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Effect of germination on protein fractions of corn cultivars

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Ten corn cultivars obtained from Shambat Farm were used in this study. The percentages of the protein fractions—albumin + globulin, prolamin zein, G_1-glutelins, G_2-glutelins, G_3-glutelins and insoluble protein—for the ten corn cultivars were in the ranges 19.5–26.2%, 18.3–35.4%, 20.9–35.3%, 0.9–1.8%, 15.2–23.8% and 1.5–2.4%, respectively. Two selected corn cultivars, Giza and Variety 113, according to their albumin + globulin and zein contents, were soaked in distilled water for 10–18 h and then germinated for 1, 2 and 3 days. The protein fractions in the germinated seeds were determined. Results indicated that the albumin + globulin fraction increased significantly ($P \leq 0.05$) for both cultivars during germination, accompanied by a decrease in the prolamin zein fraction. For both cultivars, the G_1-glutelin fraction decreased significantly ($P \leq 0.05$) during germination. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Corn is an important staple food in many less well-developed countries. Around 10% or more of the corn consumed in these countries, particularly in Central and South America and Africa, is harvested at immature stages of kernel development and boiled or roasted.

After wheat and rice, corn ranks as the most important cereal grain in the world, and provides over one-half of the total calories and the total protein for all the peoples in the developing countries (Hanson, 1974). In the USA, corn is used for animal feed in large quantities, while in other parts of the world it is used primarily for human consumption (Inglett, 1970). In the Sudan the use of corn as Kisra bread is not common unless dura is scarce. There is an increased usage of corn for poultry feed, for forage and fodder. Cereal grains have a low protein content and the protein quality is limited by deficiencies in some essential amino acids, mainly lysine. The protein quality of corn is similar to other cereals except rice. One of the feasible methods of improving the protein quality of corn is by germinating the seeds (Wang & Fields, 1978). Cameron & Hofvander (1971) stated that it is usual to ferment or germinate legumes and corn in African and Indian cultures. Hashim & Fields (1979) reported that the relative nutritive value (RNV) of corn meal made from germinated corn was higher (86%) than that made from control corn meal (69%). The improved RNV was attributed to an increase of essential amino acids, which improved the amino acid balance in the meal.

Tsai *et al.* (1975) found that, during the germination of corn, lysine and tryptophan increased, whereas the protein zein decreased. Study of corn endosperm proteins by the method of Landry & Moureaux (1970), followed by separation of the glutelin-like proteins and true glutelins into subfractions using pH buffer solution and the reducing agent β-mercaptoethanol, gives much more information about the nature of the proteins accumulated in corn than the Mendel & Osborne (1914) method.

Skotch *et al.* (1970), using a traditional procedure, only extracted an average of 31% of the protein in sorghum grain, whereas Jambunathan & Mertz (1973), using a Landry & Moureaux (1970) procedure, extracted an average of 89%.

Luk'yanenko (1974) and Pukrushpan *et al.* (1977) demonstrated that the protein composition, in terms of soluble fractions, changes with maturity of the grain. In kernel analysed shortly after pollination, free amino acids and salt-soluble proteins predominate, whereas in mature kernels zein and glutelins are the major proteins. At the milky stage used for food, an intermediate composition occurs. These changes in composition during maturation were reflected in decreased available lysine in the older grain.

The objective of this investigation was to study the effect of germination on protein fractions of corn cultivars and to investigate protein fractions for corn cultivars using a Landry & Moureaux technique.

Table 1. Protein fractions of ten corn cultivars according to the Landry & Moureaux (1970) method

Cultivar	Total protein (%)	Albumin, globulin and NPN[a] (%)	Zein (%)	G_1-glutelin (%)	G_2-glutelin (%)	G_3-glutelin (%)	Residue (%)	Total protein recovered (%)
Muneng 8934	9.19(±0.09)b	22.7(±0.20) c	32.9(±0.00)b	23.1(±0.00) d	1.73(±0.00) a	21.2(±0.20) b	1.72(±0.06) e	103
Namulonge 8934	8.98(±0.05)	19.5(±0.00) d	28.1(±0.42) d	26.4(±0.56) c	1.78(±0.42) a	23.8(±0.21) a	1.45(±0.00) c	101
Sakha 8934	9.07(±0.29) b	25.2(±0.43) b	25.5(±0.00) c	35.3(±2.87) a	0.91(±0.43) b	15.2(±0.86) e	2.40(±0.00) a	105
Kameny amiggo 8942	9.34(±0.18) b	25.6(±0.28) ab	18.6(±0.42) b	30.2(±1.39) b	1.33(±0.21) a	18.0(±0.42) c	1.81(±0.00) d	95.6
Pirsabak 8942	10.06(±0.13) a	22.7(±0.19) c	30.6(±0.39) c	20.9(±0.52) e	1.38(±0.00)a	19.4(±0.19) b	1.82(±0.10) d	96.8
Pirsabak (1) 8947	8.46(±0.60) c	26.2(±0.31) a	18.3(±0.00) b	32.3(±0.00)b	1.80(±0.23) a	18.5(±0.23) c	1.92(±0.03) c	98.9
Tlaltizapan 8647	9.22(±0.28) b	26.1(±0.00) a	20.5(±0.33) f	25.2(±0.00) c	1.80(±0.00) a	20.6(±0.21) b	2.01(±0.03) c	96.3
Tlaltizapan 8947RE	9.72(±0.11) a	19.9(±0.27) d	27.9(±0.81) d	23.5(±0.00) d	1.57(±0.20) a	19.6(±0.40) b	2.12(±0.00)b	94.7
Giza	9.16(±0.07) c	20.9(±0.23) b	35.4(±0.43) a	22.4(±2.80) d	1.82(±0.43) a	16.3(±0.00) d	2.38(±0.03) a	99.2
Variety 113	8.52(±0.07) c	25.2(±0.23) b	28.0(±0.92) d	21.0(±0.31) d	1.26(±0.00) a	20.4(±0.22) b	1.71(±0.00) c	97.6
Overall mean	9.17	23.40	26.58	26.03	1.54	19.31	1.94	–

[a]Percentage of total protein.
Values are means (±SD). Means not sharing a common letter in a column are significantly different at $P \leq 0.05$, as assessed by Duncan's multiple-range test.
NPN, non-protein nitrogen.

Table 2. Effect of germination on protein fractions of corn cultivar Giza

Germination (h)	Total protein (%)	Albumin, globulin and NPN (%)	Zein (%)	G_1-glutelin (%)	G_2-glutelin (%)	G_3-glutelin (%)	Residue (%)	Total protein recovered (%)
0	9.16(±0.21) c	20.9(±0.29) d	35.4(±0.43) a	22.4(±0.28) a	1.82(±0.43) b	16.3(±0.00) c	2.38(±0.03) b	99.2
24	9.75(±0.12) b	28.1(±0.19) c	36.2(±0.30) a	15.4(±0.00) c	2.06(±0.19) b	18.5(±0.19) a	2.01(±0.00) c	102
48	10.1(±0.03) ab	33.0(±0.00) b	31.9(±0.37) b	18.1(±0.25) b	1.58(±0.00) b	17.4(±0.00) b	1.35(±0.03) d	103
72	10.3(±0.0 9) a	40.4(±0.18) a	24.5(±0.37) c	11.2(±0.24) d	5.3(±0.18) a	16.3(±0.37) c	2.54(±0.03) a	100

NPN, non-protein nitrogen.

Table 3. Effect of germination on protein fractions of corn cultivar Variety 113

Germination time (h)	Total protein (%)	Albumin, globulin and NPN (%)	Zein (%)	G_1-glutelin (%)	G_2-glutelin (%)	G_3-glutelin (%)	Residue (%)	Total protein recovered (%)
0	8.52(±0.08) c	25.2(±0.23) c	28.0(±0.92) c	21.0(±0.31) a	1.26(±0.00) b	20.4(±0.22) a	1.71(±0.00) d	97.6
24	12.7(±0.03) b	18.6(±0.30)d	42.5(±0.30) a	20.0(±0.20) b	1.47(±0.30) b	15.4(±0.44) b	2.43(±0.00) c	100
48	12.7(±0.06)a	26.1(±0.00) b	32.0(±0.30) b	19.7(±0.20)b	5.02(±0.00) a	16.3(±0.00) b	3.40(±0.06) a	103
72	13.1(±0.06) a	37.2(±0.22) a	26.3(±0.21) c	13.3(±0.19) c	4.65(±0.00) a	15.9(±0.14) b	3.23(±0.06) b	101

NPN, non-protein nitrogen.

MATERIALS AND METHODS

Materials

Ten corn cultivars—Maneng 8934, Namulonge 8934, Sakha 8934, Kameny amiggo 8942, Pirsabak 8947, Pirsabak (1) 8947, Tlaltizapan 8947, Tlaltizapan 8244 RE, Giza and Variety 113—obtained from Shambat Farm were used in this study. These samples were carefully cleaned and freed from foreign materials; the grains were ground to pass a 0.4 mm screen.

Protein fractionation

The nitrogen from the defatted meal was extracted stepwise by a series of solvents according to the Landry & Moureaux (1970) procedure.

Thus triplicate 3.5 g samples were kept in suspension with 35 ml of extractant by magnetic stirring in 50 ml centrifuge tubes.

Step 1: To obtain the first fraction, 0.5 M NaCl was added to the sample powder and the mixture was stirred three times: 60, 30 and 30 min at 4°C.

Step 2: The residue was extracted with the same volume of distilled water twice for 15 min at 4°C.

Step 3: To obtain the second fraction, the residual material was stirred with 60% ethanol twice for 30 min at 20°C and then at 60°C for 30 min, followed by extraction with 55% isopropanol (Pr-OH) at 20°C three times (60, 30 and 15 min with stirring).

Step 4: To obtain the third fraction, the residue was extracted with 60% ethanol plus 0.6% 2-mercaptoethanol (2-ME) and stirred twice for 30 min (20°C), then extracted with 55% Pr-OH containing 2-ME (0.6%) at 20°C twice for 30 min.

Step 5: To obtain the fourth fraction, borate buffer, pH 10 (0.0125 M $Na_2B_4O_7 \cdot 12H_2O$ and 0.02 M NaOH) with 0.6% 2-ME and 0.5 M NaCl was used with stirring for 60, 30 and 30 min (20°C).

Step 6: To obtain the fifth fraction, borate buffer, pH 10 with 0.6% 2-ME and 0.5% sodium dodecyl sulphate (SDS) was used with stirring for 60, 30 and 15 min (20°C)

Fraction I contained the albumins and globulins, the free amino acids and small peptide fragments. Fraction II contained the prolamin zein. Fraction III contained the zein-like protein (G_1-glutelin). Fraction IV contained the glutelin-like (G_2-glutelin) protein. Fraction V contained the true glutelin (G_3-glutelin). The solid material was isolated from extractants by centrifugation at 30000*g* for 15 min. For each solvent, supernatants were combined to give the total extract. The nitrogen content of each of these five fractions was detemined by the micro-Kjeldahl method. The residue left after extraction was also analysed for nitrogen content.

Germination of corn samples

Germination was carried out according to the method of Bhise *et al.* (1988). Broken grains were removed by hand. The seeds were soaked with about 3 volumes of distilled water overnight at room temperature, with two changes of water during the day to remove dirt and extra husks. The wet grains were then soaked in 1–2 volumes of 0.2% formaldehyde solution for 40 min to retard mold growth during germination. The soaked grains were then washed with distilled water several times and soaked in water for 20 min to remove residual formaldehyde. These seeds were spread evenly (about 1 cm thick) in a plastic tray, with plenty of air space, covered with cheese cloth and germinated in an air-circulating incubator at 30±2°C for 1, 2 and 3 days. Water was sprinkled on the grains every 12 h to avoid drying; non-germinated and moldy seeds were discarded. Sprouted corn samples were dried at 50°C to constant weight and finely ground. Samples were taken to fractionate the protein on the basis of solubility.

Statistical analysis

Each sample was analysed in triplicate and the figures were then averaged. Data were assessed by analysis of variance (ANOVA) (Snedecor & Cochran, 1987) and by the Duncan's multiple range test with a probability $P \leq 0.05$ (Duncan, 1955).

RESULTS AND DISCUSSION

Distribution of protein groups

The percentages of protein fractions of ten corn cultivars are shown in Table 1. Water and salt-soluble proteins, albumin and globulin fractions made up 19.5% of the total protein for Namulonge 8934 and 26.2% for Pirsabak (1) 8947. The prolamin zein fraction was 18.3% for Pirsabak (1) 8947 and 35.4% for Giza. Zein was the predominant fraction for most corn cultivars, while the zein-like (G_1-glutelin) fraction varied from 20.9% for Pirsabak 8947 to 35.3% for Sakha 8934; the glutelin-like fraction (G_3-glutelin) ranged from 15.2% to 23.8% for Sakha 8934 and Namulonge 8934, respectively. Residual protein for the varieties of corn studied ranged from 1.45% to 2.40%.

Pirsabak (1) 8947 cultivar had the lowest amount of zein and the highest amount of albumins and globulins; the reverse was true for the Giza cultivar. Esen (1980) indicated that the zeins, as a percentage of total extractable protein, or the ratio of zein to non-zein protein, can be used to estimate the protein quality.

Germination and the protein fractions

Table 2 shows the variation in protein fractions during the germination of cultivar Giza. The albumin + globulin fractions increased significantly ($P \leq 0.05$) as a result of germination for 72 h, while the zein fraction slightly increased during the first day of germination and then started to decrease significantly ($P \leq 0.05$) until it reached a minimum value after 72 h of germination.

The G_1-glutelin fraction decreased significantly ($P \leq 0.05$) during the period of germination; it reached its minimum value on the third day of germination, while the G_2-glutelin fraction fluctuated during the germination process. The G_3-glutelin fraction was not affected after 72 h of germination, but it increased significantly ($P \leq 0.05$) after 2 days of germination and then slightly increased at the end of germination.

Wu & Wall (1980) fractionated germinated sorghum protein and found that the 3 day sprouted sorghum had a higher albumin, and lower prolamin, with a small increase in globulin and no definite trend for glutelin and residue fractions. This would seem to agree with our results. The increase in albumin fraction would result in the improvement of the nutritive value of the grains as the albumin fraction is rich in the amino acid lysine. Thus, the nutritive value of corn would be expected to increase due to the increase in the albumin and globulin fractions as a result of germination.

Table 3 shows the variations in protein fractions during germination of corn cultivar Variety 113. The albumin and globulin fractions decreased on the first day of the germination process but then started to increase significantly ($P \leq 0.05$), reaching a maximum value after 72 h of germination.

The prolamin zein fraction increased significantly ($P \leq 0.05$) during the first 2 days of germination. The G_1- and G_3-glutelin fractions decreased significantly ($P \leq 0.05$) during germination. The G_1-glutelin reached a minimum value after 72 h of germination, while the G_3-glutelin had its minimum value on the first day of germination. G_2-glutelin and residue fractions increased significantly ($P \leq 0.05$) as a result of germination, reaching a maximum value after 2 days of germination.

CONCLUSIONS

The results obtained in this study have shown a large variation in the protein fractions of the ten cultivars of corn studied, especially in the albumin and globulin fractions and prolamin zein fractions. Corn cultivar Pirsabak (1) 8947 was superior in this respect compared to all the others.

Two cultivars of corn, one rich in albumin and globulin fractions and the other cultivar rich in prolamin zein fraction, were germinated for 3 days and proteins were fractionated. The results showed that, for both cultivars, the albumin and globulin fractions were significantly increased as a result of germination at the expense of the prolamin zein fraction.

Thus it could be concluded that germination greatly improves the nutritive value of corn.

REFERENCES

Bhise, V. J., Chavan, J. K. & Kedum, S. S. (1988). Effect of malting on proximate composition and *in vitro* protein and starch digestibilities of grain sorghum. *J. Food Sci. Technol.*, **25**, 327–329.

Cameron, M. & Hofvander, Y. (1971). *Manual of Feeding Infant and Young Children*. Protein Advisory Group of the UN, New York, p. 73.

Duncan, B. O. (1955). Multiple range and multiple *F*-tests. *Biometrics*, **11**, 1–42.

Esen, A. (1980). Estimation of protein quality and quantity in corn (*Zea mays* L.) by assaying protein in two solubility fractions. *J. Agric. Food Chem.*, **28**(3), 529–532.

Hanson, H. (1974). Role of maize in world food needs to 1980. In *Symposium Proceedings: Worldwide Maize Improvement in the 70's and Role for CIMMYT*, pp. 1–10.

Hashim, N. B. & Fields, M. L. (1979). Germination and relative nutritive value of corn meal and corn chips. *J. Food Sci.*, **44**, 936–937.

Inglett, G. E. (1970). Food uses of corn around the world. In *Corn: Culture, Processing Products*, ed. G. E. Inglett. Avi Publishing, Westport, CT, p. 123.

Jambunathan, R. & Mertz, E. T. (1973). Relationship between tannin levels, rat growth and distribution of proteins in sorghum. *J. Agric. Food Chem.*, **21**, 691–696.

Landry, J. & Moureaux, T. (1970). Heterogeneity of the glutelins of the grain of corn: selective extraction and composition in amino acids of the three isolated fractions. *Bull. Soc. Chim. Biol.*, **52**, 1021–1037.

Luk'yanenko, E. Kh. (1974). Change in nitrogenous substances in ripening sweet corn kernels. *Dokl. Vses. Akad. Skh. Nauk*, **4**, 16.

Mendel, L. B. & Osborne, T. B. (1914). Nutritional properties of proteins of maize kernel. *J. Biol. Chem.*, **18**, 1–4.

Pukrushpan, T., Mitchell, H. L. & Greig, J. K. (1977). Effect of stage of maturity on protein fractions of sweet corn cultivars. *J. Food Sci.*, **42**, 851–852.

Skotch, I. V., Deyoe, C. W., Shoup, F. K., Bathurst, J. & Liang, D. (1970). Protein fractionation of sorghum grain. *Cereal Chem.*, **47**, 472–481.

Snedecor, G. W. & Cochran, W. G. (1987). *Statistical Methods*, 17th edn. The Iowa State University Press, Ames, pp. 221–222.

Tsai, C. Y., Dalby, A. & Jones, R. A. (1975). Lysine and tryptophan increase during germination of maize seed. *Cereal Chem.*, **52**(1-61), 356–358.

Wang, Y. D. (1977). Enrichment of ingredients for fabricated foods by fermenation and germination of corn and sorghum. Ph.D Thesis, University of Missouri, Columbia, MO.

Wu, Y. V. & Wall, J. S. (1980). Lysine content of protein increased by germination of normal and high lysine sorghums. *J. Agric. Food Chem.*, **28**, 455–458.