

Effect of Various Acids and Sulfites in Steep Solution on Yields and Composition of Corn Fiber and Corn Fiber Oil

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ABSTRACT

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The addition of six acids (organic and inorganic) and four sulfite compounds (including gaseous SO₂) during the conventional corn wet-milling steeping process of two yellow dent corn hybrids were evaluated for the effect on corn fiber yield, corn fiber oil yield, and the composition of three phytosterol compounds (ferulate phytosterol esters [FPE], free phytosterols [St], and phytosterol fatty acyl esters [St:E]) in the corn fiber oil. No significant effect of different sulfite compounds and acids were observed on corn fiber yields. However, a significant effect was observed on corn fiber oil yield and the composition of corn fiber oil for phytosterol compounds. Three of the sulfite compounds (including gaseous SO₂) caused very little effect on the levels of phytosterol compounds compared with the control sample (corn steeped with sodium metabisulfite and lactic acid). However, for one hybrid, ammonium sulfite gave a significantly higher yield of FPE and St:E and had no effect on the yield of St. For the

other hybrid, it gave a significantly higher yield of FPE and had no effect on the yield of St and St:E compared with the control sample. This indicates that the effect of these sulfite compounds on yields of these phytosterol compounds in corn fiber oil is probably hybrid-dependent. No significant effect of acids was observed on corn fiber yields, but significant effects were observed on corn fiber oil yields and yields of phytosterol compounds in the corn fiber oil. The effect also seems to be hybrid-dependent because different acids affected the two hybrids differently. Overall, it seems that weak acids have a positive effect on increasing the individual phytosterol compounds in the corn fiber. When comparing the effect of experimental acids and sulfites on the two hybrids, acids have a more positive effect than sulfites in increasing the yield of phytosterol compounds in corn fiber oil.

Recently, process modifications to the conventional dry grind ethanol process were developed that facilitate recovery of germ and fiber as valuable coproducts (Singh and Eckhoff 1996, Singh et al 1999). The process modifications consist of a short soaking step (up to 12 hr) of raw corn in plain water, followed by a coarse grind, then recovery of germ by flotation, and then fine grind and fiber recovery also using flotation (Singh et al 1999). No chemicals (SO₂ or lactic acid) are needed during the soaking process because, unlike the conventional corn wet-milling steeping process, the objective is to recover germ and fiber and not starch and protein. Germ can be used for recovery of corn germ oil and fiber can be used for recovery of corn fiber oil and corn fiber gum. Recently, research on corn fiber has shown that corn fiber oil is very different from regular corn oil (oil from germ) because it contains some unique compounds that lower serum cholesterol levels in blood and, therefore, has nutraceutical value (Moreau et al 1996).

Preliminary results in our laboratory showed that if we added conventional steep chemicals (SO₂ in the form of sodium metabisulfite and lactic acid), the fiber yields and the amount of nutraceutical compounds recovered from fiber in the "Quick Fiber" process increase considerably (Singh et al 1999). Another study (Wang et al 1998) also showed that SO₂ affects the recovery of tocopherols and tocotrienols (vitamin E isomers) in corn. It is not known how these chemicals increase the yield of other nutraceutical compounds. A plausible explanation is that these chemicals react with the matrix surrounding these compounds and increase or decrease the extraction efficiency. Similar increases in the amounts recovered of certain lipids due to processing (thermal) changes have been noticed in rice bran (Lane et al 1997) and corn fiber

(Moreau et al 1999). In the conventional corn wet-milling steeping process sulfur dioxide is added because SO₂ species (bisulfite and sulfite) cleave the disulfide bonds and loosen the protein matrix surrounding the starch particles, so that when subsequent milling is done, starch particles can be easily recovered (Watson 1984). Lactic acid also has a beneficial effect on starch recovery (Watson et al 1951, Eckhoff and Tso 1991, Singh et al 1997). However, in a modified dry grind ethanol process, we are not only interested in starch recovery. Our objective is to get maximum germ and fiber yield and maximum yields of nutraceutical compounds from fiber. Therefore, during the soaking process in a modified dry grind ethanol plant, we are not restricted to adding conventional steep chemicals but also can add other sulfite compounds or other organic or inorganic acids that might increase the recovery of these nutraceutical compounds from corn fiber. Therefore, there is a need to determine the effect of different sulfites and organic and inorganic acids during steeping on corn fiber and corn fiber oil yields and the composition of corn fiber oil to optimize recovery.

In this study, the effects of addition of different sulfite compounds and organic and inorganic acids during a laboratory corn wet-milling steeping process were evaluated for corn fiber yield, corn fiber oil yield, and composition of phytosterol compounds in the corn fiber oil.

MATERIALS AND METHODS

Two yellow dent corn hybrids (33R87 and 3394) grown during the 1998 crop season at the Agricultural Engineering Farm, University of Illinois at Urbana-Champaign, were field-dried to 14–16% moisture content and combine-harvested. Both hybrids were hand-cleaned to remove broken corn and foreign material, packaged in plastic bags, and stored at 4°C until wet-milling. The whole kernel moisture content of the samples was measured using the 103°C convection oven method (AACC 2000). Wet-milling was done using the 100-g laboratory corn wet-milling procedure as outlined by Eckhoff et al (1996), and both fine and coarse fiber fractions were recovered separately. The collected fiber samples were dried for moisture content determination using the two-stage convection-oven procedure (AACC 2000).

For both hybrids, corn fiber oil and its composition were studied for two different steeping conditions: 1) steeping was done with 0.2% SO₂ (using sodium metabisulfite) and one of the six acids (acetic, citric, hydrochloric, oxalic, *o*-phosphoric, and sulfuric) and

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2) steeping also was done with 0.55% lactic acid and one of the sulfite compounds (K_2SO_3 , Na_2SO_3 and $(NH_4)_2SO_3$) and a gaseous SO_2 solution. The acids that were added to the steep solution had the same normality (0.0634 meq/L) as 0.55% lactic acid, and the three sulfite compounds and gaseous SO_2 added to the steep solution had the same concentration as 0.2% SO_2 (based on the titrations for active sulfur equivalents).

Use of gaseous SO_2 or sodium metabisulfite are the methods of choice (by the corn wet-milling industry) to provide sulfurous acid in the steep solution during the conventional corn wet-milling steeping process. In our 100-g laboratory, we used sodium metabisulfite and lactic acid as conventional steep chemicals for our control samples.

Dried fiber samples were ground to 20 mesh in a Wiley mill. Ground fiber samples (4 g) were placed in screw-top vials (55 mL) and 40 mL of hexane was added. The tubes were shaken horizontally for 1 hr in a wrist-action shaker. After extraction, the hexane

extracts were filtered through a Whatman GF/A glass fiber filter (Whatman Laboratory Products, Clifton, NJ) fitted in a Buchner funnel with gentle vacuum. A part of the sample was removed for HPLC analysis as previously outlined by Moreau et al (1996). The rest of the solvent sample was dried under nitrogen and heat (using an N-EVAP analytical Evaporator, Organomation Associates Inc., Berlin, MA). The dried sample was transferred to 2-dram vials in an 85:15 chloroform-methanol mixture and dried again under nitrogen to measure oil dry weight. The fiber yields, fiber oil recoveries, and yield of the three phytosterol compounds (FPE, St and St:E) were compared with the fiber yield, fiber oil recovery and yield of the three phytosterol compounds from the control sample (sample steeped with sodium metabisulfite and lactic acid). Total individual phytosterol compounds in the corn hybrids were determined by grinding dried, intact corn kernels and extracting the ground samples using the above mentioned procedure (Table I).

Analysis of variance (ANOVA) and Duncan's multiple range test were used (SAS Institute, Cary, NC) for corn fiber oil and phytosterol compound yields. The level selected to show statistical significance was 5% ($P < 0.05$).

TABLE I
% Oil Yield and Phytosterol Compounds^a
from Two Corn Hybrids^b

Hybrid	Oil (%)	FPE	St	St:E	Total
3394	2.96	9.41 (100) ^c	26.79 (100)	30.03 (100)	66.23
33R87	3.06	11.23 (100)	27.26 (100)	31.83 (100)	70.32

^a Ferulate phytosterol esters (FPE), free phytosterols (St) and phytosterol fatty acyl esters (St:E) measured in mg/100 g of kernels.

^b Content in whole corn kernels.

^c % recovery of total phytosterol compound originally present in corn kernels are in parentheses.

RESULTS AND DISCUSSION

Effect of Different Sulfite Compounds on Fiber Yield, Fiber Oil Yield and Yields of Phytosterol Compounds in Fiber Oil

For both hybrids, 3394 and 33R87, the fiber yields, fiber oil yields, and the levels of three phytosterol compounds (FPE, St, and St:E) obtained at conventional steeping conditions (with addition of sodium metabisulfite and lactic acid) were in agreement with

TABLE II
Yield of Fiber, Fiber Oil, and Phytosterol Compounds^a from Fiber for Two Corn Hybrids Treated with Various Sulfites in Steep Solution^b

Hybrid	Treatment	Fiber Yield	Oil Yield	FPE	St	St:E	Total
3394	$Na_2S_2O_5$ (control)	13.72a ^c	1.51ab	7.00 (74.4)b ^d	2.85 (10.6)a	8.45 (28.1)b	18.29
	K_2SO_3	13.08a	1.14b	6.69 (71.1)b	2.22 (8.3)a	5.90 (19.6)d	14.81
	$(NH_4)_2SO_3$	13.80a	1.73ab	9.03 (96.0)a	2.53 (9.4)a	9.50 (31.6)a	21.06
	Na_2SO_3	13.32a	1.39ab	7.05 (74.9)b	2.20 (8.2)a	6.93 (23.1)c	16.17
	Gas SO_2	13.19a	1.77a	6.12 (65.0)b	2.46 (9.2)a	7.29 (24.3)c	15.87
33R87	$Na_2S_2O_5$ (control)	12.62a	1.62a	7.30 (65.0)b	2.25 (8.3)a	7.54 (23.7)a	17.09
	K_2SO_3	12.92a	1.78a	7.51 (66.9)b	2.34 (8.6)a	7.36 (23.1)a	17.21
	$(NH_4)_2SO_3$	13.39a	1.56a	8.35 (74.4)a	2.47 (9.1)a	7.38 (23.2)a	18.21
	Na_2SO_3	12.87a	1.90a	7.77 (69.2)ab	2.41 (8.8)a	6.04 (19.0)a	16.22
	Gas SO_2	12.39a	2.14a	7.77 (69.2)ab	2.56 (9.4)a	8.02 (25.2)a	18.35

^a Ferulate phytosterol esters (FPE), free phytosterols (St) and phytosterol fatty acyl esters (St:E) measured in mg/100 g of kernels.

^b Different sulfite compounds or gaseous SO_2 (with same active sulfur equivalents) and 0.55% lactic acid.

^c Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

^d % Recovery of total phytosterol compound originally present in corn kernels are in parentheses.

TABLE III
Yield of Fiber, Fiber Oil, and Phytosterol Compounds^a from Fiber for Two Corn Hybrids Treated with Various Acids^b in Steep Solution

Hybrid	Treatment ^c	Fiber Yield	Oil Yield	FPE	St	St:E	Total
3394	Control ^c	13.72a ^d	1.51c	7.00 (74.4)a ^c	2.85 (10.6)a	8.45 (28.1)c	18.29
	Acetic acid	13.66a	1.96ab	6.87 (73.0)a	2.80 (10.5)a	9.83 (32.7)ab	19.50
	Citric acid	13.52a	1.88a-c	7.11 (75.6)a	2.89 (10.8)a	9.00 (30.0)bc	19.00
	Hydrochloric acid	13.56a	2.05a	6.85 (72.8)a	3.29 (12.3)a	10.59 (35.3)a	20.72
	Oxalic acid	13.48a	1.56bc	7.18 (76.3)a	2.78 (10.4)a	9.02 (30.0)bc	18.98
	<i>o</i> -Phosphoric acid	14.17a	1.55bc	7.79 (82.8)a	2.60 (9.7)a	8.44 (28.1)c	18.82
	Sulfuric acid	13.21a	1.76a-c	6.69 (71.1)a	2.65 (9.9)a	9.13 (30.4)bc	18.47
	Control	12.62a	1.62d	7.30 (65.0)a	2.25 (8.3)c	7.54 (23.7)d	17.09
	Acetic acid	12.86a	2.50a	7.48 (66.6)a	3.44 (12.6)a	10.19 (32.0)a	21.11
33R87	Citric acid	13.16a	2.62a	7.59 (67.6)a	3.16 (11.6)ab	10.21 (32.1)a	20.96
	Hydrochloric acid	13.01a	2.19b	6.93 (61.7)a	2.88 (10.6)b	9.25 (29.1)ab	19.07
	Oxalic acid	13.33a	1.85cd	7.46 (66.4)a	2.92 (10.7)b	8.27 (26.0)b-d	18.66
	<i>o</i> -Phosphoric acid	13.32a	2.03cb	6.60 (58.8)ab	2.47 (9.1)c	9.06 (28.5)a-c	18.13
	Sulfuric acid	12.93a	2.06cb	5.67 (50.5)b	2.45 (9.0)c	7.77 (24.4)cd	15.89

^a Ferulate phytosterol esters (FPE), free phytosterols (St) and phytosterol fatty acyl esters (St:E) measured in mg/100 g of kernels.

^b Normality 0.0634 meq/L.

^c Steeped with 0.55% lactic acid and 0.2% SO_2 generated from sodium metabisulfite.

^d Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

^e % Recovery of total phytosterol compound originally present in corn kernels are in parentheses.

a previous study (Singh et al 1999). The yields of corn fiber from different sulfite compounds were not significantly different from yields obtained by the conventional steeping process (Table II). With the four experimental sulfite compounds (including gaseous SO_2 , fiber yields for hybrid 3394 were 13.2–13.8%, compared with 13.7% obtained by conventional steeping (with sodium metabisulfite). For hybrid 33R87, fiber yields were 12.4–13.4%, with the four experimental sulfite compounds, compared with 12.6% with conventional steeping. No significant effect of different sulfite compounds on oil yields was observed compared with the conventionally steeped samples. However, significant effects between the different sulfite compounds on oil recoveries was observed for one of the hybrids. The oil yield, for hybrid 3394, with K_2SO_3 treatment was significantly lower (by 0.6%) when compared with gaseous SO_2 treatment. For hybrid 33R87, no significant difference in the oil yield was observed between the different sulfite treatments.

Steeping with the various sulfite compounds resulted in significantly different yields (Table II) of the FPE and St:E in the corn fiber oil. No significant effect of sulfite compounds was observed on the yield of St. Percent recovery of FPE (of the total FPE measured in the corn kernels) was higher by 21.6% for hybrid 3394 and was higher by 9.4% for hybrid 33R87, compared with the conventionally steeped sample for $(\text{NH}_4)_2\text{SO}_3$. No significant difference in percent recovery was observed with Na_2SO_3 , K_2SO_3 , and gaseous SO_2 compared with conventionally steeped samples for either hybrid.

Percent recoveries of St:E (of the total St:E measured in the corn kernels), for hybrid 3394, significantly decreased for K_2SO_3 , Na_2SO_3 , and gaseous SO_2 by 8.5, 5.0, and 3.8%, respectively, and significantly increased for $(\text{NH}_4)_2\text{SO}_3$ by 3.5% compared with the control sample. For hybrid 33R87, no significant differences were observed between the different sulfite treatments and the control sample. Overall, $(\text{NH}_4)_2\text{SO}_3$ gave the highest recoveries for two (FPE and St:E) of the three phytosterol compounds compared with the other sulfite compounds tested.

Total phytosterol recovery (Table II) in mg per 100 g of kernels for all of the sulfite compounds tested decreased (2.12 to 3.48 mg) for all treatments of hybrid 3394, except for $(\text{NH}_4)_2\text{SO}_3$, for which it increased by 2.77 mg compared with the control sample. For hybrid 33R87, the total phytosterol recovery in mg per 100 g of kernels, increased for all sulfite compounds tested anywhere from 0.12 to 1.26 mg, except for Na_2SO_3 , for which it decreased by 0.87 mg, compared with the control sample (Table II).

These results suggest that overall there is not a significant effect of these sulfite compounds on corn fiber yield, oil yield, and yield of phytosterol compounds in fiber oil compared with the control sample. Most of the sulfite compounds tested in the steep solution do not give a high yield of phytosterol compounds compared with the control sample, except ammonium sulfite, which for one hybrid gave a high yield of FPE and St:E, and for the other hybrid a high yield of FPE compared with the control sample. This indicates that the effect of these sulfite compounds on yields of these phytosterol compounds in corn fiber oil is probably hybrid-dependent.

Effect of Different Acids on Fiber Yield, Fiber Oil Yield and Yields of Phytosterol Compounds in Fiber Oil

The effect of different acids on corn fiber yields was not significant (Table III). Depending on the acid, fiber yields for hybrid 3394 were 13.2–14.2%, compared with 13.7% with conventional steeping (with lactic acid). For hybrid 33R87, the fiber yields were 12.9–13.3%, depending on the acid added, compared with 12.6% with conventional steeping. A significant effect of certain acids, however, was noticed on the oil recoveries from the fiber. For hybrid 3394, acetic and hydrochloric acid had 0.45 and 0.54% higher oil recoveries, respectively, compared with the control sample. For hybrid 33R87, all acids except oxalic acid had higher oil recoveries (0.41–1.0%) compared with the control sample. A significant effect of various acids was noticed on the composition of phytosterol

compounds in the oil, especially for St and St:E (Table III). In the FPE recovery for hybrid 3394, no significant effect of different acids was observed compared with the control sample. For hybrid 33R87, no significant effect of acetic, citric, hydrochloric, oxalic, and orthophosphoric acids was observed on FPE recovery, but sulfuric acid gave significantly lower (by 14.5%) FPE recovery (of the total FPE measured in the corn kernels) compared with conventional steeping.

Percent St recoveries (of the total St measured in the corn kernels) for hybrid 3394 were not significantly different between the different acid treatments compared with the control sample. For hybrid 33R87, acetic, citric, hydrochloric, and oxalic acid gave 2.3–4.3% higher St recoveries compared with the control sample. Orthophosphoric and sulfuric acid resulted in no significant differences compared with the control sample.

Percent recoveries of St:E (of the total St:E measured in the corn kernels) for both of the hybrids were either comparable or significantly higher (up to 8.4%) for all acids compared with the control sample. The highest recovery of St:E for hybrid 3394 was with hydrochloric acid and for hybrid 33R87 was with acetic and citric acid.

For hybrid 3394, the total phytosterol recovery in mg per 100 g of kernels increased by 0.18 to 2.43 mg for all acids compared with the control sample (Table III). For 33R87, the total phytosterol recovery in mg per 100 g of kernels increased for all acids by 1.04–4.02 mg, except for sulfuric acid, for which it dropped 1.2 mg compared with the control sample.

These results suggest that there is not a significant effect of different acids on corn fiber yields, but there is a significant effect on corn fiber oil yields and levels of phytosterol compounds in the corn fiber oil. The effect seemed to be hybrid-dependent because different acids affected the two hybrids differently. It seems that, overall, weak acids have a positive effect on increasing the individual phytosterol compounds in the corn fiber. When comparing the overall effect of experimental acids and sulfites (on total phytosterol compounds in mg/100 g of kernels) on the two hybrids, acids have a more positive effect than sulfites in increasing the yield of phytosterol compounds in corn fiber oil.

CONCLUSIONS

Significant effects (increases as well as decreases, depending on the sulfite or acid used) were observed on the yield and phytosterol composition of corn fiber oil when either sulfite compounds and acids were added to the steep solution. The effect was hybrid-dependent, depending on the sulfite or acid used. Most of the sulfite compounds added to the steep solution had no effect or a slightly detrimental effect on the phytosterol composition of the oil, except ammonium sulfite, which significantly increased the yield of FPE and St:E for one hybrid and FPE alone for the other hybrid. With acids, the maximum increase in the yield of any of the phytosterol compounds in corn fiber oil was 8.4% (increase in St:E with citric acid). Only sulfuric acid had a detrimental effect on FPE recovery of total phytosterol compounds for one of the hybrids. Ammonium sulfite, among different sulfite compounds, and acetic acid, citric acid, and hydrochloric acid among different acids, gave the largest overall positive effect of increasing the phytosterol composition of corn fiber oil. Economic assessment needs to be done to determine whether the increase in yield of these phytosterol compounds justifies the addition of these chemicals into the steep solution.

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