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Series on the Safety of Novel Foods and Feeds, No. 6

**CONSENSUS DOCUMENT ON COMPOSITIONAL CONSIDERATIONS FOR NEW VARIETIES OF
MAIZE (Zea Mays): KEY FOOD AND FEED NUTRIENTS, ANTI-NUTRIENTS AND SECONDARY
PLANT METABOLITES**

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No. 2, Consensus Document on Compositional Considerations for New Varieties of Soybean: Key Food and Feed Nutrients and Anti-nutrients (2001)

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OECD Environmental Health and Safety Publications

Series on the Safety of Novel Foods and Feeds

No. 6

**Consensus Document on Compositional
Considerations for New Varieties of Maize
(*Zea Mays*): Key Food and Feed Nutrients,
Anti-nutrients and Secondary Plant
Metabolites**

Environment Directorate

Organisation for Economic Co-operation and Development

Paris 2002

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FOREWORD

The OECD's Task Force for the Safety of Novel Foods and Feeds decided at its first session, in 1999, to focus its work on the development of science-based *consensus documents*, which are mutually acceptable among member countries. These consensus documents contain information for use during the regulatory assessment of a particular food/feed product. In the area of food and feed safety, consensus documents are being published on the nutrients, anti-nutrients or toxicants, information of its use as a food/feed and other relevant information.

This consensus document addresses compositional considerations for new varieties of maize by identifying the key food and feed nutrients, anti-nutrients and secondary plant metabolites. A general description of these components is provided. Also included are considerations to be taken when assessing new maize varieties, including suggested analyses.

The Netherlands and the United States served as the lead countries in the preparation of this document.

The Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology has recommended that this document be made available to the public. It is published on the authority of the Secretary-General of the OECD.

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Preamble

Food and feed products of modern biotechnology are being commercialised and marketed in OECD Member countries. The need has been identified for detailed technical work aimed at establishing appropriate approaches to the safety assessment of these products.

At a Workshop held in Aussois, France (OECD 1997), it was recognised that a consistent approach to the establishment of substantial equivalence might be improved through consensus on the appropriate components (e.g., key nutrients, key toxicants and anti-nutritional compounds) on a crop-by-crop basis, which should be considered in the comparison. It is recognised that the components may differ from crop to crop. The Task Force therefore decided to develop consensus documents on compositional data. These data are used to identify similarities and differences following a comparative approach as part of a food and feed safety assessment. They should be useful to the development of guidelines, both national and international and to encourage information sharing among OECD Member countries.

These documents are a compilation of current information that is important in food and feed safety assessment. They provide a technical tool for regulatory officials as a general guide and reference source, and also for industry and other interested parties and will complement those of the Working Group on Harmonisation of Regulatory Oversight in Biotechnology. They are mutually acceptable to, but not legally binding on, Member countries. They are not intended to be a comprehensive description of all issues considered to be necessary for a safety assessment, but a base set for an individual product that supports the comparative approach. In assessing an individual product, additional components may be required depending on the specific case in question.

In order to ensure that scientific and technical developments are taken into account, Member countries have agreed that these consensus documents will be reviewed periodically and updated as necessary. Users of these documents are invited to provide the OECD with new scientific and technical information, and to make proposals for additional areas to be considered.

The Role of Comparative Approach as Part of a Safety Assessment

In 1990, a joint consultation of the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) established that the comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessment (WHO, 1991).

In 1993 the Organisation for Economic Co-operation and Development (OECD) further elaborated this concept and advocated the approach to safety assessment based on substantial equivalence as being the most practical approach to addressing the safety of foods and food components derived through modern biotechnology (as well as other methods of modifying a host genome including tissue culture methods and chemical or radiation induced mutation). In 2000 the Task Force concluded in its report to the G8 that the concept of substantial equivalence will need to be kept under review.

The Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology in 2000 concluded that the safety assessment of genetically modified foods requires an integrated and stepwise, case-by-case approach, which can be aided by a structured series of questions. A comparative approach focusing on the determination of similarities and differences between the genetically modified food and its conventional counterpart aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy for the safety and nutritional assessment of genetically modified foods. The concept of substantial equivalence was developed as a practical approach to the safety assessment of genetically modified foods. It should be seen as a key step in the safety assessment process although it is not a safety assessment in itself; it does not characterise hazard, rather it is used to structure the safety assessment of a genetically modified food relative to a conventional counterpart. The Consultation concluded that the application of the concept of substantial equivalence contributes to a robust safety assessment framework.

A previous Joint FAO/WHO Expert Consultation on Biotechnology and Food Safety (1996) elaborated on compositional comparison as an important element in the determination of substantial equivalence. A comparison of critical components can be carried out at the level of the food source (i.e., species) or the specific food product. Critical components are determined by identifying key nutrients and key toxicants and anti-nutrients for the food source in question. The comparison of critical components should be between the modified variety and non-modified comparators with an appropriate history of safe use. The data for the non-modified comparator can be the natural ranges published in the literature for commercial varieties or those measured levels in parental or other edible varieties of the species (FAO, 1996). The comparator used to detect unintended effects for all critical components should ideally be the near isogenic parental line grown under identical conditions. While the comparative approach is useful as part of the safety assessment of foods derived from plants developed using recombinant DNA technology, the approach could, in general, be applied to foods derived from new plant varieties that have been bred by other techniques.

Section I - Background

A. Production of maize for food and feed

Maize is the world's third leading cereal crop, following wheat and rice. It is grown as a commercial crop in over 25 countries worldwide. Field maize has been grown for 8000 years in Mexico and Central America and for 500 years in Europe. Maize is naturally cross-pollinated and until about 1925 mainly open pollinated varieties were grown. Today mainly hybrids are grown. To produce hybrid seed the tassels are removed from the plants prior to pollen shedding, so that only one sort of pollen will be received by the silks. The hybrid plants grown from this seed give more vigorous growth and higher yields. Sweet maize, derived from field maize by crossbreeding, introducing a sugar gene, has been grown in the US since 1930 and in Europe since 1979. Maize for popcorn is a minor crop. The cultivation and use is mainly takes place in the US (Jugenheimer, 1976).

Worldwide production of maize is about 600 million tons a year (Corn Refiners Association, USA 2001; Pingali, 2001). In the EU, the annual total production of maize is 38.9 million tons. The major producers, the US and China, account for 43.2 and 17.9 % of the field maize production respectively. In the EU, 6.6 % of the total amount of field maize is grown. The US accounts for 81% of the production of sweet maize, whereas in the EU, only 7% is grown.

Table 1. World maize grain production 2000/2001

	Production (Mt)	% of total
US	253.2	43.2
China	105.0	17.9
EU	38.9	6.6
Brazil	38.5	6.6
Mexico	18.5	3.2
Argentina	15.0	2.6
India	12.0	2.0
South Africa	8.0	1.4
Canada	6.8	1.2
Indonesia	6.2	1.1
Egypt	5.8	1.0
Yugoslavia	5.5	0.9
Hungary	4.5	0.8
Thailand	4.4	0.8
Philippines	4.3	0.7
Romania	4.0	0.7

Source: Corn Refiners Association, USA 2001¹

1. Source: U.S. Department of Agriculture, Foreign Agricultural Service. Based on local marketing years in thousands of metric tons. Updated May 17, 2001 as reported by Corn Refiners Association, Inc. Washington DC at website www.corn.org

In the EU, 2.9 million tons of field maize are used as food, and 21 million tons as feed (Eurostatistics, 1994). In 1995-97, 66 % of all the maize produced worldwide was used for animal feed and 17 % for human consumption. In the developing countries, 30 % of the maize produced was used for human consumption and 57 % for animal feed, whereas in Western Europe, North America and other high-income countries, 4 % was used for human consumption and 76 % for animal feed during the same period (Pingali, 2001)². The consumption of sweet maize was 79 thousand ton (frozen), 298 thousand ton (canned) and 45 thousand tons (fresh) in 1995 in Europe (AGPM, 1996).

Field maize and its products are used in food products (oil, grits, meal, flours, ethanol, syrup, starch) and feed (hulls, gluten, hominy). Sweet maize and its products are used in food (kernels, meal) and feed (hulls, 60-65 % of volume). Popcorn maize kernels are used for popcorn and as basis for confections.

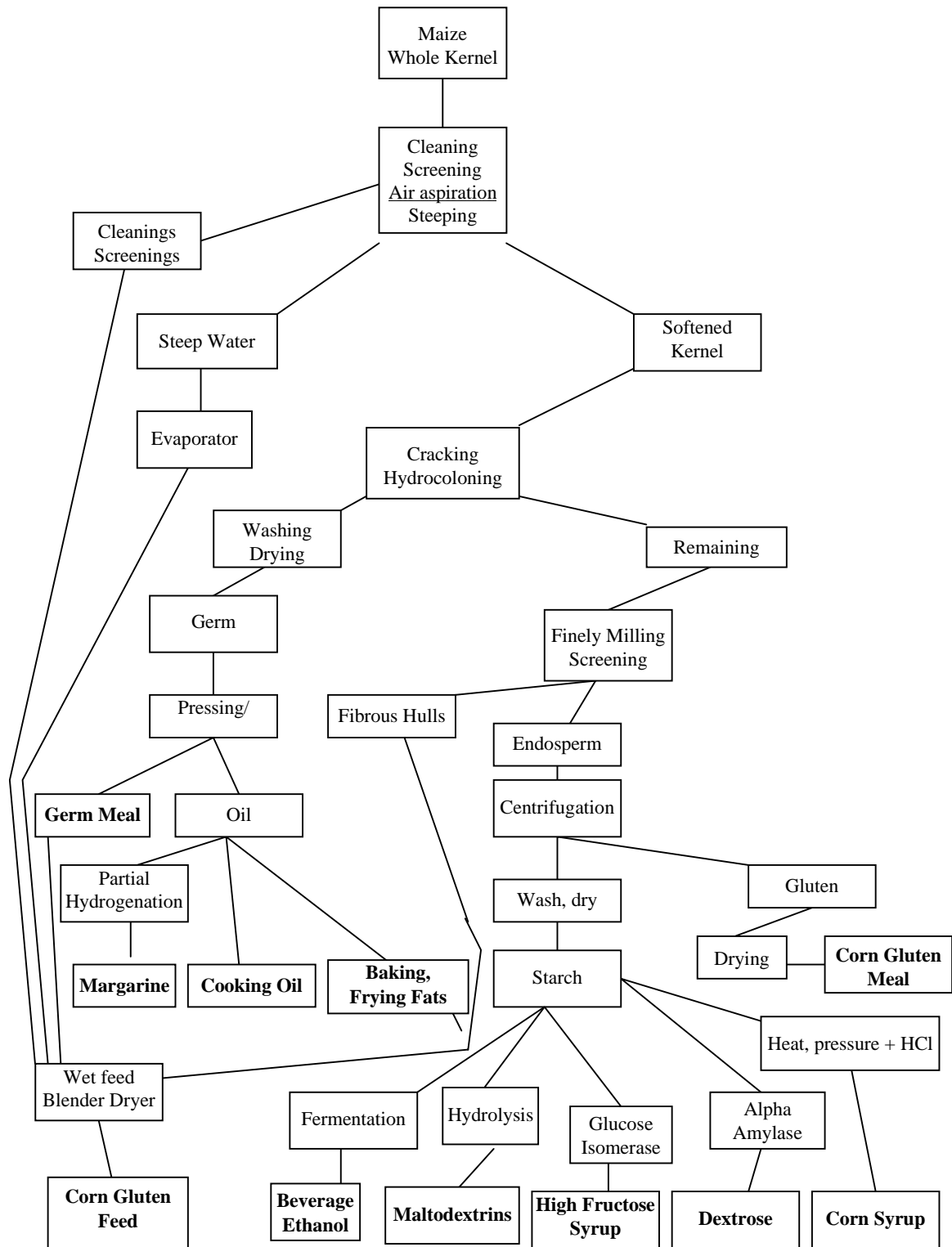
B. Processing of maize

Wet Milling

The maize kernel is composed of a hard outer layer (pericarp), the germ and endosperm (NCGA, 1999). The pericarp is a very hard fibrous coat of cellulose and hemicellulose that must be broken or removed in order for the kernel to be beneficial for consumption or for processing (Eckhoff and Paulsen, 1996). The tip of the pericarp that attaches the kernel to the cob is softer and easily broken providing an access into the kernel, particularly in the steeping process. The germ, the only living part of the corn kernel, contains about 50 % oil on a dry weight basis, while the endosperm contains 70 % starch (White and Pollak, 1995). Processes have been devised to separate these components of the maize kernel, and, in the process derive many food (67 %) and feed (33 %) products (Newcomb, 1995). The wet milling process is the most important one and it employs modern technology as shown in Figure 1.

Generally the type of corn used for wet milling is yellow dent. However, it is estimated that 'waxy type' corn may make up as much as one third of the corn processed, while a very small amount of high amylose corn is also processed (White and Pollak, 1995). Maize prepared for wet milling must be cleaned as thoroughly as possible. It is then steeped in hot water (49°C - 54°C) and sulfur dioxide (0.1-0.2 %) to soften the pericarp. Water-soluble nutrients adhering to the surface of the maize enter the steep water that is drawn off and evaporated, leaving solubles which are mixed with cleanings and screenings to make maize gluten feed. The softened kernel is cracked by machine and hydrocloned, a flotation process that separates the germ portion from the endosperm. The germ portion is pressed to separate the oil that is used for margarine, cooking oil and baking and frying fats for human use. The pressed germ is dried and added to maize gluten feed. The endosperm portion is finely milled and passed through screens to remove the fibrous hulls. Fibrous hulls are also added to maize gluten feed. The screened endosperm portion is centrifuged to separate the starch portion from the gluten. The gluten portion is dried and used as maize gluten meal. Maize starch, the primary product of wet milling, is obtained by washing and drying the starch portion. About 40 % of the starch is consumed directly as food or used for other industrial purposes, while about 60 % is converted to various sweeteners (White and Pollak, 1995). The primary sweeteners (maize syrups) are regular, high fructose, dextrose and maltodextrins. The major use is for syrup containing approximately 55 % fructose that is much sweeter than sucrose. Maltodextrins are not sweet, but contribute viscosity, mouthfeel, and body to food products. Starch also serves as a major source of sugar for the fermentation of beverage alcohol. Dextrose that is enzymatically produced from starch has many food uses.

Figure 1. Wet Processing of Maize



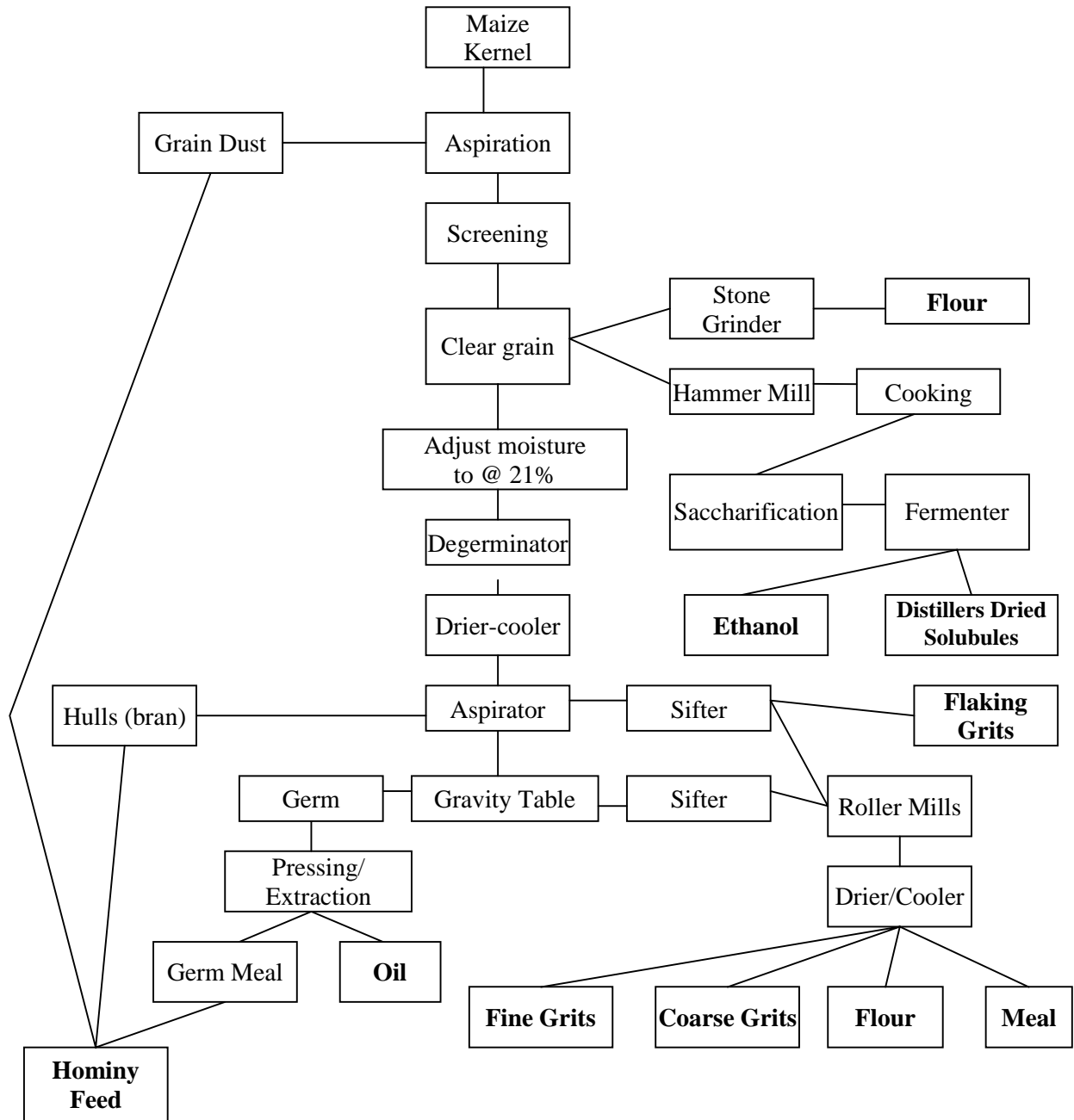
Dry Milling

Dry Milling is the oldest way of processing the corn kernel for human and animal food use. Dry milling is a term that usually refers to one of three different processes. The first process is stone grinding after screening and cleaning. Stone grinding is widely used in Africa, Latin America, Asia, and by small mills in the U. S. and Canada (White and Pollak, 1995). Because of the oil content, the storage life and flavour stability of whole cornmeal is short. So, the industry has devised processes that remove the oil, producing more refined products.

The second process is the dry-grind ethanol process for producing ethanol for commercial purposes (Eckhoff and Paulsen, 1996). Maize kernels are cleaned, ground, cooked, saccharified, and put into a fermenter to convert starch to ethanol. The by-product, distillers dried solubles, is an important livestock feed.

The third process is called the tempering degerminating system (TD), and is the most widely used in the food processing industry. Maize kernels are cleaned and tempered by soaking in water, strengthening the pericarp and the germ to protect them from shattering in subsequent mechanical separation procedures. Tempering is followed by degerminating, drying and mechanical separation. The preferred degerminating equipment is the Beall type degerminator, though several other types of machines are used, i.e., Entoleter, granulator, disc mill, roller mill or decorticator (Eckhoff and Paulsen, 1996). The tempering and degerminating steps are the most important because the clean separation of the germ is paramount to obtaining high quality products in the down stream separation process. The usual products and yields of the TD process are flaking grits (12 %), coarse grits (15 %), regular (fine) grits (23 %), meal (6 %), flour (4 %), oil (1 %), and hominy feed (35 %). Corn bran is high in fibre, low in calories, and readily absorbs water, making it a useful additive in human prepared foods. Flaking grits are used almost exclusively in the manufacture of corn flakes. Fine grits are frequently utilised by the snack, breakfast cereal and brewing industries. Cooked coarse grits are eaten as a breakfast food. Maize flour is used as an ingredient in muffins, breadings, batters, pancakes, doughnuts, breakfast foods, and as binders in processed meats. Dried-milled maize products serve as a substrate for brewing beer. Corn grits and whole kernels are used to produce many distilled hard liquors. A minimum of 51 % maize is used in the fermentation of the mash that is distilled into Bourbon (White and Pollak, 1995).

Figure 2. Dry Milling of Maize

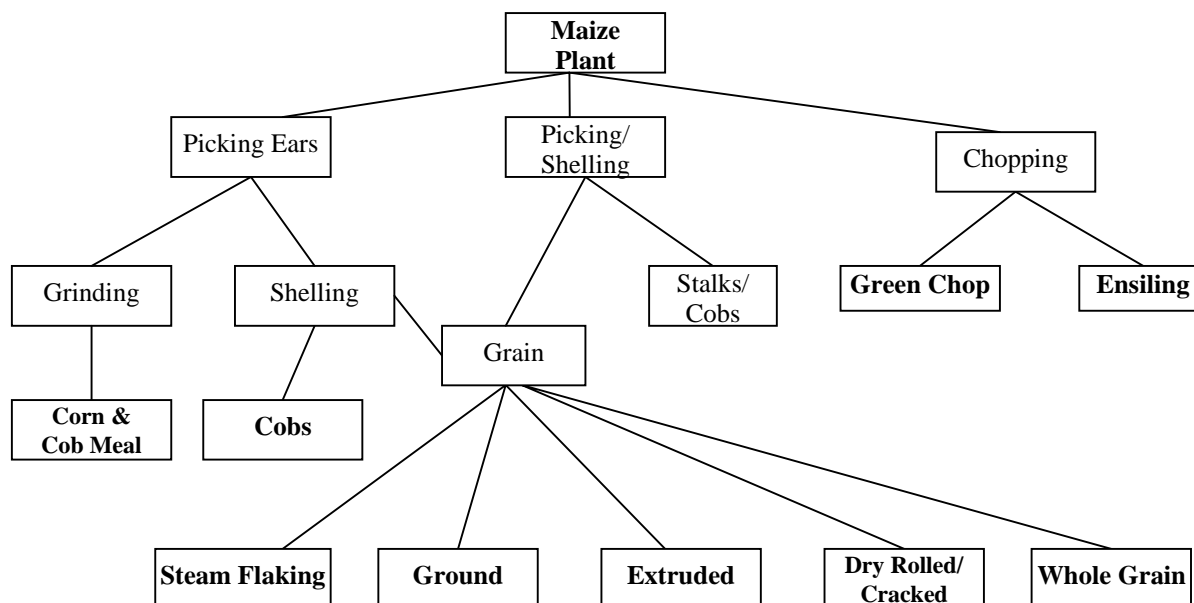


Masa production

Cooking (85 - 95° C) maize in the presence of alkali (lime) and fine grinding it produces a dough material called Masa. Masa is the starting material for tortillas, taco shells, tortilla chips and maize chips, that are widely consumed in South-western U.S., Mexico, Central America and South America. Both white and yellow corn are used to make Masa. Totally hard endosperm dent corn is preferred because its superior cooking characteristics maximise the handling and mechanical qualities of the finished products. However, good quality Masa can be produced from soft endosperm corn by altering the cooking time (Eckhoff and Paulsen, 1996).

Feed processing

As described earlier, animal feed is produced as a by-product of milling. Alternatively, the whole corn plant may be used for animal (primarily ruminant) feed. It is harvested at various stages of growth, usually after the ear is formed, but is usually mechanically chopped prior to full maturity of the ear when the plant contains about 35 - 40 % moisture. The material can be fed directly or preserved as silage in an upright sealed silo or in a trench or bunker, so as to limit the exposure to oxygen. The resulting silage is allowed to age under anaerobic conditions producing a palatable feed that retains up to 90 % of its nutrients (Ensminger *et al.*, 1990). When the ear is allowed to go to maturity and the moisture content recedes to around 15 %, it can be harvested by mechanically picking, or by mechanically picking and shelling in one operation. Stalks can be grazed in the field by ruminants, or harvested for roughage or animal bedding. Dry ear corn can be mechanically ground and fed to ruminants or it can be shelled. If the moisture content of the harvested maize grain is above about 13 %, it is either dried or sometimes stored in an airtight silo and fed to ruminants or swine as high-moisture corn. The cobs can be used in animal feed or for commercial uses. Corn grain is the feed of choice. It can be fed as is to ruminants as whole grain, rolled (cracked), ground or steam flaked with there being little difference in digestible and net energy in diets containing less than 20 % roughage (NRC, 1996). Maize grain is usually ground or rolled when fed to swine and poultry, but pelleting is becoming more popular with poultry producers (Newcomb, 1995). For use in pet foods, maize is usually ground, cooked, pelleted or extruded. Common feed processing methods are shown in Figure 3.

Figure 3. Feed Processing of Maize

C. Appropriate comparators for testing new varieties

This paper suggests parameters that maize developers should measure. Measurement data from the new variety should ideally be compared to those obtained from the near isogenic non-GMO line grown under identical conditions. A developer can also compare values obtained from new varieties with the literature values of conventional counterparts present in this paper. Critical components include key nutrients and key toxicants for the food source in question. Key nutrients are those components in a particular product, which may have a substantial impact in the overall diet. These may be major constituents (fats, proteins, and carbohydrates) or minor compounds (vitamins and minerals). Key toxicants are those toxicologically significant compounds known to be inherently present in the species, i.e. compounds whose toxic potency and level may impact on human and animal health. Similarly, the levels of known anti-nutrients and allergens should be considered. As part of the comparative approach, selected secondary plant metabolites, for which characteristic levels in the species are known, are analysed as further indicators of the absence of unintended effects of the genetic modification on the metabolism.

D. Traditional characteristics screened by maize developers

Phenotypic characteristics provide important information related to the suitability of new varieties for commercial distribution. Selecting new varieties is started based on parent data. Plant breeders developing new varieties of maize evaluate many parameters at different stages in the developmental process. In the early stages of growth, breeders evaluate stand count and seedling vigour. As the plant matures, disease data is evaluated, e.g., grey leaf spot, anthracnose, fusarium and head smut infestations. At near maturity or maturity, root lodging, stalk lodging, brittle snap, time to silk, and time to shed are evaluated. The mature plant is measured for plant height, ear height, drop ear, and husk cover. The harvested grain is measured for yield, moisture, and test weight. In some cases, plants are modified for specific increases in certain components, and the plant breeder would be expected to analyse for such components (UPOV, 1994).

Section II- Nutrients in maize and maize products

A. Kernels

Dent field maize is harvested at maturity. The kernel goes through maturity stages denoted by 'milk', 'dough' and 'dent'. Maize kernels consist of endosperm (containing starch), and germ (containing oil). They are wrapped in the pericarp, a cellulose layer. At maturity of field maize, which usually occurs about 50-60 days after pollination, moisture content is 30 % (White and Pollack, 1995). Sweet maize is harvested when the kernels are in the 'milk'-stage, when moisture content is about 75 %. The moisture content of dried popcorn maize kernels is about 10 %. It should be noted that values for some components (e.g. minerals) could vary considerably as result of differences in genetics and environmental and agronomic conditions (FAO, 1992). In addition to these general types of maize, several maize variants have been developed with specific improvements in composition. Quality Protein Maize (QPM) variants have been developed with improved levels of lysine and tryptophan, the two limiting essential amino acids in maize protein. Other specialty types of maize are characterised by a higher oil content, higher amylose content or higher amylopectin content (waxy maize) (Jugenheimer, 1976). If the characteristic level for a specific component, which is altered in a specialty type of maize, is outside the general range of values found in scientific literature, the comparison with the parent line will be decisive.

Table 2. Proximate analysis of field maize kernels

Reference		Wat82	Wat87	USDA01 ^a	Sou00 ^a	NRC ^{ab}	Commercial range ^c	Range
Moisture	% of fw		7 – 23	10.37	12.0-13.0	10-11.9	9.4-14.4	7-23
Protein	% of dw	8.1-11.5	6 – 12	10.5 ^d	9.37-12.1 ^e	9.3-9.8	9.57-12.7	6-12.7
Total fat	% of dw	3.9-5.8	3.1 – 5.7	5.29 ^d	3.66-4.91 ^e	4.1-4.4	3.6-5.3	3.1-5.8
Ash	% of dw	1.27-1.52	1.1 – 3.9	1.34 ^d	1.28-1.73 ^e	1.5	1.28-1.5	1.1-3.9
Neutral detergent fibre (total fibre) ^f	% of dw	8.3-11.9	8.3 – 11.9			9.5-10.8	10.1-11.7	8.3-11.9
Acid detergent fibre (cellulose) ^f	% of dw	3.0-4.3	3.3 – 4.3			3.1-3.3	3.7	3.0-4.3
Total dietary fibre ^f	% of dw				11.1 ^e			11.1
Carbohydrates	% of dw			82.85 ^d			82.2-82.9	82.2-82.9

Source: Watson, 1982 and 1987; USDA, 2001; Souci *et al.*, 2000; NRC 1994, 1998, 2000 and 2001

^a: possibly including GMO-varieties

^b: values taken from NRC (1994), NRC (2000), NRC (1998) and NRC (2001). Values from NRC (1994) and NRC (1998) are calculated from given values on total weight-basis, using reported moisture content of 11.00 %,

^c: commercial range on non-GMO controls, compiled from data from AgrEvo (1998), Dow AgriSciences (2000), Monsanto (1997 and 2000) and Pioneer Hi-Bred (1998)

^d: values calculated from given % of total weight, using the reported moisture content of 10.37 %

^e: values calculated from given % of total weight, using 12.50 % as the average moisture content (reported values range from 12.0-13.0 %)

^f: Proximate analysis of maize usually includes acid detergent fibre (ADF) and neutral detergent fibre (NDF). The terms ADF and NDF are still commonly used in the feed industry and values for comparison are readily available. For food use, however, the concept of dietary fibre is preferred, although different definitions and methods of analysis are being used (see: USA Panel on the Definition of Dietary Fibre, 2001). The value for total dietary fibre from Souci *et al.* is obtained using a modification of the analytical method recommended by the Association of Official Analytical Chemists (AOAC). Total Dietary Fibre determined this way includes lignin and non-starch polysaccharides (including cellulose, hemicellulose and pectin).

Table 3. Proximate analysis of sweet maize and popcorn maize kernels

Reference		Sweet maize				Popcorn maize	
		NEVO01 ^{ab}	USDA01 ^a	Sou00 ^a	Range	NEVO01 ^{ac}	USDA01 ^{ad}
Moisture	% of fw	84	75.96	74.70	74.70-84	10	4.10
Protein	% of dw	15.6 ^c	13.4 ^c	11.3-14.6 ^f	11.3-15.6	12.2 ^c	12.5 ^c
Total fat	% of dw	8.75 ^c	4.91 ^c	4.86 ^f	4.86-8.75	4.4 ^c	4.38 ^c
Ash	% of dw		2.58 ^c	2.77-3.86 ^f	2.58-3.86		1.88 ^c
Total Dietary Fibre	% of dw	15.6 ^c	11.2 ^c		11.2-15.6	5.56 ^c	15.7 ^c
Carbohydrates	% of dw	72.5 ^c	79.18 ^c		72.5-79.18	79 ^c	81.2 ^c

Source : USDA, 2001; NEVO, 2001; Souci *et al.*, 2000

^a: possibly including GMO-varieties

^b: values for boiled kernels

^c: dried kernels

^d: air-popped kernels

^e: values calculated from given % of total weight, using the indicated moisture content

^f: values calculated from given % of total weight, using average moisture content of 74.70 % (reported values range from 73.90-75.60 %)

Table 4. Levels of minerals and vitamins in field maize kernels

Reference		Wat82	Wat87	USDA01 ^{ab}	Sou00 ^{ac}	NRC ^{ad}	Commercial range ^e	Range
Na	mg/100g	0-150	0-150	39	1.1-11	10-22		0-150
K	mg/100g	320-720	320-720	320	340	340-440	360-370	320-720
Ca	mg/100g	10-100	10-100	7.8	4.4-22	22-40	3-5	3-100
P	mg/100g	260-750	260-750	234	190-290	300-320	290-320	234-750
Mg	mg/100g	90-1000	90-1000	142	82-140	120-130	120-130	82-1000
Fe	mg/100g	0.1-10	0.1-10	3.02	1.7	3.3-5.5	2.3-2.5	0.1-10
Cu	mg/100g	0.09-1.0	0.09-1.0	0.35	0.27	0.25-0.34	0.19-0.21	0.09-1.0
Se	mg/100g	0.0045	0.001-0.1	0.017	0.005-0.018	0.0034-0.014		0.001-0.1
Zn	mg/100g	1.2-3.0	1.2-3.0	2.47	1.9	2.0-2.7	2.0-3.0	1.2-3.0
<hr/>								
Vit. A	mg/kg RE ^f	2.5 IU ^f /g	2.5 mg/kg	0.52	0.49-2.18			0.49-2.18
Vit. B1 (Thiamin)	mg/kg	3.0-8.6	3.0-8.6	4.3	2.3-6.9	3.9	3.5	2.3-8.6
Vit. B2 (Riboflavin)	mg/kg	0.25-5.6	0.25-5.6	2.2	1.1-2.7	1.1-1.3	5.6	0.25-5.6
Vit. B6 (Pyridoxine)	mg/kg	9.6	5.3	6.9	4.6	5.6-7.9		4.6-9.6
Vit. C (Ascorbic acid)	mg/kg			0	0			
Vit. E	mg/kg	3.0-12.1	17-47 IU/kg	8.4 mg/kg ATE ^f	4.1-31.1mg vit.E act ^f .	9.3-25 ATE		
Folate, total	mg/kg			0.21 (folic acid=0)	0.23-0.46 mg/kg folic acid	0.17-0.45		
Niacin (Nicotinic acid)	mg/kg	9.3-70	9.3-70	40.5	11-23 mg/kg nicotinamide	27		9.3-70

Source: Watson, 1982 and 1987; USDA, 2001; Souci *et al.*, 2000; NRC, 1994, 1998, 2000 and 2001

Please Note: All values are expressed on a dry weight-basis

^a: possibly including GMO-varieties

^b: values calculated from given values on total weight-basis, using reported moisture content of 10.37 %

^c: values calculated from given values on total weight-basis, using average moisture content of 12.50 % (reported values range from 12.0-13.0)

^d: values taken from NRC (1994), NRC (2000), NRC (1998) and NRC (2001). Values from NRC (1994), and NRC (1998) calculated from given values on total weight-basis, using the reported moisture content of 11.00 %.

^e: commercial range on non-GMO controls, compiled from data from AgrEvo (1998), Dow AgriSciences (2000), Monsanto (1997 and 2000) and Pioneer Hi-Bred (1998)

^f: RE = retinol equivalents, IU = international units, ATE = alpha tocopherol equivalents = vit E act

Table 5. Levels of minerals and vitamins in sweet maize and popcorn maize kernels

Reference		Sweet maize				Popcorn maize	
		NEVO01 ^{ab}	USDA01 ^a	Sou00 ^c	Range	NEVO01 ^{ad}	USDA01 ^{ae}
Na	mg/100g	6.3	62	0.59-1.98	0.59-62	5.6	4.2
K	mg/100g	1560	1120	900-1150	900-1560	278	314
Ca	mg/100g	69	8.3	8.6-13.7	8.3-69	22	10
P	mg/100g	625	370	320-328	320-625	278	313
Mg	mg/100g	281	154	106-120	106-281		137
Fe	mg/100g	3.1	2.2	1.6-2.3	1.6-3.1	3.3	2.77
Cu	mg/100g	0.25	0.22	0.08-0.18	0.08-0.25		0.44
Se	mg/100g	trace	0.025	0.0025-0.011	0.0025-0.025		0.10
Zn	mg/100g	6.25	1.9	2.21-3.95	1.9-6.25		3.59
Vit. A	mg/kg RE	0.44	1.16	0.40	0.40-1.16	0.89	0.21
Vit. B1 (Thiamin)	mg/kg	7.5	8.3	5.9	5.9-8.3	3.3	2.1
Vit. B2 (Riboflavin)	mg/kg	4.4	2.5	4.7	2.5-4.7	0.89	3.0
Vit. B6 (Pyridoxine)	mg/kg	6.3	2.3	8.7	2.3-8.7	2.4	4.7
Vit. C (Ascorbic acid)	mg/kg	0	283	470	283-470	0	0
Vit. E	mg/kg	56	3.7 mg/kg ATE ^g	3.75 mg/kg vit.E act. ^g			1.25 mg/kg ATE
Folate, total	mg/kg	2.1	19.2 (folic acid=0)	1.7 mg/kg folic acid		0.12	2.4
Niacin (Nicotinic acid)	mg/kg	106	70.8	67.2 mg/kg nicotinamide		11	20.3

Source: USDA, 2001; NEVO, 2001; Souci *et al.*, 2000

Please Note: All values are expressed on a dry weight-basis

^a : possibly including GMO-varieties

^b : values calculated from given values on total weight-basis, using moisture content indicated in Table 3

^c : values calculated from given values on total weight-basis, using average moisture content of 74.70 % (reported values range from 73.90-75.60 %)

^d : values for boiled kernels

^e : dried kernels

^f : air-popped kernels

^g : ATE = alpha tocopherol equivalents = vit E act

Table 6. Amino acid composition of maize kernels in % of kernel d.w.

Reference	Field maize							Sweet maize	Popcorn maize
	Wat82 ^a	Whi95 ^b	USDA01 ^{cd}	Sou00ce ^{ce}	NRC ^{cf}	Comm. Range ^g	Range	USDA01 _{cde}	USDA01 _{cdh}
Essential amino acids									
Methionine	0.10-0.21	0.16-0.25	0.22	0.10-0.46	0.19-0.20	0.17-0.28	0.10-0.46	0.28	0.26
Cysteine	0.12-0.16	0.20-0.27	0.19	0.08-0.32	0.20-0.21	0.17-0.26	0.08-0.32	0.11	0.23
Lysine	0.20-0.38	0.26-0.34	0.30	0.05-0.55	0.27-0.30	0.21-0.38	0.05-0.55	0.57	0.35
Tryptophan	0.05-0.12	0.04-0.06	0.07	0.05-0.13	0.07-0.07	0.05-0.08	0.04-0.13	0.10	0.09
Threonine	0.29-0.39	0.28-0.39	0.39	0.37-0.58	0.33-0.33	0.27-0.49	0.27-0.58	0.54	0.47
Isoleucine	0.26-0.40	0.27-0.38	0.38	0.40-0.71	0.31-0.33	0.22-0.50	0.22-0.71	0.54	0.45
Histidine	0.20-0.28	0.24-0.32	0.32	0.15-0.38	0.26-0.29	0.21-0.38	0.15-0.38	0.37	0.38
Valine	0.21-0.53	0.39-0.52	0.53	0.49-0.85	0.38-0.45	0.30-0.61	0.21-0.85	0.77	0.63
Leucine	0.79-1.54	0.98-1.38	1.29	1.04-2.41	1.05-1.14	0.84-1.84	0.79-2.41	1.45	1.54
Arginine	0.29-0.60	0.36-0.51	0.52	0.22-0.64	0.42-0.43	0.27-0.57	0.22-0.64	0.54	0.62
Phenylalanine	0.29-0.58	0.39-0.54	0.52	0.37-0.58	0.43-0.44	0.32-0.64	0.29-0.64	0.62	0.62
Glycine	0.26-0.47	0.32-0.41	0.43	0.49	0.38	0.29-0.45	0.26-0.49	0.53	0.51
Non-essential amino acids									
Alanine	0.65-1.00	0.59-0.79	0.79	0.88-0.95		0.56-1.04	0.56-1.04	1.23	0.94
Aspartic acid	0.59-0.73	0.52-0.71	0.73	0.67-0.72		0.48-0.85	0.48-0.85	1.01	0.87
Glutamic acid	1.25-1.98	1.46-2.01	1.97	1.99-2.15		1.26-2.58	1.25-2.58	2.65	2.35
Proline	0.67-1.04	0.71-0.99	0.92	1.06-1.36		0.63-1.16	0.63-1.36	1.21	1.09
Serine	0.42-0.56	0.35-0.49	0.50	0.57-0.61	0.42	0.37-0.91	0.35-0.91	0.64	0.60
Tyrosine	0.29-0.47	0.22-0.34	0.43	0.22-0.79	0.28-0.34	0.12-0.48	0.12-0.79	0.51	0.51

Source: Watson, 1982; White & Pollack, 1995; USDA, 2001; Souci *et al.*, 2000; NRC, 1994, 1998 and 2001

^a: values calculated from given % of total amino acids (10.1 % total protein)

^b: values calculated from given % of total amino acids (8.74 % total protein)

^c: possibly including GMO-varieties

^d: values calculated from given values on total weight-basis

^e: values calculated from given values on total weight-basis, using average moisture content of 12.50 %

^f: values taken from NRC (1996), NRC (1998) and NRC (2001). Values from NRC (1994) and (1998) calculated from given values on total weight-basis, using reported moisture content of 12.00 % and 11.00 %, respectively. Values from NRC (2001) were calculated from reported % of crude protein, using given crude protein content of 9.4 % on dry basis.

^g: commercial range on non-GMO controls, compiled from data from AgrEvo (1995, 1998), Dow AgriSciences (2000), Monsanto (1995, 1997 and 2000) and Pioneer Hi-Bred (1998)

^h: values for air-popped kernels

Table 7. Fatty acid composition of maize kernels in % of kernel d.w.

Reference	Field maize					Sweet maize	Popcorn maize
	USDA01 ^{ab}	Sou00 ^{ac}	NRC ^{ad}	Comm. Range ^e	Range	USDA01 ^{ab}	USDA01 ^{abf}
16:0 Palmitic	0.63	0.29-0.79	0.70	0.30-0.37	0.29-0.79	0.71	0.52
18:0 Stearic	0.084	0.04-0.17	0.11	0.05-0.08	0.04-0.17	0.046	0.073
18:1 incl. Oleic	1.39	1.26	1.31	0.70-1.03	0.70-1.39	1.44	1.15
18:2 incl. Linoleic	2.34	0.67-2.81	2.04	1.80-2.21	0.67-2.81	2.25	1.92
18:3 incl. Linolenic	0.073	0.03-0.08	0.10	0.03-0.04	0.03-0.10	0.067	0.063

Source: USDA, 2001; Souci et al., 2000; NRC, 1994

^a: possibly including GMO-varieties

^b: values calculated from given values on total weight-basis

^c: values calculated from given values on total weight-basis, using average moisture content of 12.50 %

^d: values taken from NRC (1994) are calculated from given values on total weight-basis, using average moisture content of 11.00 %.

^e: commercial range compiled from data from Aventis (1999), Monsanto (1996b) and Monsanto (2000).

^f: values for air-popped kernels

B. Oil

Oil is produced from the field maize germ by wet milling. Maize oil in the germ consists mostly of triglycerides (TG) (75-92 %). Crude maize oil contains 95.6 % TG and 1.7 % free fatty acids (FFA). Refined oil contains 98.8 % TG and 0.03 % FFA (oleic acid) (Anderson and Watson, 1982). The fatty acids, linoleic acid, oleic acid and palmitic acid form the major part of the TG (Watson, 1987). In table 6, fatty acids consistently present at levels below 1 % are not included. Maize oil is used in salad- and cooking oil, mayonnaise and margarine, baking and frying fat and in sauces and soups. In the production process for refined maize oil, protein is reportedly reduced to amounts below 100 micrograms per ml (SCF, 1999), or to amounts below the level of detection (Federal Register, 2000; EPA, 2001).

Table 8. Fatty acid composition of refined maize oil in % of total fatty acids

	USDA01 ^{ab}	Codex99 ^a	And82	Ort87
16:0 Palmitic	11.4	8.6-16.5	11.5	11.0 ± 0.5
18:0 Stearic	1.9	0-3.3	2.0	1.8 ± 0.3
18:1 incl. Oleic	25.3	20.0-42.2	24.1	25.3 ± 0.6
18:2 incl. Linoleic	60.7	34.0-65.5	61.9	60.1 ± 1.0
18:3 incl. Linolenic	0.73	0-2.0	0.7	1.1 ± 0.3

Source: USDA, 2001; Codex Alimentarius, 1999; Anderson & Watson, 1982; Orthofer & Sinram, 1987

^a: possibly including GMO-varieties

^b: values calculated from given % of oil

C. Grits, Meal, Flour, Bran

Grits, meals and flours are products of the dry milling process of field maize with de-germination. Bran is a by-product of this process. Grits are used to make cereals and snacks and also to produce alcoholic beverages; meal is used for bread and muffins, flour for pancakes and snacks. Bran is used as a dietary source of fibre. Typical composition (% as-is basis) of dry milled corn products is 7-8 % protein, less than 1 % fat, ash or fibre, and 77-79 % starch (88-90 %, dry basis) (Alexander, 1987).

Table 9. Proximate analysis of grits, flour and meal in % of d.w.

Reference	Grits			Flour				Meal	
	Ale87	And82	USDA01 ^a	Ale87	And82	USDA01 ^a	Sou00 ^a	And82	USDA01 ^a
Moisture ^b	11.5	12	10	13	12	9.81	12.00	12	11.59
Protein	8.47	9.9	9.78	6.0	8.9	6.13	7.65-11.38	9.0	9.59
Carbohydrates	90.2	88.8	88.4	90.7	87.3	90.7		89.1	87.9
Fat	0.79	0.91	1.33	2.3	3.0	1.52	1.77-4.43	1.36	1.87
Crude Fibre	0.23	0.45		0.57	0.80				
Total dietary fibre ^c			1.78			2.08	10.7	0.68	8.4
Ash	0.34	0.45	0.44	0.46	0.91	0.50	1.30-1.36	0.57	0.68

Source: Alexander, 1987; Anderson & Watson, 1982; USDA, 2001; Souci et al., 2000

^a : possibly including GMO-varieties

^b : values are % of total weight (all other values are % of d.w., calculated from given % of total weight)

^c : measured according to AOAC method

Table 10. Levels of minerals and vitamins in grits, flour and meal

Reference		Grits		Flour		Meal
		USDA01	USDA01	Sou00	USDA01	
Na	mg/100g	1.1	1.1	0.80	3.4	
K	mg/100g	152	99	136	183	
Ca	mg/100g	2.2	2.2	11-30	5.7	
P	mg/100g	81	66		95	
Mg	mg/100g	30	20	53	45	
Fe	mg/100g	1.1	1.0	2.7	1.2	
Cu	mg/100g	0.083	0.16		0.088	
Se	mg/100g	0.19	0.088		0.088	
Zn	mg/100g	0.46	0.41		0.81	
Vit. A	mg/kg RE ^a	4.9	0.55	0.57	4.6	
Vit. B1 (Thiamin)	mg/kg	1.44	0.81	4.3-5.6	1.58	
Vit. B2 (Riboflavin)	mg/kg	0.44	0.64	1.3-1.9	0.57	
Vit. B6 (Pyridoxine)	mg/kg	1.63	1.06	0.68	2.91	
Vit. C (Ascorbic acid)	mg/kg	0	0	0	0	
Vit. E	mg/kg ATE ^a	2.9	3.6		3.7	
Folate, total	mg/kg	0.56	5.3	0.11 mg/kg folic acid	5.4	
Niacin (Nicotinic acid)	mg/kg	13.3	29.1	19-23.5 mg/kg nicotinamide	11.3	

Source: USDA, 2001; Souci *et al.*, 2000

Please Note: All values are expressed on d.w. basis, calculated from given values per 100 g of total weight, data possibly include GMO-varieties

^a : RE = retinol equivalents, ATE = alpha tocopherol equivalents

D. Starch

Starch is derived from field maize by the wet milling process. About 60 % of the starch is converted (by acid or enzyme hydrolysis) to sweeteners (syrops) and ethanol. The remaining 40 % is used for foods and industrial uses. The lipids in starch are mainly free fatty acids (Anderson and Watson, 1982).

Starch is used in a variety of products that include bakery products, baby foods, sauces, dressings and soups. Typically, maize starch contains residual protein at 0.4 % (SCF, 1999) or 0.6 % (Federal Register, 2000), whereas starch hydrolysates contain 100-200 ppm of protein (SCF, 1999).

Table 11. Proximate analysis of corn starch in % of d.w.

	And82	USDA01 ^a	Sou00 ^a
Moisture (% of total weight)	11	8.32	11-12.6
Protein	0.39	0.28	0.30-0.78
Lipids	0.61	0.055	0-0.23
Carbohydrate	98.9	99.55	
Fibre	0.11 ^b	0.98 ^c	
Ash	0.11	0.098	0.07-0.34

Source: Anderson & Watson, 1982; USDA, 2001; Souci *et al.*, 2000

^a: possibly including GMO-varieties

^b: measured as crude fibre

^c: measured as total dietary fibre (AOAC method)

E. Feed

Gluten meal and gluten feed are by-products of the wet processing of maize. Hominy feed and distillers' grain with solubles are products of the maize dry milling industry. Gluten meal is high in protein (65 - 69 %) and carotenoids. Gluten feed is medium in protein (24 - 25 %) and is higher in fibre. Hominy feed is lower in protein with about the same fibre content as gluten feed. Distillers' grain with solubles is a medium protein (29 %) higher fibre product. All maize products are relatively low in the amino acid lysine, and in calcium. When maize and maize products are used in diets containing soybean meal, the amino acid composition of the feed meets nutritional requirements of most domestic animals.

Table 12. Proximate of common maize animal feed products

Parameter		Gluten Meal	Gluten Feed	Hominy Feed	Distillers Grain w/ Solubles	Maize Silage	Maize Grain ^b
Moisture	% of fw	86 - 90	90	90	90.2 - 93.0	62 - 78	7-23
Protein	% of dw	65.0 - 68.9	23.98-24.4	11.4-11.56	29.7 - 29.5	4.7 - 9.2	6-12.7
NDF	% of dw	9.17-14.00	33.5 - 37.0	23.0 - 38.8	45.0	40 - 48.2	8.3-10.8
ADF	% of dw	5.00 - 5.11	11.89-12.1	6.2 - 9.0	17.53 - 19.7	25.6 - 34	3.0-4.3
Fat	% of dw	2.5 - 3.22	2.77 - 3.33	5.7 - 8.89	9.03 - 10.00	1.5 -3.2	3.1-5.8
Ash	% of dw	1.9	6.8 - 6.9	2.2 - 2.7	5.2 - 7.7	2.9 - 5.7	1.1-3.9

Source: BNF³; NRC, 1994, 1998, 2001; Ensminger *et al.*, 1990

^a: Monsanto (1995), Monsanto (1996a), Monsanto (1996b), Monsanto (1997) Aventis (1999), Monsanto (1999) Dow Agrisciences (2000) and Monsanto (2000).

^b: values taken from Table 2.

Table 13. Levels of minerals, amino acids and fatty acids in common maize animal feed products

Parameter		Gluten Meal	Gluten Feed	Hominy Feed	Distillers Grain w/ Solubles	Maize Silage	Maize Grain ^a
Calcium	% of dw	0.06 - 0.08	0.04 - 0.27	0.05 - 0.06	0.22 - 0.32	0.15 - 0.31	0.003 - 0.15
Phosphorus	% of dw	0.49 - 0.56	0.55 - 1.00	0.48 - 0.57	0.83 - 1.40	0.20- 0.27	0.23 - 0.75
Argenine	% of dw	2.02 - 2.14	0.91 - 1.16	0.52 - 0.62	1.21 - 1.22	0.17 - 0.34	0.22 - 0.64
Histidine	% of dw	1.33 - 1.42	0.68 - 0.79	0.22 -0.31	0.74 - 0.74	0.16 - 0.17	0.26 - 0.37
Isoleucine	% of dw	2.67 - 2.76	0.74 - 0.98	0.40 - 0.44	1.10 - 1.11	0.29 - 0.34	0.22 - 0.71
Leucine	% of dw	10.9 -11.3	2.10 - 2.44	0.93 - 1.09	2.76 -2.85	0.75 - 0.76	0.79 - 2.41
Lysine	% of dw	1.10 - 1.14	0.65 - 0.71	0.42 - 0.44	0.67 - 0.67	0.22 - 0.33	0.05 - 0.55
Methionine	% of dw	1.54 - 1.66	0.38 - 0.50	0.14 - 0.20	0.54 - 0.54	0.135 - 0.15	0.10 - 0.46
Phenylalanine	% of dw				1.44 - 1.45	0.34 - 0.40	0.29 - 0.64
Threonine	% of dw	2.20 - 2.31	0.82 - 0.99	0.44 - 0.44	1.01 - 1.02	0.28 - 0.37	0.27 - 0.58
Tryptophan	% of dw	0.34 - 0.40	0.08 - 0.13	0.11 - 0.12	0.26 - 0.27	0.04- 0.09	0.04 - 0.13
Valine	% of dw	3.02 - 3.10	1.06 - 1.16	0.54 - 0.58	1.40 -1.40	0.39 - 0.47	0.48 - 0.59
Cysteine	% of dw	1.21 - 1.22	0.51 - 0.57		0.55 - 0.56	0.118 - 0.12	0.08 - 0.32
Glycine	% of dw						0.26 - 0.49
Palmitic 16:0	% of dw						0.29-0.79
Stearic18:0	% of dw	0.07					0.04 - 0.17
Oleic 18:1	% of dw	0.68					0.70 - 1.39
Linoleic 18:2	% of dw	1.29 - 1.30					0.67 - 2.81
Linolenic 18:3	% of dw						0.03 - 0.10

Source: Monsanto, 1995, 1996; NRC, 1994, 1998, 2001; Ensminger *et al.*, 1990

^a: Values for Ca and P taken from table 4, for amino acids, taken from table 6, and for fatty acids, taken from table 7.

Section III- Anti-nutrients and allergens in maize

A. Phytic acid

Phytic acid (myo-Inositol 1,2,3,4,5,6-hexakis [dihydrogen phosphate]) is present in maize and binds about 60 - 75 % of the phosphorus in the form of phytate (NRC, 1998). Because of phytate binding, bioavailability of phosphorus in maize is less than 15 % for nonruminant animals. Ruminants utilise considerably more phosphorus since the rumen microbes produce the enzyme phytase that breaks down phytate and releases phosphorus (Ensminger *et al.*, 1990). It is becoming common for feed formulators to add phytase to swine and poultry diets to improve the utilisation of phosphorus. Phytic acid levels in maize grain vary from 0.45 to 1.0 % of dry matter (Monsanto, 1995; Watson, 1982).

B. DIMBOA

2,4-Dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA) belongs to a group of metabolites, hydroxamic acids and benzoxazinoids, commonly found in cereal plants). The glycoside of DIMBOA, DIMBOA-glc, is the most prominent of these compounds in green aerial- and root- tissues of maize during initial plant development (Cambier *et al.*, 2000).

Levels of DIMBOA and related compounds in green- and root- tissues of maize seedlings vary by orders of magnitude (approximately 0-1000 ppm fresh weight) among maize varieties (Xie *et al.*, 1992). High levels are associated with elevated resistance of conventional maize varieties against insects, such as European Corn Borer (Sicker *et al.*, 2000). In addition, these levels change in the course of green tissue development, reaching a maximum within several days after germination and then declining to a fraction within weeks (Cambier *et al.*, 2000).

DIMBOA-glc is enzymatically deglycosylated in injured plant tissues to DIMBOA, which is toxic to insects. The mechanism of DIMBOA's toxicity to insects has not been elucidated yet. In addition, data on the possible toxic and physiological effects of DIMBOA and related compounds on humans and domestic animals are scarce. One report, for example, describes the *in vitro* mutagenicity of DIMBOA in the Ames test (Hashimoto *et al.*, 1979). In addition, a number of reports document hormonal effects of MBOA, a metabolite of DIMBOA, in wild rodents (Korn, 1988). Data on hormonal effects of MBOA in domestic animals are, however, fragmentary.

Analysis of DIMBOA in maize silage is not recommended in Table 15, because of the high variability of its levels among maize varieties, and the fragmentary knowledge on its toxicology.

C. Raffinose

Raffinose is a non-digestible oligosaccharide (NDO), i.e. it cannot be broken down by enzymes in the gastro-intestinal tract. Raffinose is considered an anti-nutrient due to gas production and resulting flatulence caused by its consumption (Maynard *et al.*, 1979). A daily dose of 15 g NDO is considered to be

safe (Voragen, 1998). Raffinose is not a toxicant but may cause discomfort. It can be removed from food and feed by soaking, cooking, enzyme or solvent treatment and by irradiation.

Percentages of raffinose in field maize are 0.21-0.31 %, and in sweet maize 0.1 %. (Naczka *et al.*, 1997; Aung *et al.*, 1993 ; NOTIS Plus, 1999).

D. Other anti-nutrients

Maize contains low levels of trypsin and chymotrypsin inhibitors, neither of which is considered nutritionally significant (White and Pollak, 1995).

E. Identification of allergens

Maize is not a common allergenic food, although in some case-studies, allergic reactions were reported (Hefle, 1996). These reported allergic effects for maize include skin -, gastrointestinal -, and respiratory complaints.³

3. Recently, using sera from 22 maize allergic patients, Pastorello *et al.* (2000) identified two proteins as the major food allergens in maize, *i.e.* a 9-kd lipid transfer protein (LTP) and a 16-kd trypsin inhibitor. The 9-kd LPT represents a significant fraction of the amount of soluble protein in maize and has a high physicochemical stability, thus possessing important general characteristics of food allergens. In another report, zeins, water-insoluble proteins from maize, were implicated as causative agents of allergic responses to a hypoallergenic, cow's milk based infant formula containing maize starch (Frisner *et al.*, 2000). The clinical relevance of these findings is, however, uncertain.

Section IV – Secondary plant metabolites

Secondary plant metabolites are neither nutrients nor anti-nutrients. They are important though for compositional analysis and the comparative approach (OECD, 1997). As part of the comparative approach, selected secondary plant metabolites, for which characteristic levels in the species are known, are analysed as further indicators of the absence of unintended effects of the genetic modification on the metabolism. Characteristic plant metabolites in maize are furfural and phenolic acids (ferulic acid and p-coumaric acid). The biological function is not always known, but furfural might play a role in toxicity and the phenolic acids might influence digestion, while other data suggest beneficial effects.

A. Furfural

Furfural is a heterocyclic aldehyde. It occurs in several vegetables, fruits and cereals. It is used as a pesticide, but also in foodstuff as flavouring. Furfural is generally recognised as safe (GRAS) by FEMA under conditions of intended use as a flavour ingredient, i.e. at levels 100 times lower than the occurrence of furfural as a natural ingredient in traditional foods. Field maize contains < 0.01 ppm (mg/kg) furfural (Adams *et al.*, 1997).

The acute toxicity of furfural is moderate, with LD_{50 (oral)} 50-149 mg/kg bw (rats), 250-500 mg/kg bw (mice), and 650-950 mg/kg bw (dogs) (Adams *et al.*, 1997). In acute and sub-chronic studies in rodents, effects were seen mainly in the liver. Evidence of genotoxicity and carcinogenic activity after oral administration is limited. Furfural is considered an oral genotoxic carcinogen of low potency. An increase of the furfural level in food stuff should be avoided (Feron *et al.*, 1991). Furfural can partly be removed from products by heating.

B. Ferulic acid and p-coumaric acid

The phenolic acids, ferulic acid and p-coumaric acid are structural and functional components of plant cells (Kroon and Williamson, 1999). Their function is, amongst others, to act as a natural pesticide against insects and fungi. Ferulic acid and p-coumaric acid are found in vegetables, fruit and cereals. Also they are used as flavouring in foods, as supplements and in traditional Chinese herbal medicine.

Daily intake of phenolic acids by humans is estimated to be 0.2 - 5.2 mg/day (Clifford, 1999; Radtke *et al.*, 1998). There are indications that phenolic acids may play a role in the beneficial health effects of vegetables and fruits. The anti-oxidative action of phenolic acids might be involved in prevention of chronic diseases. Ferulic acid and p-coumaric acid are weak anti-oxidants. In vitro tests are equivocal as to whether ferulic acid enhances or inhibits the effects of mutagenic substances (Sasaki *et al.*, 1998; Stich, 1992).

Reported concentrations of ferulic acid in field maize kernels are 0.02 to 0.03 % (Notis Plus), 0.02 to 0.1 % (Classen *et al.*, 1990; Rosazza, 1995) or 0.3 % (Dowd and Vega, 1996). Concentrations of p-coumaric acid in field maize kernels are reported to be 0.003 to 0.03 % (Classen *et al.*, 1990; Notis Plus).

Section V- Food use

A. Identification of key maize products consumed by humans

In the EU, 2,9 million tons of field maize is consumed as food along with 21 million tons as feed (Eurostatistics, 1994). Field maize products (Starch, oil, grits, meal and flour) are used in many foods. Starch is mostly fermented to sweeteners (syrups) and ethanol. It is also used for foods, such as bakery products, baby foods, sauces, dressings and soups. Maize oil is used in salad- and cooking oil, mayonnaise, margarine, baking and frying fat and in sauces and soups. Grits are used to make cereals and snacks and also to produce alcoholic beverages. Meal is used for bread and muffins, and flour is used for pancakes and snacks. Bran is used as a dietary source of fibre. Field maize is also used as a raw material for the production of paper, fuel, glue, textiles, pharmaceuticals and soap.

During 1995, consumption of sweet maize (mostly the whole kernel is consumed as vegetable) amounted to 76,000 ton frozen, 298,000 ton canned, and 45,000 ton fresh in the EU (AGPM, 1996).

Popcorn maize kernels are used (in dried form) as popcorn and as a basis for confections (Juggenheimer, 1976).

B. Identification of key products and suggested analysis for new varieties

Since all maize-derived food products are produced from kernels, analysis of the composition of kernels is the most appropriate test for food use. If only agronomical traits are influenced by the genetic modification, derived products need not be analysed separately. In other cases, the additional analysis of derived products can be useful, depending on the nature and purpose of the modification (e.g. deliberately changing the oil composition). This can apply to the following products: maize oil, starch, grits, meal, and flour. The parameters to be analysed are discussed in detail in sections II, III and IV of this document.

Table 14. Suggested nutritional and compositional parameters to be analysed in maize matrices for human food use

Parameter	Oil	Starch	Grits/Meal/Flour	Kernels (Field maize, Sweet maize, Popcorn)
Proximate analysis ^a		X	X	X
Minerals				X
Vitamins				X
Amino acids			(X)	X
Fatty acids	X		X	X
Phytic acid				X
Raffinose				X
Furfural				X
Ferulic acid				X
p-coumaric acid				X

^a: Proximate includes protein, fat, total dietary fibre, ash and carbohydrates

Section VI - Feed use

A. Identification of key maize products consumed by animals

Maize is the preferred feedstuff in livestock production either as a processed whole grain, as a by-product of the milling industry, or as a whole plant silage (Newcomb, 1995). The preference results from its high nutrient value and relative low cost. Yellow dent maize and flint dent maize are the primary types that are fed, though other types of maize such as white, waxy, or popcorn, may be fed under certain economically feasible circumstances. The maize kernel contains the most energy of all the grains used for livestock feed, but also has the lowest crude protein content (9 - 11 %) (Ensminger *et al.*, 1990). However, since maize grain is usually included in high percentage in animal diets, a substantial amount of protein containing essential amino acids is provided by corn. The corn milling industry, as previously mentioned, produces several animal feed products such as gluten feed, gluten meal, distillers grains, distillers solubles, germ meal, and hominy, that are economically attainable in specific areas. The products of major significance are maize gluten feed and maize gluten meal. Most corn gluten feed is fed to ruminants, but some is fed to swine. The major use of gluten meal is in poultry diets because the gluten contains carotenoid pigments that express themselves in skin and eggs of poultry.

B. Identification of Key Products and Suggested Analysis for New Varieties

Maize grain is fed to animals as a source of energy from carbohydrates and oils and provides a source of essential and nonessential amino acids. From the oil, essential fatty acids are also provided. The kernel (grain) is generally fed at moisture levels of 10 to 15 %, which is considered safe for storage. Corn grain is sometimes fed to cattle and swine at moisture levels up to 30-35 % where the maize has either been ensiled or treated with an organic acid. The kernel contains about 83 % carbohydrate that is in the form of starch, pentosans, dextrans, sugars, cellulose, and hemicellulose. Starch makes up the biggest part of the carbohydrate fraction and provides most of the energy. The fibre portion includes the cellulose and hemicellulose portions that are generally unavailable to nonruminants. Maize grain is rich in linoleic acid, one of the essential fatty acids needed by swine and poultry. Maize also has a favorable content of essential amino acids with the exception of lysine and tryptophan that are the most limiting amino acids in corn, particularly for swine. Maize provides an important source of methionine which is the most limiting amino acid in poultry. In cattle and sheep where microbial protein from the rumen is considered the primary protein source for the animal, there is increased interest in proteins that escape rumen fermentation, particularly in high producing dairy cattle. Thus, nutritionists are taking a closer look at the potential for cattle to also have certain limiting essential amino acids. Methionine and lysine have been found to be the two most limiting amino acids for lactating dairy cattle fed corn based diets (NRC, 2001). The ten traditional essential amino acids are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Glycine is considered essential for poultry. Cystine, tyrosine and serine are also important amino acids as they can partially substitute for methionine, phenylalanine and glycine, respectively. Proline has also been shown to be an important amino acid for young chicks (NRC, 1994). Calcium and phosphorus are important minerals in animal nutrition. Maize grain is extremely low in calcium, and thus, not a big contributor to the calcium in animal diets. Maize, on

the other hand is a fair source of phosphorus, yet a substantial amount of the phosphorus is bound in the form of phytic acid - a form of phosphorus that is of little value to nonruminant animals such as swine and poultry (Ensminger *et al.*, 1990). However, many producers are now adding the enzyme phytase to the diet to release some of the bound phosphorus from the phytic acid. Other minerals such as selenium are also important, but the amount in plants has been shown to reflect the amount of the mineral in the soil. Nutritionists incorporate supplemental sources of calcium, phosphorus, sodium chloride, magnesium, iron, zinc, copper, manganese, iodine and selenium as needed to balance diets. Maize grain is a source of vitamins A, E, thiamin, riboflavin, pantothenic acid, and pyridoxine. While niacin occurs in relative high concentration, it is in the form of niocytin that is biologically unavailable. Again, nutritionists supplement swine diets with vitamins A, D, E, K, B12, riboflavin, niacin and pantothenic acid (NRC, 1998); and ruminant diets with vitamins A, D, E, and K.

Maize silage is a very important feed ingredient for feedlot cattle and dairy cattle. In the U.S., approximately 10 % of the maize crop is harvested as silage. It is regarded highly as a palatable energy source (Newcomb, 1995). The whole corn plant contains about one and one-half times the nutrients of the grain, and the ensiling process preserves more than 90 % of the nutrients (Ensminger *et al.*, 1990). In that silage is fed to lactating dairy cows, nutritionists are becoming more interested in the amino acid content of silage, particularly for high producing animals. Concerning minerals and vitamins in silage, a similar situation exists as described for maize grain; although silage contains more calcium, levels are not enough to meet an animal's needs and should be supplemented.

As previously mentioned, most corn gluten feed is fed to ruminants, but some is fed to swine. The major use of gluten meal is in poultry rations because the gluten contains high amounts of protein and carotenoid pigments that express themselves in the skin and eggs of poultry. Cattle nutritionists are including corn gluten meal and dried corn distillers in diets because they are thought to contain by-pass rumen protein (Newcomb, 1995). Thus, the amino acid composition of these maize products has become important in addition to total protein content.

Proximate analyses are commonly conducted on animal feedstuffs, including the amounts of nitrogen, ether extract, ash, and crude fibre. Carbohydrates are measured as starch or nitrogen free extract. Nitrogen free extract, which includes starch, sugars, some cellulose, hemicellulose and lignin, is calculated by subtracting the total of the determinates from 100. Crude protein is calculated by multiplying the nitrogen content by 6.25, a conversion factor based on the average amount of nitrogen in protein. Fat is considered to be acid ether extractable material (Ensminger *et al.*, 1990). In the case of ruminants and swine, the traditional analysis for crude fibre is considered obsolete and has been replaced by analyses for acid detergent fibre and neutral detergent fibre. For amino acids, the ten essential amino acids plus glycine, cystine, tyrosine, serine and proline are the key nutrients. Linoleic is the fatty acid of key importance for the kernel, while the fatty acid spectrum is more important for the oil.

In considering the anti-nutrients and natural toxins in maize, only phytic acid is significant to the animal feed. With the use of the enzyme phytase, it is possible to break down part of the phytic acid and release bound phosphorus and calcium. Hence, the phytic acid content of the grain is beneficial to know.

When one considers the remainder of the maize products that might be fed to animals, their nutrient content would not be expected to change if the whole maize plant and the maize kernel are not changed. Hence, only the whole plant (silage) and the kernel are suggested to be analysed (Table 15).

Table 15. Suggested nutritional and compositional parameters to be analysed in maize matrices for animal feed

Parameter	Kernel	Silage
Proximate	X	X
Amino Acids	X	
Fatty Acids	X	
Calcium	X	X
Phosphorus	X	X
Phytic Acid	X	

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