Quality of Corn Germ Oil Obtained by Aqueous Enzymatic Extraction

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Aqueous enzymatic extraction of corn germ oil was investigated. By applying hydrothermal pretreatment of corn germ, it was possible to inactivate native enzymes present in the germ and to loosen its structure. The corn was then ground and treated with enzymes. After the oil had been released by the enzyme reaction, it was separated by centrifugation. The quality of oil obtained by enzymatic extraction (Pectinex Ultra SP-L at 37°C, pH 5.2, for 6 h) was good. The oil had 1.5% free fatty acids; a total content of oxidation product value of 8.1; light yellow color (AOCS photometric color, 12.7; dominant wavelength λ_D = 575 nm); 0.022% phosphatides; 1350 mg/kg total tocopherol; and an oxidative stability value (Rancimat test, 100°C) of 14.6 h.

KEY WORDS: Aqueous enzymatic extraction, corn germ, hydrothermal pretreatment, oil quality.

Corn germ oil is characterized by high contents of essential fatty acids and tocopherols. The presence of γ-tocopherol makes corn oil more stable to oxidation as compared with many other oils. Usually, corn oil is produced from corn germ after wet milling in the course of starch production. Wet-milled corn germ contains more than 50% moisture and about 20% oil. To enable processing (by pressing or by combined pressing and extraction) the germ needs to be dried to 2–7% moisture. The oil content in dry corn germ is 40–50%, depending on the corn type and the process used for corn germ separation (1).

A long-term study of corn germ oil available on the market has shown that quality varies widely (2). This is mainly because during corn steeping and germ storage, enzymes, such as peroxidase and others present in the germ, initiate degradation reactions (3). It is difficult to reduce the rate of these reactions or eliminate them completely. Drying and conditioning of corn germ are usually carried out under conditions that are far from optimal for oil quality. Germ prepared in the typical way has high levels of oxidation and colored products that are transferred to the oil during pressing and/or extraction. Thus, the process of oil refining is difficult (1,4–6).

An alternate way of oil production, aqueous enzymatic extraction based on mechanical and enzymatic degradation of the cell walls of oilseeds, has recently attracted interest. This process liberates the oil of lipid bodies under mild processing conditions (7). The process is especially suitable to raw materials high in moisture content, where conventional procedures (pressing and extraction) cannot be directly applied. Corn germ obtained in starch production by centrifugation or cyclone separation of the steeped and coarsely ground corn contains about 50% moisture, making it particularly suitable for enzymatic oil extraction.

Experiences with enzymatic processes for coconut and olive oil extraction have indicated that enzymatic processing is simple and has lower energy requirements. Enzyme-extracted oil is recovered by milder processing routes compared with conventional processing (pressing or extraction), and has excellent quality compared with traditionally processed corn oil. Because the enzyme-extraction process is carried out in an aqueous medium, phospholipids are separated from the oil so that there is no need for degumming (7,8).

Enzymatic extraction was first applied to olives (9–12) and later to rapeseed, flax, cottonseed, sunflower, melon seed and coconut, palm kernel and avocado fruit (13–18). Hitze *et al.* (19) were the first to apply enzymatic extraction to obtain oil from corn germ.

Our previous investigations (8,20–22) showed that the efficiency of aqueous enzymatic oil extraction is influenced by several factors. Oil yield depends greatly on pH of the medium, time of hydrothermal pretreatment, degree of grinding, time of enzymatic conversion, enzyme preparation and the speed of centrifugation. Due to pH during hydrothermal pretreatment, proteins, starch and other compounds are partially dissolved, thereby loosening the germ structure. Tissue rupture during milling releases oil. Additionally, oil is released by the action of cell wall-degrading enzymes. Depending on the pH of the medium, proteins may also dissolve, which contributes to oil liberation. Thus, liberated oil is associated with fine cell particles in an emulsion state. The yield of free oil depends greatly on the efficiency of destroying this emulsion. The effects of various enzymes on oil yield have been studied in considerable detail (23). We have obtained as much as 84.7% of the available oil by using Celluclast 1.5 L (Novo Industries, Bagsvaerd, Denmark).

In addition to attaining high oil yield, it is essential to ensure minimal oil deterioration in all stages of the process. The aim of the present work was to establish the extent of changes in oil quality occurring during thermal and enzymatic treatments of corn germ, and to compare it to the quality of oil obtained by conventional procedures.

MATERIALS AND METHODS

Samples. Corn germ was obtained from commercial wet-milling in a local starch plant (IPOK-Zrenjanin, Vojvodina, Yugoslavia). Samples were taken immediately after separation of water by pressing, and the samples were frozen at −18°C. The corn germ samples were thawed in a refrigerator at 4°C the day before the experiment. The wet corn germ contained 53.4% moisture and 19.1% oil. Calculated on a dry solids basis, the germ contained 41% oil, 14.9% protein, 6.2% starch and 0.7% pectin. On a fat-free dry solids basis, the germ contained 11.2% cellulose. Samples of crude oil were taken from the same plant and used as control samples.

Degummed corn germ oil was prepared by a laboratory procedure at 60°C with the addition of 3% (vol/vol) water to the oil (5).

Procedure. The experimental scheme for enzymatic extraction of corn germ oil is shown in Figure 1. To

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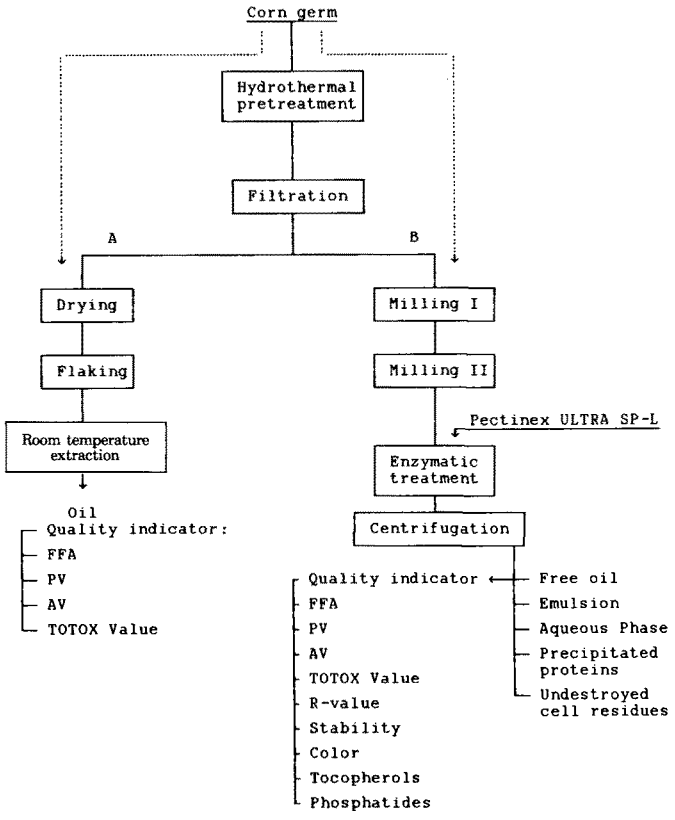

FIG. 1. Block diagram of corn germ oil separation. Abbreviations: FFA, free fatty acids; PV, peroxide value; AV, anisidine value; TOTOX, total content of oxidation products.

establish the effect of hydrothermal pretreatment on oil quality, the extraction process with hexane was carried out as outlined in stream A (Fig. 1). The dotted lines indicate oil isolation from hydrothermally untreated germ. The route of enzymatic processing of corn germ is shown in stream B (Fig. 1), which was carried out in four steps. In the first step, the germ was cooked to loosen the germ structure and to inactivate native enzymes. In the second step, size reduction was performed by two steps of wet-milling (vibration milling and colloid milling). To release the oil, the germ/water suspension was treated with an enzyme preparation. The last step was separation of oil from the suspension by centrifuging at 5000 rpm (1984 $\times$ *g*) for 20 min.

Hydrothermal pretreatment. Hydrothermal pretreatment of corn germ was carried out in a pressure cooker equipped with a pressure regulator. The cooking temperature was 112°C. To study the effect of pH of the aqueous phase and the duration of hydrothermal treatment on oil quality, a series of experiments were carried out according to the experimental plan presented in Table 1. Corn germ samples were boiled in buffer solutions pH 4.0 ($CH_3COONa/CH_3COOH/H_2O$, 0.2 mol/L), pH 5.5 ($CH_3COONa/CH_3COOH/H_2O$, 0.2 mol/L) or pH 7.0 ($NaH_2PO_4H_2O/NaOH/H_2O$, 0.2 mol/L) for 20, 40 or 60 min (3^2 factorial design) (24). Buffer solutions were used to ensure boiling at a constant pH. The corn germ obtained by wet-milling is acidic (pH of the suspension water/germ in the mass ratio 15:1 was about 4), and the

TABLE 1

Experimental Conditions Selected for 3^2 Factorial Design to Estimate the Effect of Hydrothermal Corn Germ Pretreatment on Oil Quality[a]

Independent variables (factors: X_1, X_2)	Factor level			Variation interval
	+1	0	−1	
pH of solution	7.0	5.5	4.0	1.5
Time of hydrothermal treatment (T), min	60	40	20	20

[a]Response function Y: quality of oil, free fatty acid content, peroxide value (PV), anisidine value (AV), total content of oxidation products value (2 PV + AV).

diffusion of acid (H_2SO_3) from the germ during the hydrothermal treatment continually changed the pH of the solution. After hydrothermal pretreatment, the germ samples were filtered from the aqueous medium. Part of each germ sample was dried and extracted with hexane (procedure A). From the remaining part, oil was isolated by the aqueous enzymatic extraction (procedure B).

Oil extraction with hexane. Sample of hydrothermally treated and untreated germ were dried in a thin bed at room temperature for 36 h to about 7% moisture. The dried germ samples were flaked to 0.4-mm thickness in a roller mill (Miag, Braunsweig, Germany). Oil was extracted in a laboratory tube extractor with commercial hexane (25). A tube was filled with 20 g of flaked germs and extracted for 1 h at a constant flow rate of miscella (20 mL/min) at 20°C. The miscella was collected and evaporated in a rotary evaporator at 40°C. Oil quality was determined by analyzing free fatty acids (FFA; % oleic acid) content, peroxide value (PV), content of carbonyl compounds by determining *p*-anisidine value (AV) and total content of oxidation products (TOTOX value; 2 PV + AV) (26).

Oil extraction by aqueous enzymatic process. Hydrothermally treated and untreated corn germ samples were wet-milled in a Foss-let type 15320 vibratory mill (A/S N. Foss-Electric, Hillerod, Denmark) for 2 min, in the presence of citrate buffer pH 5.2 (germ/buffer ratio, 1:2). The suspension was finely milled by passing three times through a toothed colloid mill (type ZO/50; FRYMA-Machinen AG, Rheinfelden, Switzerland) with the clearance between the rotor and stator adjusted to 0.04 mm. The suspension of ground germ (mass ratio germ/citrate buffer, 1:3.5) was then treated with the enzyme preparation Pectinex Ultra SP-L (Novo Nordisk Ferment AG, Dittingen, Switzerland) (2% calculated on germ mass) at 37°C, pH 5.2, for 6 h. The enzymatic reaction was carried out in a temperature-controlled glass reactor with a low speed stirrer set at 80 rpm. The oil released after enzymatic reaction was recovered by centrifuging. The centrifuged suspension was separated into liquid and solid phases. The liquid phase consisted of three layers (free oil, emulsion and syrup of dissolved carbohydrates and proteins). The solid phase consisted of an upper layer of sedimented proteins and a lower layer of undestroyed cell debris. Oil samples were taken from the top layer for quality determination. All experiments were replicated at least two times.

Analytical methods. Oil and moisture contents of the initial corn germ and the centrifuged sludge were

TABLE 2

Analytical Methods for Determining Quality of Corn Germ Oil[a]

Analytical method	Principle/method	Reference
FFA content	Titrimetric, NaOH	33
Peroxide value (PV)	Iodometric titration	34
Anisidine value (AV)	Spectrophotometric	35
TOTOX = 2 PV + AV	Arithmetic	26
Spectra absorbance at 232/270 nm	Spectrophotometric	36
Phosphatides	Spectrophotometric	37
Tocopherols	Emmerie Engel spectrophotometric	38
Oil color	Spectrophotometric tristimulus colorimeter	39–41
Oil stability	Automated AOM-test (Rancimat test, 100°C)	42

[a]FFA, free fatty acid; TOTOX, total content of oxidation products; AOM, active oxygen method.

measured by the ISO gravimetric methods (27,28). Nitrogen was determined by the Kjeldahl method (29). The factor 6.25 g of protein per g N was used to estimate protein content. Starch was measured according to procedures of Ewers cited by Radley (30), pectin according to McComb and McCready (31) and cellulose by an AOCS method (32). Oil quality was estimated by the methods cited in Table 2.

Extraction yield of total oil was calculated as the difference between the initial oil content of corn germ suspension taken before the aqueous enzymatic extraction and the residual oil in the centrifuge sludge.

Because it is almost impossible to quantitatively separate free oil from the top of the liquid phase in the centrifuge tube, we used the following procedure to establish the content of free oil. Free oil, together with the emulsion layer, was collected with a spoon and transferred to a beaker. Then the material was dissolved in hexane and transferred to a separatory funnel. Residual oil on the wall of the centrifuge tube was wiped away with filter paper. Oil was extracted from the filter paper three times with 60 mL hexane. The extract was also transferred to the funnel. After separation of the upper oil–hexane phase and the lower emulsion–water phase, the lower phase was removed. The upper phase was collected in a flask, evaporated and dried. This oil was termed free oil. All data represent means of at least four analytical determinations.

Statistical treatment of results. The results of the 3^2 factorial design were subjected to the RSM (Response Surface Method) program developed by Walker and Parkhurst (43), and the response surfaces were drawn with the aid of the commercial program Statgraphics v.2.1 (44).

RESULTS AND DISCUSSION

In the course of obtaining oil by the aqueous enzymatic extraction, only the hydrothermal pretreatment was performed at high temperature. Thus, it is a critical step to preserving oil quality. We also believe that germ milling before hydrothermal treatment adversely affects the quality of oil. So, the sequence of milling and hydrothermal pretreatment in our study was reversed as compared with previously proposed procedures (5). The effects of hydrothermal and enzymatic treatments on extraction yield of oil are presented in our previous work (23).

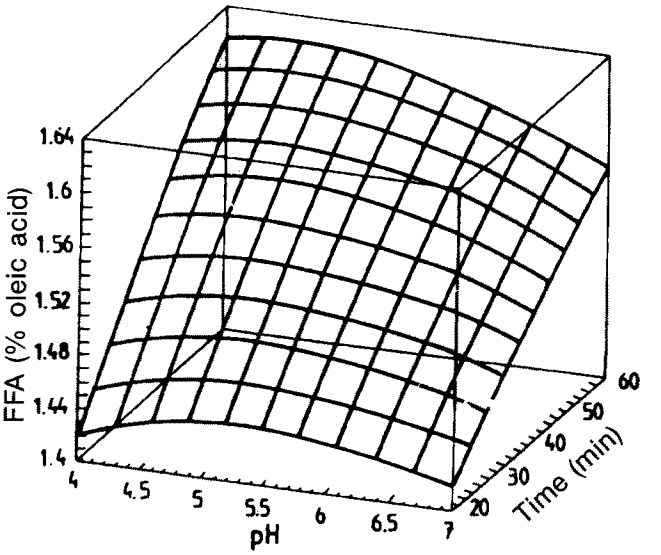

FIG. 2. Effects of pH of medium and duration of hydrothermal treatment of corn germ on free fatty acid (FFA) content in oil (content of FFA in untreated germ was 1.95 ± 0.15).

Effect of hydrothermal treatment of germ on oil quality. Changes in FFA contents of the oil extracted at room temperature from the hydrothermally treated germ are illustrated in Figure 2. This graphical presentation is based on a second-order polynom obtained by statistical treatment of the experimental data. The correlation between measured and calculated values of FFA was 0.8812, and the standard error of mean was 0.071.

The results clearly indicate that the time of hydrothermal treatment had a substantially greater effect than pH of the aqueous medium on the FFA content of the oil. An increase in FFA content from 1.4 to 1.6% when the time of hydrothermal treatment was increased from 20 to 60 min was regarded as acceptable. The FFA content in the oil extracted from thermally untreated germ was even higher (1.95%), which was attributed to the action of lipase during drying of the corn germ. At the same time, this indirectly indicated that hydrothermal treatment efficiently inactivated lipase, as has been established by Gardner and Inglett (45).

Peroxide contents of all oil samples were relatively low (Table 3). Time and pH of the hydrothermal treatment in the observed ranges did not significantly increase PV. The results indicate that the hydrothermal treatment was efficient in eliminating peroxidases and hydroperoxide isomerases, which are generally difficult to inactivate (45). The PV of the oil obtained from untreated germ was significantly higher than from any other sample (Table 3).

A complete picture of the oil quality may be obtained only if the total content of primary and secondary products of oxidation is known, *i.e.*, if the TOTOX value (2 PV + AV) of the oil is determined. Results (Table 3) indicated that pH in the range of 4–7 and time of hydrothermal treatment for 20 to 60 min have no significant effects on the contents of oxidation products. Oil from untreated corn germ exhibited approximately twofold higher contents of oxidation products. The results obtained in this

TABLE 3

Effects of pH and Time of Hydrothermal Treatment on Peroxide Content (mmol/kg) and TOTOX Value in Corn Germ Oil[a]

pH of aqueous medium	Time of hydrothermal treatment (min)					
	20		40		60	
	PV	TOTOX value	PV	TOTOX value	PV	TOTOX value
4.0	0.96 ± 0.18[b]	5.23 ± 0.30	1.78 ± 0.05	6.57 ± 0.08	1.42 ± 0.14	5.77 ± 0.48
5.5	0.94 ± 0.05	5.22 ± 0.30	0.80 ± 0.10	4.46 ± 0.37	0.82 ± 0.16	4.33 ± 0.26
7.0	1.36 ± 0.09	6.29 ± 0.49	0.96 ± 0.13	5.21 ± 0.33	1.07 ± 0.22	5.97 ± 0.21

[a]Abbreviations as in Table 1. PV of untreated corn germ, 2.7 ± 0.7; TOTOX value of untreated corn germ; 12.3 ± 1.7.
[b]Estimated with confidence coefficient 0.95.

investigation have demonstrated that the hydrothermal pretreatment of corn germ may be carried out over a wide range of pH with no effect on oil quality.

Quality and stability of oil obtained by extraction with the aid of Pectinex Ultra SPL. The qualities and oxidative stabilities of oils obtained by the enzymatic extraction are presented in Table 4. For comparison, data are also presented for expeller crude oil and degummed oil. The latter oil was used for comparison because the oil obtained by the enzymatic extraction was more similar to degummed oil. The content of phosphatides in oil obtained by aqueous enzymatic extraction was extremely low, which suggested the possibility of employing physical refining (46,47). In spite of the low level of phosphatides, which are known to improve oil stability, the oil obtained by aqueous enzymatic extraction was relatively stable. The observed induction period was in the range of 14.6 ± 3.3 h. The most likely reason for this oxidative stability was the high content of tocopherols.

Contents of FFA and primary and secondary oxidation products in the oil obtained by aqueous enzymatic extraction were in the range of values for oil obtained by conventional procedures.

Contents of conjugated dienes and trienes, and therefore the corresponding R values, were practically identical for all the samples because the contents of oxidation products in the oils were approximately the same. The visible spectra of corn germ oils obtained by the conventional procedure (spectra 1 and 2) and by aqueous enzymatic extraction (spectrum 3) are shown in Figure 3.

Oil obtained by enzymatic extraction had lower contents of colored substances. This becomes especially apparent

TABLE 4

Qualities and Oxidative Stability of Oils Obtained by Standard Industrial Procedures and Aqueous Enzymatic Extraction[a]

Analytical characteristic	Procedure		
	Standard industrial		
	Expeller crude oil	Degummed oil	Oil isolated with Pectinex Ultra SP-L[b]
Content of FFA (% oleic acid)	1.11 ± 0.71[c]	1.17 ± 0.73	1.50 ± 0.16
Peroxide value (mmol/kg)	1.10 ± 0.84	2.10 ± 0.18	0.00 ± 0.00
Anisidine value (100 $A^{1\%}_{1\,cm}$)	7.75 ± 3.77	9.00 ± 5.40	8.10 ± 0.23
TOTOX value (2 PV + AV)	9.95 ± 5.10	13.2 ± 5.20	8.10 ± 0.23
Tocopherols (mg/kg)	1145 ± 320	1264 ± 55	1350 ± 54
Phosphatides (P × 30)%	1.40 ± 0.80	0.135 ± 0.04	0.022 ± 0.02
Oxidative stability [IP at 100°C (h)]	33.0 ± 1.24	18.5 ± 0.70	14.6 ± 3.30
$A^{1\%}_{232}$	2.8 ± 0.18	2.8 ± 0.25	3.2 ± 0.46
$A^{1\%}_{270}$	0.70 ± 0.04	0.07 ± 0.06	0.80 ± 0.04
R-value	3.82 ± 0.18	3.97 ± 0.04	3.96 ± 0.04
AOCS color	32.8 ± 3.2	26.1 ± 5.0	12.7 ± 2.5
Dominant wavelength λ_D (nm)	577	576	575

[a]Abbreviations as in Tables 1 and 2. IP, induction period.
[b]From Novo Nordisk, Ferment AG, Dittingen, Switzerland.
[c]Estimated with confidence coefficient 0.95.

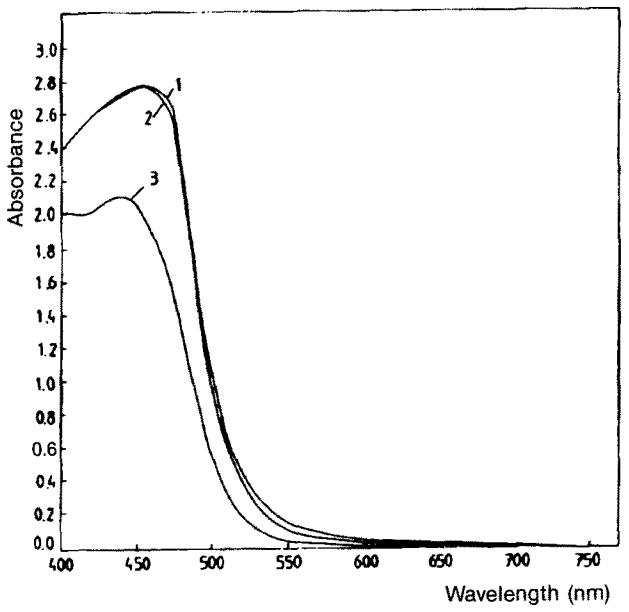

FIG. 3. Visible spectra of corn germ oils obtained by different procedures: 1, expeller crude oil; 2, degummed oil and 3, oil isolated by aqueous enzymatic extraction.

after calculating the AOCS color parameters (Table 4). The oil was light yellow in color, which was evident from the dominant wavelength determined with the aid of a tristimulus colorimeter (40). This indicated that the oil may be refined with less bleaching earth, and that a procedure of heat decoloration might be used (48,49).

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