Asian Journal of Chemistry; Vol. 28, No. 11 (2016), 2457-2460

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2016.20012

Physico-Chemical, Pasting and Morphological Characterization of Grain Amaranth Starch

NARENDER K. CHANDLA*, D.C. SAXENA and SUKHCHARN SINGH

Department of Food Engineering and Technology, S.L. Institute of Engineering & Technology (Deemed University), Longowal-148 106, India

*Corresponding author: E-mail: chandla84@yahoo.co.in

Received: 4 April 2016; *Accepted*: 30 June 2016; *Published online*: 10 August 2016; AJC-18017

Amaranth cultivars *e.g.*, *Amaranthus* chaulai, *Amaranthus hypochondricus* anapurna, *Amaranthus hypochondricus* durga and *Amaranthus paniculates* rajgeera have been explored for starch yield (31.47-37.20 %), purity of 99.54-99.74 (%) and other properties. Scanning electron microscopy revealed that amaranth starch granules isolated were of very small size (1.182-1.431 µm) and of well packed (polygonal) angular shapes. All amaranth cultivars had observed higher swelling power and water binding capacity in a range of (9.76-10.29 g/g) and (199.23-199.47 %), respectively. Among all starches, *Amaranthus hypochondricus* anapurna and *Amaranthus hypochondricus* durga starches had shown higher crystallinity, swelling power and water binding capacities. This suggests that *Amaranthus hypochondricus* anapurna and *Amaranthus hypochondricus* durga starches could be better utilized in where higher cracking type properties along with greater binding capacities are required. In contrary to other (*Amaranthus* chaulai, *Amaranthus hypochondricus* anapurna, *Amaranthus hypochondricus* durga) starches, *Amaranthus paniculates* rajgeera starch has shown higher solubility (54.60 %) and higher peak viscosity (1896cP) by Rapid Visco Analyzer.

Keywords: *Amaranthus* starches, Pasting, PSA, SEM.

INTRODUCTION

Amaranthus grain (pseudo-cereal) is well known for its high nutritional value and functional starch properties. *Amaranthus* grain has malleability to resist drought, heat, pests and have adaptation to new environmental conditions. *Amaranthus* grain belongs to the family *Amaranthaceae* which include 60 species across the globe wherein *Amaranthus hypochondricus, Amaranthus caudatus, Amaranthus paniculates L.* and *Amaranthus cruentus* are the species which are superior to cereal grains in term of proximate composition and quality attributes [1]. *Amaranthus* was originally cultivated in South and Latin America, adaptation trials and commercial production of this plant is currently being conducted in Ontario, Canada to meet the high consumer demand. In India, *Amaranthus* is commercially growing and cultivated in Himalayan foothills *viz.* Himachal Pradesh, Uttrakhand, Punjab and in many parts of Maharashtra [2,3]. The *Amaranthus* grain encompass high protein content (12-18 %), carbohydrate content (62-68 %), oil content (8 %) than most of the other cereal grains [1,4].

The major fraction component prevailed in this grain *Amaranthus* is the starch. The demand of starch has increased enormously in recent years as starch is being used widely in production of biodegradable plastics, edible films and bio-ethanol, apart from its general use in food processing industries [5,6]. Although, corn, potato, wheat and rice starches are the most commonly used in the food industry for the transformation of the functionality of the products. But a new starch like *Amaranthus* starch has found its application in cosmetics, textile, paper coatings and as laundry starch due to is functional characteristics [3,7]. In food processing industries starch contributes for a variety of characteristics that include thickening, gelling and shelf stability in varied applications [8-10]. Therefore, many authors have explored the starch for variety of applications.

Within the *Amaranthus* starch granules average amylose content 5.5 %, Hoover *et al*. [11] and Kong *et al.* [12] reported that amylose content is an important factor which affects the starch functional properties. Waxy starches (specific *spp.* of corn, rice & *Amaranthus)* can lower the gelatinization temperature, enhance the greater pastes clarity of starch, provide the starch with stability against retro-gradation and allow it to withstand freeze–thaw processes [13]. *Amaranthus* starches have shown smallest granular diameter, good gelatinization and moderated peak viscosity, elastic properties and paste clarity which is appreciated in food industry [12,14-20]. Due to smallest size and highest amylopectine content, this starch could be explored for technological textural interventions.

Therefore, this study was investigated to compare the starches in term of physico-chemical and morphological characteristics of starches.

EXPERIMENTAL

Amaranthus cultivars were procured from different locations of India. *Amaranthus* chaulai (AC) was procured from the local market of Sangrur, Punjab, India. *Amaranthus hypochondricus* anapurna (AHA) and *Amaranthus hypochondricus* durga (AHD) were collected from National Bureau of Plant Genetic Resources (NBPGR), regional station, Phagli, Shimla, Himachal Pradesh, India. *Amaranthus paniculates* rajgeera (APR) a commercial available variety was procured from Nasik, Maharashtra, India.

Isolation of starch: The starch was isolated by modified method of Choi *et al*. [8]. The *Amaranthus* flour was prepared in laboratory-stone mill and passed through 250 µ sieve (British Standard Size). The flour-slurry was prepared in NaOH solution and steeping done at refrigerated temperature (4 °C) for 20 h. The slurry steeped was blended for 2.5 min and successive filtration was applied. The filtrate obtained was centrifuged (Eltek 4100 F) at a speed of 3000 ×g for 15 min and supernatant was discarded. Repeated washing was applied with distilled water till complete removal of protein layer. Process was standardized at alkali (NaOH) concentration of (0.25 % (w/v)), alkali to flour ratio of 1:5 and screens size (mesh sizes); (70 (210 µ)), (100 (149 µ)), (200 (74 µ)), (300 (50 µ)) of British Sieve Size (BSS). The isolated white mass of starch was dried at 40 °C and passed through 100 mesh (149 µ) size screen for powder formation.

Physico-chemical properties of starch

Colour of starches: Hunter colorimeter (Model i5 Green Macbeth, USA) was used for estimation of optical properties of starch from *Amaranthus* Cultivars. Data was recorded as L*, a* and b* values. (L*= black to white); (a* = green to red); and (b* = blue to yellow).

Amylose content: Starch sample (70 mg) was mixed with 10 mL of urea and dimethyl sulphoxide solution in 1:9 ratio and heated for 10 min at 100 °C with continuous stirring. The mixed sample was incubated at 100 °C for 1 h and then cooled to room temperature. Addition of 0.5 mL solution of above mixed incubated sample was taken with subsequent addition of 25 mL distilled water and 1 mL solution of iodine and potassium iodide. The 1 mL solution was made by addition of iodine (2 mg) and potassium iodide (20 mg) and volume was made up to 1 mL by distilled water. Blank sample was also prepared without addition of starch sample and absorbance was taken at 635 nm [21].

$$\text{Blue value (\%)} = \frac{\text{Absorbance} \times 100}{2 \times \text{g of solution} \times \text{Weight of sample}}$$

$$\text{Amylose content (\%)} = 28.414 \times \text{Blue value}$$

Swelling power and solubility: Swelling power (SP) and solubility of starches were standardized [22-25] at 95 °C and latter a modified method was developed. Briefly, a homogeneous mixture of starch (1 g, dry basis) and distilled water (35 mL) was heated in 80 mL centrifuge tube at 95 °C for 0.5 h. The starch (1.0 g) suspension was heated in 25 mL of water with gentle stirring for first 15 min and remaining water was added thereafter. Samples were then cooled in an ice bath for 1 h and centrifuged at 12,500 rpm for 0.5 h. The suspended cloudy layer was poured through double folded cheese cloth by gravitation for 2 min and soluble matter which passes on the cheese cloth (filtrate) was considered as supernatants while gel retained on filter cloth was collected back inside the tube as sediments. The weight of sediment was recorded for swelling power and supernatant collected was poured in previously weighted petri dish and dried in oven at 100 °C for 3.5 h and weighted for the solubility determination.

The swelling power (g/g, dry basis) and solubility (%) were calculated as below:

$$\text{Solubility (\%)} = \frac{\text{Mass of dried solids}}{\text{Weight of starch taken}} \times 100$$

$$\text{Swelling power (g/g)} = \frac{\text{Sediment weight (wet mass)}}{\text{Sample weight of starch taken}}$$

Water/oil binding capacity (WBC/OBC): Water and oil binding capacities of starch isolated was determined by method described by Medcalf and Gills [26]. 5 g starch was taken and dissolved in 75 mL distilled water and oil for water and oil binding capacity, respectively. The sample was agitated for 1 h and centrifuged at 3000 rpm for 10 min. The free water and oil recovered from the sentimental starch sample was removed and tubes were drained for 10 min to separate out the surface water and oil. The water/oil binding capacity was calculated as follows:

$$\text{WBC/OBC (\%)} = \frac{\text{Weight of sendiments}}{\text{Weight of sample}} \times 100$$

Pasting properties: The pasting properties of the starch powder (3 g, 12.5 % moisture basis) obtained after passing through 150 µm was determined by Rapid Visco Analyzer (RVA, Starch Master TM; Model N17133; Newport Scientific Pvt. Ltd., Warriewood, Australia). The starch samples were programmed within Rapid Visco Analyzer and hold at 50 °C for 1 min and then heated to 95 °C within 4 min, held at 95 °C for 3 min and then cooled to 50 °C within 3 min and hold at 50 °C for 2 min. From the curve, pasting temperature and viscosity profile was obtained.

Particle size distribution (PSD): Laser diffraction particle size analyzer, Shimadzu SALD-2300 was used to determine the partial structural characteristics (particle size distribution) in term of percentage volume of the equivalent spheres. The starch samples were dispersed in cuvette and fixed to the sample port of equipment which measure in range of 17 nm to 2500 µm. The starch sample was added to sample port drop-wise till the obscuration to about 40 %.

Scanning electron microscopy (SEM): The samples were mounted on aluminum stub using a double backed cellophane tape, coated in auto fine coater, JEOL-JFC-1600, with gold palladium (60:40, w/w). All the starch samples were analyzed by scanning electron microscope (SEM), JEOL, Tokyo, Japan, Model No. JSM 6610-LV. The granule shape as a major morphological characteristic of the sample was absorbed at a moisture content of 5-6 %. The starch samples were examined at magnifications of 5000 and 10000 X for the alkali (native) starch isolated.

Statistical evaluation: The mean value and standard deviation were reported for the statistical analysis. All the

analysis were determined in the triplicates and subjected to one way analysis of variance (ANOVA), followed by Duncan's by Mini Tab Statistica 7. (Statesoft Inc., OK, USA).

RESULTS AND DISCUSSION

Starch isolation: The alkali treatment has been given to solubilize the protein so as to liberate starch held within the *Amaranthus* grain. The highest starch yield and purity obtained by alkali treatment were observed for *Amaranthus paniculates* rajgeera and *Amaranthus* chaulai, respectively (Table-1).

All the *Amaranthus* starches have shown colour value (L*) higher than 95.20 which is appreciable and better than starch isolated by alkali process. All the *Amaranthus* starches (L*) value were in range from 95.20 (*Amaranthus paniculates* rajgeera)–97.23 (*Amaranthus hypochondricus* durga) which is in accordance with (L*) value (96.64) as reported by Villareal *et al*. [27] for *Amaranthus* starches extracted by alkali method. Authors have acknowledged that L* values greater than 90 give an acceptable whiteness for starch purity while the (L*) value of extracted starches was better than the acknowledged starches (Table-1).

The amylose content of *Amaranthus* starches were found in range from 1.87 % (*Amaranthus* chaulai) to 3.43 % (*Amaranthus hypochondricus* durga) and have shown significant difference, due to variation in *Amaranthus* cultivars (Table-1). The amylose content values reported were in range with early reported value of Choi *et al*. [8] and Hoover *et al*. [11] for different amaranth genotypes (3 to 8 %). Our results are in agreement with the finding reported by the various authors. Amylose content could vary according to the specific lines of particular *spp.* undertaken for cultivation and moreover the demographical and soil condition may be responsible for the variation in amylose content.

Starches of *Amaranthus hypochondricus* durga showed the highest swelling power, while the lowest was observed in *Amaranthus paniculates* rajgeera and this have shown positive co-relation due to high amylose content in *Amaranthus hypochondricus* durga and lower in *Amaranthus paniculates* rajgeera. The solubility of the starches ranged from 35.60 (*Amaranthus hypochondricus* durga) to 54.60 % (*Amaranthus paniculates* rajgeera), with the lowest found in *Amaranthus hypochondricus* durga whereas *Amaranthus paniculates* rajgeera showed the highest solubility ($p < 0.05$) as shown in Table-1. Differences in the swelling power and solubility of the starches could be attributed to the variations in the amylose and amylopectin contents of the starch and associative bonding forces within the starch granules [28].

Water binding capacity and oil binding capacity of *Amaranthus* starches were observed. The highest water binding capacity and oil binding capacity were observed for *Amaranthus paniculates* rajgeera and *Amaranthus hypochondricus* anapurna respectively (Table-1). Water binding capacity of starch granule is the tendency to absorb water (bonding with hydrophic exposed groups) and the degree of association of water molecules within starch granule [29].

Pasting properties: The pasting temperature (PT) of *Amaranthus* starches isolated varied in a range of 75.17 to 76.74 °C. Highest peak viscosity (PV) was observed in *Amaranthus paniculates* rajgeera (1896cP), followed by, *Amaranthus hypochondricus* durga (1796cP), at a temperature of 95 °C (Fig. 1). Singh *et al*. [14] have reported pasting temperature and peak viscosity of *Amaranthus hypochondricus* starches ranged from 70.40 to 75.05 °C and 1582 to 2331 cp, respectively. *Amaranthus hypochondricus* anapurna have shown highest final viscosity (FV), followed by *Amaranthus hypochondricus* durga. The highest break down viscosity (BD) was seen in case of *Amaranthus hypochondricus* durga (702cP) starch paste for alkali and this indicates its measure of fragility during processing which revealed its utility over other *Amaranthus* starches [25].

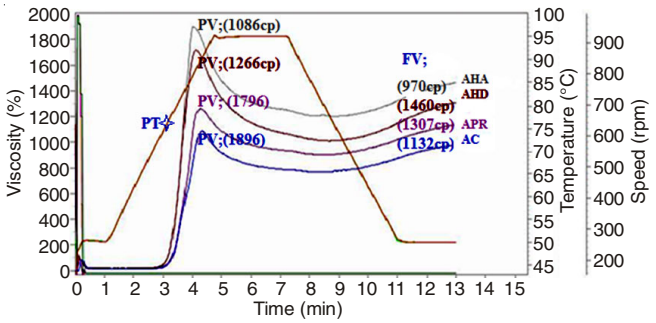


Fig. 1. Rapid Visco Analyzer curve of starches isolated from *Amaranthus* cultivars; where; PV; peak viscosity, FV; final viscosity and PT; pasting temperature (°C)

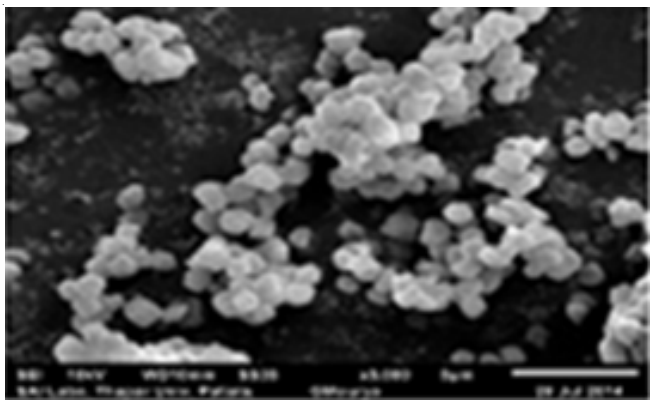
Scanning electron microscopy (SEM): The shape and size of *Amaranthus* starches have been studied by scan electron microscopy (SEM). As microscopy have played important role in understanding the internal structure of the granules and the effect of granule during the process applied to the isolation of the starch. The scan electron micrograph has revealed that *Amaranthus* starch granules were polygonal, aggregated like

TABLE-1
FUNCTIONAL PROPERTIES OF STARCHES ISOLATED FROM *Amaranthus* CULTIVARS

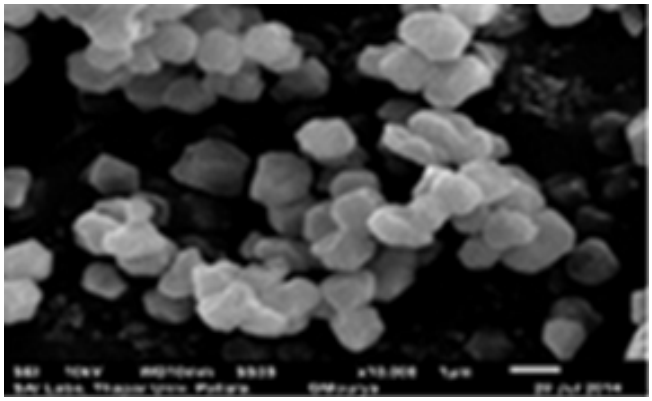
Amaranthus cultivars	Optical value			Amylose content (%)	Swelling power (g/g) (90 °C)	Solubility (%) (90 °C)	Water binding capacity (%)	Oil binding capacity (%)
	L	a*	b*					
AC	96.03±0.06^{c}	-2.57±0.06^{c}	3.53±0.06^{ab}	1.87±0.03^{d}	8.40±0.05^{c}	36.47±0.06^{c}	179.60±0.92^{bc}	170.33±0.76^{cd}
AHA	97.23±0.06^{a}	-2.70±0.07^{ab}	3.17±0.06^{d}	3.13±0.03^{b}	9.76±0.0^{b}	38.50±0.10^{b}	199.23±0.35^{ac}	236.02±0.45^{a}
AHD	97.13±0.06^{ab}	-2.83±0.06^{a}	3.47±0.06^{bc}	3.43±0.02^{a}	10.29±0.08^{a}	35.60±0.30^{d}	199.47±0.76^{abc}	185.67±3.40^{bc}
APR	95.20±0.06^{d}	-2.50±0.06^{d}	3.50±0.06^{ac}	2.81±0.01^{c}	8.10±0.1^{d}	54.60±0.20^{a}	198.41±0.78^{a}	193.40±2.03^{b}

Results are expressed as mean value±standard deviation of three determinations; Means in column with different superscript differ significantly ($p < 0.05$); AC = *Amaranthus* chaulai; AHA = *Amaranthus hypochondricus* anapurna; AHD = *Amaranthus hypochondricus* durga; APR = *Amaranthus paniculates* rajgeera

grape bunch and angular in shape (Fig. 2). The average diameter varying from 0.221 of 2.351 µm within all *Amaranthus* starches in particle size analysis (Fig. 3). The surface area per unit weight of *Amaranthus* starch obtained could be utilized in encapsulation and coating purposes of food ingredients and this finding is in agreement with the Villareal *et al.* [27].



(5000 X)



(10000 X)

Fig. 2. Surface morphology of starches isolated from *Amaranthus* starches

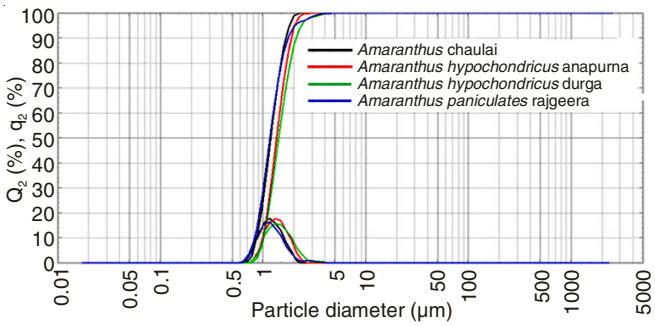


Fig. 3. Overlay particle size distribution graphs of starches isolated *Amaranthus* cultivars

Conclusion

Amaranth Starch obtained was better in terms of purity, colour and in physico-chemical properties. A significant difference among yield of starch content have been found due to variation in *cultivars*. All *Amaranthus* starches granules were of small size, low in amylose content and resulted thinning type of behaviour. Furthermore, SEM analysis and particle analysis revealed that the granules were polygonal, angular and tightly packed structure, which suggest its application in edible and biodegradable films or as filler in bio-plastics for better tensile strength in comparison to strength imparted by existing starches.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to NBPGR, New Delhi for providing the seeds through MTA, analytical support from SAIL, Thapar University, Patiala and Indian Institute of Technology, Roopnagar, India.

REFERENCES

1. D.M. Brenner, D.D. Baltensperger, P.A. Kulakow, J.W. Lehmann, R.L. Myers, M.M. Slabbert and B.B. Sleugh, *Plant Breed. Rev.*, **19**, 227 (2000).
2. J.C. Rana, K. Pradheep, S.K. Yadav, V.D. Verma and P.C. Sharma, *Indian Farming*, **57**, 27 (2007).
3. N. Singh, B.K. Tiwari, A. Gowen and B. McKenna, Pulse Foods: Processing, Quality and Nutraceutical Applications, Academic Press, Elsevier, London, pp. 101–111 (2011).
4. C. Silva-Sánchez, A.P.B. de la Rosa, M.F. León-Galván, B.O. de Lumen, A. de León-Rodríguez and E.G. de Mejía, *J. Agric. Food Chem.*, **56**, 1233 (2008).
5. M. Li, P. Liu, W. Zou, L. Yu, F. Xie, H. Pu, H. Liu and L. Chen, *J. Food Eng.*, **106**, 95 (2011).
6. M. Kaseem, K. Hamad and F. Deri, *Polym. Sci. Series A*, **54**, 165 (2012).
7. Á.L. Santana and M.A.A. Meireles, *Food Public Health*, **4**, 229 (2014).
8. H. Choi, W. Kim and M. Shin, *Starke*, **56**, 469 (2004).
9. A.A. Wani, P. Singh, M.A. Shah, U. Schweiggert-Weisz, K. Gul and I.A. Wani, *Comprehensive Rev. Food Sci. Food Safety*, **11**, 417 (2012).
10. I.A. Wani, M. Jabeen, H. Geelani, F.A. Masoodi, I. Saba and S. Muzaffar, *Food Hydrocolloids*, **35**, 253 (2014).
11. R. Hoover, A. W. Sinnott and C. Perera, *Starch/Starke*, **50**, 456 (1998).
12. X. Kong, S. Kasapis, E. Bertoft and H. Corke, *Starke*, **62**, 302 (2010).
13. D. Kalita, N. Kaushik and C.L. Mahanta, *J. Food Sci. Technol.*, **51**, 2790 (2014).
14. N. Singh, S. Kaur, A. Kaur, N. Isono, Y. Ichihashi, T. Noda and J.C. Rana, *Starch*, **66**, 457 (2014).
15. A. Lopez-Rubio, B.M. Flanagan, E.P. Gilbert and M.J. Gidley, *Biopolymers*, **89**, 761 (2008).
16. P.N. Bhandari and R.S. Singhal, *Carbohydr. Polym.*, **48**, 233 (2002).
17. D. Kanakdande, R. Bhosale and R.S. Singhal, *Carbohydr. Polym.*, **67**, 536 (2007).
18. A.C. Resio and C. Suarez, *Int. J. Food Sci. Technol.*, **36**, 441 (2001).
19. J. Pal, R.S. Singhal and P.R. Kulkarni, *Carbohydr. Polym.*, **48**, 49 (2001).
20. L.A. Bello-P´Erez, M.G.M. Montealvo and E.A. Acevedo, in eds.: F.M. Lajolo and E.W. Menezes, Starch: Definition, Structure and Properties, Carbohydrates in Food, Carbohidratos em alimentos regionales iberoamericano, São Paulo: Edusp, p. 19 (2006).
21. W.R. Morrison and B. Laignelet, *Cereal Sci.*, **1**, 9 (1983).
22. L. Wang, B. Xie, J. Shi, S. Xue, Q. Deng, Y. Wei and B. Tian, *Food Hydrocolloids*, **24**, 208 (2010).
23. O.C. Adebooye and V. Singh, *Innov. Food Sci. Emerg. Technol.*, **9**, 92 (2008).
24. T.J. Schoch, in ed.: R.L. Whistler, Fatty Substances in Starch, In: Methods in Carbohydrates, Academic Press, pp. 56-61 and 101-103, vol. IV, New York, NY, USA (1964).
25. X. Kong, J. Bao and H. Corke, *Food Chem.*, **113**, 371 (2009).
26. D.G. Medcalf and K.A. Gilles, *Cereal Chem.*, **42**, 558 (1965).
27. M.E. Villarreal, P.D. Ribotta and L.B. Iturriaga, *LWT-Food Sci. Technol.*, **51**, 441 (2013).
28. A. Kaur, N. Singh, R. Ezekiel and H.S. Guraya, *Food Chem.*, **101**, 643 (2007).
29. H.W. Leach, L.D. McCowen and T.J. Schoch, *Cereal Chem.*, **36**, 534 (1959).