Table III. Biological Oxygen Demand of Treated Soybean Whey

Treatment	Protein (N X 6.35), Mg./Ml.	Protein Precipitated, %	BOD, P.P.M.	Decrease of BOD, %
None	4.26	..	13,730	...
Kelgin (1.87 g./l.)	0.32	93	12,590	8.3
Nacconol FSNO (1.7 g./l.)	0.88	79	11,230	18.2
Duponol C (2.55 g./l.)	0.88	79	11,500	16.2
Heat, 80° C., 15 min.	2.39	44	12,320	10.3

tase activities of whey were found in the gum-protein complexes. These low activities are attributed partly to the insolubility of the complexes at the assay pH of 6.0.

Table III compares BOD of the original whey with that of whey preparations treated in various ways to remove proteins. Although Kelgin precipitated more protein, the decrease in BOD was less than with Nacconol. This may result from incomplete precipitation of Kelgin in the complex at this ratio of Kelgin to protein. Apparently protein removal does not solve the BOD problem, but recovery of valuable products, such as lipoxidase or high-quality edible protein, should contribute to solving the waste disposal problem.

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Literature Cited

(1) American Public Health Association, "Standard Method for the Examination of Water and Sewage," 9th ed., pp. 139–144, New York, 1946.
(2) Belter, P. A., Beckel, A. C., Smith, A. K., *Ind. Eng. Chem.* **36,** 799 (1944).
(3) Circle, S. J., Smith, A. K., *J. Phys. Chem.* **45,** 916 (1941).
(4) Hofreiter, B. T., Bennie, J., Hamerstrand, G. E., Mehltretter, C. L., *J. Chem. Eng. Data* **5,** 480 (1960).
(5) Holman, R. T., in "Methods of Biochemical Analysis," D. Glick, ed., Vol. **II,** p. 113, Interscience, New York, 1955.
(6) Kunitz, M., *J. Gen. Physiol.* **30,** 291 (1947).
(7) Liener, I. E., Wada, Shohachi, *J. Biol. Chem.* **222,** 695 (1956).
(8) Lowry, O. H., Rosebrough, W. J., Farr, A., Randall, R. J., *Ibid.,* **193,** 265 (1951).
(9) McKinney, L. L., Weakley, F. B., Campbell, R. E., Eldridge, A. C., Cowan, J. C., Picken, J. C., Jacobson, N. L., *J. Am. Oil Chemists' Soc.* **34,** 461 (1957).
(10) Mayer, F. C., Campbell, R. E., Smith, A. K., McKinney, L. L. *Arch. Biochem. Biophys.* **94,** 301 (1961).
(11) Mehltretter, C. L., Rankin, J. C. Watson, P. R., *Ind. Eng. Chem.* **49,** 350 (1957).
(12) Noelting, G., Bernfeld, P., *Helv. Chim. Acta* **31,** 286 (1948).
(13) Pallansch, M. J., Liener, I. E. *Arch. Biochem. Biophys.* **45,** 366 (1953).
(14) Putnam, F. W., in "Advances in Protein Chemistry," M. L. Anson, J. T. Edsall, eds., Vol. **IV,** p. 79, Academic Press, New York, 1948
(15) Rackis, J. J., Anderson, R. L., Sasame, H. A., Smith, A. K., Van Etten, C. H., J. Agr. Food Chem. **9,** 409 (1961).
(16) Rackis, J. J., Sasame, H. A., Anderson, R. L., Smith, A. K., *J. Am. Chem. Soc.* **81,** 6265 (1959).
(17) Sloan, J. W., Mehltretter, C. L., Senti, F. R., *J. Chem. Eng. Data,* to be published.
(18) Smith, A. K., Schubert, E. N., Belter, P. A., *J. Am. Oil Chemists' Soc.* **32,** 274 (1955).
(19) Smith, H. E., Russell, C. R., Rist, C. E., *Cereal Chem.* to be published.
(20) Wolf, W. J., Briggs, D. R., *Arch. Biochem. Biophys.* **85,** 186 (1959).

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WHEAT MALTING

Effects of Gibberellins and Certain Synergists on Enzyme Production during the Malting of Wheat

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Numerous workers have studied the response of cereal grains to treatment with gibberellins during the malting process (*9*). Enzyme activities, extract yield, degree of modification, and other criteria of malt quality were improved by the proper use of gibberellic acid (GA). Dickson, Shands, and Burkhart (*7*), Bawden, Dahlstrom, and Sfat (*2*), Kringstad, Busengdal, and Rasch (*15*), and Linko and Enari (*16*) have investigated the effects of various other plant regulators on the malting process and malt quality. These substances, however, did not stimulate growth processes, but reduced growth. The results suggested that such materials might be useful in curtailing malting losses. Coumarin (*14*) and potassium bromate (*19*) have also been used for this purpose.

Moffatt and Radley (*24*) found that copper, silver, and manganese salts of GA were more effective than those of the alkaline earth elements or GA when applied to pea plants.

Investigators employing excised plant parts and tissue cultures (*5, 11, 31*) have demonstrated synergistic responses when GA and other growth substances were present at proper levels. Comparatively few attempts have been made, however, to use other substances with GA during malting. Macey (*18*) reported that combinations of GA and potassium bromate were effective in increasing enzyme activities, while curtailing malt loss. Similar results were obtained when 2,4-dichlorophenoxyacetic acid and 2(3)-benzoxazolone were used in combination with GA (*16, 17*). Pollock (*26*) found that the effect of GA on the germination of some dormant barleys could be enhanced by the use of hydrogen sulfide. Mastovsky, Karel, and Kahler (*21*) reported that combinations of glucose and GA were more effective than GA alone.

This study investigated the possibility of further increasing the effect of gibberellins on the production of α-amylase and protease during the malting of wheat through the use of GA in combination with other chemicals.

Experimental

Malts were prepared on a laboratory scale, using 50-gram samples of Triumph (Hard Red Winter) wheat. Grain was steeped at 50° F. to 42 % moisture con-

The effect of potassium gibberellate (GA-K) on production of α-amylase and protease during wheat malting was enhanced by concomitant use of appropriate levels of indole-3-acetic acid, 2,4-dichlorophenoxyacetic acid, or 1-naphthaleneacetic acid. Responses were of a synergistic nature. Synergistic responses also were elicited by combinations of GA-K and kinetin or certain thiol compounds. Treatments with combinations of GA-K and low levels of succinic or fumaric acids increased enzyme activities. Iron, copper, manganese, and silver gibberellates were more effective than the free acid or its alkaline earth salts. High concentrations of indole-3-acetic acid, 2,4-dichlorophenoxyacetic acid, and naphthaleneacetic acid or comparable amounts of maleic acid hydrazide, coumarin, or 2,3,5-triiodobenzoic acid reduced the stimulatory effects of gibberellin. Certain combinations of GA-K and growth substances also reduced malting loss by restricting growth.

Table I. Effect of Auxins and Auxin Antagonists with Gibberellic Acid on the Malting of Wheat

GA Concn., P.P.M.	Growth Substance, P.P.M.	α-Amylase Activity Change, %	Protease Activity Change, %	Malt Yield, %
		AUXINS		
	Control			
0	0			91.0
1	0	+11	+12	90.6
5	0	+27	+26	89.8
10	0	+47	+31	89.3
	Indole-3-acetic acid			
0	3		+4	91.5
0	5	−5		92.0
1	3	+15	+22	91.5
1	5	+13	+13	91.3
5	3	+39	+37	90.5
5	5	+29	+32	91.5
10	3	+54	+40	90.5
10	5	+50	+32	90.8
	2,4-Dichlorophenoxyacetic acid			
0	3	−2		92.0
0	5	−6	−4	92.0
1	3	+17	+16	91.8
1	5	+9	+16	91.8
5	3	+37	+37	90.5
5	5	+31	+30	92.0
10	3	+53	+40	91.4
10	5	+44	+29	91.7
		AUXIN ANTAGONISTS		
	Control			
0	0			90.4
1	0	+10	+9	90.5
5	0	+27	+26	89.6
10	0	+43	+30	89.0
	Maleic hydrazide			
0	3	−4	+6	90.5
0	5	−7	+3	91.0
1	3	+10	+4	90.4
1	5	+6	+7	91.1
5	3	+24	+20	90.5
5	5	+20	+24	91.0
10	3	+41	+30	91.0
10	5	+35	+20	91.4
	Coumarin			
0	25	−4	−2	89.7
0	50	−8	−1	90.0
0	75	−17	−16	91.3
5	25	+2		90.3
5	50	−2	−7	91.4
5	75	−10	−11	91.3
10	25	+12	+6	90.5
10	50	+2		91.1
10	75	−3	−6	91.5

Table II. Effect of Thiol Compounds with Gibberellic Acid on the Malting of Wheat

Concn., GA P.P.M.	Treatment Thiol Compound, Molarity	α-Amylase Activity Change, %	Protease Activity Change, %
0	0	..	..
10	0	+45	+28
	Glutathione		
0	0.0005	+3	+4
0	0.001	+3	+4
10	0.0005	+50	+35
10	0.001	+53	+37
	2,3-Dimercapto-propanol		
0	0.0005	+2	+7
0	0.001	+5	+5
10	0.0005	+52	+35
10	0.001	+56	+37
	Thiourea		
0	0.005	+1	+4
0	0.01	+1	+3
0	0.02	+4	+1
0	0.04	+2	+4
10	0.005	+50	+32
10	0.01	+53	+35
10	0.02	+56	+37
10	0.04	+53	+35

tent in the solutions indicated in Tables I to IV. Damage to embryos by asphyxiation or through the accumulation of inhibitory substances in the steep liquor was minimized by changing the liquid at 6-hour intervals. Seeds were germinated in perforated polyethylene containers placed in a germination cabinet (*10*) in which a saturated atmosphere and a temperature of 62° ± 1° F. were maintained. The germination period throughout was 4 days. During germination the grain was sprayed with 5 ml. of the solutions indicated in Table V. Malts were kilned in a forced-convection oven for 24 hours at 104° F.

The salts of gibberellic acid were prepared as described by Moffatt and Radley (*24*). Except for these experimentally prepared salts, the gibberellin em-

Table III. Effect of Kinetin with Gibberellic Acid on Enzyme Production during Malting

Treatment			
GA concn., p.p.m.	Kinetin, p.p.m.	α-Amylase Activity Change, %	Protease Activity Change, %
0	0	..	..
0	1	+2	0
0	5	−2	+2
5	0	+26	+27
10	0	+50	+33
5	1	+32	+33
10	1	+56	+40
5	5	+34	+35
10	5	+57	+40

Table IV. Effect of Fumaric and Succinic Acids with Gibberellic Acid on Enzyme Production during Malting

Treatment[a]			
GA concn., p.p.m.	Acid molarity	α-Amylase Activity Change, %	Protease Activity Change, %
0	0	..	..
10	0	+52	+34
	Fumaric		
0	0.0001	+10	+12
10	0.0001	+60	+43
0	0.0005	+8	+6
10	0.0005	+60	+40
	Succinic		
0	0.0001	+7	+6
10	0.0001	+60	+42
0	0.0005	+9	+10
10	0.0005	+63	+45

[a] Fumaric and succinic acids added to steep water 12 hours before conclusion of steep period; GA added 1 hour before end of steep period.

Table V. Increasing Enzyme Activities of Malt by Spray

Treatment, Mg.	α-Amylase Activity Change, %	Protease Activity Change, %	Malt Yield, %
Control, no spray			90.5
Control, sprayed with water	+2	+3	90.0
GA-K			
0.5	+25	+20	89.5
0.75	+32	+23	88.7
1.0	+29	+20	89.3
Glucose			
5			91.2
1	+1		90.5
0.5	+3		90.7
Urea			
5	+3	+6	90.7
1	+6	+8	90.5
0.5	+6	+8	90.5
GA-K + glucose			
0.75 5	+30	+23	90.0
0.75 1	+32	+21	89.5
0.75 0.5	+32	+23	89.5
GA-K + urea			
0.75 5	+35	+25	89.9
0.75 1	+38	+28	90.2
0.75 0.5	+35	+28	90.3

ployed was a commercially prepared potassium gibberellate (GA-K).

α-Amylase activities were determined by the procedure of Sandstedt, Kneen, and Blish (*29*). Protease activity was measured by the method of Miller (*23*).

Results and Discussion

The growth - regulating substances (other than GA-K) used in this study consisted of those which may be classed as auxins, antiauxins, or auxin antagonists. The auxins were indole-3-acetic acid, 2,4 - dichlorophenoxyacetic acid (2,4-D), and 1-naphthaleneacetic acid, while the antiauxins or auxin antagonists were maleic hydrazide, coumarin, and 2,3,5-triiodobenzoic acid. Results typical of those obtained by the use of auxins, auxin antagonists, GA-K, and combinations with GA-K are recorded in Table I. Relatively low auxin levels (less than 5 p.p.m.) had little or no effect on enzyme production or malt yield. Higher levels (above 10 to 15 p.p.m.) drastically reduced growth rate and enzyme activities (*8*). The highest level reported here (5 p.p.m.) was slightly inhibitory. These data agree with those of previous investigators (*2, 7, 17*). The results obtained by the use of 1-naphthaleneacetic acid (*8*) were similar to those produced with indole-3-acetic acid and 2,4-D. The use of GA-K alone appreciably increased enzyme activities and growth, but reduced the malt yield. Treatments involving simultaneous use of GA-K at 5 to 10 p.p.m. and indole-3-acetic acid or 2,4-D at 1 to 5 p.p.m. in most instances increased enzyme activities over those which were induced by treatment with GA-K alone. The response to these treatments, when enzyme production was considered, was synergistic. Malting losses were reduced by curtailed growth of acrospires and rootlets. Clor, Currier, and Stocking (*6*) found that combinations of 2,4-D and GA induced synergistic responses with cotton seedlings. These data appear to confirm the synergistic responses obtained with combinations of auxins and gibberellin reported by Galston and McCune (*11*) and Brian and Hemming (*5*). Linko and Enari (*16*) used high concentrations of 2,4-D and low levels of GA and obtained no increase in α-amylase activity over that of the control. Their results indicated, however, that GA was able to reverse partially the depressing effect of 2,4-D on amylase production, while simultaneously increasing malt yield. The responses obtained in the present study when higher levels of auxins were used in combination with GA-K were similar to those previously reported (*16*). Reactions of other varieties of wheat to these treatments were similar, but demonstrated some dependence on variety (*8*). The contention of Brian and Hemming (*5*) that the responses elicited by a given treatment depended on the level of endogenous auxin in the tissues may explain the observed variations.

Maleic hydrazide did not stimulate growth or enzyme production at any of the concentrations employed, thus confirming the results of other workers (*2*). 2,3,5 - Triiodobenzoic acid was found to be slightly more inhibitory than maleic hydrazide (*8*). The results obtained from the use of coumarin agree with those of Kirsop and Pollock (*14*), who found that it restricted growth and reduced enzyme activities. These chemicals appear to counteract the effect of GA-K to varying degrees, depending on the concentration of each component present. Brian and Hemming (*4*) previously reported that maleic hydrazide blocks the action of GA. Mayer (*22*) found that GA could not reverse the effect of coumarin on the growth of seedlings, but was able to reverse, in part, its germination-inhibiting ability.

The effect of thiol compounds alone and in combination with GA-K on enzyme production is given in Table II. The use of $5 \times 10^{-4}M$ glutathione, 2,3-dimercaptopropanol, or $2 \times 10^{-2}M$ thiourea was found to increase enzyme activities slightly. The protease was affected slightly more than α-amylase. The combinations of thiol substances and GA-K increased the activities of both α-amylase and protease. The responses to these combinations appeared to be moderately synergistic. The molar centrations of thiourea, which were required to induce a given response, were appreciably higher than those of the other compounds in this group. The glutathione and 2,3 - dimercaptopropanol used were in the reduced form, while the thiourea probably was a mixture of both tautomeric forms and, hence, lacked the reducing power of BAL. Haber and Luippold (*12*) recently reported a synergistic effect of gibberellic acid and thiourea on lettuce seed germination. Marré and Arrigoni (*20*) suggested that some relation exists between auxin activity and the ratio of the oxidized to the reduced form of glutathione and that growth was correlated with the redox potential. While the interaction between compounds of this type and GA-K is of theoretical interest, its practical importance is doubtful because of objectionable odors contributed by sulfhydryl compounds and the carcinogenic property of thiourea.

The effects of combinations of GA-K and kinetin on enzyme activity are given in Table III. Kinetin alone had little or no effect on seed germination or enzyme production. A rather striking stimulation was noted, however, when kinetin was combined with GA-K. Interactions of this type have been reported by workers using tissue cultures (*31*) and by Skinner, Talbert, and Shive (*30*) and

Haber and Luippold (*12*) on seed germination.

Data concerning the effect of steeping wheat in fumaric and succinic acids are given in Table IV. Earlier studies (*8*) had indicated that these substances and α-ketoglutaric and malic acids caused increased growth and enzyme production when present in the steep liquor at approximately the same concentrations.

The increase caused by these treatments was significant, although slight as compared with that which may be caused by GA. When combined with GA-K, fumaric or succinic acids induced higher levels of enzyme activity than by the use of GA-K alone. The response to these combinations appeared to be additive rather than synergistic. The stimulation of growth caused by the use of these acids confirmed the work of Blagoveschenskiĭ and Petrochenko (*3*). Ruge (*27*) stated that the degeneration of seeds on aging was due to the loss of readily oxidizable substances, thus reducing respiration. The responses noted during the current study could result from the increased respiration rate of GA-K-treated seeds over that of the controls (*8, 25*). The use of these acids, in dilute concentrations, particularly in combination with gibberellin, may have some practical application in malting.

Studies by several investigators (*9, 28*) indicated that significant increases in enzyme activities are induced when the grain is sprayed with GA during the germination period. Previous work (*8, 9*) had indicated that the wheat was most responsive to this type of treatment at the start of the germination period. Results obtained from application of GA-K alone and in combinations with glucose and urea as a spray are reported in Table V. Optimum levels of GA-K, when applied in this manner, appeared to be 0.75 to 1.0 mg. per 50 grams of wheat. Mastovsky *et al.* (*21*) found a greater stimulation of the malting process when glucose was added with GA than that induced by GA alone. The present work did not confirm this. Urea, however, applied singly or in combination with GA increased enzyme production. It is assumed that urea hastens growth in this instance as it does when applied as a commercial fertilizer. The complementary action of urea when added with GA-K may reflect the need of GA-treated seeds for additional nitrogenous material to sustain growth. Alvim (*1*) found that combinations of urea and GA were more effective than GA alone when applied to bean plants. Further studies appear to be warranted.

α-Amylase and protease activities of wheat malts produced after treatment with various salts of GA are given in Table VI. The experimentally produced alkaline earth salts induced the formation of approximately the same levels of enzyme activitity as those obtained following treatment with GA or commercial GA-K. These results agree with those reported for these salts by Moffatt and Radley (*24*) and Halevy and Cathey (*13*). Cobalt gibberellate appeared to be slightly less effective than the other compounds tested. Silver, copper, iron, and manganese gibberellates were more effective than other salts. These results agree with those of Moffatt and Radley (*24*) for the copper, manganese, and silver salts. Combinations of gibberellic acid and metal carbonates or hydroxides were used in steep water to check the possibility that metallic ions, per se, were responsible for the increased efficiency of some gibberellates. These combinations were found to cause essentially the same responses obtained with GA or GA-K; hence, the metallic ions apparently must be combined with GA to cause the effects summarized in Table VI.

Table VI. Comparison of Gibberellic Acid and Some Derivatives on the Production of Enzymes during Malting

Compound[a]	α-Amylase Activity, % of Control	Protease Activity, % of Control	Melting Points, °C. Observed	Melting Points, °C. Reported by Moffatt and Radley (24)
Control	100	100		
GA	130	121		
GA-K, commercial	130	126	227–230 d.	225–229 d.
GA-K, experimental	128	117	226–228 d.	225–229 d.
GA-Na	125	117	232–236 d.	232–238 d.
GA-Ca	133	129	261–265	260
$GA-NH_4$	128	119	165–168 d.	167–170 d.
GA-Ag	140	134	203–205 d.	198–199 d.
GA-Cu	143	134	248–251 d.	245–248 d.
GA-Co	121	126	203–205 d.	204–206 d.
GA-Fe	147	134		
GA-Mn	138	129	258–264 d.	261–266 d.
GA-Mg	131	121	174–176	

[a] Gibberellins were present in steep water at 0.0005% for final hour only of steep period.

Conclusion

This study has indicated further possibilities for the use of gibberellins during malting. Combinations of GA and auxins at levels which induce moderately large increases in enzyme activities without stimulating growth excessively would appear to be of potential commercial importance. Specific recommendations cannot be made because of the complexity of the problem, but it would seem possible for malt users to develop acceptable treatments tailored to their needs. More detailed studies are necessary to elucidate the mechanisms involved in the action of GA and the interactions between GA and other substances.

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Literature Cited

(1) Alvim, P. T., *Plant Physiol.* **35,** 285–92 (1960).
(2) Bawden, R. F., Dahlstrom, R. V., Sfat, M. R., *Am. Soc. Brewing Chemists Proc.* 137–41 (1959).
(3) Blagoveschenskiĭ, A. V. Petrochenko, U. A., *Fiziol. Rasteniĭ* **6,** 53–60 (1959).
(4) Brian, P. W., Hemming, H. G., *Ann. Appl. Biol.* **45,** 489–97 (1957).
(5) Brian, P. W., Hemming, H. G., "Plant Growth Regulation," pp. 645–53, Iowa State Univ. Press, Ames, 1961.
(6) Clor, M. A., Currier, H. B., Stocking, C. R., *Botan. Gaz.* **120,** 80–7 (1958).
(7) Dickson, A. D., Shands, H. L., Burkhart, B. A., *Cereal Chem.* **26,** 13–23 (1949).
(8) Fleming, J. R., M. S. thesis, Kansas State University, Manhattan, 1961.
(9) Fleming, J. R., Johnson, J. A., J. Agr. Food Chem. **9,** 152–5 (1961).
(10) Fleming, J. R., Johnson, J. A., Miller, B. S., *Cereal Chem.* **37,** 363–70 (1960).
(11) Galston, A. W., McCune, D. C., "Plant Growth Regulation," pp. 611–25, Iowa State University Press, Ames, 1961.
(12) Haber, A. H., Luippold, H. J., *Plant Physiol.* **35,** 486–94 (1960).
(13) Halevy, A. H., Cathey, H. M., *Botan. Gaz.* **122,** 63–7 (1960).
(14) Kirsop, B. H., Pollock, J. R. A., *J. Inst. Brewing* **65,** 259–65 (1959).
(15) Kringstad, H., Busengdal, H., Rasch, S., *Ibid.*, **66,** 477–80 (1960).
(16) Linko, M., Enari, T. M., *J. Inst. Brewing* **66,** 480–6 (1960).
(17) Linko, M., Linko, P., Enari, T. M., *Cereal Chem.* **38,** 60–6 (1961).
(18) Macey, A., *Brewers Dig.* **35** (10), 49–53 (1960).

(19) Macey, A., Stowell, K. C., *J. Inst. Brewing* **63,** 391–6 (1957).
(20) Marré, E., Arrigoni, O., *Physiol. Plantarum* **10,** 289–301 (1957).
(21) Mastovsky, J., Karel, V., Kahler, M., *Kvasný průmysl* **5,** 81–6 (1959).
(22) Mayer, A. M., *Nature* **181,** 826 (1959).
(23) Miller, B. S., *J. Assoc. Official Agr. Chemists* **41,** 241–2 (1958).
(24) Moffatt, J. S., Radley, M., *J. Sci. Food Agr.* **11,** 386–90 (1960).
(25) Nielsen, N., Bergquist, G., *Physiol. Plant.* **11,** 329–31 (1958).
(26) Pollock, J. R. A., *Chem. & Ind. (London)*, **1958,** 387–8.
(27) Ruge, V., *Planta* **25,** 397–418 (1947).
(28) Sandegren, E., Beling, H., *European Brewery Conv. Proc.* Rome, Italy, pp. 278–89 (1959).
(29) Sandstedt, R. M., Kneen, E., Blish, M. J., *Cereal Chem.* **16,** 712–23 (1939).
(30) Skinner, C. G., Talbert, F. D., Shive, W., *Plant Physiol.* **33,** 190–4 (1958).
(31) Skoog, F., Miller, C. O., *Symposia Soc. Exptl. Biol.* **11,** 118–31 (1957).

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CORN FLOURS

The Nutritional Evaluation of Processed Whole Corn Flours

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A processing method for preparing whole corn flours for human consumption under heat and pressure without the use of lime was developed. A nutritional evaluation was made of samples produced experimentally by this new method to determine the best conditions for preparing a product of maximum nutritive value. Nine processed whole corn flours, prepared under different conditions, were evaluated by means of chemical analyses, microbiological assay of amino acids, and biological experiments with rats. None of the treatments of the flours had any effect on composition and true protein digestibility, but all reduced the solubility of the nitrogen and high pressure reduced the nutritive value of the flour. Processed whole corn flour with higher nutritive value can be prepared by cooking moist corn at 5 p.s.i.g. for 15 or 30 minutes.

In the Central American countries, tortillas made with corn form the basic diet for the majority of the population and, up to the present, have been prepared at home from raw corn by traditional methods (*4, 5*). Recently, interest has arisen in the industrial production of whole corn flours suitable for home use. Important problems include production costs, physical and organoleptical characteristics, and the nutritive value of the flour.

Numerous investigations (*17, 18, 22, 26*) have shown that experimental animals fed lime-treated corn grow better than animals fed raw corn, probably because of greater niacin availability (*17*) or the absorption of a better pattern of amino acids from lime-treated corn (*5, 26*). Pearson and coworkers (*25*) found that simple boiling of maize released bound niacin and gave a product causing rat growth responses equal to those obtained from the feeding of lime-treated corn. Harper, Punekar, and Elvehjem (*13*) found, on the other hand, that prolonged boiling was not so good as short-duration alkali treatment of maize in releasing bound niacin and promoting growth. Both groups of authors (*13, 25*) concluded that the beneficial effect of alkali treatment and of water cooking could not be attributed to the correction or prevention of an amino acid imbalance, but only to release of niacin from an unavailable form.

Taking the preceding experimental findings into consideration, the Instituto Centroamericano de Investigaciones y Tecnología Industrial (ICAITI) developed a method for preparing whole corn flours under heat and pressure without lime. Because heat affects the nutritive protein value of cereal grains and other foods (*19, 24*), a nutritional evaluation was made of flour samples produced experimentally by this new method in order to determine the best conditions for preparing a product of maximum nutritive value. Successful production of corn flours on an industrial basis could greatly benefit the Central American population, for such flours could at the same time be enriched with vitamins and other essential nutrients (*10*).

Materials and Methods

A starchy-type, white hybrid corn was used for flour production in a pilot plant of ICAITI. The dry whole corn was steam-cooked at a pressure of 5 or 15 p.s.i.g. (227° and 249° F., respectively) for 15, 30, and 60 minutes at each pressure. The cooked grain was then ground to 100 mesh, and a 75-pound sample from each preparation (six in all) was brought to the INCAP laboratory for chemical and microbiological analyses and biological studies with animals. In addition, three flour samples were prepared using whole corn previously soaked in water to raise the moisture content to approximately 30% and cooked at 5 p.s.i.g. (227° F.) for 15, 30, and 60 minutes.

Using 25 pounds of whole raw corn, two masa samples were prepared by the lime-treatment cooking method de-