Consecutive Aqueous Extractions of Wet-milled Corn Germ Cake

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Corn germ cake is an abundant and inexpensive residue of corn germ pressing. The permanent increase of corn processing – due to the recent growing demand for bioethanol – has resulted in a surplus of this by-product, making it unmarketable as feed. Our goal was to find an alternative way to utilize this by-product. We could successfully extract 86 % of the polysaccharide content of the squeezed germ by using only hot distilled water and 1 % dilute sulphuric acid consecutively. The 14.7 % oil content of the squeezed germ was concentrated to 46.25 % in the remaining solid fraction, which is high enough to be pressed. (Oil content of less than 20 % can only be extracted with organic solvents, which is not attractive for food safety and environmental reasons.) The sterol concentration of this oil was 8200 mg kg^{-1}, which is significantly more than the sterol concentration of commercial germ oils (4500 mg kg^{-1}).

Key words:
Pressed corn germ, dilute acid treatment, phytosterols, bioethanol, oil extraction

Introduction

Corn-based bioethanol production is increasing worldwide and the limit of this increase seems to be very far above present production. Environmental and economical reasons motivate scientists and engineers to improve the productivity and economics of bioethanol production. From the environmental point of view, we can state that the use of bioethanol instead of fossil based gasoline reduces carbon-dioxide emissions.[1] By reducing carbon-dioxide emissions we can reduce global warming, which is desirable for several ecological reasons. From the economic point of view, the main advantage of producing bioethanol is the reduction of dependency on crude oil imports. By having two sources (biomass and crude oil) of liquid automotive fuels the supply and the price become more stable and balanced. Another advantage of producing bioethanol is the promotion of agriculture and other local businesses. The profit from bioethanol production is shared among more companies – and thus more people – than that of fossil fuel production.

In Europe, the most promising raw material for ethanol production is corn (*Zea mais*). The corn kernel contains about 74 % starch, 12 % proteins, 5 % lipids, 6 % fiber (cellulose and hemicelluloses) and 1.4 % ash (by mass, based on dry substrate). There are two corn milling processes, dry milling and wet milling. Wet milling is more complicated. Using it, starch, gluten, oil, and fibre fractions are separated; the two most important and valuable are starch and oil. This process begins with mechanical purging to eliminate dust and other particulate impurities. Then the corn is steeped in dilute sulphurous acid (at 50 °C for 48 hours) to hydrate and swell it. The corn is then milled in two steps, and fractionated by screening, sedimentation, and flotation to the following fractions: germ, fibre, starch and gluten. The germ is ground and heated and oil is pressed from it.

Finally, there are two main products: starch and oil, and four feed components: corn steep liquor, fiber, gluten, and the germ cake (Table 1).

Table 1 – *Products of the corn wet milling process*

Fraction	Total mass [g/100 g corn]	Starch content [%]	Protein content [%]	Oil content [%]	Ash content [%]
original corn	100	74	12	5.1	1.4
starch	68.6	99.0	0.3	0.65	0.08
oil	3.78	0	0	100	0
corn steep liquor	7.6	0	46.1	0	18.0
fiber	9.5	20.0	14.0	4.0	1.0
gluten	5.8	25.8	70.0	3.7	0
germ cake	3.52	16.0	23.0	15.0	3.0

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The starch can be hydrolysed and further processed into many valuable products, like sweetening agents or bioethanol. The germ oil is refined and deodorized, and marketed as cooking oil. The corn steep liquor is concentrated by evaporation. The concentrate is either used as a nitrogen source for fermentations, or mixed with fibre and gluten, dried and then sold as "corn gluten feed" (CGF). The germ cake is also sold as feed. The residual oil content of germ cake (ca. 15 %) could be extracted by solvent extraction. Hexane is the preferred solvent, which extracts the most oil, but it causes food safety and environmental problems. The solvent must be recovered from the oil, and the concentration of the residual hexane in the product must be lower than 1 mg kg^{-1}.[2] Furthermore, hexane is a volatile pollutant (VOC), and its total loss is limited to 1 kg hexane per a ton of raw material. Biodegradable and neutral solvents – like ethanol, ethyl-acetate, acetone, or supercritical carbon-dioxide – are not as strictly regulated, but these solvents extract significantly less oil than hexane. It is predictable that the environmental and food safety regulations are going to be ever stricter. For these reasons, Hungarian processors do not solvent-extract germ cake. Thus, it is reasonable to search for alternative ways to extract oil from germ cake.

An increasing problem is that the bioethanol by-products (germ, fibre, gluten, steep liquor) production can easily exceed demand. It seems that Hungary has already reached this condition, thus if bioethanol production is going to increase, new markets for the by-products are needed. The United States has already passed this point, since some of their DDGs and CGF have been exported to Europe for many years. Europe and many other countries plan to increase bioethanol production, so export of the by-products is not a solution. It is reasonable to search for alternative ways of utilizing these feed components.

The economics of wet milling depend on the starch (glucose) and oil yields. The germ cake contains about 16 % starch and 15 % oil, and these components are poorly used. Considering the relatively low ratio of germ cake (3.52 g/100 g corn) it could be argued that it is not a significant loss. In fact, the quantities of corn being converted to ethanol and by-products are so huge that fractionally small increases in the starch and oil separation are worthwhile. Hungarian wet mills capacity is about 550 thousand tons of corn per year, which produces 19 thousand tons of germ cake per year. Hungary is not unique, since France, Italy, Spain and Germany mill amounts of corn germ of the same order of magnitude, producing similar quantities of germ cake.

We can conclude that germ cake is an abundant and inexpensive by-product of corn wet milling, and it would be beneficial to use it in other ways. The existing uses for germ cake are combustion – and cogeneration of heat and electricity – and composting. The disadvantage of the first is the high capital cost, and of the second the low price of compost.

This paper reports on a study, which searched for a new use for germ cake. Our goal was to separate glucose solution and oil fractions from the germ cake, thereby increasing the overall yield of these products in a wet milling plant and reducing the problem of by-product disposal. We measured the phytosterol contents of the produced oils. Phytosterol is the collective noun for those sterols and sterol esters primarily found in plants. These compounds are beneficial to human health, since their consumption reduces LDL (low density lipoprotein) cholesterol levels.[3] High LDL-cholesterol levels are associated with heart and circulatory diseases.

Materials and methods

Raw material

The corn germ was kindly donated by Corn Drop Ltd. The corn was part of the 2005 Hungarian corn crop, processed by wet milling. The germ was heated to a maximum 120 °C before pressing. According to the processor, the dry content of the substrate was min. 95 % w/w, thus it did not need drying before the experiments, but it was analysed and processed within 60 days of receipt to avoid biological decay, since it did not contain any antioxidants.

Analysis

Glucan, xylan, and arabinan contents were measured by the following procedure, based on Hägglund's method.[4] The fiber was first milled with a coffee mill. Dry material content of the milled fiber was measured by drying at 105 °C until the sample mass was constant. 5 mL sulphuric acid (72 % w/w) was added to 1.0 g of milled fiber. The suspension was incubated and stirred from time to time at room temperature for 1.5 hours. Then it was diluted with 145 mL of distilled water and heated to, and kept at 120 °C for 1.5 hours. The suspension was then chilled and the supernatant sampled. The sample was analysed for monosaccharides by HPLC. Glucan, xylan and arabinan contents of the raw material were calculated according to equation 1:

$$C = \frac{c \cdot V}{f \cdot m} \cdot 100 \text{ [\% w/w]} \qquad (1)$$

where:

C – polysaccharide content, % w/w

c – monosaccharide concentration measured by HPLC, g L^{-1}

V – volume of the suspension, L

f – hydration factor (glucose: 180/162; cellobiose: 342/324; xylose: 150/132; arabinose: 150/132)

m – dry mass of the analyzed raw material, g.

The HPLC analysis was performed using an Aminex HPX-87H column at 65 °C. The eluent, 5 mmol L^{-1} of H_2SO_4 was added at a flow rate of 0.5 mL min^{-1}. Glucose, xylose, arabinose and cellobiose were detected and quantified by refractive index measurement.

The protein content was measured according to Dumas' method.[5]

Milled fiber, 5–15 g, was placed into a paper or glass vial and extracted with hexane in a soxhlet--extractor for five hours. Afterwards the solvent was evaporated with a laboratory evaporator (Rotadest), and the oil fraction was determined as the oil mass divided by the dry mass of the sample.

First, the non-saponifiable constituent (NSC, which contains the phytosterols as well) was separated. The extracted oil, 5 g, was placed in a flask with 5 g of KOH and 50 mL of ethanol. The mixture was boiled for an hour under nitrogen flow. After boiling, 50 mL distilled water (DW) was added. After the mixture was chilled, the NSC was extracted three times with 75 + 50 + 50 mL hexane. Base (KOH) was then extracted from the solvent mixture with DW. The hexane was screened through Na_2SO_4 and evaporated by a laboratory film evaporator (Rotadest). The residue (NSC) was dried in an exsiccator and weighed. Then the NSC was dissolved in 10 mL chloroform, then 250 μL internal standard (cholesterol), 500 μL pyridine and one ampule of sililizing agent was added, and 1 μL of the sample was injected into the GC.

Set type:	HP 6890 GC System
Column:	HP 1 (19091Z-002) (25 m · 0.2 mm · 0.11 μm)
Temp. program:	240 °C: 1 min; 240 – 260 °C: 5 °C/min; 260°C: 30 min
Flow rate:	0.6 mL min^{-1}
Pressure:	95.62 kPa; constant
Injector:	300 °C, split
Split ratio:	50:1
Detector:	300 °C, Flame Ionization Detector

Sterol content was calculated by equation 2.

$$\text{Sterol content} = \frac{m_{\text{int}} \cdot A_{\text{sterol}}}{O \cdot A_{\text{int}}} \text{ [mg kg}^{-1}\text{ oil]} \quad (2)$$

where:

m_{int} – mass of internal standard, mg

A_{sterol} – total area of chromatographic peaks of sterols

O – mass of the oil sample, kg

A_{int} – area of chromatographic peak of internal standard.

The ash content was measured by burning 1–5 g dry sample at 600 °C for 6 hours. Ash content was calculated as the ash mass divided by the dry mass of the sample.

Fractionation

Step 1 (extraction with distilled water)

The air-dried raw material was suspended in enough DW so that the dry material concentration of the suspension was 8 % w/w. The suspension was incubated at 120 °C for 2 hours. After cooling, the suspension was filtered through a 150 μm mesh nylon filter, separating the solid fraction and the supernatant. The solid fraction was washed with 80 °C DW three times to remove soluble substances, then dried at room temperature (less than 40 °C), and sampled for analysis. The supernatants were combined, homogenized and the volume measured. A 20 mL sample of the combined, homogenized supernatant was diluted 1:1 with 8 % (w/w) dilute sulphuric acid and incubated at 120 °C for 10 minutes to hydrolyse the suspended starch and dextrin. The hydrolysate was chilled and sampled for HPLC analysis.

Step 2 (dilute acid hydrolysis)

After step 1, the air-dried solid fraction was suspended in 1 % dilute sulphuric acid so that the dry material concentration of the suspension was set to 8 % w/w. The suspension was incubated at 120 °C for 2 hours. The suspension was cooled and filtered through a 150 μm mesh nylon filter, thus the solid fraction and the supernatant were separated. The solid fraction was washed with 80 °C DW three times to remove soluble substances, and dried at room temperature (less than 40 °C), and sampled for analysis. The supernatants were combined, homogenised, the volume measured and sampled for HPLC analysis.

Results and discussion

Dry content of the raw material was 96.7 % w/w. The average composition of the germ residue – based on dry mass – can be seen in Table 2.

Table 2 – *Composition of germ cake*

Component	[%]
glucan	16.1
hemicelluloses	19.0
xylan	7.8
arabinan	11.2
protein	28.1
lipids	14.7
sterols [mg kg^{-1} germ]	*911*
sterol esters [mg kg^{-1} germ]	*985*
ash	2.8
sum	80.7

Glucan in the germ cake can originate from three polysaccharides: starch, cellulose and hemicelluloses. Our previous experience is that the methods for the determination of these polysaccharides are biased if these polysaccharides coexist in a certain raw material,[6] so here we decided to determine and publish only the total glucan content.

The oil content of the germ cake also seems to be noteworthy and valuable, since its sterol content is 6200 mg kg^{-1} oil, while the sterol content of commercial corn germ oil is only 4500 mg kg^{-1} oil.

Composition of the solid phase after hot DW extraction (step 1) can be seen in Table 3. Components are indicated as grams per 100 grams native dry germ cake.

Table 3 – *Composition of the germ cake after 2 h hot DW extraction (step 1)*

Component	g/100 g nat. germ cake	Change to prev. (%)
dry mass	57.6	-42.4
glucan	8.0	-50.3
hemicellulose	8.98	-52.7
xylan	5.33	-31.7
arabinan	3.65	-67.4
protein	18.45	-34.3
lipids	11.61	-21.0
sterols [mg kg^{-1} germ]	*1022*	*+12.2*
sterol esters [mg kg^{-1} germ]	*882*	*-10.5*
sum	47.04	-41.7

The first step, a 120 °C distilled water extraction, dissolved 42.4 % of the original dry matter. More than half of the glucan and the hemicellulose content were extracted, while ca. 80 % of the lipids remained in the solid fraction. The arabinan content of the hemicelluloses is easier to hydrolyse than the xylan content, which is in accordance with our previous results[6,7] of corn fiber and corn-stover fractionation.

Total phytosterol yield increased (1022 + 882 = 1904 mg kg^{-1} germ vs. 911 + 985 = 1896 mg kg^{-1} germ), which means that we could gain more phytosterols by extracting the oil content after step 1, than by direct extraction of the germ cake. The oil content of the solid phase increased to 20.2 %. The sterol concentration of the oil extracted after step 1 was 8800 mg kg^{-1}, which is nearly two times higher than the sterol concentration of commercial germ oils (4500 mg kg^{-1}).

The steep liquor from the hot DW extraction contained 8.1 g of glucan, and ca. 10 – 10 g of hemicelluloses and protein /100 g of germ cake. The glucan content can be used for ethanol fermentation, but prior to that the steep must be concentrated, since the ca. 0.65 % glucose concentration is too dilute to be fermented. 3.52 g of germ cake is produced from 100 g of corn (see Table 1), thus the steep liquor of the hot DW extraction adds 3.52 · 0.081 ·180 / 162 = 0.317 g of glucose to the 75.46 grams glucose yield of the wet milling process. This would be a 0.42 % increase of the glucose yield. It is noteworthy that only distilled water and no chemical reagents were applied for step one, thus this might be an inexpensive, convenient process except at industrial scale.

Composition of the solid phase after hot dilute sulphuric acid extraction (step 2) can be seen in Table 4. Components are indicated as grams per 100 grams of native dry squeezed germ.

Table 4 – *Composition of the germ cake after dilute sulphuric acid extraction (step 2)*

Component	g/100 g nat. germ cake	Change to prev. (%)
dry mass	20.0	-65.3
lipids	9.25	-20.3
sterols [mg kg^{-1} germ]	*759*	*-25.7*
sterol esters [mg kg^{-1} germ]	*934*	*+5.9*
protein	2.78	-84.9
sum	12.03	-74.4

After the second step, the dry mass of the solid phase decreased to 20 % of the original substrate. It can be seen that we lost some part of the lipids

(yield: 9.25 g/100 g nat. germ cake vs. 14.7 g/100 g nat. germ cake), but the lipid content of the solid phase increased to (9.25/20 · 100 =) 46.25 %. This oil content is high enough for pressing. Thus, solvent extraction is not necessary to recover some of the oil from germ cake. Theoretically, the traces of sulphuric acid can occur in the oil, but it is not likely considering the low solubility of sulphuric acid in lipids. We emphasize that these results were obtained by using only 1 % w/w dilute sulphuric acid and 8 % w/w dry cake content.

In the second step, we lost some phytosterols, but the loss of total phytosterols was only 10.7 % relative to hexane extraction of germ cake. The sterol concentration of the oil extracted after step 2 was 8200 mg kg^{-1}.

We have previously concluded and reported[6] that solvent extraction of corn fiber results in high-phytosterol oil (1.84 g total phyt.st./kg corn fiber). Presumably, the fibrous parts of seeds contain high concentrations of these components. We have previously proved[6] that the hydrolysis of fibrous parts can further increase the phytosterol yield of the solvent extraction. Hydrolysis can liberate lipids and lipophilic constituents by cleaving bonds between lipopolysaccharides and lipoproteins. Germ cake is also a fibrous material, and supposedly, that is why the solvent extraction of the hydrolysed residue results in a relatively high yield of phytosterols.

In the steep liquor, we found 3.13 g glucan/100 g germ cake, 5.33 g xylan/100 g nat. germ cake, and 3.65 g arabinan/100 g nat. germ cake. This mixture can be used for ethanol fermentation and hemicelluloses isolation or as a substrate for other fermentations. The glucose content of this liquor adds (3.52 · 0.0313 ·180 / 162 =) 0.122 g of glucose to the 75.46 g glucose yield of wet milling. The supernatants of step 1 and step 2 altogether add (0.317 + 0.122 =) 0.439 g of glucose to the glucose yield, a 0.58 % increase.

The composition of phytosterols in the extracted oils was also measured. The results can be seen in Table 5.

The compositions of phytosterols in the oils extracted from germ cake are similar to the composition of phytosterols in commercial corn germ oil. Table 5 intimates that the determination of phytosterols composition (like the determination of fatty acid composition) is an adequate way of the verification of origin.

Conclusions

Corn germ cake is an abundant and inexpensive by-product of corn wet milling, which contains about 15 % oil and 16 % glucan. The oil content may be valuable, since our analytical results show that it contains 6200 mg kg^{-1} sterols, significantly more than the 4500 mg kg^{-1} average sterol content of commercial corn germ oil. Phytosterols are non-saponifiable, heat insensitive compounds of plant oils, and they are valuable for cholesterol lowering. The glucan content of the germ cake could be used for ethanol fermentation, thus it could increase the ethanol yield of a wet mill.

The applied methods (extraction with hot distilled water and then with hot dilute sulphuric acid) were adequate to separate the germ cake into three

Table 5 – *Composition of phytosterols in the extracted oils [% of total phytosterols]*

Component	Commercial corn germ oil [%]	Oil extracted from native germ cake [%]	Oil extracted from the solid phase after step 1 [%]	Oil extracted from the solid phase after step 2 [%]
brassicasterol	0	0.5	0.7	0.3
campesterol	17.5	21.1	21.2	21.1
campestanol		1.4	1.6	1.4
stigmasterol	6.8	7.0	6.9	6.5
Δ5,23-stigmastadienol	0	0.8	0.8	0.8
B-sitosterol	61.1	58.8	58.1	59.5
Δ5-avenasterol	7.6	7.2	7.9	7.3
Δ7-stigmasterol	3.9	2.1	1.9	2.1
Δ7-avenasterol	3.1	1.1	0.9	1.0

fractions: the steeps of the two extractions were dilute glucose solutions, and the residual solid phase after the second extraction contained 46.25 % oil. The steeps altogether contained 12.4 g of glucose per 100 g of native germ cake in the form of poly-, oligo-, and monosaccharides. This liquor must be concentrated before further treatment, since the concentration of saccharides is < 1 %, which is too dilute to be directly hydrolysed and fermented. If the poly-, and oligosaccharides are hydrolysed to glucose (for example with commercial amylases), the combined steep liquors can be used for ethanol fermentation.

Corn wet milling produces 3.52 g of (dry) squeezed germ per 100 g of processed (dry) corn. Steps 1 and 2 add (0.317 + 0.122 =) 0.439 g of glucose to the glucose yield of a wet milling plant, a 0.58 % increase.

The solid residue after the second step contained 46.25 % oil. This oil content is high enough to be pressed, so solvent extraction, which causes food safety and environmental problems, would be avoided. This is a new result, since the oil content of the germ cake has never been concentrated to this value. We conclude that the consecutive aqueous extractions of wet-milled corn germ cake result in a fibrous solid residue, from which valuable edible oil with significantly high phytosterol content can be pressed.

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List of symbols

C – polysaccharide content, % w/w

c – monosaccharide concentration measured by HPLC, g L^{-1}

V – volume of the suspension, L

f – hydration factor (glucose: 180/162; cellobiose: 342/324; xylose: 150/132; arabinose: 150/132)

m – dry mass of the analyzed raw material, g

m_{int} – mass of internal standard, mg

A_{sterol} – total area of chromatographic peaks of sterols

O – mass of the oil sample, kg

A_{int} – area of chromatographic peak of internal standard

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