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Title: Distribution of bioactive compounds in maize fractions obtained in two different types of large scale milling processes.

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Abstract

Maize kernels contain different bioactive compounds that are important for human health. The aim of this study was to analyze the distribution of the bioactive compounds in maize fractions derived from two industrial dry-milling processes, characterized by a dry-degermination (DD) system and a tempering-degermination (TD) system.

The bioactive compounds in maize resulted unevenly distributed in the milling fractions of the kernel. By-products such as the germ and the animal feed flour, had higher total antioxidant capacity (TAC), total polyphenol content (TPC) and total dietary fibre content (TDF) than the whole grains, while xanthophyll and resistant starch resulted to be higher in the fractions derived from the vitreous endosperm. The germ fraction showed also the highest folate content. Results also showed that the type of degermination process influences the bioactive compound contents in the milling fraction, in accordance to the effectiveness of the germ and bran removal from the endosperm fractions. In particular, the animal feed flour obtained by means of TD system resulted in an higher TAC, TPC and TDF than the same fraction obtained by means of the DD system. Conversely, the extraction rate do not affect the recovery of bioactive components in particular fractions.

KEYWORDS: maize dry-milling, total antioxidant capacity, polyphenols, xanthophylls

ABBREVIATIONS

ABTS, 2,2'-azino-bis/3-ethylbenzthiazoline-6-sulphonic acid; ANOVA, analysis of variance; DD, dry degermination; DM, dry matter; FAE, folic acid equivalents; FTL, floating test; LE, lutein equivalents; RS, resistant starch; TAE, tannic acid equivalent;

25 TAC, total antioxidant capacity; TD, tempering-degermination; TDF, total dietary
26 fibre; TE, Trolox equivalents; TME, total milling energy; TPC, total polyphenol
27 content; TW, test weight; XPC, xanthophyll content.
28

1. Introduction

Maize (*Zea mays* L.) is one of the world's leading crops, along with rice and wheat. In 2014 the estimated world maize production was 1037 millions of tonnes, 8 of which were produced in Italy (Food and Agriculture Organization of the United Nations, 2017). Most of the maize produced throughout the world is used for animal feeds, but this cereal is part of the staple diet of more than 200 million people in Latin America, Asia and Africa, and is used for the preparation of traditional foods, such as tortillas, arepas, couscous and porridge (Rooney and Serna-Saldivar, 2003). Furthermore, the consumption of this crop for food has also recently increased in developed countries, since it is used as an ingredient for breakfast products, snacks, dietetic products and, in particular, for gluten-free foods, whose consumption is rising. From a nutritional point of view, maize is a good source of starch, proteins and lipids, but it also contains different bioactive compounds that are important for human health (Nuss and Tanumihardjo, 2010). The most important groups of bioactive compounds found in whole maize grains are polyphenols, carotenoids, vitamins and dietary fibre.

The global action of all antioxidant substances present in a raw material is generally expressed as total antioxidant capacity (TAC). Increasing evidence suggests that the consumption of food characterized by a high content of antioxidants might have effects on the prevention of various oxidative stress-associated diseases, such as cancer and cardiovascular diseases (Adom and Liu, 2002). Maize has been reported to have the highest TAC among different cereal grains, including rice, oat and wheat (Adom and Liu, 2002). The antioxidant activity of maize is mainly related to the presence of high concentrations of polyphenols. Other compounds that play important role as antioxidants are carotenoids, which are responsible for the yellow-orange colour of maize (Kurilich and Juvik, 1999). In particular, xanthophylls, the

oxygenated hydrocarbon derivatives of carotens, have shown a potential role in the protection against age-related macular degeneration (Botella-Pavía and Rodríguez-Concepción, 2006).

Maize-derived food products could also be a source of vitamins. Folates are a group of water-soluble vitamins that are characterized by a biological activity similar to folic acid; an insufficient folate intake is usually associated with a large number of health disorders and with an increased risk of neural tube defects in the developing foetus (Wald et al., 1996).

Dietary fibre includes cellulose, hemicellulose, lignin, inulin, resistant starch (RS) and other constituents, and plays a beneficial role in the maintenance of gut health and in the control of body weight and cholesterol levels (Liu, 2007). RS has been defined as a starch that cannot be digested in the small intestine, and which passes to the colon where it is fermented by the microflora (Perera et al., 2010). The physiological effects of RS include improved glycemic response and colon health, lower calorie intake, modulated fat metabolism and the prevention of cardiovascular diseases (Liu, 2007). The consumption of whole cereal grains allows the synergistic effect of these biologically active compounds to be taken advantage of (Liu, 2007).

Milling processes transform cereals into more palatable and shelf-stable food ingredients, but they could result in the loss of most of the nutritional compounds. The way in which maize is processed and consumed varies greatly from country to country. Dry-milling is the main milling procedure adopted in the maize food chain, and it produces refined endosperm products with various particle sizes, and other by-products. Different dry-milling approaches could be applied, mainly according to the degermination system (Kent and Evers, 1994; Eckhoff, 2004):

- In dry-degermination (DD) systems, maize grains are broken by an impact degerminator, which works at a storage moisture content of 13-15%; the grain components are then separated by sifting and progressively ground into fractions of various particle size through further repeated milling and sifting steps;
- In tempering-degermination (TD) systems, maize grains are initially tempered to a moisture content of 18-22%, in order to facilitate the separation of the germ, pericarp and endosperm by means of a Beall type conic degerminator, using kernel-to-kernel shear.

The former process is generally employed for the production of maize meal and flour, while the latter is considered the best one to produce flaking grits (Eckhoff, 2004) and hominy grits, although further grinding and refining processes could be applied to these products in order to obtain maize meal and flour. Flaking grits are mainly used for the production of cornflakes, while medium and small hominy grits are used for the production of snacks, breakfast cereals and alcoholic beverages (Kent and Evers, 1994). Conversely, maize meal, which is characterized by a smaller particle size than grits, is used for polenta and several leavened baked and fried products. Finally, maize flour, which has a fine particle size, is used as an ingredient in many baking formulations (Rooney and Serna-Saldivar, 2003). In both processes, the high particle size fractions (maize meal or flaking grits) that can easily be refined and reduced to a desired size, have a high economic value. Grits and meal are essentially derived from the vitreous part of the endosperm, while the softer parts are mainly broken down to flour, and kernel hardness is the main grain quality attribute that influences the efficiency of the extraction yield in dry-milling processes. Grains with high coarse/fine endosperm ratios result in higher flaking grit yields (Blandino et

al., 2013a). Conversely, the main by-products are the germ, which is generally dispatched to the oil industry, and the animal feed flour, which is a mixture of impurities, bran and a part of the mealy endosperm produced during the milling process.

Several authors have investigated the distribution of bioactive compounds in the milling fractions of wheat (Liyana-Pathirana and Shahidi, 2006), barley (Sullivan et al., 2010) and rye (Gómez et al., 2009). However, only limited information is currently available on the impact of milling processes on the bioactive content of maize products and by-products (Kean et al., 2008; Locatelli and Berardo, 2014).

Considering the increasing use of maize milling derivatives for the production of gluten-free foods for people affected by celiac disease, which may result in some nutritional deficiencies, the aim of this study was to analyze the content of bioactive compounds of maize fractions derived from two industrial dry-milling processes. Moreover, the study considered the possible interaction between the extraction yield, as influenced by maize kernel hardness, and the distribution of the bioactives in different products and by-products of the milling processes.

2. Material and methods

2.1 Maize samples

The present study has analysed nine different commercial maize lots (Table 1), cultivated from 2011 to 2013 in the same growing area (North West Italy, province of Turin). All the maize lots were processed in an industrial mill: seven lots were constituted by single hybrids, which were chosen among the ones commonly processed in the industrial mill, while the remaining lots were a mixture of two or three of these hybrids.

2.2 Analysis of the kernel hardness and extraction yield of lots

According to Blandino et al. (2013a), each lot was characterized for its kernel hardness by determining the following parameters: test weight (TW), total milling energy (TME), and floating test (FTL). Moreover, during the milling process the yield of the large grits (sum of flaking and medium hominy grits obtained in the TD process) was recorded and expressed as a percentage of processed grain weight. The maize lots were then grouped as high (> 48%) or medium (44-48%) extraction yield (Table 1), in order to evaluate the possible role of the extraction yield, related to kernel hardness, on the distribution of the bioactive compounds in the milling fractions.

2.3 Maize milling processes and sampling

All the maize lots were processed in an industrial mill by means of two separate dry-milling lines based on different degermination processes.

143 The first process was based on a dry-milling technology, coupled to a dry-
144 degermination (DD) system (Figure 1). Maize kernels were cleaned through a dry
145 stoner, an intensive horizontal scourer and a vibrating aspirator, then they were sent
146 to the degermination plant, which was composed of three types of machines: an
147 impact degerminator (DGF-impact degerminator), plansichters and a gravity
148 separators (Ocrim, Cremona, Italy). The first machine broke down the kernels into
149 germ, bran and endosperm fractions, and all these fractions were successively
150 separated in the plansichter and gravity separator steps. The endosperm fraction
151 was then progressively refined through a series of passages in a grinding and
152 classification system, which was composed of roller mills, plansichters and flour
153 purifiers (Sangati, Padova, Italy), that allow to obtain maize flour and meal. These
154 additional grinding and classification steps led to a further separation of the maize
155 flours from the germ, bran and finer particle size endosperm fractions. Maize meal
156 and flour differ for their particle size as detailed in Table 2.
157 The second process was based on a dry-milling technology coupled to a tempering-
158 degermination (TD) system (Figure 2). The maize kernels were cleaned as reported
159 above and water was then added to increase the moisture content to approximately
160 20%, by adding 50-70 kg of water for each maize tonne, according to the moisture
161 content of the stored kernels. A Beall type degerminator (DGC/X conical
162 degerminator, Ocrim, Cremona, Italy) was then used to remove the bran and germ
163 through an abrasive action and the endosperm was broken into particles of various
164 sizes, as indicated in Table 2 (Kent and Evers, 1994). Finally, the endosperm
165 fractions were separated by means of plansichters and gravity separators (Ocrim,
166 Cremona, Italy).

In both processes, the main by-products were the germ and the animal feed flour, a mixture of bran and a part of the mealy endosperm. The usual expected yield of these by-products, in comparison to whole grain before cleaning, is 10% for the germ and 35% for the animal feed flour.

Each maize lot (200 t) was simultaneously subjected to both processes for 40 h. A total of 198 samples (11 milling fractions X 9 lots X 2 replications) were collected including the whole grain before and after cleaning (2 fractions), and each product and by-product obtained from DD (4 fractions) and TD milling (5 fractions) processes, so that their sum represented the lot of origin. The sampled fractions are reported in an oval shape in Figure 1 and 2. Samples were collected from the opening slits of the milling plant, and the adopted sampling method was derived from European Commission Regulation (EC) No. 401/2006. Considering that the industrial plant mills an average 5 t/h of maize grains, a dynamic sampling procedure was set up in which each aggregate sample was the result of a careful blending of 40 incremental samples of 100 g each, collected over a period of 1 hour at regular intervals. A sampling lasting 1 h was performed twice for each lot and each dry-milling process in order to obtain two replications.

The whole grain and hominy grit samples were ground using a ZM 200 Ultra Centrifugal Mill (Retsch GmbH, Haan, Germany). A second milling step was performed for all the samples using a CT 193 Cyclotec^{TM}, in order to obtain a fine and homogeneous particle size (<250 μm) and to improve the complete extraction of bioactive compounds. The samples were stored at -25°C until the analyses were performed, with the exception of an aliquot of each sample, which was stored at 4°C before the RS analysis.

2.4 Chemical analyses

2.4.1 Analysis of the moisture

The moisture content, determined in order to express the results on a dry matter (DM) basis, was obtained using a Sartorius MA30 thermo-balance (Sartorius AG, Goettingen, Germany).

2.4.2 Analysis of the total antioxidant capacity (TAC)

TAC was determined by means of a direct method (ABTS assay), as described by Alfieri et al. (2014). TAC was expressed as mmol of Trolox equivalents (TE)/kg DM by means of a Trolox-dose response curve.

2.4.3 Analysis of the total polyphenol content (TPC)

TPC was determined on methanol extracts by means of the Folin-Ciocalteu colorimetric method (Blandino et al., 2013b). The results were expressed as mg tannic acid equivalents (TAE)/kg DM.

2.4.4 Analysis of the xanthophyll content (XPC)

XPC was determined according to the AOAC Method 970.64 (1974), with spectrophotometric determination at 470 nm. The results were expressed as mg of lutein equivalents (LE)/kg DM.

2.4.5 Analysis of the total dietary fibre (TDF)

TDF was measured using the Megazyme total dietary fibre analysis kit (AOAC Method 985.29 (Prosky et al., 1985) - Megazyme International, Wicklow, Ireland). The results were expressed as g/100 g DM (%).

2.4.6 Analysis of the resistant starch (RS)

The RS content of uncooked maize fractions was measured using the Megazyme resistant starch kit (AOAC Method 2002.02; McCleary and Monaghan, 2002), checking that the standard error was ≤5%. The analysis was not carried out on pre-cleaned whole grain samples and on germ samples. The results were expressed as g/100 g DM (%).

2.4.7 Analysis of the total folate content

The total folate content was determined on the maize samples, obtained by means of the DD system, taken from lot no. 8 (Pioneer 3245, 2011 growing season). The total folate content was determined through a microbiological assay according to the AOAC Method 2004.05 (DeVries et al., 2005), with a few modifications, as reported by Giordano et al. (2016a). The amount of folate in each sample was determined through a comparison with calibration curves of folic acid (Sigma-Aldrich, Saint Luis, Missouri). The results were expressed as ng folic acid equivalents (FAE)/g DM.

2.5 Statistical analysis

The normal distribution and homogeneity of variances were verified by performing the Kolmogorov–Smirnov normality test and the Levene test, respectively. Rank

237 transformation of the data, relative to the total folate content, was performed, since
238 the previous assumptions had not been verified.
239 One-way analysis of the variance (ANOVA) was applied in order to compare the
240 bioactive compound contents in different milling fractions obtained through the two
241 dry-milling processes. The maize extraction yield of the lots, grouped as medium or
242 high, was set as a further factor for the TAC, TPC, XPC, TDF and RS. Multiple
243 comparison tests were performed, using the Ryan-Einot-Gabriel-Welsh F (REGW-F)
244 test.
245 Simple correlation coefficients were obtained through Pearson's two-tailed test for all
246 the compared bioactive compounds, relative to each another, by joining the data sets
247 that referred to different maize lots.
248 SPSS for Windows statistical package, Version 21.0 (SPSS Inc., Chicago, IL, USA)
249 was used for the statistical analyses.
250

3. Results and Discussion

3.1 Extraction yield and kernel hardness

The extraction yield of the compared maize lots was found to be positively related to the kernel hardness measured as TW, TME or FLT (Table 1). As expected, the lots characterized by a high extraction yield resulted in greater values of TW (on average 80.4 vs 77.5 kg/hl) and TME (1849 vs1631 J), whereas a lower FLT (2420 vs 2713), than the ones characterized by a medium extraction yield.

As far as the effect on bioactive compounds is concerned, a slightly higher TAC was observed in high extraction yield lots (P=0.038), while no differences were reported for any of the other bioactives analyzed (Table 3). Moreover, no significant interaction was observed between the milling fractions of the compared dry-milling processes and the extraction yields, for any of the considered bioactive compounds. Thus, although the physical and chemical properties associated to kernel hardness in the considered maize lots led to different extraction yields, the results underline that this factor did not influence the distribution of the bioactive compounds in the maize milling fractions.

3.2 Antioxidant compound distribution in the maize milling fractions

Both phenolic compounds and xanthophylls act as antioxidant compounds in cereal grains (Adom and Liu, 2002; Nuss and Tanumihardjo, 2010). In the present study, no significant difference was observed between the grain before and after the cleaning step for both TAC, TPC and XPC (Table 3). Thus, impurities and broken kernels removed trough the cleaning step and collected into the animal feed flour contributed little to the bioactive content of the latter fraction.

The germ fraction on average had more than double TAC and TPC than the whole grain; no significant difference was observed for these parameters when the germ fractions obtained from the two different degermination processes were compared. After the germ, the second milling fraction with the highest TAC and TPC was the animal feed flour. The animal feed flour fraction obtained through the TD system showed a higher TAC (+52%) and TPC (+55%) than the same by-product obtained through the DD system. As far as the TPC content is concerned, the germ fraction showed a significantly higher content (+44%) than the animal feed flour fraction in the DD system, while no significant difference was observed between these by-products in the TD system.

In the TD system, the products derived from the endosperm (small and medium hominy grits and flaking grits) showed a significantly lower TAC (-53%) and TPC (-61%) than the whole grain. No difference was observed between these three products and the maize meal obtained through the DD system. Conversely, the maize flour, which is characterized by a finer particle size, resulted in a higher TAC and TPC, but did not differ significantly from the whole grain for either parameter.

The lowest XPC was observed in the germ fraction (on average 55% less than the one observed in the whole grain), regardless of which milling process was employed. Conversely, the XPC of the whole grain did not differ significantly from any of the fractions mainly derived from the endosperm. In both processes, the animal feed flour resulted in a significantly lower XPC than the whole grain and the endosperm products.

Xanthophylls, which are predominantly made up of lutein and zeaxanthin, are the main pigments responsible for the yellow-orange colour of maize grains and they also play an important role as antioxidants. Other studies performed on botanical

300 grain fractions, have shown that lutein and zeaxanthin are mainly concentrated in the
301 germ of wheat, barley and oat, whereas they are concentrated in the endosperm and
302 aleurone layer of yellow maize (Ndolo and Beta, 2013). Similarly, Kean et al. (2008)
303 showed that carotenoids are concentrated more in maize flour than in bran.
304 A significant positive correlation was clearly observed between TPC and TAC ($r = 0.945$, $P < 0.01$), thus confirming the results of previous studies performed on whole
306 grain maize samples (Žilić et al., 2012). Conversely, a strong negative correlation
307 was observed between XPC and TAC ($r = -0.734$, $P < 0.01$), in accordance with a
308 previous study conducted on maize inbred lines (Alfieri et al., 2014). In fact, other
309 components, such as polyphenols and tocopherols, may play a major role in the
310 antioxidant capacity of maize milling fractions (Kurilich and Juvik, 1999; Žilić et al.,
311 2012).
312
313 *3.3. TDF and RS distribution in the maize milling fractions*
314 The highest TDF content was observed in the animal feed flour and in the germ
315 fractions: about twice the content of the whole grain. As previously reported for both
316 TPC and TAC, the TDF content in the animal feed flour fraction, obtained by means
317 of the TD system, was significantly higher than the same fraction obtained from the
318 DD system (+17%). Moreover, the TDF content of the animal meal fraction was on
319 average 15% higher than the one observed in the germ fraction.
320 As expected, the TDF content of the endosperm fractions was much lower than that
321 of the whole grain (-66%), a result that is comparable with other studies performed on
322 maize (Rosin et al., 2002), wheat (Haskåa et al., 2008) and barley (Sullivan et al.,
323 2010) flour.

On average, RS was higher in the milling fractions derived from the endosperm. Even though RS is generally considered to be one of the components that contributes to TDF, its distribution in maize milling fractions follow the one of starch (Nuss and Tanumihardjo, 2010). As far as the DD system is concerned, the maize meal fraction, which mainly derived from the vitreous endosperm, contained more RS than the maize flour derived from the floury endosperm (1.5% vs 0.5%). The hominy grit fractions obtained from the TD system resulted in an intermediate RS content (1.1%), without any significant differences from the whole grain. In both processes, the animal feed flour was characterized by the lowest RS content (on average 0.7%).

3.4 Total folate distribution in the milling fractions obtained through the dry-milling procedure coupled to the DD system

The whole grain of the Pioneer 3245 hybrid showed a total folate content of 358 ng FAE/g. Previous studies reported that whole maize contains about 280 ng/g folate, and that this content is reduced by 64% after degerming (Hegedüs et al., 1985). This is the first report that showed the distribution of folate in industrial maize milling fractions obtained by means of a dry-degermination system (Figure 3). In accordance with previous studies, the highest total folate content was observed in the germ fraction (851 ng FAE/g), while lower concentrations were observed in the endosperm fractions. The maize flour, which mainly derived from the floury endosperm, was characterized by a significant higher folate concentration (509 ng FAE/g) than the maize meal (303 ng FAE/g), which mainly derived from the vitreous endosperm. The total folate content of the animal feed flour (312 ng FAE/g) did not differ significantly from the one observed in the whole kernel and in the maize meal.

3.5. Influence of the dry-milling processes on the bioactive compound content of maize fractions

The results of the present study clearly show that the employment of a specific dry-milling procedure influences the bioactive compound content of the milling fractions. The most relevant results concerns the animal feed flour. The employment of a TD system results in an animal feed flour characterized by a higher TAC, TPC and TDF contents than the same fraction obtained by means of the DD system. In fact, as also demonstrated by the bioactive compound contents of the endosperm products, the two processes differ in their effectiveness in the removal of the bran and fine endosperm fractions from the endosperm. In particular, the TD system was more efficient than the DD system in separating bran and fine endosperm fractions, that converge in the animal feed flour, from the vitreous endosperm. Thus, no difference was observed in the grit fractions in function of their particle size for any of the bioactives. Conversely, as far as the meal and flour fractions obtained by means of the DD system are concerned, a negative relationship between the TPC, TAC and folate contents and the particle size was observed. These results are in accordance with previous studies that showed that the meal derived from the vitreous endosperm portion is characterized by a lower fat content than the flour derived mainly from the floury endosperm that surrounds the germ (Locatelli and Berardo, 2014; Vanara et al., 2009).

3.6. Nutritional advantages of selected dry-milling fractions

In agreement with previous studies performed on maize botanical fractions (Das and Singh, 2015; Ndolo and Beta, 2013 and 2014), the analyses of the industrial maize milling fractions clearly showed that the by-products, referred here as germ and

374 animal feed flour, could be valuable functional ingredients in terms of total antioxidant
375 capacity and total polyphenol, fibre and folate contents.
376 The increase in maize production for food, together with the relative high germ
377 percentage in the maize kernel, could provide a good source for the expanded use of
378 maize germ for food production, as a valuable alternative to the oil extraction
379 process. Dry-heat treatments could be used to obtain full-fat maize germ
380 characterized by a high nutritional value and storage stability suitable for food
381 purposes (Giordano et al., 2016b). Conversely, the presence of high concentrations
382 of contaminants, such as mycotoxins (Vanara et al., 2009), makes the animal feed
383 flour unsuitable for food production.
384 As far as the endosperm products are concerned, the maize flour showed the highest
385 TAC, TPC and total folate content, although the TDF and XPC were similar to those
386 of other endosperm derived fractions. Thus, this product is the one that should be
387 most valued as a functional ingredient through the milling of maize genotypes
388 naturally rich in bioactive and antioxidant compounds. At the same time, the highest
389 XPC and RS contents were observed in the maize meal and in the grit fractions. The
390 distribution of these compounds was found to be moderately influenced by the milling
391 operations, and their contents resulted to be similar or even slightly higher than those
392 of the whole kernels. Thus, the nutritional advantages of the use of maize cultivars
393 particularly rich in these bioactives would not be negatively restricted by the
394 employed milling system.
395

2. Conclusions

This study confirms that, in the same way as in other cereals, bioactive compounds are unevenly distributed in the industrial milling fractions of maize grains. The distribution of the bioactive compounds is primarily related to the class of nutrient and to the milling fraction, although the difference among the fractions derived from the endosperm appears to be moderate. The bioactive compound content of different fractions also results related to the type of milling process employed, according to its effectiveness in the removal of the germ and bran residuals from degerminated endosperm fractions. On the contrary, the extraction yield, which is related to the kernel hardness, does not seem to affect the bioactive content to any great extent.

The effect of cooking, extrusion or other food processing steps on the bioactive compound contents in maize milling fractions could be of interest for future researches, to obtain a holistic evaluation of the functional role of these ingredients that are largely used to produce gluten-free food.

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TABLES

Table. 1.

Maize lots processed in the industrial mill, ranked according to their flaking and medium hominy grit yields.

Lot	Growing season	Hybrid	Kernel hardness[a]			Extraction yield[b]	
			TW (kg/hl)	TME (J)	FLT	(%)	Type
1	2013	Pioneer P1758	75.6	1405	3096	45	medium
2	2011	DKC 6795	77.6	1711	2665	45	medium
3	2013	Pioneer P1547	76.6	1516	2623	46	medium
4	2013	Mixture Pioneer 3245, P1543 and DKC6795	77.9	1723	2560	46	medium
5	2012	Pioneer P1547	79.7	1801	2619	46	medium
6	2012	Mixture Pioneer P1543 and P1547	80.1	1819	2442	49	high
7	2011	Pioneer P1543	80.2	1834	2463	49	high
8	2011	Pioneer 3245	79.9	1859	2393	53	high
9	2012	Pioneer 3245	81.3	1882	2380	56	high

[a] kernel hardness: TW = test weight, TME = total milling energy, FLT = floating test.

[b] extraction yield: sum of the flaking grits and the medium hominy grits expressed as a percentage of the processed grain weight.

Table. 2.

Particle size and expected extraction yield for maize fractions obtained through different dry-milling processes in the studied industrial mill.

Dry-milling process[a]	Milling fractions	Particle size (µm)	Particle size (mesh)	Expected extraction yield[b] (%)
DD	germ			10
	animal feed flour			35
	maize flour	< 350	> 45	5
	maize meal	350 - 800	20 - 45	50
TD	germ			10
	animal feed flour			35
	small hominy grits	1500 - 2500	8 - 14	10
	medium hominy grits	2500 – 4000	5 - 8	25
	flaking grits	> 4000	< 5	20

[a] dry-milling process: DD, dry-degermination system; TD, tempering-degermination system.

[b] extraction yield expressed as a percentage of the whole grain weight.

Table. 3.

Total antioxidant capacity (TAC), total polyphenol content (TPC), xanthophyll content (XPC), total dietary fibre (TDF) and resistant starch (RS) content in the milling fractions obtained from lots with different extraction yields and through different dry milling processes.

Factor	Dry milling process[a]	Source of Variation	TAC (mmol TE/kg)	TPC (mg/kg TAE)	XPC (mg/kg)	TDF (%)	RS (%)
Milling fraction		whole grain pre-cleaning	11.2 cd	1051.5 c	14.3 ab	11.0 c	nd[d]
		whole grain post-cleaning	11.2 cd	1046.7 c	14.4 a	10.8 c	1.0 b
	DD	germ	23.2 a	2096.5 a	6.5 d	23.0 b	nd
		animal feed flour	13.1 c	1452.9 b	11.2 bcd	24.1 b	0.8 c
		maize flour	9.4 d	793.2 cd	16.2 a	3.2 d	0.5 d
		maize meal	4.5 e	577.2 de	16.3 a	4.8 d	1.5 a
	TD	germ	25.6 a	2412.4 a	6.4 d	21.5 b	nd
		animal feed flour	19.9 b	2250.4 a	8.4 cd	28.1 a	0.6 cd
		small hominy grits	6.2 e	528.5 de	18.8 a	3.6 d	1.2 b
		medium hominy grits	4.9 e	398.3 de	16.8 a	3.5 d	1.0 b
		flaking grits	4.7 e	305.1 e	18.1 a	3.2 d	1.1 b
		P (F)	<0.001	<0.001	<0.001	<0.001	<0.001
		sem[c]	3.3	300.9	5.1	3.6	0.3
Extraction yield[b]		Medium	11.7 b	1204.6 a	13.7 a	12.5 a	1.0 a
		High	12.4 a	1129.7 a	13.5 a	11.9 a	1.0 a
		P (F)	0.038	0.135	0.901	0.248	0.860
		sem[c]	3.0	272.2	4.6	3.3	0.2
Milling fraction X extraction yield		P (F)	0.474	0.090	0.996	0.786	0.218

Means followed by different letters are significantly different (the level of significance is shown in the table). The reported milling fraction values are based on 9 lots, while the grit yield values are based on 5 and 4 lots for low and high yields, respectively. See Table 1 for details on the maize lots.

[a] dry milling process: DD, dry-degermination system; TD, tempering-degermination system.

[b] extraction yield, expressed as a percentage of the processed grain weight. Medium = sum of flaking grits and medium hominy grits < 48% of wholegrain; high = sum of flaking grits and medium hominy grits > 48% of wholegrain.

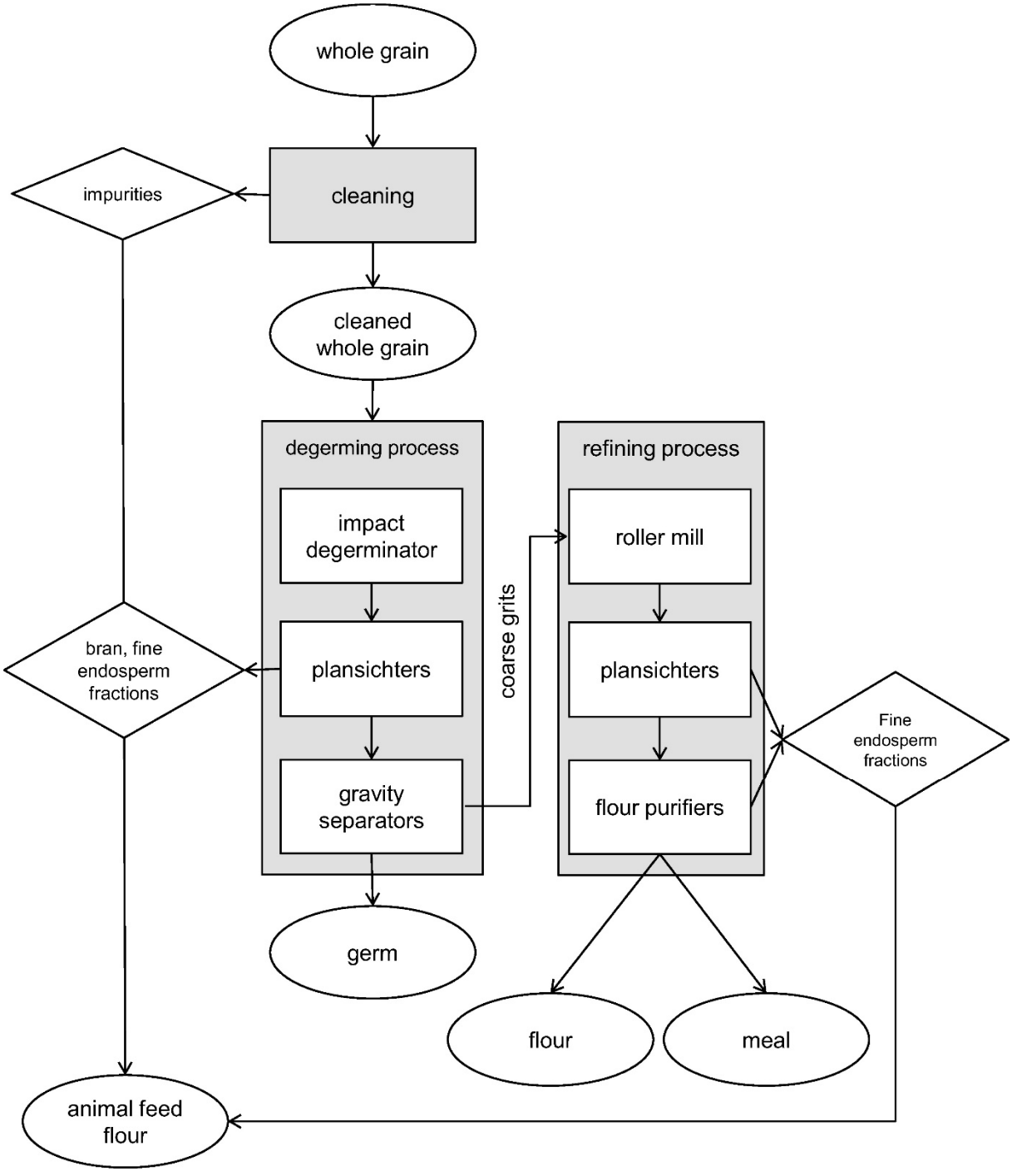
[c] sem = standard error of the means

[d] nd: not determined.

548 **FIGURES**

549 **Figure 1.**

550 Flow diagram of the dry-milling process with a dry-degermination (DD) system.



551

552 The raw materials, products and by-products collected and analysed in the study are
553 reported in the oval shape.
554

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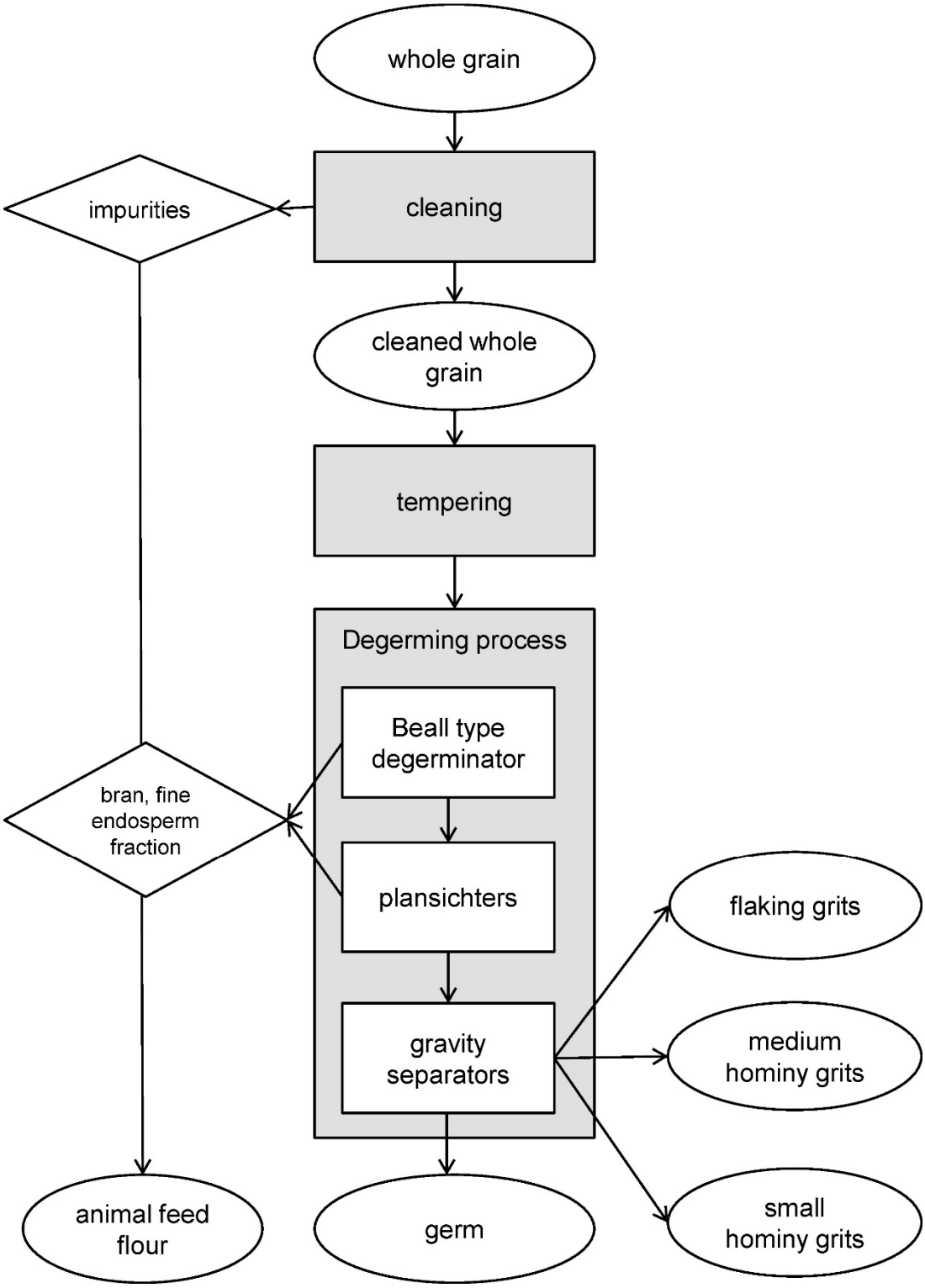
Figure 2.

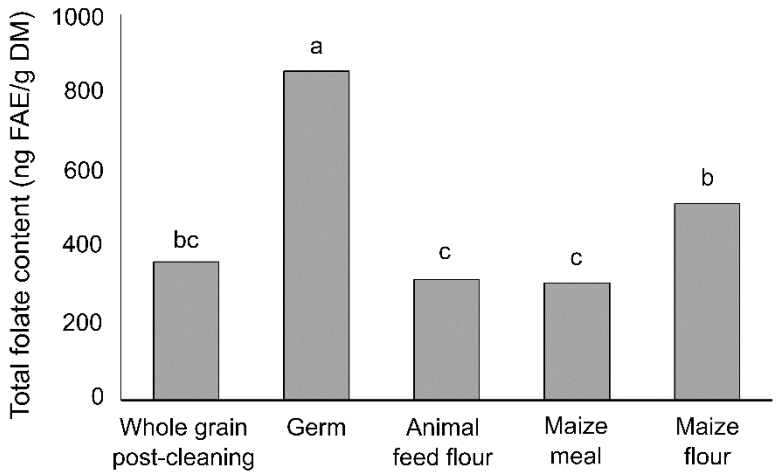
Flow diagram of the dry-milling process with a tempering-degerming (TD) system.



The raw materials, products and by-products collected and analysed in the study are reported in the oval shape.

563 **Figure 3.**

564 Average folate contents in the maize milling fraction obtained from the DD system.



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566 Values with different letters differ significantly ($P<0.001$).

567 The reported data are based on lot n°8 (Pioneer 3245 hybrid as specified in Table 1).

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