqueous processing. The suspension was stirred, cooled at 25–30°C, and centrifuged to remove the phenolic compounds and sugars released in the aqueous phase. This procedure was performed four times which was enough to reduce the chlorogenic acid content in the meal.

Enzymatic treatment

The hydrolytic enzymatic treatment to enhance oil extractability was performed during the mixing step of the ground kernels, either with Multifect or with a 2 : 1 (w/w) mixture of Celluclast + Pectinex, which in previous experiments was found to enhance the oil extractability (Dominguez *et al.*, 1991). The effect of the enzymatic treatment was always compared with a control sample (without enzymes). Optimum pH for these enzymatic activities is in the range 4·5–5; phenolics are also easily extracted in acid media and this is a suitable pH for the production of a proteinic concentrate. Temperature was maintained at 50°C, to preserve the quality of the products, and to favour both the activity and stability of the enzymes.

RESULTS AND DISCUSSION

The kernels used for experimentation contained (on a dry basis) 63% oil, 20% protein, 5·7% total fibre as NDF (neutral detergent fibre), 2·6% ash, 2·5% ethanol-soluble sugars and 1·3% chlorogenic acid.

The particle size and the water : kernel ratio used along the experimental study were selected during the aqueous process without enzymatic treatment; their effect on the oil extraction yield was determined. The oil extraction in the liquid phase was calculated as difference between the total oil content of the kernels and the residual in the solid product (measured by Soxhlet). Particle size reduction from more than 1 mm to less than 0·75 mm gave a slight improvement in the oil extraction yield for the smaller sizes (from 39 to 46%).

Since small differences were found in the oil extraction with water : kernel ratios ranging from 5 to 25 (v/w), and a 10 : 1 ratio provided a better oil removal than lower values, this ratio and particle size < 0·75 mm were chosen for further experiments.

The oil extraction yield was 40% of the total oil extractable with the Soxhlet method. This value is in the range of those reported by other authors for a similar particle size and under analogous separation conditions in a batch centrifuge (Hagenmaier, 1974). The efficiency is low due to the short mean path of the oil in this system (Kim, 1989). Higher oil extraction yields can be reached with other centrifugation systems or in pilot plant (Lanzani *et al.*, 1983).

Enzyme : kernels ratio and hydrolysis time

The effect of the enzymatic hydrolysis time during mixing was studied, and compared with control samples (without enzyme) processed under the same conditions. The mixture (2 : 1) Celluclast + Pectinex at enzyme : kernels ratios in the range 0·5–4 g/100 g kernel (DB) was employed. Figure 1 illustrates the effect of both variables on the oil extraction yield; 2 g/100 g kernel seems to be the most favourable, because higher values do not produce additional increases. For this value, an incubation time of 2–3 h gives the maximum oil yield, about 70% of the total extractable oil.

The extension of the enzymatic reaction was followed by measuring the concentration of reducing sugars in the whey resulting from the first centrifugation. As the enzyme : kernel ratio and incubation time increase, the sugar concentration rises with a much more pronounced tendency than that presented by control samples (Fig. 2). Conclusions are analogous to those from Fig. 1, although a beneficial effect was noticed if treatment time was prolonged until 5 h. Reducing sugars concentration increased from 20–25 mg/g (dry defatted basis) in control samples, to 70–75 mg/g in the samples treated with the optimum enzyme concentrations for

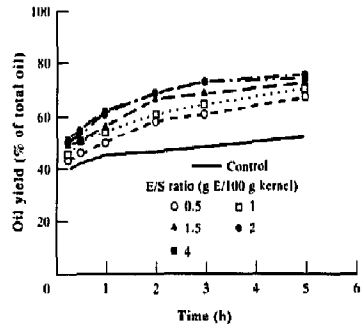

Fig. 1. Effect of enzyme : kernel ratio on the oil extraction yield. Each result is the average value of two measurements.

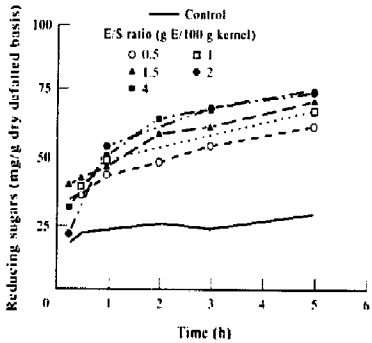

Fig. 2. Effect of the enzyme : kernel ratio on the reducing and total sugars solubilized in the liquid phase resulting from the first stage of separation. Each result is the average value of at least two measurements.

oil extraction during periods up to 5 h, representing an increase of more than three times.

Mass balance

Figure 3 presents the mass balance for oil, protein and chlorogenic acid during the aqueous processing of sunflower kernels to obtain oil and protein, for both control and enzyme-treated samples. Mixing-extraction was performed for 3 h at 50°C and 200 rpm, these being the most favourable conditions found in previous experiments. The processes with the specific stages of chlorogenic acid removal are indicated as (a) and (b).

Worth mentioning is the removal of free chlorogenic acid in the aqueous process (90% removal), and the solid end-product contains less than 12% of that initially present in the kernels. The residual oil content in the proteinic product obtained from enzyme-treated samples was considerably reduced as compared to that obtained from untreated ones.

The main advantage of the removal of chlorogenic acid was the enhanced purification of the proteic product. The removal of other soluble compounds was responsible for this higher purity. However, the protein recovery in the solid product was lower (78%) in the procedure (b) than for the aqueous process (90% of the initial).

The interest of the incorporation of a specific stage of extraction of phenolic compounds lays mainly in the higher quality of the proteic product caused by the removal of phenolic compounds which darken the meal and reduce its nutritious and functional properties. Although phenolic compounds are known to inhibit the cellulolytic and pectinolytic enzymes (Bolaños *et al.*,

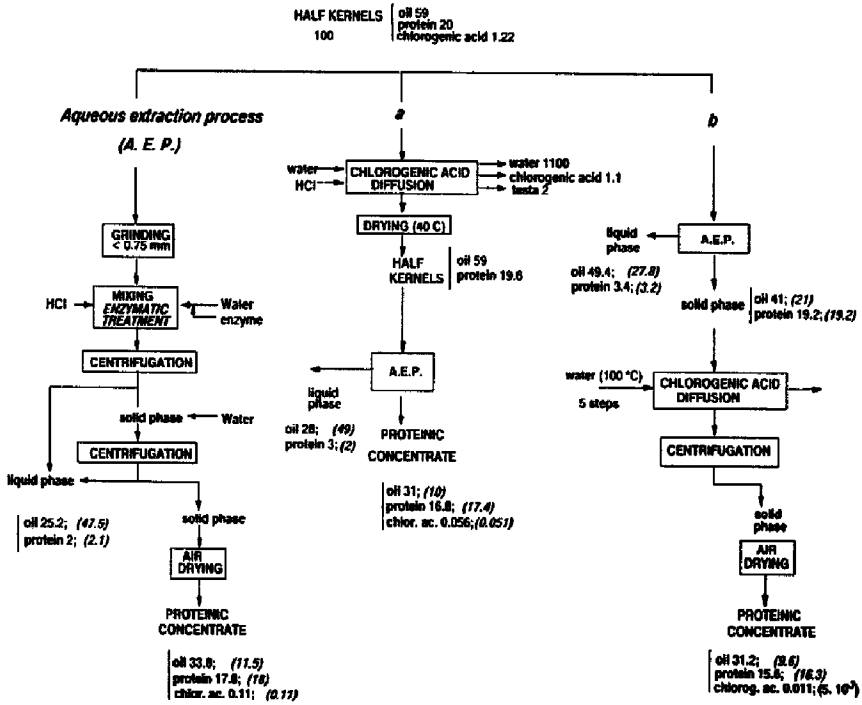

Fig. 3. Mass balance during aqueous processing of ground sunflower kernels. Values corresponding to the products from enzymatic trials are indicated in italics.

1989; Nyman & Björck, 1989), no significant effect was noticed as a result of removing these compounds before the enzyme treatment, on the enzyme efficiency, measured as increased oil extractability. Probably, at the levels present, the effect on the enzyme activity was not sensitively reduced.

Although in the aqueous processes the liquid phase is usually recycled to the mixing step, this possibility has not been considered here, because 80% of the chlorogenic acid initially present in the kernels was removed in this phase. Studies should be done to determine whether the reduction in the water : kernels ratio, beneficial from the operational point of view (the de-emulsification step would be favoured, and the effluent disposal would be reduced) would affect the enzymatic action, since it was observed that, at 0·45% (w/v), the presence of chlorogenic acid reduced to 75% the filter paper activity, and at a concentration near 0·6% (the saturation value at 20°C) the activity was reduced to almost 50%. On the other hand, the diffusion would be seriously hindered. Therefore, the separation of phenolics and active enzyme from the whey would be an interesting alternative.

Composition and characterisation of products

Oil

Table 1 summarises some properties of the oil obtained from the aqueous processing. Each value is the average of at least two measurements with a difference between them of 5–7%. The free fatty acid content (FFA) of the oil obtained with enzymatic technology was slightly superior to that of the untreated samples. This characteristic was also observed by other authors, for different seeds and extraction technologies: extraction of canola oil by pressing (Sosulski & Sosulski, 1990), aqueous extraction of rapeseed oil or coconut (Cintra *et al.*, 1986).

The phosphorus content, measured as phospholipids (μg P/g crude oil), was sensitively lower than that from solvent-extracted oil (≈ 100 ppm), so the refining losses will be reduced in oils from aqueous treatment that are partly degummed (Laiho *et al.*, 1990). The fact that values are lower than 10 ppm indicates that, during the aqueous treatment, these oils were degummed, although not completely, probably because the conditions for degumming require temperatures higher than 50°C (70–90°C) for a short period of time. Another reason could be the presence of nonhydratable phospholipids because no thermal conditioning to inactivate endogenous enzymes (lipoxygenases and phospholipases) was done. Phospholipases hydrolyse lecithins to the corresponding phosphatidic acids, which constitute the greater part of nonhydratable phospholipids. Even in the alternative (b), washings at 90°C done on the defatted meal did not remove the phospholipids in the oil.

Peroxide values, more influenced by the quality of the raw materials and processing than by the enzymatic treatment, were similar for all samples, and the obtained values were always under the standard limits 10 meq/kg.

Meal

The composition of the air-dried defatted proteic products is presented in Table 2. The concentrates show a protein content (N × 6·25) under 70% dry weight, because no washing steps were used to purify them. The proteic content of the concentrate could be raised by a more efficient separation, as well as by the application of a washing stage with cold water in one or more steps, where oil, sugars and salts could be removed. This additional step would improve the product stability, as fat provokes oxidations, and reducing sugars are susceptible to reacting with the protein, causing a darker colour and reducing the nutritive quality. No significant differences were found between the samples treated with the enzymes used, but the proteic content of the concentrations from the processes with specific stages of chlorogenic acid removal was slightly higher that those of concentrates from the aqueous process.

As observed from the mass balances, the oil content in the proteic products was sensitively reduced with respect to those from untreated kernels, resulting in an increased protein percent in the concentrate obtained from the enzymatic treatment, although the overall

Table 1. Characteristics of the oil obtained from the different aqueous processes[a]

Characteristic	Control	Treated	
		Celluclast + Pectinex	Multifect
Aqueous extraction (without specific stage to remove phenolics)			
FFA (% oleic)	0·79	0·82	0·86
Phosphorus (μg/g)	8·89	7·31	8·26
Peroxide (meq/kg)	5·27	5·12	5·06
(a) Diffusion from kernels transversally cut in halves			
FFA (% oleic)	1·23	1·34	1·40
Phosphorus (μg/g)	4·15	8·72	3·76
Peroxide value (meq/kg)	5·50	4·33	5·47
(b) Diffusion from semi-defatted ground kernels			
FFA (% oleic)	1·03	1·29	0·91
Phosphorus (μg/g)	6·26	8·39	6·36
Peroxide value (meq/kg)	5·76	4·93	3·85

[a]Values are averages of duplicate samples.

Table 2. Characteristics of the meal obtained after the aqueous extraction using different procedures for chlorogenic acid removal (% dry and defatted basis

Characteristic	Control	Enzyme-treated	
		Celluclast + Pectinex	Multifect
Aqueous process			
Residual oil (%)[a]	54·59	28·24	30·06
Chlorogenic acid	0·41	0·44	0·40
Protein	63·6	63·5	65·8
NDF[b]	20·08	18·0	17·91
Ash	5·36	5·61	6·05
Soluble protein (pH 9)	87·4	88·3	87·9
ADC[c]	82·1	83·5	84·6[d]
Available lysine (mg/16 g N)	3·40	3·62	3·40
CPER[e]	1·86	1·88	1·91
Diffusion from kernels transversally cut in halves			
Residual oil (%)[a]	54·96	28·20	27·43
Chlorogenic acid	0·22	0·20	0·18
Protein	66·1	67·5	66·4
NDF	19·23	18·66	17·82
Ash	6·69	6·06	5·31
Soluble protein (pH 9)	85·2	84·4[d]	87·7
ADC	84·7[d]	85·9[d]	84·9[d]
Available lysine (mg/16 g N)	3·18	3·23	3·27
Diffusion from ground semi-defatted kernels			
Residual oil (%)[a]	57·27	26·86	30·22
Chlorogenic acid	0·047	0·002	0·045
Protein	67·0	67·3	68·0
NDF	21·00	19·39	18·75
Ash	6·87	7·31	6·04
Soluble protein (pH 9)	78·3[d]	78·2[d]	79·8[d]
ADC	85·5[d]	86·4	88·1[d]
Available lysine (mg/16 g N)	3·53	3·75	3·68

[a]Dry basis.
[b]NDF, neutral detergent fibre.
[c]ADC, apparent digestibility coefficient.
[d]Significant difference between the digestibilities of the proteic product respect to the control from the aqueous process with a *t*-test ($P<0·05$).
[e]C-PER, calculated protein efficiency ratio.

proteic contents (DB) of the products were similar. In the diffusion from semi-extracted meal, more oil was removed from both untreated and enzymatically-treated samples as the proteic product was submitted to several washings. Remarkable was the reduction in the fat content for the solid product obtained in the process (b) with Celluclast + Pectinex (26·8% residual oil): the enzyme treatment causing a reduction in the residual oil by an average value of 27% of the total oil.

The aqueous process allows reduction of the polyphenolic compounds, measured as chlorogenic acid content, from the initial value in the defatted meal of 3·5% (w/w) to 0·41%, indicating more than 88% removal. This was also observed when the enzymatic treatment was incorporated. Procedures (a) and (b) yielded meals with considerably reduced contents of chlorogenic acid. Diffusion from transversally cut (in halves) kernels, followed by aqueous extraction reduced chlorogenic to 0·22% (93·7% removal); when the enzymatic technology was employed, the reduction was slightly superior, 94 and 94·8%. The diffusion from the semi-defatted meal reached even higher percentages of removal (98% in control samples and 99·9 and 98·7% in enzyme-treated samples).

The neutral detergent fibre (NDF) content, corresponding to cellulose, hemicellulose and lignin, was reduced by approximately 10% after enzymatic treatment. The total content in the procedure (b) was raised due to the purification of the product by removal of soluble substances in the washing liquids.

The method (b) for chlorogenic acid removal affected the protein solubility of the concentrate. This property, required for food uses especially for drinks, was reduced by 10% in spite of the reduced period of contact of the meal with water at high temperatures. It must be considered that after the 10 s of contact and stirring to extract the phenolics, the cooling stage with water at 15°C probably was not quick enough and denaturation could take place to some extent thus diminishing solubility.

The apparent digestibility coefficient (ADC) reflects the effect of the thermal denaturation or the interaction of polyphenols with proteins on the digestibility. The extreme conditions ($T = 100$°C) used during the procedure (b) did not reduce the meal quality, and the subsequent reduction in chlorogenic acid content raised the digestibility values. A slight increase in protein digestibility was observed in the concentrate from enzymatically-treated samples, compared to that from untreated, but it

was significant only in the aqueous processing without specific stages of chlorogenic acid removal. This improved digestibility could be ascribed to the enhanced accessibility of the proteins (entrapped in the polysaccharide matrix) to the digestive enzymes (Walsh *et al.*, 1993). The above-mentioned reduction in the proteic solubility can be ascribed to a mild denaturation whereby the nutritional value of the proteins increases due to modification of tertiary structure, making them more digestible (Barry, 1989; Gómez & Torre, 1989).

Available lysine was not observed to be significantly affected, either by enzymatic treatment or by incorporation of the diffusion–extraction steps. The proteic products, light coloured at neutral pH, once resuspended at alkaline pH maintained this colour if meals were derived from processes (a) and (b), while a creamy colour appeared in meals from the aqueous process.

CONCLUSIONS

The enzymatic treatment of sunflower kernels during aqueous extraction efficiently enhanced oil extractability. The degradative effect on the cell wall, measured as reducing sugars in the liquid phase could be correlated with the increased oil yield, revealing an increase with hydrolysis time and enzyme : substrate ratio up to 2 g enzyme/100 g kernels and 3–4 h treatment.

Aqueous extraction, even without specific stages of phenolics removal, reduced the chlorogenic acid content in the solid product by almost 90%. The removal of chlorogenic acid from the cut kernels before enzymatic treatment did not significantly enhance the enzyme efficiency. In the process where ground semi-defatted kernels were submitted to extraction of chlorogenic acid, the percentage elimination of this phenolic was higher, due to the washings at elevated temperature, which slightly reduced protein solubility, but did not affect other properties.

The main oil quality parameters were unaffected by the enzymatic treatment during the mixing of the paste. The final proteinic product resulting from the three proposed processes had a light colour, that made it suitable for human consumption.

REFERENCES

AOAC (1990). *Official Methods of Analysis* (15th edn), ed. K. Helrich. Association of Official Analytical Chemists, Arlington, VA, USA.

AOCS (1985). *Official and Tentative Methods of the American Oil Chemists' Society*, ed. R. G. Walker. American Oil Chemists' Society, Champaign, IL, USA.

Barry, J. L. (1989). L'influence des traitments technologiques sur la digestibilité des aliments. *Biofutur.*, **62**, 51–5.

Bau, H. M., Mohtadi-Nia, D. J., Mejean, L. & Debry, G. (1983). Preparation of colourless sunflower protein products: effect of processing on physicochemical and nutritional properties. *J. Am. Oil Chem. Soc.*, **60**(6), 1141–7.

Bhatnagar, S. & Johari, B. N. (1987). Microbial enzymes in the processing of oil seeds. *Curr. Sci.*, **56**(15) 775–6.

Bolaños, G. Heredia, J. F. & Guillén, B. R. (1989). Inhibidores de enzimas celulolíticos. *Grasas y Aceites*, **40**(2), 146–9.

Cintra, M. O. López-Munguía, C. A. & Vernon, C. J. (1986). Coconut oil extraction by a new enzymatic process. *J. Food Sci.*, **51**(3), 695–7.

Cruz Madueño, E. (1990). Extracción acuosa. *Alimentación, Equipos y Tecnología*, **9**(3), 51–8.

Domínguez, H., Sosulski, K., Núñez, M. J. & Lema, J. M. (1991). Preselección de preparados comerciales para el tratamiento enzimático de semillas de soja y girasol. III World Congress of Food Technology. Barcelona, Spain, 20–23 February.

Fan, T. Y. & Sosulski, F. W. (1976). New techniques for preparation of improved sunflower protein concentrates. *Cereal Chem.*, **53**(1), 118–25.

Flemming, S. E. & Sosulski, F. W. (1977). The utilization of sunflower protein in milk-like beverages. *J. Inst. Can. Sci. Technol. Aliment.*, **10**(4), 229–32.

Fullbrook, P. D. (1983). The use of enzymes in the processing of oilseeds. *J. Am. Oil Chem. Soc.*, **60**, 476–8.

Fullbrook, P. D. (1984). Extraction of vegetable oils. UK Patent Application GB 2 127 425A. No 8227661. 28 September 1982, 11 Apr 1984.

Goering, H. K. & Van Soest, P. J. (1970) *Forage Fiber Analyses (Apparatus, Procedures and some Applications)* (Agriculture Handbook No 379). USDA (Agricultural Research Service).

Gómez, P. J. M. & Torre, B. C. (1989). Influencia de la tecnologia en el valor nutritivo de los alimentos. I: Proteinas. *Alimentaria*, **Jul–Aug**, 15–21.

Hagenmaier, R. D. (1974). Aqueous processing of full-fat sunflower seeds: yields of oil and protein. *J. Am. Oil Chem. Soc.*, **51**(10), 470–1.

Hron, R. J. Sr, Koltun, S. P. & Graci, A. V. Jr (1982). Biorenewable solvents for vegetable oil extraction. *J. Am. Oil Chem. Soc.*, **59**(9), 674A–82A.

Hsu, H. W., Vavak, D., Satterlee, L. D. & Miller, G. A. (1977). A multienzyme technique for estimating protein digestibility. *J. Food Sci.*, **42**(5), 1269–73.

Hsu, H. W., Sutton, N. E., Banjo, M. O., Satterlee, L. D. & Kendrick, J. G. (1978). The C-PER and T-PER assays for protein quality. *Food Technol.*, **12**, 69–73.

Hurrell, R. F. & Finot, P. A. (1985). Effects of food processing on protein digestibility and amino acid availability. Chap. 8. in *Digestibility and Amino Acid Availability in Cereals and Oilseeds*, eds. J. W. Finley & D. T. Hopkins, American Association of Cereal Chemists Inc, St. Paul, MN, USA.

James, N. A. & Ryley, J. (1986). The rapid determination of chemically reactive lysine in the presence of carbohydrates by modified trinitrobenzensulphonic acid procedure. *J. Sci. Food Agric.*, **37**, 151–6.

Johnson, L. A. & Lusas, E. W. (1983). Comparison of alternative solvents for oils extraction. *J. Am. Oil Chem. Soc.*, **60**(2), 229–42.

Kim, S. H. (1989). Aqueous extraction of oil from palm kernel. *J. Food Sci.*, **54**(2), 491–2.

Laiho, S., Tulisao, U., Oksanen, H. & Nystrøm, R. (1990). A process for the production of a vegetable-oil product. WO-A-91 13956 (Öljyynpuristamo OY).

Lanzani, A., Cardillo, M. & Petrini, M. C. (1979). Preparazione di concentrati proteici da semi di girasole per via umida. *La Riv. Ital. delle Sostanze Grasse*, **56**, 48–51.

Lanzani, A., Camurati, F., Cardillo, M., Cortesi, N., Mariani, C., Fedeli, E., Ponzetti, A. & Pieralisi, G. (1983). Tecnologia di estrazione di farine a partire da semi di girasole. Nota II. *La Riv. Ital. delle Sostanze Grasse*, **60**(52), 353–63.

Lawhon, J. T., Rhee, K. C. & Lusas, E. W. (1981). Soy protein ingredients prepared by new processes—Aqueous processing and industrial membrane isolation. *J. Am. Oil Chem. Soc.*, **58**, 377–84.

Leung, J., Fenton, T. W. & Clandinin, D. R. (1981). Phenolic components of sunflower flour. *J. Food Sci.*, **46**, 1386–93.

Lowry, R. R. & Tinsley, I. J. (1976). Rapid colourimetric determination of free fatty acids. *J. Am. Oil Chem. Soc.*, **53**(7), 470–2.

Marek, E., Schalinatus, E., Weigelt, E., Mieth, G., Kerns, G. & Kude, J. (1990). On the application of enzymes in the production of vegetable oil. *Prog. Biotechnol.*, **6**, 471–4.

Nagel, C. W., Herrick, I. W. & Graber, W. R. (1987). Is chlorogenic acid bitter? *J. Food Sci.*, **52**(1), 213.

Nyman, M. A. & Björck, I. M. (1989). In vivo effects of phytic acid and polyphenols on the bioavailability of polysaccharides and other nutrients. *J. Food Sci.*, **54**(5), 1332–5.

Raheja, R. K., Kaur, C., Singh, A. & Bathia, I. S. (1973). New colourimetric method for quantitative estimation of phospholipids without acid digestion. *J. Lipid Res.*, **14**, 695–7.

Rhee, K. C., Cater, C. M. & Mattil, K. F. (1972). Simultaneous recovery of protein and oil from raw peanuts in an aqueous system. *J. Food Sci.*, **37**, 90–3.

Sabir, M. A., Sosulski, F. W. & Finlayson, A. J. (1974). Chlorogenic acid-protein interactions in sunflower. *J. Agric. Food Chem.*, **22**(4), 575–8.

Shamanthaka, S. M. C. & Sastry, R. N. M. S. (1990). Binding of chlorogenic acid by the isolated polyphenol-free 11S protein of sunflower (*Helianthus annuus*) seed. *J. Agric. Food Chem.*, **38**(12), 2103–10.

Sodini, G. & Canella, M. (1977). Acidic butanol removal of colour-forming phenols from sunflower meal. *J. Agric. Food Chem.*, **25**(4), 822–5.

Somogyi, M. (1952). Notes on sugar determination. *J. Biol. Chem.*, **195**, 19.

Sosulski, F. W. (1979). Organoleptic and nutritional effects of phenolic compounds on oilseed protein products: a review. *J. Am. Oil Chem. Soc.*, **56**(8), 711–15.

Sosulski, K. & Sosulski, F. W. (1990). Quality of oil and meal from enzyme treated canola seeds. Proceedings of *33rd Annual Conference on the Canadian Institute of Food Science and Technology*, 3: 656.

Sosulski, F. W., Sabir, M. A. & Fleming, S. E. (1973). Continuous diffusion of chlorogenic acid from sunflower kernels. *J. Food Sci.*, **38**, 468–70.

Staron, T. & Guillaumin, R. (1979). Une méthode d'extraction des protéines et de l'huile de colza, par l'eau. *Revue Française des Corps Gras*, **26**(11), 441–6.

Walsh, G. A., Power, R. F. & Headon, D. R. (1993). Enzymes in the animal-feed industry. *Tibtech*, **11**, 424–30.