COMPARATIVE STUDY OF MAIZE CULTIVARS 30 DAYS AFTER POLLINATION (30 DAP): CARBOHYDRATES AND PROTEIN

Félix G. R. Reyes[1], *Antonio M. Iguti*[2] *and Valdemiro C. Sgarbieri*[3]

Departamento de Ciência de Alimentos
Faculdade de Engenharia de Alimentos (FEA),
Universidade Estadual de Campinas (UNICAMP)
Campinas, São Paulo, Brasil

SUMMARY

A comparative study of the carbohydrates and protein of five maize cultivars at 30 days after pollination (DAP) showed a higher protein content for the Supersweet variety, and better protein quality for the Maya Opaque-2, Nutrimaiz, the Supersweet, and the Hybrid L90 x L41, when compared to the Maya Normal. The lipid content also proved to be higher in the four genetically-modified cultivars in relation to the Maya Normal. In contrast, the Maya Normal and the Opaque-2 were higher in starch content, lower in total and reducing soluble sugars, and exhibited only very small amounts ($\doteq$ 1%) of phytoglycogen, a water-soluble polysaccharide. Nutrimaiz and the Hybrid L90 x L41 had approximately half the starch of the Maya Normal cultivar, but presented an appreciable content of phytoglycogen (23-26%), which is typical for sugary maize mutants, and a higher amount of soluble sugars, mainly sucrose, in relation to Maya Normal. The Supersweet had about half the starch content and a very high ($\doteq$ 24%) amount of soluble sugars, mainly sucrose, in relation to the Maya Normal cultivar. The Supersweet also had a negligible amount of phytoglycogen. Nutrimaiz and the Hybrid L90 x L41 showed a much higher proportion of grain in the ears and, as a consequence, a higher yield of dry solids at 30 DAP, when compared with the Maya Normal cultivar.

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1 Professor, School of Food Engineering, State University of Campinas, Campinas, SP, Brazil.

2 Research Assistant, School of Food Engineering of the above-mentioned University.

3 Chief, Department of Food and Nutrition Planning, School of Food Engineering, State University of Campinas, Caixa Postal 1170, 13100 Campinas SP, Brazil.

INTRODUCCION

Maize is a very important cereal grain for animal feeding all over the world, as well as for human nutrition in many countries of South, Central and North America. As a dietary staple, it provides mainly calories (approximately 400 kcal/100 g), 80o/o of which come from starch. Normal corn contains around 72o/o starch, 10o/o protein and 4.4o/o total lipids (1, 2).

Brazil, in spite of being one of the largest world corn producers (25 million metric tons in 1982), consumes much less corn than other American countries, as human food. The main reason is that rice and beans constitute the main staples, rather than corn.

In countries where the cereal is consumed in large quantities, it contributes with a significant percentage of the protein intake. However, Normal corn protein is low in biological value due to its high zein content and, consequently, limiting concentrations of lysine and tryptophan.

The discovery by Mertz, Bates and Nelson (3) of the mutant Opaque-2 (O2) with higher lysine and tryptophan contents, stimulated a great amount of research aimed at producing corn varieties with better composition and higher nutritional values (4, 5). All nutritional studies have revealed that the O2 –containing cultivars possess a much higher nutritive value than Normal corn.

Misra, Mertz and Clover (6, 7) reported that the endosperm genes which reduce starch synthesis, could also decrease the zein level in the seed, when combined with the 02 gene, causing a substantial increase in the lysine and tryptophan proportion.

Da Silva, Teixeira and Lovato (8) notified a double mutant sweet corn (Nutrimaiz) with suo2 endosperm, high lysine and tryptophan contents and outstanding agronomical properties. The overall protein value (9) and the protein composition (10) of this new cultivar were also studied.

The interest and major purpose of the authors (8-10) have been the development of maize cultivars with adequate composition and nutritive value and good processing characteristics for use as special products in human nutrition. In the present paper, a comparison is made of the amino acid composition, protein value and composition of the carbohydrate fractions of five maize cultivars at 30 days after pollination (30 DAP).

MATERIAL AND METHODS

Cultivars — The five maize cultivars utilized in this study were: Maya Normal, Maya Opaque-2 (O2), Nutrimaiz (suo2), a Hybrid L90 x L41 (suo2), obtained from isogenic lines selected from Nutrimaiz, and the Supersweet (Population SRR "Duro" – sh2). For the purpose of the experiment all five cultivars were produced at the experimental farm of the State University of Campinas (Campinas, SP., Brazil) in the same experimental plot and under identical conditions. The ears were harvested 30 days after pollination, and prepared for subsequent studies.

Preparation of the samples — Immediately after harvesting, the fresh ears were taken to the laboratory and the husk removed. The grains –still on the cob– were frozen to -20°C and freeze-dried. After dried, the grains were removed from the cob and ground to flour (100–mesh screen).

Per cent proximate composition and preparation of diets — Determination of total protein, total lipids, ash, fiber, moisture content and preparation of rat diets were done according to AOAC (11). The diets were prepared to contain 9.5% protein (N x 6.25) provided by the freeze-dried corn flour or by casein. The oil and carbohydrate contents in the corn cultivars were subtracted from the amount to be added to the diets, whereas the vitamins and minerals, naturally present in the cultivars, were not taken into consideration.

Amino acids determination — Amino acid composition was determined on the acid hydrolysate (6*N* HCl, 105°C, 22 h) using a Beckman Model 119CL amino acid analyzer and following the procedure recommended by the manufacturer. Tryptophan was determined colorimetrically according to the W procedure of Spies (12).

Fractionation of the carbohydrates — Fractionation of the various carbohydrates was performed essentially by the procedure of González, Rhodes and Dickinson (13) with only minor modifications. The procedure is illustrated in the diagram (Figure 1).

Determination of total and reducing sugars — Total sugars were determined colorimetrically by the phenol/sulphuric acid reaction (14), and the reducing sugars by the colorimetric method of Somogy (15). Sucrose was established by subtracting reducing sugars from total sugars.

Identification of soluble sugars — Fifty milliliters of the fraction containing the soluble sugars (supernatant I, Fig. 1) were percolated through a cation exchange resin (Bio-Rad Ag 50W-X_4, 200-400 mesh, H^+ form) and on anion exchange resin (Bio-Rad AG 1-X8, 200-400 mesh, OH^- form). The system was washed with deionized water until negative reaction for sugars (Molish test). The percolated solution was vacuum concentrated to approximately 2 ml divided into three vials and evaporated to dryness, washed in the same vials with chloroform, and stored under vacuum overnight in a desiccator over phosphorus pentoxide. The purified samples were silylated and analyzed by gas-liquid chromatography according to the procedure of Reyes, Wrolstad and Cornwell (16).

RESULTS AND DISCUSSION

The proximate composition (% dry basis) of the five freeze-dried cultivars is shown in Table 1. The main differences among the cultivars were a higher protein content in the Supersweet and higher lipid contents in Nutrimaiz and Supersweet, as compared to the Maya Normal.

Table 2 depicts the amino acid composition and the net protein ratio (NPR) for the five cultivars. The distinguishing features of the genetically modified cultivars, when compared to Maya Normal, were the higher contents of lysine and tryptophan and a lower ratio of leucine/isoleucine.

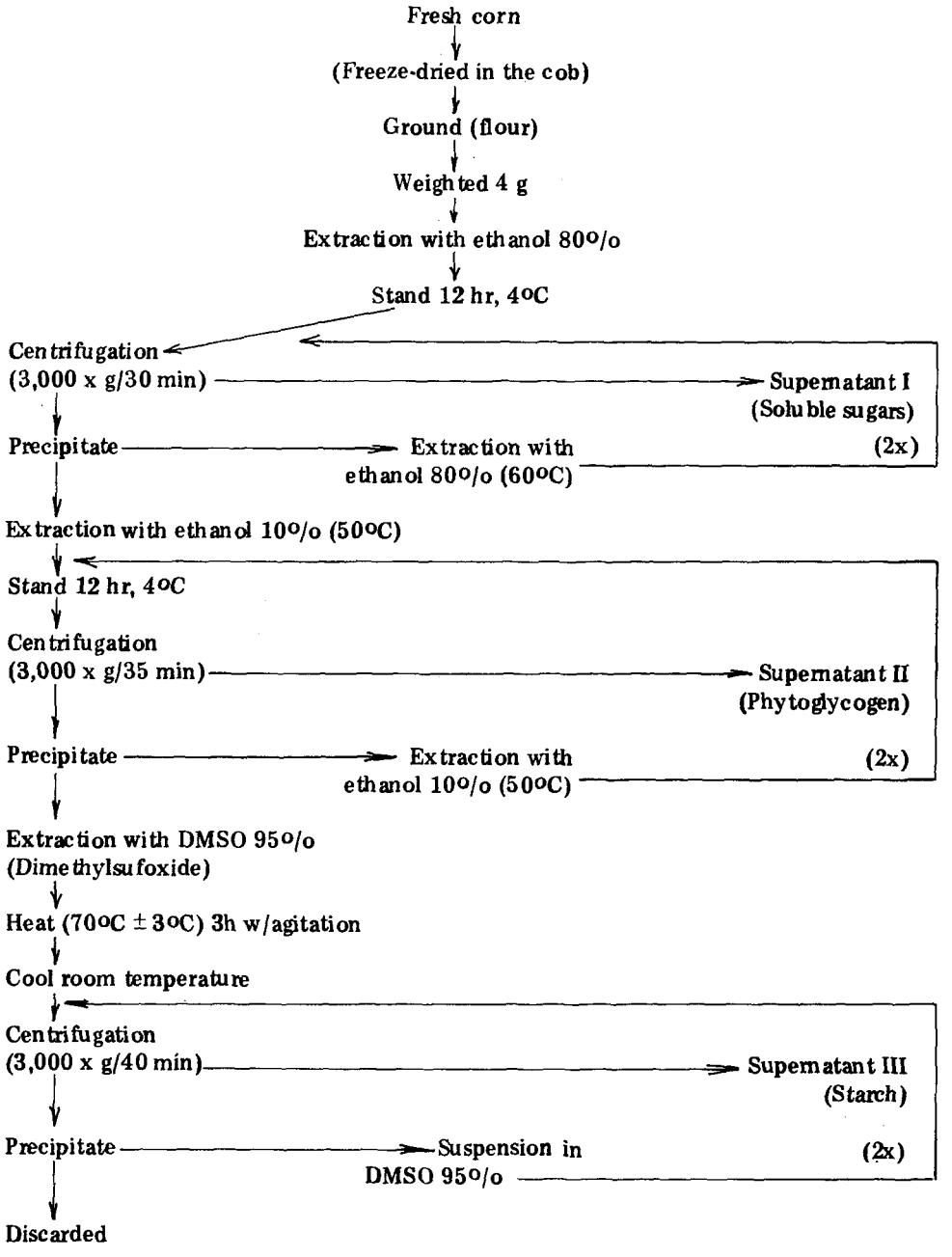

FIGURE 1

Extraction and determination of the carbohydrate fractionation of corn cultivars.

TABLE 1

PER CENT PROXIMATE COMPOSITION (DRY BASIS) OF FIVE MAIZE CULTIVARS 30 DAYS AFTER POLLINATION (30 DAP)

	Protein (%o N x 6.25)	Total lipid (%o)	Ash (%o)	Crude fiber (%o)	Carbohydrate (difference) (%o)
Maya Normal	11.87	3.6	1.8	4.6	78.1
Maya Opaque-2 (02)	10.27	4.0	1.4	4.7	77.6
Nutrimaiz (suo2)	11.43	6.7	3.1	5.2	72.6
Hybrid L90 x L41 (suo2)	10.67	4.6	2.0	5.4	77.3
Supersweet-SRR "Duro" (sh2)	14.90	5.4	1.9	4.8	74.0

Lysine and tryptophan are limiting amino acids in the Normal immature corn protein, becoming even more limiting as maturation develops.

The NPR for the cultivars (Table 2) indicate that the protein quality of Opaque-2, Nutrimaiz, the Hybrid L90 x L41 and of the Supersweet are superior, when compared with the Maya Normal. All genetically-modified cultivars studied had similar NPR values. The NPR of all five cultivars can be compared with the value of 3.90 obtained with a 9.5%o casein diet used as a control, in the same experiment.

Sgarbieri *et al.* (9, 17) have demonstrated that the protein quality of Nutrimaiz and Opaque-2 cultivars is maintained as maturation progresses to "dryness", while that of Maya Normal decreases considerably with maturity. The decrease in protein quality of the Normal corn was explained by a proportional increase in the zein content as the ripening advances.

Gas chromatographic analysis of the soluble sugars revealed the presence, in all samples, of sucrose, glucose and fructose. Reducing sugars varied from 1.6%o to 4.5%o for Maya Normal and Supersweet, respectively. Total sugar was low (4-5%o) for Maya Normal and Opaque-2, slightly higher (6.8-9.3%o) for Nutrimaiz and the Hybrid L90 x L41, and much higher (24.3%o) for the Supersweet. Taking into account that sucrose, glucose and fructose were the only soluble sugars detected in the extracts of all the cultivars studied, the indirect determination of sucrose was considered to be valid. Therefore, sucrose accumulation was considered to be responsible for the higher total sugar content of all cultivars in relation to Maya Normal. These results agree well with those published by González, Rhodes and Dickinson (13).

In agreement with results reported in the literature (13, 18, 19), only small amounts were found (1-1.4%o of phytoglycogen [a water soluble glucan that is more highly branched than the amylopectin component of maize starch (20)] in the starchy Maya Normal (suo2) and Maya Opaque-2 (suo2) as well as in the Supersweet (sh2) genotypes. The phytoglycogen content was higher, 23 and 26%o, for the Nutrimaiz and for the Hybrid L90 x L41, respectively. These levels of phytoglycogen have been reported to be typical for the sugary (su) genotype (18). As

TABLE 2

AMINO ACID COMPOSITION OF FIVE MAIZE CULTIVARS 30 DAYS AFTER POLLINATION (30 DAP)

Amino Acid (g/16 g N)	Maya Normal	Maya Opaque-2	Nutrimaiz	Hybrid (L90 x L41)	Supersweet SRR-"Duro"
Lysine	3.50	4.75	4.35	4.58	3.83
Histidine	3.07	2.71	3.33	2.98	2.32
Threonine	4.20	4.18	3.85	4.01	3.92
Methionine	1.49	1.92	1.74	1.38	1.44
Valine	4.52	5.31	5.22	5.16	4.87
Leucine	15.60	7.12	8.32	6.53	7.89
Isoleucine	2.81	3.39	3.35	3.09	3.25
Phenylalanine	4.10	3.84	4.10	4.44	4.52
Tryptophan	0.40	0.80	0.94	1.15	0.65
Aspartic acid	9.32	8.48	8.45	8.59	8.59
Glutamic acid	31.07	20.00	18.51	20.50	17.40
Serine	6.33	4.52	3.85	4.35	5.22
Proline	10.50	7.57	7.45	7.10	6.38
Glycine	4.08	4.41	4.60	4.35	4.87
Alanine	12.21	11.30	12.55	14.32	12.65
1/2 Cystine	1.11	0.93	0.90	0.70	0.93
Tyrosine	2.20	2.83	3.23	1.72	3.60
Arginine	3.21	4.41	4.35	3.78	4.52
Ammonia	3.51	2.37	2.73	2.29	2.20
Total recovery	123.23	100.77	101.84	100.08	99.06
NPR	2.74 ±0.93	3.88 ± 0.42	3.46 ±0.79	3.22 ±0.22	3.50 ± 0.58

expected, starch content was high (60-65o/o) in Maya Normal and Maya Opaque-2, dropping to less than half (20.8–28.5o/o) in Nutrimaiz, Hybrid L90 x L41 and Supersweet cultivars (Table 3).

Nutrimaiz and the Hybrid L90 x L41 have been studied for the purpose of producing dehydrated fresh corn solids. Table 4 indicates the various proportions of husk, cob and grain in the whole ears, as well as the final percentages of dehydrated solids. At this stage of ripeness the normal corn has about 35o/o solids in the grain, but due to the low proportion of grain, renders the lowest yield. On the other hand, the Hybrid L90 x L41 with 27o/o solids in the grains, gives the highest yield of dry solids, due to the high proportion of grain in the ears. The higher yields of grains and dry solids associated with the high protein quality, the high digestibility of the carbohydrate fraction, and the slow ripening in the field are highly favorable properties of these genetically-modified cultivars for industrialization as fresh corn.

TABLE 3

STARCH, PHYTOGLYCOGEN AND SOLUBLE SUGARS CONTENT OF FIVE MAIZE CULTIVARS 30 DAYS AFTER POLLINATION (30 DAP)*

	Reducing sugars (o/o)	Total sugars (o/o)	Phyto-glycogen (o/o)	Starch (o/o)
Maya Normal	1.6	3.8	1.4	60.3
Maya Opaque-2 (o2)	2.0	5.0	1.0	65.8
Nutrimaiz (suo2)	2.3	6.8	23.3	24.7
Hybrid L90 x L41 (suo2)	2.6	9.3	26.2	28.5
Supersweet (sh2)	4.5	24.3	1.0	20.8

* Values expressed as percentage of dry weight.

TABLE 4

PROPORTION OF GRAINS, COB, HUSK AND DEHYDRATED SOLIDS FROM THREE CULTIVARS OF MAIZE, 30 DAYS AFTER POLLINATION (30 DAP)

Proportions	Maya Normal	Nutrimaiz	Hybrid (L90 x L41)
Huks (o/o)	40.5	51.7	34.5
Cob (o/o)	30.5	6.5	11.8
Grain (o/o)	29.0	41.8	53.7
Dry solids* (o/o)	10.0	12.6	16.0

* Dry solids (o/o) represent industrial yield in relation to the whole corn ear.

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RESUMEN

ESTUDIO COMPARATIVO DE CULTIVARES DE MAIZ 30 DIAS DESPUES DE LA POLINIZACION (30 DAP): CARBOHIDRATOS Y PROTEINAS

Un estudio comparativo de las proteínas y los carbohidratos de cinco cultivares de maíz, 30 días después de la polinización (30 DAP) reveló que, con relación al Maya

Normal, el cultivar Superdulce contenía un mayor porcentaje (14.9o/o) de proteína, la que también era de mejor calidad. Por otra parte, los cultivares Maya-Opaco-2, Nutrimaíz y el Híbrido L90 x L41 también mostraron proteína de mejor calidad. En comparación con el Maya Normal, el contenido lipídico fue mayor en los cuatro cultivares genéticamente modificados. El Maya Normal y el Opaco-2 acusaron alto contenido de almidón, bajo de azúcares solubles totales y reductores, y sólo pequeñas cantidades ($\cong$ 1o/o) de fitoglicógeno, un polisacárido soluble en agua. En contraste con el Maya Normal, el Nutrimaíz y el Híbrido L90 x L41 contenían, aproximadamente, la mitad del almidón. En compensación, estos últimos cultivares mostraron un apreciable contenido de fitoglicógeno (23-26o/o), y un alto contenido de azúcares solubles, principalmente sacarosa. El Superdulce acusó un alto contenido de azúcares solubles ($\cong$ 24o/o, principalmente sacarosa) y una cantidad de almidón igual a la mitad del Maya Normal. El contenido de fitoglicógeno en el Superdulce fue ínfimo. El Nutrimaíz y el Híbrido L90 x L41 tuvieron mayor proporción de granos en la mazorca y, en consecuencia, mayor producción de sólidos secos a los 30 DAP que el Maya Normal.

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