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Chemical composition and phytosterols profile of degermed maize products derived from wet and dry milling

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Abstract

Maize is part of the diet of populations of all socio-economic classes in many countries around the world and is an excellent source of bioactive compounds such as carotenoids, phytosterols and lignans. Phytosterols have gained considerable interest because they possess properties that significantly reduce cholesterol levels in the blood by acting as antagonists. The purpose of this study was to characterise the fractions of maize flour obtained from Italian wet and dry milling processes from a chemical viewpoint (crude proteins, crude lipids and starch) and the contents of three main phytosterols. Whole parameters exhibited a broad variability in their content. The crude protein, crude lipids and starch contents ranged from 7.43 to 18.38% dry matter (dm), 0.61 to 8.53% dm and 23.77 to 80.41% dm, respectively, and the total phytosterol content ranged from 4.6 to 53.48 mg 100 g^{-1} dm. Significant differences were observed in the phytosterol compositions measured in degermed maize products obtained from wet and dry milling. The campesterol content ranged from 0.54 to 6.19 mg 100 g^{-1} dry weight (dw), respectively, for maize flour III and dry milling maize meal feed. The stigmasterol content ranged from 0.55 to 4.77 mg 100 g^{-1} dw for hominy grits II and broken degermed type I, respectively. In addition, the β-sitosterol content varied significantly from 2.51 to 44.37 mg 100 g^{-1} dw for hominy grits II and dry milling maize meal feed, respectively.

Keywords: phytosterols, maize, wet milling, dry milling, HPLC-ELSD

Introduction

The economic importance of maize (*Zea mays* L) has increased in recent decades due to major improvements in the dry matter production of the seed oil content as well as in the oil and meal quality. More recently, attention has focused on minor oil constituents such as tocopherols, phytosterols, and carotenoids, which are recognised for their antioxidative, cholesterol-lowering, and other health-benefiting potentials (Shewmaker et al, 1999).

Phytosterols are a collective term for plant sterols and stanols, which are similar in structure to cholesterol, differing only in the side chain groups. The most common plant sterols are sitosterol, campesterol, and stigmasterol, and the most common plant stanols are sitostanol, campestanol, and stigmastanol. The most important natural sources of plant sterols in human diets are oils and margarines, although they are also found in a range of seeds, legumes, vegetables and unrefined vegetable oils (Dutta and Appelqvist, 1996). Phytosterols are known to have a wide range of biological activities and physical properties. The efficacy of phytosterols in reducing blood cholesterol levels in humans has been known since the 1950s (Best et al, 1955). Furthermore, phytosterols may have beneficial effects against colon cancer (Awad et al, 2000) and are considered to have anti-inflammatory, antibacterial, anti-ulcerative and anti-tumour properties (Beveridge et al, 2002).

Many different sterols may be present in plant species (over 200 have been characterized), and the amounts and relative proportions of these sterols are dependent on the plant species. In general, a typical plant sterol mixture would contain 70% sitosterol, 20% stigmasterol and 5% campesterol. Some data from some representative commercial seed oils indicate that maize oil contains the highest level of phytosterols (Gunstone et al, 1994). The oil extracted from whole maize kernel is very rich in phytosterols - typically ranging between 0.8% and 1.6% (Weihrauch and Gardner, 1978). Nuts, seeds and cereal products are significant sources of phytosterols, whereas the phytosterol content of vegetables, given on a fresh weight basis, is considerably lower (Hearty et al, 2008). Although cereals and cereal products are generally regarded as significant plant sterol sources, the availability of recent data on sterol contents is scarce (Chung and Ohms, 2000). Ryan et al (2007) reported the following phytosterol values in maize seed: β-sitosterol 34.1 ± 1.1 mg 100 g^{-1}; campesterol 9.1 ± 0.5 mg 100 g^{-1}; stigmasterol 0.4 ± 0.0 mg 100 g^{-1}. Harrabi et al (2008) determined the distribution of sterols among the corn kernel fractions (endosperm, germ and pericarp) to evaluate the seed parts for their potential use as a low-cost source of these high-value-added compounds; the resulting endosperm fractions revealed its potential.

Maize use as a commodity for industrial pro-

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cesses produces a selection of different byproducts that are generally sold for preparing feed products. Instead of this utilization, Moreau et al (1996) reported that maize fibre oil has several unique components and functional properties not observed in any current commercial phytosterol product. The levels of total phytosterols in maize fibre oil range from approximately 15% to more than 50%, depending on the extraction and fibre pretreatment conditions. These results have suggested the use of this byproduct as a natural source of phytosterols.

Milling transforms cereals into more palatable and more desirable food ingredients. Milling involves the separation of the anatomical parts of the grain as cleanly as possible. Subsequently, some of the parts are reduced in particle size. In maize milling, the desired products are low-fat grits rather than flour. Milling generally involves recovery of the main tissue (*i.e.*, the starchy endosperm) and the concomitant removal of the material called «bran». Thus, the miller wants to remove the bran (*i.e.*, pericarp, seed coat, and aleurone layers) and germ without reducing the endosperm to a small particle size. After removal of the germ and bran, the endosperm is reduced to grits of a desired size. The bran is sold as animal feed, and the germ is processed to recover the valuable oil. In milling, the particle size distribution of the resulting endosperm products is dictated by the end-use of the product. In general, degermed maize products are primarily destined for human consumption within a limited number of commercial categories. These categories include ready-to-eat (RTE) cereals, brewing adjuncts, extruded and sheeted snacks, breading, batters, and prepared mixes, fortified foods, and non-food applications (Duensing et al, 2003).

The aim of this study was to characterise two commercial maize hybrids and their milling products (obtained by Italian millers from wet and dry processes) from the main chemical viewpoint (crude proteins, crude lipids and starch) and the content of the three main phytosterols.

Materials and Methods

Plant material

The whole grain of two commercial maize hybrids (*Zea mays* L) and the corresponding fractions derived from wet and dry processes were provided by Molino Favero of Padova (Italy). Based on their specific features, as determined by Molino Favero, one hybrid was chosen specifically for wet milling and the other *vice versa* for dry grinding. Table 1 provides a general overview of the range of products available from the maize wet milling and dry milling industries. Each milling company, however, might offer many additional products, as the product granulation profiles can be tailored to meet individual customer requirements.

Chemical analysis

Whole grains of maize and their milling fractions were ground to increase the homogeneity of the material for chemical analysis using a mill (ZM200, Retsch) with a 0.5-mm sieve. The main chemical components of the flours were characterized: i) moisture content in an oven at 105°C to constant weight; ii) crude proteins using the modified Dumas method (Kirsten, 1982); iii) crude lipids using the Soxtec method; iv) starch using the Nelson method (1944).

Table 1 - Granulation profiles for degermed maize products derived from wet milling and dry milling.

Product name	Dimension (μm)	Process[$]
Bran	100 – 500	wm
Maize meal feed	900 – 1900	wm, dm
Hominy grits I	> 4500	wm
Hominy grits II	4000 - 4500	wm
Hominy grits III	2200 - 4000	wm
Broken degermed type I	> 4500	dm
Broken degermed type II	4000 – 4500	dm
Broken degermed type III	2200 – 4000	dm
Maize flour I	400 – 900	dm
Maize flour II	250 – 600	dm
Maize flour III	< 250	dm

[$]wm - wet milling; dm - dry milling

Phytosterol extraction and purification

The phytosterol content was determined on all the fractions derived from both milling technologies. The total phytosterol content was extracted according to the method described by Moreau et al (1996) with minor modifications. Briefly, 2 g of maize meal was suspended with 20 ml of hexane containing 0.01% of butylated hydroxytoluene (BHT), which was added using an internal standard and extracted during stirring for 18 hours at room temperature. Cholesterol was used as the internal standard. The homogenized sample was filtered through a Buchner funnel using a glass fibre filter (Whatman 934-AH). The filtered sample was transferred to a round-bottom flask and evaporated to dryness in a rotary evaporator at 40°C and re-dissolved with 5 ml of chloroform.

An aliquot of the sample, corresponding to 25 mg of extract, was purified on a Hypersep column - NH_2 500 mg (Thermo Scientific). The purification process involved the following steps: one column was conditioned with 12 ml of hexane. The solution containing the extract was applied by adjusting the vacuum to obtain a suction time of approximately 1 ml min^{-1}. The neutral lipids fraction was eluted with 2 ml of chloroform:isopropyl alcohol 2:1. The collected fraction was evaporated and re-dissolved with 1 ml of hexane. Two columns were connected in series and packed with 22 ml of hexane; the neutral lipids fraction was then added. The SPE column in the head was disconnected and from this column, the cholesteryl esters were eluted with 1 ml of hexane. The triglycerides fraction was eluted from the 2 columns train relocated series with 1 ml of a solution containing 89% hexane, 1% diethyl ether, and 10% methylene chloride. The fraction containing the sterols was eluted from the two separate columns with a solution of 2 ml each of chloroform:methanol 2:1.

The purified component, evaporated to dryness

under a gentle stream of nitrogen, was re-dissolved using 1 ml acetonitrile and, before HPLC analysis, was filtered with a 0.22-µm nylon filter (Albet). All of the samples and analyses were performed in duplicate.

HPLC-ELSD analysis

All the experiments were performed using a Beckman Coulter HPLC system equipped with a separation module (model 126), a UV detector (model 166), an autosampler (Perkin Elmer) model 200 and a C8 column Biosil PRC 90-3S 3 µm 150 x 4, 6 mm (Bio-rad). The chromatographic elution was accomplished isocratically with acetonitrile - water (95:5 v/v) at a flow rate of 1.2 ml min^{-1}. The temperature was maintained at 23 ± 1°C, and the injection volume was 10 µl. UV detection was selected at 205 nm, and the results were used for quantitative purposes. After the UV detection, the chromatographic column effluent was subjected to detection by ELSD (Alltech 3300, Alltech Associates Inc, Deerfield, USA). Nebulization of the effluent in the ELSD was provided by a stream of pressurized nitrogen (1.5 ml min^{-1}), and the nebulised effluent was evaporated at 40°C. The detector was set at a gain of 16x, with the output interfaced with a 32 Karat software (Beckman Coulter).

Reagents and chemicals

All the organic solvents used (HPLC Grade), stigmasterol (product n° 47132, analytical standard, 99.9%), campesterol (from *Glycine max*, soybean, product n° C5157, purity of approximately 98%), β-sitosterol (product n° 47133, analytical standard, 99.9%), cholesterol (product n° 47127-U analytical standard, 99.9%) and all the reagents used for analysis by GC-MS were purchased from Sigma (St. Louis, MO, USA). Water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA), and all the samples were filtered using a 0.22-µm nylon filter (Albet).

Standard solutions

The phytosterols were identified based on the relative retention times of commercially available standards and literature data. In addition, gas chromatography-mass spectrometry (GC-MS) was used to confirm the identities of the three main phytosterols.

GC-MS analysis

Each sterols fraction was silylated with 150 µl BSA (bis N,O - trimethylsilyl acetamide) agent in 150 dry pyridine at 70°C for 20 minutes. The derivatized sterols fractions were immediately injected separately into a GC (Hewlett Packard 6890) coupled to a HP5973 mass selective detector (Agilent technologies), set to scan from 50 to 600 m/z. The system was fitted with a capillary HP-5 column (5% phenyl methyl siloxane, 30 m x 0.25 mm, 0.25-µm film thickness) and helium was used as the carrier gas at 1 ml min^{-1}. The GC–MS operating temperatures were as follows: injector, 260°C; detector, 280°C; and the oven temperature was programmed from 150°C to 320°C at 10°C min^{-1}. The ionisation energy was 70 eV. Manual injection of 1 µl of the sterol solution was performed in the split mode at a 1:50 split ratio. The sterols were identified by comparing the relative retention times (to a cholesterol, β-sitosterol, campesterol and stigmasterol standard mix) and mass spectra. The peaks were also confirmed using the Wiley 275.L Mass Spectral Library. The compounds were quantified by directly comparing their total ion chromatogram peak areas with those of an internal standard (betulin). The GC–MS response factor of the sterols, calculated using betulin, was 0.94 ± 0.05.

Statistical analysis

Statistical analysis was performed using the Proc GLM of the STATISTICA software (Windows Statsoft Inc, 2325 East 13th Street, Tulsa, Oklahoma, 74104, USA, 1984-1999). The means were also compared using the Duncan multiple-comparisons test. For each sample, between 10 and 32 determinations were performed.

Table 2 - Chemical composition profile (% dry matter ± SE) of degermed maize products derived from wet and dry milling.

Milling	Product name	No. of samples	Crude proteins	Crude lipids	Starch
wet	Whole grain	16	9.84±0.17 e	4.14±0.08 d,e	66.47±1.84 d,e
wet	Bran	32	8.2±0.34 d	4.56±0.65 e	23.77±0.66 a
wet	Maize meal feed	32	11.69±0.08 f-h	8.53±0.06 f-h	42.66±0.54 c
wet	Germ	10	18.38±0.50 f-h	26.53±0.48 f-h	36.16±1.89 b
wet	Hominy grits I	10	8.81±0.10 d	0.73±0.04 a	80.41±0.57 h
wet	Hominy grits II	10	8.44±0.09 d	0.61±0.03 a	73.6±0.68 f
wet	Hominy grits III	10	8.46±0.12 d	0.86±0.06 a	74.47±0.99 f,g
dry	Whole grain	24	8.54±0.11 d	3.55±0.08 c,d	67.56±0.38 e
dry	Maize meal feed	32	8.18±0.11 c,d	2.64±0.20 b	63.7±1.89 d
dry	Germ	12	16.94±0.32 f-h	22.12±0.60 f-h	32.61±2.02 b
dry	Broken degermed type I	12	7.53±0.10 b	0.97±0.11 a	75.75±1.15 f-h
dry	Broken degermed type II	12	7.43±0.06 b	0.77±0.12 a	78.63±1.12 g,h
dry	Broken degermed type III	12	8.72±0.04 d	2.84±0.22 b,c	74.46±1.15 f,g
dry	Maize flour I	12	8.29±0.18 d	0.92±0.07 a	78.82±0.94 g,h
dry	Maize flour II	12	7.58±0.22 b,c	1.54±0.23 a	75.24±1.41 f,g
dry	Maize flour III	12	6.03±0.12 a	1.59±0.20 a	75.96±3.38 f-h

Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$.
SE - standard error

Results and Discussion

Chemical composition profile of degermed maize product derived from wet and dry milling

As each milling company might offer many additional products, as the product granulation profiles can be tailored to meet individual customers requirements, we detected the chemical composition of the derived products. The compositions of the degermed maize products obtained from the wet and dry processes are summarized in Table 2. Significant differences were observed in the whole chemical components measured in the maize degermed products obtained using wet and dry milling. The crude protein content ranged from 6.03% to 18.38% dry matter (dm) for maize flour III and wet milling germ, respectively. The crude lipids content ranged from 0.61% to 26.53% dm for hominy grits II and dry milling germ, respectively. In addition, the starch content varied significantly from 23.77% to 80.41% dm for bran and hominy grits I of the wet-milling product, respectively.

Within the family of degermed maize products described in Table 1, there exists a wide difference in the proximate composition, Table 2. These differences occur because the various products originate from different areas within the maize kernel. The products from the horney (or vitreous) endosperm portion of the kernel, for example, exhibit the lowest fat content, whereas the products primarily from the soft flour endosperm portion of the kernel (*i.e.*, break flour) have higher fat contents as reported by Duensing et al (2003). The protein differences in these degermed products are related to the presence or absence of endosperm proteins (primarily zeins and glutelins) rather than germ proteins, as confirmed by Duensing et al (2003). The starch content in most maize dry-milling products is similar, greater than 70% dm, and only maize meal feed and germ have lower starch contents. This result could be attributed to the fact that the maize dry miller can control only three attributes of the milled product: the granulation profile, moisture content and relative crude lipids content. The wet milling process produces primary crude starch that is purified through three steps (primary separation, purification and finishing).

HPLC analysis of degermed maize products

An HPLC system was developed to separate (Figure 1) and quantify (using the standard curve) the free main phytosterol components in the hexane extracts of the milling corn fraction. A sample (10 µl) after the purification was injected into the HPLC. Using two detectors in series, it was possible to identify the peaks on the ELSD that were also UV205 nm-absorbing peaks.

In previous papers from different authors (Singh et al, 2000), there has been a reported study of the variability of commercial corn hybrids for corn fibre yields and of the amount of oil in the total phytosterol component levels. Winkler et al (2007), using several different extraction methods, characterized

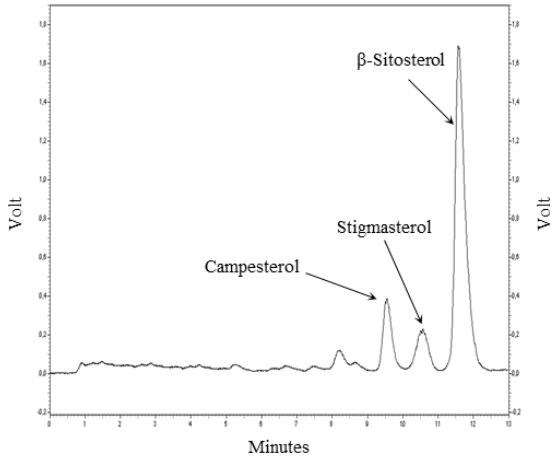

Figure 1 - HPLC-ELSD chromatogram of the bran fraction.

corn distiller's dried grain (DDG), a major co-product of ethanol fermentation from corn processed by dry milling that is primarily sold as livestock feed, for its phytosterol, ferulate phytosterol ester, tocopherol, and tocotrienol content.

The performance of the method was verified with recovery and repeatability studies. The performance of the HPLC-ELSD method was verified daily by analyzing a phytosterol standard mixture and was demonstrated to be good and stable. The retention time of plant sterols was: 9.51 ± 0.3 min (n = 12) for campesterol, 10.60 ± 0.4 min (n = 12) for stigmasterol, and 11.76 ± 0.4 min (n = 12) for β-sitosterol.

The resulting flour from the maize bran was used as an in-house reference flour. The recovery of plant sterols was determined by adding 50 µg cholesterol equal to the bran maize flour as an internal standard. The mean recovery expressed as cholesterol, determined for 24 extractions, was observed to be 92.54%. The retention time of cholesterol was 8.20 ± 0.2 min (n = 12). The performance of the method was evaluated by analyzing the in-house reference flour once in each sample batch. The total phytosterol content of the in-house reference flour was also very stable, 34.74 ± 3.66 mg 100 g^{-1} dry weight (dw) (n = 24), implying that the repeatability of the phytosterol analysis was good. The contents of β-sitosterol, campesterol and stigmasterol of the in-house reference flour were 28.81 ± 3.67 mg 100 g^{-1} dw (n = 24), 3.48 ± 0.29 mg 100 g^{-1} dw (n = 24), and 2.45 ± 0.39 mg 100 g^{-1} dw (n = 24), respectively. All the results are given as the mean of the replicate samples on a dw basis.

The data obtained from triplicate injections of the mixture of standard solutions over three days of analysis were processed for linearity. The ELSD mode resulted in a determination coefficient of $r^2 > 0.9769$ for a range of 0.1 - 1.5 µg. Calibration curves for the three phytosterols were measured and observed to be linear over the range studied (Lampi et al, 2004).

The limits of detection (LOD, signal/noise > 3) and

Table 3 - Phytosterols (mg 100 g^{-1} dw ± SE) profile of degermed maize products derived from wet and dry milling.

Milling	No. of samples	Product name	Campesterol	Stigmasterol	ß-sitosterol
wet	32	Bran	3.45±0.05 d	2.39±0.07 e	27.72±0.67 d
wet	32	Maize meal feed	4.88±0.09 e	3.48±0.07 f,g	31.81±0.35 d
wet	10	Hominy grits I	1.55±0.07 b,c	0.81±0.05 b,c	3.20±0.18 a
wet	10	Hominy grits II	1.54±0.12 b,c	0.55±0.09 a,b	2.51±0.11 a
wet	10	Hominy grits III	1.46±0.12 b	0.68±0.04 a-c	2.67±0.11 a
dry	32	Maize meal feed	6.19±0.16 e	2.92±0.10 e,f	44.37±0.90 d
dry	12	Broken degermed type I	5.06±0.16 e	4.77±0.10 f,g	6.96±0.12 b
dry	12	Broken degermed type II	3.33±0.17 d	2.90±0.12 e,f	4.86±0.17 a,b
dry	12	Broken degermed type III	2.89±0.07 c	1.79±0.14 e	4.84±0.20 a,b
dry	12	Maize flour I	3.40±0.13 d	1.52±0.09 d,e	9.91±0.17 c
dry	12	Maize flour II	2.00±0.16 c	0.90±0.05 c	9.36±0.25 c
dry	12	Maize flour III	0.54±0.07 a	0.44±0.09 a	5.94±0.31 b

Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$.
SE - standard error

the limits of quantification (LOQ, signal/noise >10) were determined by analysing dilutions of a solution containing all the marker compounds and by establishing the level at which the analytes could be reliably detected. The LOD and LOQ were 30 mg l^{-1} and 100 mg l^{-1}, respectively, for all the compounds. The ELSD exhibited uniformity in response to the uniform response factor for all the structurally similar compounds used for quantification.

The precision of the method was determined by analyzing the degree of dispersion of a set of individual measurements using the standard deviation. Three sets of in-house reference flours were prepared as described earlier. The intra-analyses precision (n = 8) (repeatability) was between RSD 0.83% and 2.98%. The inter-analyses precision (n = 3) was at most RSD 2.47%. It was concluded that the ELSD results were similar to those obtained using other detectors. The percentage accuracy was calculated to be between 97.84% and 102.67% using the calibration curves.

The purified sterol fractions were also analyzed by GC-MS; the results obtained in these tests confirmed the data obtained using the HPLC-ELSD method.

Phytosterols profile of degermed maize products derived from wet and dry milling

In the milling of maize, the plant sterols were distributed according to the corresponding milling product as indicated in Table 3. The wet milling process is designed to efficiently take maize apart and purify its constituents (starch, oil, protein, and fibre), making them suitable for use as ingredients in food, feed and industrial products or as feedstocks for conversion into other value-added products (Anderson and Watson, 1982).

Significant differences were observed in the phytosterol compositions measured in degermed maize products obtained from wet and dry milling. The campesterol content ranged from 0.54 to 6.19 mg 100 g^{-1} dw, respectively, for maize flour III and dry milling maize meal feed. The stigmasterol content ranged from 0.55 to 4.77 mg 100 g^{-1} dw for hominy grits II and broken degermed type I, respectively. In addition, the ß-sitosterol content varied significantly from 2.51 to 44.37 mg 100 g^{-1} dw for hominy grits II and dry milling maize meal feed, respectively.

The smaller fractions of maize kernel, bran and meal feed, produced during the milling process, are removed and generally sold as animal feed. A more in-depth analysis of the chemical composition, mostly for the presence of some molecules in these maize fractions, could utilise the molecules in different ways and give them an added economic value. This study revealed that the highest levels of phytosterols were detected in the maize meal feed (53.48 mg 100 g^{-1} dw from wet and 40.17 mg 100 g^{-1} dw from dry milling) and in the bran (33.56 mg 100 g^{-1} dw).

This paper describes the characterization of the fractions of maize flour obtained from Italian milling processes. The chemical composition profiles of the degermed maize product derived from wet and dry milling exhibit a wide difference in their content (crude protein, crude lipids, and starch).

A HPLC-ELSD method to quantify the campesterol, stigmasterol and β-sitosterol contents was developed. The ELSD detection method resulted in uniformity in response, and the baseline separation was achieved within a short analysis time. The purified sterols content obtained was confirmed by GC-MS analyses. This simple, rapid, precise, and accurate method was successfully applied to detect the phytosterol contents in processed maize grain and revealed that each maize milling product had a characteristic phytosterol composition, particularly bran, and that maize meal feeds have a higher content. This result suggested that these products could potentially be used as a good source of phytosterols. In fact, all maize milling products may be a good source of phytosterols.

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