The Effect of Wet-Milling on the Tocopherols in Corn Germ Oil

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ABSTRACT

Commercial wet-milling appears to have little effect on the content of α- and γ-tocopherols in corn germ oil. The tocopherol composition of corn germ oil from hand-dissected germ was about the same as that from the germ recovered from a commercial wet-milling plant. Of the several methods examined for the extraction of oil and tocopherols from corn germ, that utilizing a 9:1 hexane-ethanol mixture at 30°C. was preferred.

Recent reports have indicated that sizable losses of tocopherols occur during the wet-milling of corn in the laboratory (1). Only 18% of the α-tocopherol and 27% of the total tocopherols in the whole grain were recovered. Since about 95% of the α-tocopherol in corn is present in the germ (2), we feared that sizable losses of the tocopherols in corn germ oil might occur during commercial wet-milling. The purpose of this study was to determine whether appreciable destruction of the α- and γ-tocopherols in corn germ occurred under practical wet-milling conditions.

MATERIALS AND METHODS

The commercial process for the wet-milling of corn has been fully described by Watson (3). Our samples of corn and germ were obtained from the CPC International Inc. wet-milling plant at Argo, Ill. Whole corn was sampled automatically as the steeps were being filled. The whole germ samples were taken just before the washed germ was sent to the dryers. The plant-dried germ was taken as it discharged from the dryers. An effort was made to take the germ samples at the appropriate time so that they corresponded to the whole corn sample. Wet germ samples were immediately dried by lyophilization and the vacuum was broken with nitrogen. Dry germ was stored in a nitrogen atmosphere in a deep freeze.

Whole corn was steeped in the laboratory in an aqueous solution containing sulfur dioxide and lactic acid according to the procedure of Watson et al. (4). The only change was that the initial steeping time was reduced to 32 hr. and the final steep was extended to 16 hr.

Corn germ oil was analyzed for α- and γ-tocopherols using gas-liquid chromatographic (GLC) separation of the acetates. The procedure is similar to that briefly described by Alfin-Slater et al. (5). This technique, which does not separate the tocotrienols from γ-tocopherol, is quite satisfactory in this case, because corn germ oil contains practically no tocotrienols (2). A gram of oil was saponified with potassium hydroxide in the presence of pyrogallol, the unsaponifiables extracted with ether, and the ether extract evaporated to dryness under vacuum. The acetates were prepared in pyridine-acetic anhydride (2:1, v./v.) by overnight reaction at room temperature. The next day the solution was taken to dryness with a rotary vacuum evaporator (40°C. maximum) and the residue dissolved in 2 ml. benzene. A 0.125-in. X 9-ft. stainless steel column packed with 1% SE-30 coated on 100–120-mesh Gas Chrom Q was used to separate α- and γ-tocopherols. The

[1]Presented at the 57th Annual Meeting, Miami Beach, Oct.-Nov. 1972.

column temperature was 220°C. with a helium flow of 60 ml. per min. The hydrogen flame detector was operated at 290°C.

Losses suffered during the analytical procedure were determined by comparing GLC responses of an α- and γ-tocopherol reference mixture carried through saponification, extraction, and derivatization and of the same mixture which was merely derivatized. The data showed that a 20% loss of each tocopherol occurred during saponification, extraction, and derivatization. Since the tocopherol recoveries were not quantitative, standards of about the same α- and γ-tocopherol ratio as corn germ oil were analyzed daily, and the response of the daily standard used to calculate the tocopherol content of the unknown. Great care was exercised to be certain that samples and standards were run identically.

Reproducibility of the acetate method is good. The standard deviation calculated from 20 duplicate analyses was 4.0% relative for both α- and γ-tocopherol. Included in this series are samples representing wide variations in tocopherol contents (from ∿0.1% to ∿5% total tocopherols). This standard deviation incorporates variation incurred in all steps of sample preparation and analyses. Standard deviation of individual GLC analyses by duplicate injections of the sample was also 4.0% relative, indicating that essentially no error was introduced by sample preparation, extraction, and derivatization.

TABLE I. EFFECT OF EXTRACTION CONDITIONS ON THE TOCOPHEROL CONTENT OF OIL EXTRACTED FROM CORN GERM

Steeped Germ[a]	Extraction Procedure[b]	Tocopherol % Based on Oil α	Tocopherol % Based on Oil γ	Oil in Residue %
July 1971	Ethanol, 1 hr., 30° C.	0.031	0.39	...
July 1971	Ethanol, 2 hr. reflux	0.030	0.15	...
August 1971	Ethanol, 1 hr., 30° C.	0.019	0.11	23
August 1971	Ethanol, 1 hr., 30° C., extd. 3X	0.014	0.08	11
August 1971	9:1 hexane-ethanol, 1 hr., 30° C., extd. 3X	0.018	0.12	3
November 1971	9:1 hexane-ethanol, 1 hr., 30° C.	0.017	0.22	...
November 1971	Oil squeezed from germ	0.016	0.20	...

[a]Corn steeped and germ separated in plant. Germ dried by lyophilization. Oil content about 50% d.b.

[b]A single extraction, unless otherwise indicated.

The method used for the extraction of oil and tocopherols from corn germ was essentially that of Slover et al. (6). Whole germ was ground finely in a Labconco mill, then extracted with anhydrous ethanol at 30°C. in the dark. (Slover et al. extracted at room temperature.) Filtration after extraction was under a nitrogen blanket and the residue was washed with additional solvent. The bulk of the solvent was evaporated from the extract under a strong stream of nitrogen while being gently heated. The last traces of solvent were removed by heating to 50° to 55°C. while under vacuum (vacuum pump). The vacuum was broken with nitrogen and the oil stored under nitrogen in the deep freeze until analyzed.

The method of Quaife and Harris (7) when applied to corn germ gave lower

TABLE II. EFFECT OF WET-MILLING ON THE TOCOPHEROL CONTENT OF CORN GERM OIL

Sample	Oil Source	Tocopherol % Based on Oil	
		α	γ
November 1971	Germ from hand-dissected corn	0.016	0.21
	Germ from laboratory-steeped corn	0.014	0.21
	Plant germ, freeze-dried	0.017	0.22
	Plant germ, plant-dried	0.015	0.21
March 1972	Germ from hand-dissected corn	0.021	0.13
	Germ from laboratory-steeped corn	0.019	0.13
	Plant germ, plant-dried	0.018	0.11
June 1972	Germ from hand-dissected corn	0.028	0.18
	Plant germ, freeze-dried	0.026	0.18
	Plant germ, plant-dried	0.033	0.18

results than those obtained by the method of Slover et al. (Table I). Following the former method, ground germ was extracted at reflux with ethanol for 2 hr. The γ-tocopherol concentration of this extract was appreciably lower than that obtained by extraction at 30°C.

A 9:1 (v./v.) mixture of hexane and ethanol extracted more oil from corn germ at 30°C. than ethanol (Table I). This fact is of significance when the amount of germ available is sharply limited, as in the case of hand-dissected germ, because 1 g. of oil is required for analysis. The relatively low values obtained on triplicate extraction with ethanol (Table I) probably illustrate the difficulty in excluding oxygen from the system during multiple extractions, even though great precautions are taken. Except for these examples, single extractions were always used.

In judging the merit of the various extraction methods, the assumption is made that the higher the tocopherol content of the oil, the milder or less destructive the extraction conditions have been. Possibly the mildest method for the recovery of oil from germ is to squeeze unground germ at room temperature. Dry germ was placed in a stainless steel basket with a perforated bottom and squeezed with a stainless steel plunger in a Carver Press. At about 11,000 p.s.i. half of the oil is expressed without appreciable change in germ temperature.

Oil squeezed from the germ had essentially the same tocopherol concentration as oil extracted with solvent at 30°C.(Table I). Thus, apparently little or no destruction of tocopherols occurs during those parts of the usual procedure that might be suspect, grinding and evaporation of the solvent.

A problem that arises in handling small samples of germ obtained by hand dissection is that much of the germ would be lost during grinding in the Labconco mill. To circumvent this problem, the germ was simultaneously ground and extracted in a Spex mixer mill. This is a small ball mill that is shaken in an eccentric pattern. On mixing even for a short time, the mill becomes warm. To avoid elevated temperatures, the mill containing the balls, germ, and solvent was cooled to 0°C. before being shaken for 10 min. Wherever possible the Labconco mill was used because extraction of the larger quantity of germ provided greater assurance that a fair sample was being assayed.

Conditions finally adopted for oil extraction from germ follow. Twenty grams

of dry germ was finely ground in a Labconco mill. The ground germ was extracted with 200 ml. 9:1 hexane-ethanol (v./v.) containing 0.13% pyrogallol. The flask containing the ground germ was thoroughly purged with nitrogen before adding the solvent. The flask was shaken gently in the dark in a 30°C. room for 1 hr. The slurry was filtered under nitrogen and the solvent evaporated with gentle heating under a strong stream of nitrogen. The flask was then held in a bath at 50° to 55°C. and evacuated (vacuum pump) to remove the last traces of solvent. All manipulations were done in a darkened laboratory.

In the case of hand-dissected germ, grinding and extraction were done simultaneously. About 5 g. of hand-dissected germ was mixed with 25 ml. 9:1 hexane-ethanol containing 0.13% pyrogallol in a Spex mixer mill, Model 8000. The mill and contents were cooled to 0°C., then shaken 10 min. The unground slurry was kept in a nitrogen atmosphere during filling and sealing the Spex mill container. The ground slurry was then filtered and treated as above.

RESULTS AND DISCUSSION

On three widely spaced dates samples of corn and the corresponding germ were taken from the plant. Analyses of the tocopherols in the oil from germ hand-dissected from the corn and from germ separated in the plant from this corn are shown in Table II. The concentration of α- and γ-tocopherols in the oil from hand-separated corn germ is not appreciably different from that in germ separated from corn by the wet-milling process and dried in the laboratory.

Drying corn germ in the plant did not reduce the tocopherol content of the germ oil. In all cases examined, the oil from hand-dissected germ and from plant-dried germ were of about the same tocopherol content.

These data show that during commercial steeping of corn and drying of the germ little or no destruction of the tocopherols occurred. This conclusion contrasts sharply with that of Grams et al. (1). Some of this difference may be caused by our samples being taken from an operating corn wet-milling plant rather than from a laboratory simulation of the plant. Another difference is that we used an oil extraction procedure that gave higher tocopherol recoveries.

Laboratory steeping of corn appears to have little effect on the tocopherol content of the germ oil. On two occasions whole corn was steeped in the laboratory. In both bases the γ-tocopherol content of the germ oil was the same as that of the oil from the hand-dissected germ (Table II). The α-tocopherol level in both steeped samples was 0.002% (absolute) lower than the values found in germ oil from unsteeped corn.

The considerable variations in the total tocopherol contents of the various corn samples is surprising (Tables I and II). Samples taken in July 1971 and June 1972 contained appreciably more tocopherols than samples taken in August or November 1971 or in March 1972. Apparently considerable variation occurs, probably depending on the type of hybrid and the conditions under which the corn was stored before processing. Quackenbush et al. (8) have found the oil in freshly harvested corn inbreds to range from 0.03 to 0.33% total tocopherols.

A major problem in this work is sampling. We have relied on estimated average times of processing to determine when samples are to be taken at each station in the plant. The general consistency of these results has been a pleasant surprise.

At elevated extraction temperatures, γ-tocopherol appears to be destroyed in preference to α-tocopherol. The July 1971 sample was extracted at reflux in a Soxhlet extractor for 17 hr. The α-tocopherol content, originally 0.031%, was reduced to 0.021%, but the γ-tocopherol content fell to 0.06 from 0.39%. This same trend is noted in the data shown in Table I.

Acknowledgments

The GLC procedure used was based on information received from A. E. Waltking, Best Foods Research Laboratories, CPC International Inc., Union, N. J. Some of the GLC analyses were performed by R. T. Armatys and S. J. Filipello.

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[Received December 6, 1972. Accepted April 17, 1973]