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Composition and technological value of genetically modified and conventional maize (*Zea mays* L.) grains

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RÉSUMÉ

Composition et qualité technologique de grains de maïs (*Zea mays* L.) normaux et génétiquement modifiés

L'évaluation de la sécurité des organismes génétiquement modifiés destinés à l'alimentation humaine implique une étude de la composition des produits et de leur adéquation aux procédés industriels (valeur technologique). Quatre hybrides de maïs qui ont été génétiquement modifiés pour conférer une résistance à la pyrale et/ou une tolérance aux herbicides (glufosinate ou glyphosate) ont été étudiés en comparaison de leurs lignées parentales respectives. Les échantillons de grains ont été transformés dans une semoulerie de maïs pilote (échelle, 150 kg) en fractions de mouture telles que : hominy, gritz, semoules et germes. La composition des grains de maïs et des fractions de mouture de chaque hybride génétiquement modifié a été comparée à celle du parent correspondant et aux valeurs couramment publiées pour les hybrides de maïs commerciaux. Les échantillons de grains ont fait l'objet d'analyses d'amidon, de cellulose, de protéines et de lipides, ainsi que d'une détermination des profils d'acides aminés et d'acides gras. On a examiné différentes caractéristiques physiques des grains (poids du grain, poids spécifique, calibrage, Promatest, rendements en hominy et en semoules). Les fractions de mouture ont été soumises à des transformations classiques à une échelle pilote : les semoules ont été transformées en produits extrudés ; les gritz ont été soumis à une fermentation pour obtenir de la bière, l'huile de maïs a été obtenue à partir des germes.

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Les paramètres technologiques des différents procédés ont été enregistrés et tous les produits intermédiaires et finis ont été recueillis pour subir les mêmes analyses que celles pratiquées sur les grains. L'ensemble des résultats collectés montre que chacun des hybrides génétiquement modifié possède une composition et une valeur technologique équivalentes à celles de la lignée parentale, et que, pour tous les paramètres analysés, les échantillons se classent dans la gamme habituelle des hybrides de maïs actuellement commercialisés.

Mots clés :

maïs ; organisme génétiquement modifié ; composition ; qualité technologique.

SUMMARY

An important aspect of the safety assessment of genetically modified crops to be used for human food and animal feed is the product composition and suitability to industrial processes (technological value). Four maize hybrids that have been modified genetically to confer levels of resistance to European maize borer and/or tolerance to glufosinate or glyphosate herbicides were investigated in comparison with their respective parental lines. Samples of maize grain were processed in a dry-milling pilot plant at a 150-kg scale into milling fractions such as hominy, grits, semolina and germs. The composition of the maize grain and milling fractions of each genetically modified hybrid was compared to that of the parent variety and to published values for conventional commercial maize hybrids. The nutrients measured on grains were: starch, cellulose, proteins and lipids. Amino acid and fatty acid profiles were also determined on grains. Physical characteristics of grains were: kernel weight, specific weight, kernel size, Promatest, hominy and semolina yields. Milling fractions were submitted to conventional processing at a pilot scale: semolina was processed into extruded products, grits were fermented and processed into beer; maize oil was extracted from germs. Technological parameters of the processes were recorded and intermediate and end-products were analyzed using methods common to maize products. On the basis of the whole set of data collected, the results indicate that each genetically transformed hybrid is compositionally and technologically equivalent to the parental line and that all samples fall into the range of conventional maize hybrids.

Keywords:

maize; genetically modified; composition; technological quality.

1 – INTRODUCTION

Although genetic transformation of cereal crops initially lagged behind other major crops, their great importance has been the impetus for the accelerated application of modern technologies to these crops (AIGLE *et al.*, 1996). This acceleration can be measured by the fact that a number of transgenic traits have been developed and commercialized in maize and many others are in advanced development in wheat and rice (BARRY, 1999).

Genetic modification of crops offers the potential to improve varieties or hybrids. For instance, the resistance to the European maize borer was achieved by introduction of the *Cry1* (b) gene of *Bacillus thuringiensis* or a synthetic equivalent (NOTEBORN *et al.*, 1995). Transgenes can also be incorporated which confer tolerance to glufosinate or the glyphosate herbicides. Field experiments carried out in France and abroad (GAY, 1993; KOZIEL *et al.*, 1993; LABATTE *et al.*, 1996) indicated particularly encouraging results.

Tolerance to glufosinate was obtained by introduction of either the BAR or PAT gene, derived from *Streptomyces viridochromogenes* and *Streptomyces hygroscopicus*, respectively. The *BAR* or *PAT* genes allow detoxification by acetylation of the herbicidal molecule within the modified plant. Its introduction into rapeseed and maize has allowed soil conservation by incorporation of reduced tillage practices (SARRAZIN *et al.*, 1995).

Tolerance to glyphosate was imparted by the two genes *CP4* and *GOX*. The former was isolated from the SP4 accession of *Agrobacterium sp.*, the latter from the LLBA accession of *Achromobacter. sp*. Whereas CP4 acts by modifying the glyphosate target site, GOX (glyphosate oxidoreductase) degrades the glyphosate molecule. Field experiments on rapeseed and maize containing herbicide resistant genes clearly showed interest of the genetic engineering technology by farmers (MARTIN and RASS, 1995).

Genetic engineering allows insertion into the plant genome genes of traits which can either control the synthesis of specific proteins, or repress synthesis of target proteins naturally present in the plant. The result is plants with new agronomic characteristics of benefits to producers. Although such biotechnological processes were demonstrated to be safe in a number of studies (FUCHS *et al.*, 1993; SIEGEL and SHADDUCK, 1989; WEHRNAN *et al.*, 1996), additional work was requested by consumers to assure that processing technologies and quality of products are unaffected.

The release of such transgenic plants into the market requires an assessment of environmental impact; agronomic behaviour, outcome of constituents resulting from the transgene when using the plant as animal or human food; toxicological and allergic risks and demonstration of analytical equivalence of grains and other consumed parts of the plant (PASCAL, 1996).

Genetically modified plants have to satisfy the current regulations that are governed by the European Guideline 90/220. Results in the literature cited above were an integral part of assessment for market release (section C of the Guideline 90/220). However, the scientific information may be difficult to interpret by non specialist consumers or manufacturers. Public acceptance plays a key role in the development of such new varieties, where farmers base adoption

of these technologies on acceptance. Accumulation and circulation of objective analytical data will allow an increased number of people to evaluate and understand those data which should dissipate to a large extent the number of unfounded concerns.

To enlighten users about the products of genetically modified plants, it was therefore desirable to carry out studies on a larger scale which should evaluate the impact of processing technologies on the quality of products. Particularly relevant is the comparison of composition characteristics and technological quality of both native and industrially processed products, when derived from parental, commercial and genetically modified maize lines.

Although the primary use of maize is for cattle feed, there is a considerable economical interest in the industrial processing of the maize grain, mainly using wet processes (starch industry) and dry processes (dry milling). In this study, focus was placed on the latter processes, a sector that has a very high added value and whose growth has exceeded 60% during the last 10 years.

The main products obtained in the dry milling process are:

- hominy, these are very large pieces of maize endosperm (larger than 3.2 mm) used for production of corn flakes;
- grits (coarse semolina particles from 1 to 3 mm), used in brewing. Grits must contain minimal amounts of lipid and thus requires complete removal of the germ;
- fine semolina (particles below 1 mm), used in extrusion-cooking, snack foods, and polenta;
- flour (particles below 200 µm), used for human consumption and feed flour;
- maize germ, from which maize oil is extracted.

In this paper, the composition and technological quality of the maize grain and of various products processed from it at a pilot scale were extensively evaluated to check whether the products derived from genetically modified maizes were equivalent to those of parental forms, as well as to conventional maize hybrids commercially available

2 – MATERIALS AND METHODS

2.1 Production of maize samples

Four pairs of maize hybrids, each represented by the parental form and by the corresponding isogenic transformed line were grown in 1997 at the same location (Lème, Pyrénées Atlantiques, France) At harvest, the 8 grain samples were obtained.

After drying at low temperature (< 40°C), each sample (150 to 200 kg at 15% humidity wet basis) was divided into 10 kg samples using a cone divider. Excess sample was processed into semolina. All samples were stored at 4°C.

The surpluses of milling products and by-products of the genetically modified samples were documented and systematically destroyed.

The grain samples submitted to the chemical, physical and technological analyses were coded as follows:

Maize Variety	Type of Endosperm	Transformation Event	Reference Sample	Genetically Modified Sample
DK 300	Flint-dent/Dent	DLL 25	W	V
ANJOU 285	Flint-Dent	TR 25	S	R
PACTOL	Flint/Flint-dent	Bt 176	N	M
Not indicated	Flint-Dent	MON 810	Z	X

Milling and extrusion experiments were carried out on all samples. Because sample size was too small for pilot experiments for brewing and oil extraction, a comparison between a pooled reference sample and a pooled genetically modified sample was conducted. The reference sample (REF) was prepared by pooling equal proportions of W, S, N and Z samples, while the genetically modified sample (TR) was prepared by pooling equal proportions of the four V, R, M and X samples.

2.2 Compositional analysis

All compositional analyses were performed at AGPM (Montardon, France) for grain, amino acid distributions and grits analyses, production and quality of the beer; at Cirad (Montpellier, France) for amylose content; at Cetiom (Paris, France) for oil analyses; and at Iterg (Pessac, France) for fatty acids.

2.2.1 Analysis of grains and milling fractions

All results are expressed as % dry basis except water content which is expressed as % wet basis.

Moisture content was determined by loss of weight in 25 g samples heated for 38 h in an oven set at 130-133°C. Protein level was estimated by determining the total nitrogen content using the Kjeldahl method. Protein was calculated from total nitrogen using N x 6.25. Starch was estimated according to the Ewers enzymatic method (3rd European Guideline CEE 72/199 of April 27th, 1972). Amylose was estimated according to a Differential Scanning Calorimetry method (MESTRES *et al.*, 1996). Fat content of grains and grits was estimated by the diethyl ether extraction method based on the European Guideline CEE 84. Cellulose was estimated according to the Weende method, based on both the French Standard NF V03-040 and on the European Guideline 73/46. Fatty acid composition was determined by the Atlantic Analyses Laboratory (La Rochelle, France), according to the Standard NF ISO 508 and expressed as g/100g fats, dry basis. Amino acid composition was determined by UCAAB (Château-Thierry and expressed in g/100g dry basis.

2.2.2 Physical characteristics of products and prediction of technological value

Thousand-Kernel-Weight was determined according to the French Standard NF V03-702. Test weight (or voluminal mass) was determined according to the French Standard NF V03-719, and expressed in $g.L^{-1}$. Granular characteristics were determined in duplicate either in a rotary screen (ROTEX, Tripette et Renaud, Paris, France) from 100-g samples, or in a 12-sieve laboratory plansifter Bühler MLU 300 (Tour Aurore, 92080 Paris Défense cedex 5, France), according to the French Standard NF X11-501. The results were expressed as median diameter of particles (d50) or log-normal mean diameter (md) and geometric standard deviation (Sg) according to the French Standards NF X11-635 and -636.

Technological value was obtained through determination of yield of raw hominy (YRH), after grain fragmentation (BENETRIX, 1997), and of yields of raw semolina (YRS) by infrared spectroscopy according to BENETRIX and LE BRAS (1999). In addition, starch value of maize grain was assessed by « Promatest », a method developed by AGPM and referenced as AGPM Q203. Promatest is based on an estimation of the effects of heat stress by colorimetric measurement of salt-soluble (heat-sensitive) proteins.

2.2.3 Grits and beer analysis

The percentage of "extract" was estimated according to the EBC Method 6.2.3, and expressed in % of the dry grits. The progress of the brewing operations was monitored by means of the wort saccharification and filtration speeds. Wort analysis at the end of the boiling step consisted of the following measurements: density (EBC Method 8.3), pH, colour (EBC Method 8.5), free amino nitrogen (continuous stream method), limit attenuation (EBC Method 8.6), amino acids (Method R-T-M-56) and fermentable carbohydrates (Method R-T-M-17).

The progress of the fermentation step was followed by measurements of density (° Plato), optical density, temperature, and diacetyl determination at the 7th and 12th days. Beer analysis consisted in measurements of apparent extract, real extract, initial extract and apparent attenuation on the initial wort (EBC Method 9.1.1), alcohol (EBC Method 9.2.1), color (EBC Method 9.4), brilliancy (EBC Method 9.16), foam stability ("Kromousse" method), bitterness (EBC Method 9.6), sodium dioxide (EBC Method 9.12), amino acids (Method R-T-M-56), and a sensory analysis (EBC Method 13.7).

2.2.4 Germ oil analysis

Peroxide value was measured according to the French Standard NF T60-220 and expressed in meq $O_2.kg^{-1}$. The acid value and the oleic acidity were determined according to the Standard NF IN ISO 660 and expressed in %. Percentage in unsaponifiable residue was determined by the quick method of hexane extraction (Standard NF T60-205-2). The phosphorus content was determined by atomic absorption, according to the IUPAC Method 2.423, and expressed in $mg.kg^{-1}$. Content and composition in fatty acids fat were determined by gas chromatography of methyl esters of fatty acids, according to the ISO 5609 and NF IN ISO 5508 Standards, and expressed in g/100 g. The composition of fatty acids fat in the position C-2 in triglycerides was determined according to the

Standard NF IN ISO 6800, with calculation of the relative percentage of the acids esterified in positions C-1 and C-3 of glycerol by difference to 100.

2.3 Processing equipments

2.3.1 Pilot semolina processing and production of maize milling fractions

The objective of this work was to prepare various products and by-products of maize semolina, comparable to those of an industrial semolina plant, from maize samples in the 150-200 kg range: hominy, semolina, grits, germ and by-products flours, offals and outer layers of grains).

All eight maize samples were processed in the pilot plant. However, considering the capacity of the machines and the amount of grains available, each sample could be only processed once (that is, unreplicated).

The milling diagram was developed in the Inra pilot plant (Montpellier). It includes the following stages: cleaning, conditioning, degermination, separation, reduction of semolinas and drying (CHAURAND *et al.*, 1999).

Grain cleaning (elimination of impurities and broken kernels) was performed in a mini cleaner Petkus [Tripette et Renaud, Paris, France]. Grain was conditioned to simultaneously aid fragmentation and separation between endosperm and outer parts, and consisted of tempering maize grains at up to 20% humidity, in two cycles.

Degermination was carried out in an impact system, or "Fragmentator" described by CHAURAND *et al.* (1993). It consisted in both splitting the grain into pieces and removing its outer envelopes (*figure 1*). Three types of products were produced: a stock flour, a by-product which corresponds to the fractions passing through to a 1.8 mm opening of the wire screen; a light "beeswing bran" pericarp product, which is recovered by aspiration; a raw hominy which consists of large endosperm fragments still contaminated by pieces of germs.

Raw hominy was further purified on a Fluidized-Bed Sorter (FBS) (Hydroméca-nique et Frottement, Andrezieux-Bouthéon, France) according to ABECASSIS *et al.* (1985) (*figure 1*). Three other types of products (QS1, QS2 and QS3) were then obtained: two fractions (QS1 and QS2) of concentrated germ, and a purer hominy product constituted only of endosperm fragments. A series of passages of the QS1 and QS2 fractions on the fluidized-bed sorter permitted collection of a germ fraction for the oil extraction process. The mechanical sieving of fractions QS3 on a 3-mm sieve allowed us to obtain a pure hominy (*i.e.* particles of endosperm particles having by definition a size larger than 3.2 mm and a lipid content less than 1%).

The reduction of fraction QS3 was done in the semolina pilot plant (flow: 150 kg/h) described by ABECASSIS and CHAURAND (1997) (*figure 1*). Two types of granulations were produced: (i) grits (endosperm particles ranging between 1 and 3 mm), and (ii) semolina (particles ranging between 0.2 and 1.0 mm). The pilot semolina plant used conventional roller mills for the reduction of particles and plansifters and a purifier for the separation and purification of products. Grits were obtained from the purifier after passage of products through the break rolls B1, B2 and B3. Semolina underwent a supplementary reduction step on a sizing roll. Flours and offals were discarded as by-products. All products were dried to a moisture content in a 14-15% w.b. range and then stored at 4°C.

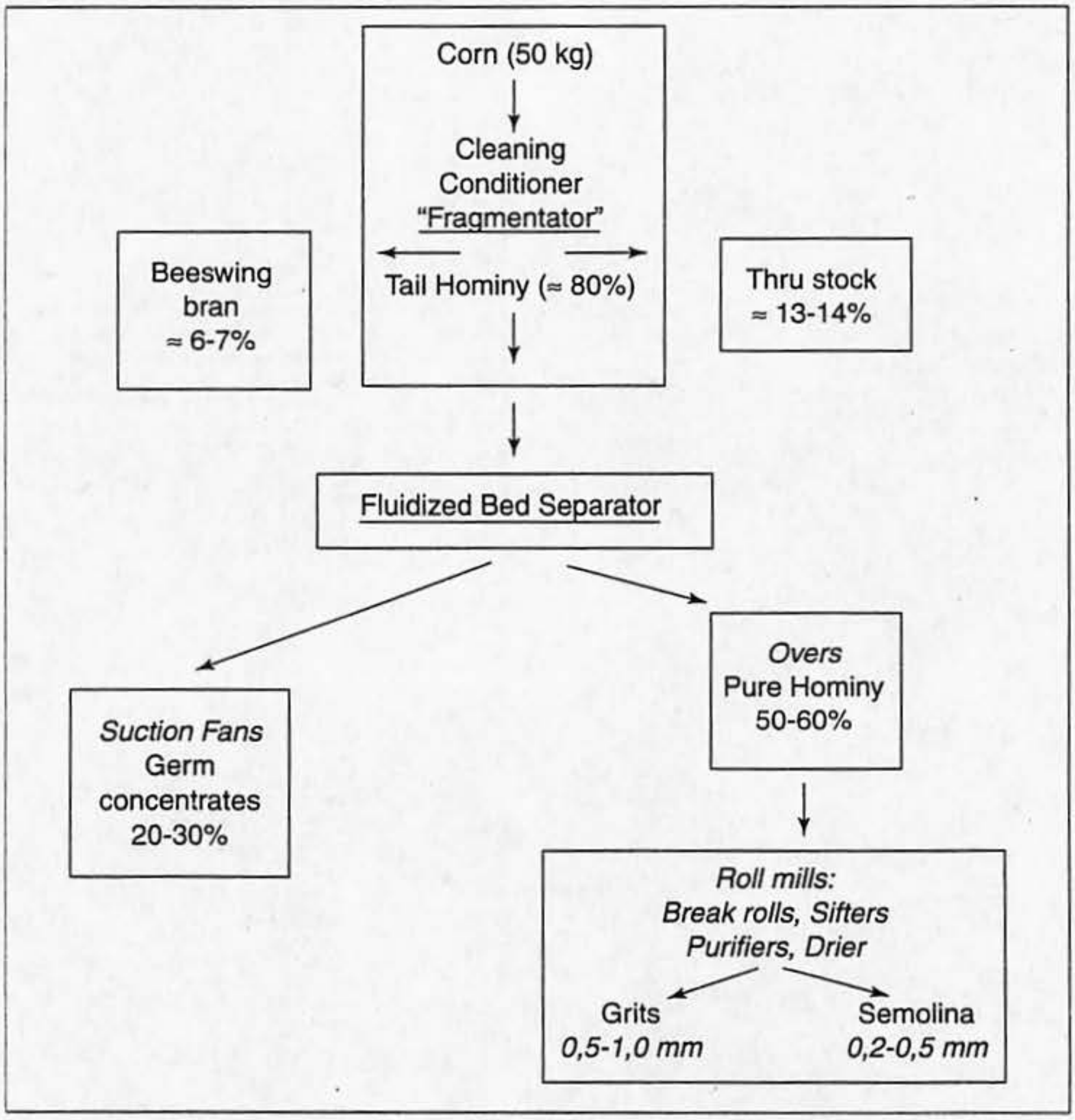

Figure 1

Flow chart for pilot dry milling of corn (From Chaurand *et al.*, 1993, and Abecassis and Chaurand, 1997)

2.3.2 Pilot extrusion process

Chart of tests

Semolina was homogenized during 7 min in a Hobart PF 800 mixer (39, rue Cambon, 75001 Paris, France). A representative test sample was taken for the granulometric determination.

Extrusion was performed in a Clextral extruder, type BC 45 (B.P. 10, 42701 Fiminy cedex, France). This extruder was equipped with: i) a two-screw feed regulator designed for powders and calibrated to prevent variations in granulation

and humidity, and ii) a piston pump FKM for the water supply. The semolina consumption by test was about 19.0 to 21.8 kg, and the effective consumption of a bearing varied in the 3.7 to 6.4 kg range depending on difficulties of stabilization. Values of the main parameters were directly noted from the operating cabinet and recorded in parallel by means of a Yew 3087 multichannel data acquisition system.

Extruded product corresponding to the consumption of a bearing was collected in a 0.28 m^3, punched bottom, air-cooled container. A fraction of the product was recovered under the shape of a cast to allow measurement of diameter expansion, whereas density was directly measured on the extruded product.

To avoid a possible influence of the adopted sequences of analyses, samples resulting from each variety pair were extruded on the morning of a first day (replicate n° 1) and the afternoon of a second day (replicate n° 2). For each pair, the parent sample was always tested before the genetically modified sample.

Controls and measurements

Values for the three variables of the extrusion were expressed either as means calculated from the control panel of the extruder, or values measured on the recording sheets and expressed as means and standard deviations in mm. The output flow ($kg.h^{-1}$) was determined (3 to 5 replicates) by weighing the amount produced in 1 min. The specific mechanical energy (that is the amount of mechanical energy required to produce one ton of extrudate) expressed in $kWh.t^{-1}$, was calculated by the equation:

$$EMS = (1.642\ N\ I - 0.8\ I^2) / Q$$

With:[6]

N = rotation speed of screws (min^{-1})

I = absorbed electric intensity (A)

Q = output flow of the extruder ($kg.h^{-1}$)

The diameter of the extrudate was measured by means of slide calipers and the diameter expansion was expressed by the extrudate diameter : die diameter ratio. The density of the loose extrudate was weighed after leveling a capacity of 1.22 L. In both cases, the measure was repeated 10 times.

2.3.3 Brewing process

Micro brewing protocol

Micro brewing was carried out once on the two pooled maize grits samples, respectively REF and TR, according to the procedure R-T-N-9. Grits were tested at a 45% level, that is 2.7 kg of grits were mixed with 3.3 kg of barley malt, for a total of 6 kg solid matters in 18 L of water.

Brewing Pattern

For the mashing phase, 2.7 kg of grits were mixed with 15% malt (405 g) and pasted in 10 L of water during 10 min at 50°C. The mixture was then raised for 10 min at 75°C, then warmed and maintained for 15 min at 100°C, then cooled to 45°C by addition of cold water (final volume, 18 L). The remainder of

malt (2.9 kg) was added into the tank and pasting continued during 20 min at 45°C. The mixture was then warmed and maintained for 20 min at 64°C, then raised and maintained for 30 min at 74°C. The final wort was filtered on a tank-filter, with a hot water wash. After adding CO_2 extracts of hops, the wort was boiled for 90 min.

The progress of the brewing operations was monitored through the rate of saccharification and of wort filtration.

Fermentation, maturation and stabilization

The main fermentation was carried out at 12°C, with the use of a dry active yeast (15 to 20.10^6 cells per mL of wort), during a time dependent on the rate of diacetyl reduction. The beer was matured for at least 7 days at 0°C. The beer was then membrane-filtered and pasteurized.

The progress of the fermentation was monitored through measurements of density (°Plato), optical density, temperature, and diacetyl content at the 7th and 12th days.

2.4 Processes of extraction and refining of germ oil

The oil extraction was carried out without replication on the two pooled maize germ samples, respectively REF and TR.

The germ was flattened as flakes by passing through the pair of rolls of a DAMMAN CROES flattener, type H 500*500 (Damman-Croes N.V., Spanjestraat 55, 8800 Roeselare, Belgium). The extraction itself was carried out by six hexane washings (each including a 5 min discharge of solvent, a 15 min recycling, and a 20 min filtration) in a GUEDU ML 750 continuous extractor (total volume, 470 L; filter surface, 0.71 m^2; Guedu, Semur-en-Auxois, 21140 France) heated by water circulation at an average temperature of 50°C.

After extraction, the cake was transferred into a two-stage purifier in which it is heated to 105°C under steam injection in the filter. The product was maintained during 30 min under partial vacuum (< 1500 mbars), then air-cooled for 15 h. The extract was concentrated in the filter for oil by heating under vacuum in a rotary evaporator maintained at 70°C for 7 h. The concentrated oil was finally heated under vacuum in a glass rotary evaporator to remove solvent.

Throughout each of the six successive operations of extraction and solvent removal of each of the germ samples, duration, temperature and pressure of all steps were recorded. The volume of hexane used was noted and a filtration index ($L.dm^{-3}.h^{-1}$) was calculated.

Oil was refined by a sequence including: mucilage removal with water, phosphoric acid conditioning, neutralization, washings with distilled water (30 min at 60°C, 3% water), drying, wax crystallisation and removal, bleaching, filtration and steam deodorization.

Volumes and weights of each product were recorded at the various stages: germs, flakes, oil, oilcake, mucilage removal, neutralization, bleaching. Amounts of "potential oil" and "potential oilcake" were then calculated.

3 – RESULTS AND DISCUSSION

The strategy employed to assess the equivalence of genetically modified maize grains to their respective parental hybrids was to process them on a pilot scale so as to allow comparison of a maximal number of parameters of composition and technological quality in both grains and various industrially processed maize products.

3.1 Milling of maize samples

The settings of the various machines in the semolina milling diagram were maintained for all maize samples. The pilot experiment allowed subsequent processings of grain (extrusion, brewing, oil extraction) without introducing any processing difficulty other than those resulting from the endosperm texture, as dent types yield more fine particles of flour and less semolina or hominy than vitreous, horny types.

The values of yield in the various products collected at the successive milling stages are presented in *table 1*, as follows:

- At the cleaning level: cleaning losses;
- In the Fragmentator: yields in overs of the wire screen and in raw hominy (YRH);
- In the Fluidized-Bed Sorter: yields in purified endosperm fragments (QS3) and in pure hominy (YPH) (overs of a 3.15 mm sieve).

Table 1

Comparative yields of the various maize products produced at the various stages of the dry-milling process

Cleaning Samples	Cleaning Losses (in % w.b.)							
	W	V	S	R	N	M	Z	X
	4.9	5.4	1.1	1.5	3.0	3.5	2.6	2.7

Fragmentator Samples	Yields in % d.b.							
	W	V	S	R	N	M	Z	X
Overs	66.1	67.3	78.2	77.7	78.7	78.9	76.7	77.9
YRH	50.1	52.7	63.4	62.2	60.5	59.9	49.3	55.1

FBS Samples	Yields in % d.b.							
	W	V	S	R	N	M	Z	X
QS3	70.0	68.6	63.8	63.6	64.6	64.8	66.7	64.9
Hominy (YPH)	31.5	31.7	35.5	37.1	37.2	38.4	30.8	34.7

Whole Milling Samples	Yields in % d.b.							
	W	V	S	R	N	M	Z	X
Endosperm fragments	31.6	31.4	36.9	35.7	37.9	37.8	37.7	36.5
Flours	29.2	26.9	18.4	19.6	19.2	20.1	21.9	21.3
Germs and offals	39.2	41.7	44.6	44.8	42.9	42.1	40.5	42.2

Finally, overall effects were calculated by taking into account all the milling operations and after grouping together the homologous fractions. Specific yields were expressed in pure endosperm fragments (that is hominy + grits + semolina), in fine fractions (that is through products of the Fragmentator + reduction flours), and in germs and offals (that is QS1 + QS2 + pericarp + overs of the purifier and of the B3 plansifter).

The yields in the various milling fractions clearly differentiated the maize samples (*table 1*). For instance, it appeared that some hybrids (S-R and N-M pairs) are characterized by a greater YRH, whereas the W-V pair yielded a greater proportion of flours.

These results are in good agreement with the physical characteristics of grains as reported in the literature. They do not totally agree, however, with the behaviour which could be deduced from the type (horny, dent) of endosperm characterizing the hybrid. For instance, the S-R pair yields a greater proportion of hominy, and a smaller proportion of flour, than could be expected from a dent type.

Within each pair of hybrids, no measurable differences could be noted between the yields in the various milling fractions, respectively the parent hybrid and the genetically transformed one.

Granulometric characteristics of grits and semolina (data not shown) of the products were also highly comparable, regardless of hybrid.

Therefore, these first results of maize milling:

- confirm the validity of the experimental plan, as all observed values are in range of values observed for commercial samples of grain, both regarding yields in fractions and granular characteristics,
- demonstrate differences of behaviour between the various pairs of hybrids, as expressed in different proportions of hominy, semolina, flours, offals, etc., which are the most relevant parameters in the specification of grain for processing in the dry milling industry,
- show that differences within each of the pair of hybrids (between the regular and the transformed hybrid) are much smaller than those observed between some flint/flint-dent, flint-dent/dent and dent types of grain.

However, the experimental chart of the milling process was limited by produce and supply, from the 150 kg of maize grains available per hybrid, sufficient amounts of products comparable to those used by the industry. In these conditions, because the pilot milling experiments could not be run in duplicate, there was no possibility to calculate standard deviation of yields in the various milling

fractions, or to suggest an indisputable ranking of the various hybrids on the basis of their milling quality.

3.2 Compositional analysis of grains

The main data on chemical composition of the grains of parent hybrids (W, S, N Z) and of the corresponding transformed ones (V, R, M, X) are presented in *table 2*. Amylose contents and gelatinization temperatures are presented in *table 3*. Fatty acid and amino acid compositions were also analyzed (data not shown). In all cases, the regular sample of the hybrid pair was analyzed before the transformed one.

Table 2

Chemical composition of grain and milling fractions from parent and genetically modified maize hybrids

Samples	W	V	S	R	N	M	Z	X
Starch (% d.b.), enzymatic method	75.8	75.2	76.0	76.6	80.6	80.3	77.4 *(75.1)*	74.7 *(75.4)*
Total Nitrogen (% d.b.), Kjeldahl method	11.16	11.08	9.00	9.04	9.03	9.51	8.65	9.30
Fat content (% d.b.), hexane extraction	4.1	4.1	4.4	4.4	4.1	4.2	3.9 *(3.9)*	4.2 *(4.1)*
Cellulose (% d.b.), Wende method	2.7	2.8	2.5	2.4	2.6	2.6	2.2	2.4

(0,0) : duplicate analysis

Table 3

Amylose content and gelatinization temperature

Samples	W	V	S	R	N	M	Z	X
Amylose content (in % d.b.)	21.6	21.0	21.7	21.7	21.5	22.7	22.4	21.6
Gelatinization temperature (°C)	65.3	63.0	61.1	62.5	63.0	60.6	61.5	62.5

From all the four previous analyses, it appeared that no significant difference could be found in terms of fat content, cellulose content, fatty acids and amino acids compositions.

In contrast, the hybrids pairs differed in their protein or starch content, the W-V pair having a significantly higher protein content than the three other pairs, whereas the N-M pair had the highest starch content, exceeding 80%.

On the other hand, within each pair of hybrids, no difference could be seen in the above mentioned parameters of chemical composition ($p>0.05$), demonstrating that respective differences between the regular and the transformed forms on protein or starch content were much less than those noted between the various flint/flint-dent or flint-dent types of hybrids. The transformed hybrid M had greater percentage of amylose (22.7) than the sister hybrid N (21.5; $p<0.05$), logically associated with a cooler (60.6°C) gelatinization temperature for M than for N (63.0°C). The analytic data of all samples clearly fall into the range of conventional commercially available maize hybrids.

3.3 Physical characteristics of grains and prediction of technological value

Thousand kernel weight and specific weight data are presented in *table 4*. Prediction of suitability to dry-milling process through Promatest, yield in raw semolina and yield in raw hominy are presented in *table 5*.

Table 4

Physical characteristics of grains from regular and genetically modified maize hybrids

Samples	W	V	S	R	N	M	Z	X
Thousand Kernel Weight (g)	354	360	276	269	292 *(311)*	320 *(330)*	383 (-)	417 (457;427)
Specific Weight (g)	822	833	797 *(793)*	821 *(816)*	809	821	759	783

(0,0) : duplicate analysis

Table 5

Prediction of suitability to dry-milling process of grains from regular and genetically modified maize hybrids

Samples	W	V	S	R	N	M	Z	X
Promatest †	28.9	31.7	38.9	37.7	40.7	41.7	43.1	42.6
YRS ‡ (%) (Infrared)	69.4	69.1	67.1	67.9	68.5	67.9	64.9	65.5
YRH § (%) (Fragmentator)	58.6	60.2	51.2	51.0	55.2	54.1	48.2	49.9

† < 9: poor; 9 to 17.9: acceptable; 18 to 26.9: rather good; 27 to 35.9: good; ≥ 36: very good
‡ < 67: poor (generally dent types); 68 to 70: good (generally flint types)
§< 50: poor; 52-55: good; ≥ 55: very good

The hybrid pairs differed in most of their physical criteria, as these hybrids had different endosperm textures: flint/flint-dent for N-M, flint-dent/dent for W-V, and flint-dent for S-R and Z-X.

Differences in Thousand Kernel Weight (for example, least values for the S-R pair) and in Test Weight (for example, least values for the Z-X pair) were therefore observed, associated with differences in cumulating calibration characteristics.

Concerning technological characteristics, so far as the specific conditions of sample production (manual harvest, low temperature drying) permit, the following observations were made:

- "very good" starch value (through "Promatest") for all samples, excepted for the W-V pair ("good" value only),
- "good" semolina value for all samples, excepted for the Z-X pair, a dent type ("poor" value)
- higher hominy value of the W-V pair, a flint-dent/dent type.

Within each pair of hybrid, no difference could be noted in most of the above mentioned parameters of chemical composition and of physical or technological characteristics, demonstrating that respective differences between the regular and the transformed form were much less than those noted between the various flint/flint-dent or flint/dent-dent types of hybrids.

Also, a difference ($p<0.05$) was observed for the specific weight, which was systematically greater (by 1.3-3.6%) in the transformed grains than in the regular ones, the former having a more dense texture of their endosperm than the latter.

3.4 Suitability to the extrusion process

3.4.1 Granulometric characteristics of semolinas

Before the extrusion experiments, semolinas, simply isolated by sieving, may have variable granular characteristics within a given granulation class. At constant grain humidity, the presence of smaller semolina particles (revealed by a greater median diameter (d_{50}), or by a greater calculated specific surface) usually indicates a more floury texture of the maize endosperm.

Regardless of the mode of expression, the granular characteristics of the semolinas (detailed data not shown) had little variance (d_{50} = 386-420 µm) and were similar to a commercial semolina used in preliminary trials (d_{50} = 394 µm). Small differences appeared between the hybrids pairs. For instance, the semolinas of the W-V were finer than those of the S-R pair. The other hybrids were intermediate. The semolinas of the genetically modified hybrids looked slightly coarser than those of the regular hybrids, except for the W-V pair for which the inverse tendency was observed.

3.4.2 Behaviour during the extrusion process

Performances of the extruder

The hourly output results from both feeding input and from water steam evaporation during expansion of the product as it exits the die. As such, it is an indication of the average thermal conditions in the extruder. The specific mechanical energy expresses the importance of the mechanical work produced

by the screws, and can be connected to a degree of macromolecular transformation of the raw material. These two parameters are valuable economical indicators for industrial production.

The mean hourly outputs ranged from a 24,8 to 25,7 kg.h^{-1} and are not different both between hybrid pairs or between any parental hybrid and the corresponding transformed one ($p<0.05$) (*table 6*). The same was true when considering the specific mechanical energy, except for the Z-X pair for which a slight average reduction of 3 to 5 kWh.t^{-1} of the consumed energy was observed. On the other hand, there was only a very slight decrease (–0.8%) of the average consumed energy in the genetically modified hybrids compared to the regular ones.

Table 6

Suitability to the extrusion process of the maize samples:
Monitoring of extrusion through Mass Flow and Specific Mechanical Energy (†)

	Samples	W	V	S	R	N	M	Z	X
Replicate 1	Flow (kg.h-^{1})	25.2	25.0	25.7	25.4	25.3	25.0	25.6	25.2
	Energy (kWh.t-^{1})	142	143	147	141	142	140	140	135
Replicate 2	Flow (kg.h-^{1})	–	–	25.0	25.0	24.9	25.5	24.8	25.7
	Energy (kWh.t-^{1})	–	–	144	144	144	141	134	141
(†) calculated from the formula: SME = (1,462 NI – 0.8 I^2) / Q									

Parameters of the extruder operation

The extruder operation was monitored using the three following parameters recorded from the control panel: electrical intensity absorbed by the principal engine, the pressure on the screw shafts, and the product temperature on contact before it goes through the die.

Very slight differences were found in the operation of the machine (data not shown). At the very most, a slightly lower pressure on the screw shaft (– 9.1 %), which is at the borderline of the significance, could be noted, in both replicates, with the Z-X pair of hybrids. Taking this difference into account, a higher apparent viscosity of the material in the extruder is expected for the Z-X pair, associated with a lower expansion rate of the extruded product. The difference in the behaviour of products deriving respectively from parent and from transformed hybrid was negligible, < 1%.

3.4.3 Characteristics of the maize extrudates

The first indicator of the degree of expansion is the diameter expansion, that expresses an extrudate diameter: die diameter ratio. The second one is the density in bulk, a practical criterion that affects the filling of bags (for example in the case of snack biscuits). These two criteria do not have the same significance and are not correlated between each other ($r = – 0,19$). For instance, differences in density indicate not only changes in volume, but also in shape and therefore in flow and spatial arrangement of the products (MELCION, 1987).

The results presented in *table 7* suggested an important variation in the bulk density between the various products, as it is the usual practice in the extrusion processes. Bulk density values range from 64.2 to 86.0 kg.m^{-3} (*i.e.* a 29% range from the mean value, and a coefficient of variation of 8%). In contrast, the expansion rate parameter was a less cause of variation as its values range from 2.98 to 3.35 mm (*i.e.* a range of 11,7% only, and a coefficient of variation of 3%). These values were quite comparable to those obtained in the same conditions from a commercial semolina used in preliminary trials, 59 to 60 kg.m^{-3} and 3,5 to 3,6 mm, respectively.

Table 7

Diameter expansion and bulk density of the extruded products
(mean values, and range of variation)

	Samples	W	V	S	R	N	M	Z	X
Rep. 1	Diameter Expansion (mm)	3.33 (0.16)	3.27 (0.08)	3.30 (0.16)	3.23 (0.11)	3.40 (0.11)	3.30 (0.15)	3.24 (0.09)	3.30 (0.09)
	Voluminal mass (kg.m^{-3})	80.5 (1.0)	76.6 (1.0)	73.2 (1.2)	64.2 (1.6)	71.3 (0.8)	75.4 (1.1)	83.4 (1.2)	78.3 (0.9)
Rep. 2	Diameter Expansion (mm)	–	–	3.26 (0.12)	3.11 (0.09)	3.25 (0.07)	3.26 (0.12)	2.98 (0.12)	3.35 (0.10)
	Voluminal mass (kg.m^{-3})	–	–	68.9 (0.7)	67.1 (0.7)	74.1 (0.8)	76.7 (0.7)	86.0 (1.3)	77.4 (1.3)

Especially on the first trial, it was apparent from an analysis of variance (data not shown), that the density was significantly affected by the type of maize hybrid. On the basis of a decreasing density, partly associated with an increasing diameter expansion, the ranking of the four pairs of hybrids was the following:

Z-X (greatest density) > W-V > N-M > S-R (least density)

No significant difference was observed for the diameter expansion parameter, the density was significantly lesser (–4,6%) in the genetically modified hybrids than in the corresponding parents.

3.4.4 Conclusion on the suitability of the maize samples to the extrusion process

With reservations due to experimental design (only two replicates for the extrusion experiments, and only one for the W-V pair of hybrids), the following observations are made:

- The small differences observed in the behaviour of the various maize semolinas during the extrusion process were more apparent between hybrids than between a parent and its genetically modified form. The very small decrease in energy consumed (average: 1%) observed with the transformed hybrids was a rather favorable industrial factor, but without practical significance in the context of the present pilot experiments.
- The samples were more clearly discriminated by their bulk density than by their diameter expansion. The differences observed for this measure

between the pairs of hybrids were significant ($p < 0.05$). They were partly associated to the trends observed during the extruder operation: to the greatest density and least diameter expansion observed for the Z-X pair of hybrids correspond to least specific energy and pressure on the screw shafts.

- In contrast to the diameter expansion, that indicated no difference between the regular and the transformed hybrids, the bulk density seemed to be slightly decreased in three of the four hybrids by about 4% (another rather favorable industrial factor) by the genetic modification.
- It was confirmed that the differences in density were connected to the amylose: amylopectin ratio in the maize starch (DELLA VALLE *et al.*, 1987). In the three hybrids in which the genetic modification seemed correlated to a decrease of the density, there was also a decrease of the amylose content. Whereas in the only hybrid (N-M) showing the opposite behaviour (increase of the density), an increase of the amylose content, with a decrease of the gelatinization temperature of starch, was observed (*table 3*).

3.5 Production and quality of the beer

In this section, the assessment of the equivalence of genetically modified maize grains to their respective parental hybrids was studied by collecting a number of parameters of composition and quality of the raw material (grits) and of the beer between a pooled reference sample processed in duplicate (REF 1 and REF 2) and a pooled genetically modified sample also run in duplicate (TR1 and TR2).

The compositional parameters of the grits are presented in *table 8*. The main parameters of the brewing process (saccharification speed at 74°C, time for wort filtration) were monitored (data not shown). The analysis of the worts at the end of the boiling step is presented in *table 9*. The composition in fermentable sugars at the end of the boiling step was investigated (data not shown). The monitoring of the fermentation process is presented in *table 10*. In addition, all parameters and curves of fermentation were recorded (data not shown).

Table 8

Analysis of grits

Samples / Analyses	REF	TR
H20 (%)	13.9	14.7
Extract (% grits d.b.)	91.2	91.6
Lipids (% d.b.)	0.54	0.46

Table 9

Analysis of worts at the end of the boiling step

Analyses \ Samples	REF 1	REF 2	TR 1	TR 2
Extract (° Plato)	12.44	12.65	12.50	12.58
pH	5.75	5.68	5.63	5.86
Color (° EBC)	5.0	4.8	10.6	4.75
Free amino nitrogen ($mg.L^{-1}$)	115	110	105	110
Limit attenuation (%)	80.7	81.1	81.6	81.2

Table 10

Monitoring of the fermentation process

Parameters \ Samples	REF 1	REF 2	TR 1	TR 2
Fermentation speed during days 1, 2 and 3 (° Plato per day)	1.34	1.41	1.57	1.57
Density at half-fermentation (° Plato)	7.38	7.50	7.38	7.33
Half-fermentation time	96	84	80	82
Diacetyl at the 7th day ($mg.L^{-1}$)	0.88	0.62	0.88	–
Diacetyl at the 12th day ($mg.L^{-1}$)	0.10	0.10	0.11	0.09
Apparent extract at the beginning of maturation (° Plato)	2.30	2.23	2.13	2.20
Apparent attenuation at the beginning of guard (%)	81.5	82.4	83.0	82.5

From all the parameters analyzed, it appears that practically none of the analyses carried out on the grits and on the beer shows a appreciable difference between the reference sample and the genetically transformed one.

Only a minor difference could be noted for the time of wort filtration that is slightly shorter (63 to 70 min, a rather favorable industrial factor) in the TR samples than in the reference samples (80 to 82 min), considering that, in the pilot process used, the usual filtration time is in the order of 60 min.

3.6 Production and quality of the maize germ oil

The oil extraction was carried out in single on the two pooled maize germ samples, respectively REF and TR .

The records of temperature and pressure during the stages of oil extraction and solvent removal (results not shown) did not reveal any technical problem. Filtration was easy (about 5 $L.dm^{-2}.h^{-1}$) and no difference was noted between the REF and TR samples.

3.6.1 Balance of the dry matters

It appears that the process allowed complete separation of oil and germ (*table 11*). The residual fat content of the cakes was less than 1% d.b. No noticeable difference could be observed between the behaviour, during oil extraction, of the REF and TR samples. The minor differences noted on the oil and the oil-cake between the potential and the effectively recovered oil quantities fall in the range of the losses usually recorded on this CETIOM pilot plant.

Table 11

Balance of dry matters during the process of oil extraction from maize germs

	REF			TR		
	Weight (kg)	Dry matter (% on raw)	Fats (% d.b.)	Weight (kg)	Dry matter (% on raw)	Fats (% d.b.)
Germs	48.6	90.03	21.50	76.3	89.3	19.97
Flakes	43.3			60.2		
Oil	9			11		
Oil-cake	29.5	91.69	0.81	44.5	90.62	0.50
Potential oil (kg)	9.1			13.3		
Potential oil-cake	33.6			47.8		

3.6.2 Physico-chemical characterization of the raw oil

The most relevant and commonly used parameters to determine the composition and quality of both the raw and the refined oils were determined and presented in *table 12*. In theory, one could have also investigated the sterol fraction. However, the sterol analysis was not retained, as this fraction has never been reported as relevant for the quality of maize oils.

Table 12

Physico-chemical characteristics of maize germ oils. Comparison of the REF and TR samples and effect of the refining process

	REF	TR
Raw oils		
Indice of peroxide	5.32 meq O_2/kg	8.82 meq O_2/kg
Oleic acidity	2.2005%	2.9705%
Unsaponifiable residues	1.1405%	1.1405%
Phosphorus content	800±1005 mg/kg	1000±1005 mg/kg
Refined oils		
Indice of peroxide	1.2 meq O_2/kg	0.4 meq O_2/kg
Oleic acidity	0.07%	0.08%
Unsaponifiable residues	1.05%	1.03%
Phosphorus content	21 mg/kg	< 5 mg/kg

It appears that the raw oil of both REF and TR samples contains very small amounts of peroxide, respectively 5.3 and 8.8 meq $O_2.kg^{-1}$. These trace amounts are not different, as they are less than the acceptable value in this type of oil (Codex Alimentarius, 23rd Session, July 1999).

The contents of free fatty acids and of phospholipids (phosphorus) are slightly greater in the oil obtained from the TR germ sample. It is well known that large values for these two parameters of oil may result in difficulties during the refining process, or in a lesser yield in refined oil (DENISE, 1983). However, for both the REF and the TR oils, the contents in free fatty acids and in phospholipids remain at a quite usual level for such raw oils processed from flattened and hexane-extracted germs, that are on the order of 2.0 to 4.0% for the oleic acidity, and of 1.0 to 1.5 $g.kg^{-1}$ for the phosphorus (Iterg data). Contents of unsaponifiable residues are identical for the two REF and TR samples.

3.6.3 Physico-chemical characterization of the refined oil

As expected from the refining stages, the refined oils from both the REF and the TR samples can be considered as "normalized" (*table 12*), as they contain extremely small levels of peroxides. For instance, the peroxide contents are respectively 1.2 and 0.4 meq $O_2.kg^{-1}$ (trace amounts, that are not measurably different); the oleic acidities, respectively 0.07 and 0.08, are also far less than the 0.3% standard; the phospholipids are practically removed, with respective values of 21 and < 5 $mg.kg^{-1}$, (trace amounts, that are not significantly different). Some constituents of the unsaponifiable fractions were also removed by refining, in an equivalent way for the two REF and TR oil samples.

3.6.4 Fatty acids composition and distribution

Fatty acid composition and the total fatty acid content of the REF and TR oils appear equivalent (*table 13*).

Table 13

Distribution (%) and total fatty acid content g/100 g in REF and TR samples

Fatty acid	REF	TR	"Reference" (†)
14:0	< 0.1	< 0.1	0-0.3
16:0	11.7	11.8	8.6-16.5
16:1	0.2	0.2	0-0.5
17:0	< 0.1	< 0.1	0-0.1
17:1	< 0.1	< 0.1	0-0.1
18:0	1.8	1.8	0-3.3
18:1	26.3	26.9	20-42
18:2	57.5	56.6	34-65
18:3	1.0	1.0	0-2.0
20:0	0.4	0.4	0.3-1.0
20:1	0.3	0.3	0.2-0.6
22:0	0.1	0.1	0-0.5

Fatty acid	REF	TR	"Reference" (†)
22:1	0.3	0.2	0-0.3
24:0	0.1	0.2	0-0.5
24:1	0.1	0.1	0-0.1
Non identified	0.2	0.4	
Content in total fatty acids (g/100 g)	98.0	97.3	
(†)Typical composition of maize germ oils (CETIOM Data).			

The position of the various fatty acids on the three respective hydroxyl groups (C-1, C-2 and C-3) of the glycerol were investigated. Fatty acids are not randomly distributed on glycerol due to the action of enzymes during lipid biosynthesis which confers to this distribution its specificity. The distribution of fatty acids on the respective positions C-2 and C1+C3 between the REF and the TR oils were not different (*table 14*).

Table 14

Respective positions of the triglycerides in REF and TR oils

Fatty acid	REF Oil		TR Oil	
	Position C – 2	Positions C1 + C3	Position C – 2	Positions C1 + C3
16:0	2.2	97.8	2.2	82.1
18:0	3.7	96.3	3.7	96.3
18:1	32.8	67.2	32.7	67.3
18:2	41.7	58.3	41.6	58.4
18:3	33.3	66.6	30.0	70.0
20:0	8.3	91.7	8.3	91.7

4 – CONCLUSIONS

This study was aimed at investigating the possible equivalence of four modified genetically hybrids, to confer levels of resistance to European maize borer and/or tolerance to glufosinate or glyphosate herbicides, with their respective parental hybrids. The strategy employed was to process maize grains and various intermediate products (semolina, grits, germs) at a pilot scale so as to allow comparison of a maximal number of parameters of composition and technological quality.

Among the numerous compositional analyses that were carried out, and that include the most important and the most relevant parameters of quality characterization, nearly all showed either no difference, or only slight differences that

always clearly fell within the range of commercially available hybrids. Obviously, the various types of genetic modifications investigated did not unsettle either the composition or the technological quality of the maize grain and of products processed from it.

This main conclusion is in general agreement with all previous studies carried out with a similar purpose on feeding value of Bt-maize (BRAKE and VLACHOS, 1998; FAUST and DeWITT, 1998), of glyphosate-tolerant maize (SIDHU *et al.*, 2000), composition of glyphosate-tolerant cottonseed (NIDA *et al.*, 1996), composition (PADGETTE *et al.*, 1996) and feeding value (HAMMOND *et al.*, 1996) of glyphosate-tolerant soybeans.

A few instances have indicated some minor differences between a parental hybrid and its genetically modified form. For instance:

- a minor difference was observed for the specific weight, which was systematically greater (by 1.3 to 3.6%) in the transformed grains than in the regular ones, the former having a more dense texture of their endosperm than the latter;
- a minor difference was found for the amylose level, the transformed hybrid M having 22.7% and the regular hybrid N having 21.5%, this was associated with a lesser (60.6°C) gelatinization temperature for M than for N (63.0°C);
- the bulk densities of the maize extrudates seemed to be slightly decreased, in three of the four hybrids, about 4%, by genetic modification.

Although these differences are minor and favorable quality factors, the data fall largely within the established ranges reported in the literature for maize. Therefore, it cannot be concluded that they result from the random insertion of synthetic constructions or possibly from pleiotropic effects interfering with the natural regulation of the maize genes.

The conclusion based on such a large number of data is that the four genetically modified maize hybrids are not different from their respective parental lines, or other commercial maize hybrids.

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ABBREVIATIONS

AFNOR, Association Française de Normalisation ; AGPM, Association Générale des Producteurs de Maïs ; CETIOM, Centre Technique Interprofessionnel des Oléagineux Métropolitains ; CIRAD, Centre de Coopération Internationale en Recherche Agronomique pour le Développement ; EBC, European Brewery Convention ; GLP, Good Laboratory Practices ; INRA, Institut National de la Recherche Agronomique ; ISO, International Standard Organisation ; ITERG, Institut des Corps Gras ; IUPAC, International Union of Pure and Applied Chemistry ; OD, optical density ; YRH, Yield in Raw Hominy ; YRS, Yield in Raw Semolina.