N. INOUCHI, K. NISHI, S. TANAKA, T. ASAI, Y. KAWASE, Y. HATA, Y. KONISHI*, S. YUE**, H. FUWA

CHARACTERIZATION OF SMALL GRANULAR SIZED STARCHES – AMARANTHUS AND QUINOA STARCHES

Abstract

Starch granules were prepared from mature grains of 9 samples of *Amaranthus* and 4 samples of *Chenopodium quinoa*. By the ordinary GPC of *Pseudomonas* isoamylase-debranched starch materials the amylose content of amaranth starches was in a range of 0-28 %. Thus we confirmed that there were normal, low-amylose, and waxy-types of amaranth starches. The amylose content of quinoa starches was 25-27 %. The ratio of short chains to long chains of amylopectin of these starches was in a range of 2.2-3.3 and somewhat lower than or similar to that of the normal maize starch. Isoamylase-debranched materials were separated by HPLC with differential refractometer (RI) and low-angle laser light scattering photometer (LALLS) as detectors in one hand, and by high performance anion exchange chromatography with pulsed amperometric detector (HPAEC-PAD) in other hand. We found that amylopectins of amaranth and quinoa had increased amounts of long B chains and decreased amounts of short chains as compared with the waxy maize amylopectin, however, they had increased amounts of short chains with degree of polymerization (DP) from 6 to 12. Amaranth starches had slightly higher temperatures of gelatinization (To, Tp, and To) and smaller heats of gelatinization (ΔH) by diferential scaning calorimetry (DSC) comparing with the normal maize starch. Quinoa starch showed lower To, Tp, and Tc and smaller ΔH. Amaranth and quinoa starch granules were digested by amylases faster than those of the noraml maize.

Introduction

The granular shape and size of starches depend upon their original plant species. As some representatives of starch granules with small sizes (mean particle size, around 1–1.5 μm), we have been studied structure and properties of starches obtained from

Department of Food Science and Technology, Fukuyama University, Higashimura-cho, Fukuyama 729-0251, Japan,

* *Department of Food and Nutrition, Osaka City University, Sugimoto, Osaka 558-0022, Japan*

** *Department of Genetics, Institute of Crop Breeding and Cultivation, Chinese Academy of Agricultural Sciences (CAAS), Baishiqiao Rd., Beijin 100081, China*

grain amaranth [1-3], konjac [4], and taro [5, 6]. Ranhotra et al. [7] reported that quinoa (*Chenopodium quinoa*) has starch granules with small size.

Amaranth and quinoa have potential agronomic importance, because their seeds are generally higher in protein, fat, ash, and fiber in comparison to common cereals [8, 9] Moreover, the amino acid balance of these seeds are better than that of wheat and maize, because the first limiting amino acid, lysine, is present in relatively higher amounts in these seeds. Nevertheless, the main components of the seeds are starches. Accordingly the objective of this study is to know the structural characteristics and functional properties of starches of *Amaranthus* and *Chenopodium quinoa*.

Sample starch or sample seeds	Original place	Obtained through
Normal maize starch	USA	Sanwa Denpun Kogyo, Co., Ltd.
Waxy maize starch	USA	Sanwa Denpun Kogyo, Co., Ltd.
Amaranthus hypochondriacus K343	USA	Shinkyo Sangyo, Co., Ltd.
Amaranthus cruentus R104	China mainland	Dr. S. Yue (1996)
Amaranthus cruentus R104	China mainland	Dr. S. Yue (1997)
Amaranthus cruentus K112	China mainland	Dr. S. Yue (1996)
Amaranthus cruentus K112	China mainland	Dr. S. Yue (1997)
Amaranthus cruentus K350	China mainland	Dr. S. Yue (1997)
Amaranthus cruentus K459	China mainland	Dr. S. Yue (1997)
Amaranthus cruentus K472	China mainland	Dr. S. Yue (1997)
Amaranthus hybridus D88-1	China mainland	Dr. S. Yue (1997)
Chenopodium quinoa Quinua Real	Bolivia	Dainihon Meiji Seito, Co., Ltd.
Chenopodium quinoa	Peru	Dainihon Meiji Seito, Co., Ltd
Chenopodium quinoa Quinua ①-B	Bolivia	Dr. Takashi Akazawa
Chenopodium quinoa Quinua ②-B	Bolivia	Dr. Takashi Akazawa

Materials and methods

Sample seeds and preparation of starches

Starch granules were prepared from mature grains of 9 samples of *Amaranthus* and 4 samples of *Chenopodium quinoa* by a modification of Schoch's method [10]. Sample seeds were obtained as shown above. Commercial normal and waxy maize starches were used as references.

General methods

High performance gel permeation chromatography (HPLC) with differential refractometer (RI) and low-angle laser light scattering photometer (LALLS) as detectors and high performance anion exchange chromatography with pulsed amperometric detector (HPAEC-PAD) of isoamylase-debranched materials of amylopectin

The procedure for HPLC-RI-LALLS and Dionex chromatography (HPAEC-PAD) were reported earlier [11] except for the following minor change in the procedure for HPAEC-PAD. Namely, PAD-SC cell was used instead of PAD-standard cell and 0.1 M $NaNO_3$ was used in the elution solution instead of 0.5 M CH_3COONa.

Other methods

Contents of amylose and chain length distributions of amylopectin were determined by gel permeation chromatography (GPC) of *Pseudomonas* isoamylase-debranched starches. The methods for debranching of starch, GPC of debranched starch, analytical methods for fractionated materials have been reported previously [12, 13]. Some chemical and physical properties of starches were also determined. The method for recording absorption spectra of starch iodine complexes [14], the method for determination of starch-granule digestibility to amylase [15], and the procedure for differential scanning calorimetry (DSC) has been described elsewhere [16].

Results and discussion

The amylose content and amylopectin chain length distribution of starches by GPC

We have shown that there were normal, waxy, and low-amylose types of amaranths [1-3] From the data shown in Tables 1 and 2 we confiremed our previous results. Namely, two amaranth starches belong to normal, three to waxy, and two to low-amylose (amylose contents; 6.6 and 12.6 %, respectively) types among 7 different kinds of amaranth starches tested (Table 2). Possibility of cross contamination of normal pollen to waxy plants was cleared by microscopic observation of iodine stained starch granules obtained from *A. cruentus* K350 which stained purple instead of blue for normal starch and red for waxy one.

The ratio of short chains to long chains of amylopectins of amaranth starches (Fr. III/Fr. II) were in a range of 2.2 to 2.6 and slightly lower than those (around 3) of maize amylopectins. These results suggest that amaranth amylopectins have increased amounts of long chains and/or decreased amounts of short chains comparing with the normal and waxy maize amylopectins.

Table 1

Some characteristics of absrption curves of iodine complexes of starches obtained from maize, amaranth, and quinoa

Starch sample	Blue value*	λmax (nm)
Normal maize	0.36 0.02	587 0.8
A. hypocondriacus K343	0.08 0.00	530 0.8
A. cruentus R104 ('96)	0.12 0.00	538 0.1
A. cruentus R104 ('97)	0.10 0.00	530 0.8
A. cruentus K112 ('96)	0.31 0.01	585 0.6
A. cruentus K112 ('97)	0.40 0.01	588 0.5
A. cruentus K350 ('97)	0.15 0.00	541 0.9
A. cruentus K459 ('97)	0.25 0.01	567 1.4
A. cruentus K472 ('97)	0.43 0.03	591 0.6
A. hybridus D88-1 ('97)	0.09 0.00	529 1.0
C. quinoa Qinua Real	0.32 0.02	591 2.7
C. quinoa	0.32 0.01	587 0.7
C. quinoa 1-B	0.40 0.06	594 3.4
C. quinoa 2-B	0.41 0.03	596 1.9

*Optical density (OD) at 680nm **Wave length at the absorption maximum

Table 2

Characteristics of isoamylase-debranched materials by GPC of starches obtained from maize, amaranth, and quinoa

Starch sample	Fr. I (%)	Int. Fr. (%)	Fr. II (%)	Fr. III (%)	Fr. III/Fr. II
Normal maize	30.4	4.9	15.8	48.9	3.1
Waxy maize	0.0	4.4	24.5	71.1	2.9
A. hypocondriacus K343	0.0	5.7	26.7	67.6	2.6
A. cruentus R104 ('96)	0.0	6.8	26.3	66.8	2.6
A. cruentus R104 ('97)	0.0	5.2	27.4	67.4	2.5
A. cruentus K112 ('96)	19.4	7.4	21.9	51.4	2.4
A. cruentus K112 ('97)	27.8	5.6	19.1	47.5	2.5
A. cruentus K350 ('97)	6.6	2.3	28.4	62.7	2.2
A. cruentus K459 ('97)	12.6	5.8	22.7	58.9	2.6
A. cruentus K472 ('97)	24.1	6.0	21.1	48.8	2.3
A. hybridus D88-1 ('97)	0.0	3.4	27.5	69.2	2.5
C. quinoa Qinua Real	27.0	5.5	20.4	47.2	2.3
C. quinoa	24.7	7.9	21.2	46.3	2.2
C. quinoa 1-B	26.7	6.6	20.2	47.5	2.4
C. quinoa 2-B	26.4	3.9	16.7	52.9	3.2

*Each fraction (Fr.) was devided according to λmax of carbohydrate-iodine complexes as follows; Fr. I, λmax 620nm, Intermediate Fr., 620nm λmax 600nm, Fr. II, 600nm λmax 540nm, Fr. III, 540nm λmax.

The amylose content of quinoa starches was in a range of 24.7 to 27.0 %, however, 7 and 15 % amylose contents were recently reported by other investigators for different quinoa samples [7, 17]. Quinoa starch has received relatively little attention. Information regarding quinoa starch has been incomplete and contradictory. The variations in the results, probably due to in part to environmental, agronomic and genetic factors, but also due to the analytical procedures employed. Fr. III/Fr. II for quinoa amylopectins tended to be lower than those of maize amylopectins.

The amylopectin chain length distribution by HPLC-RI-LALLS

We showed amaranth amylopectins have increased amounts of long B chains and decreased amounts of short chains by HPLC-RI-LALLS (Fig. 1 and Table 3). Interestingly, short chains (F.3 in Fig. 1) of amaranth amylopectins have two peaks instead of one peak for the waxy maize amylopectin. These types of F.3 curves with two peaks were reported for amylopectins of the dull (du) maize mutants [18].

Table 3

Characteristics of isoamylase-debranched materials of maize and amaranth amylopectin by HPLC-LALLS

					ACL			ACLp	
Sample starch	F.2 %	F.3 %	F.3/ F.2	MW/MN	TCL	F.2	F.3	F.2	F.3
waxy maize	27.1	72.9	2.7	1.37	29.3	54.6	20.2	32.1	15.2
*A.C.R.*104	29.6	70.4	2.4	1.46	29.4	55.2	18.5	48.5	21.4/14.7
*A.C.R.*350	27.1	72.9	2.7	1.40	28.2	53.7	18.7	48.3	15.2/14.5
*A.hyb.*D88-1	29.2	70.8	2.4	1.37	28.7	54.4	18.2	47.6	19.2/14.4

*F.2 and F.3 are long and short chains of amylopectin, respectively. MW and MN are weight average and number average molecular weights, respectivly. TCL, ACL, and ACLp are total chain length, average chain length, and ACL at the apieces of the curve, respectively .

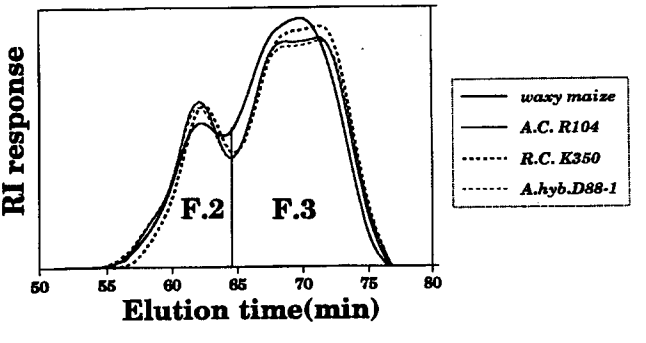

Fig. I. HPAEC-PAD traces for isoamylase-debranched materials of starches obtained from rice plants grown under different temperature conditions after anthesis. (a) group I-5; and (b) group II-5.

The amylopectin short chain-length distribution by HPAEC-PAD

Figure 2 shows the short chain-length distrubutions of isoamylase-debranched materials of amaranth and quinoa amylopectins with comparison to waxy maize amylopectin by Dionex chromatography. The amaranth and quinoa amylopectins have increased amounts of chains with degree of polymerization (DP) from 6 to 12 and some decreased amounts of chains with DP from 13 to 20 in comparision to the waxy maize amylopectin.

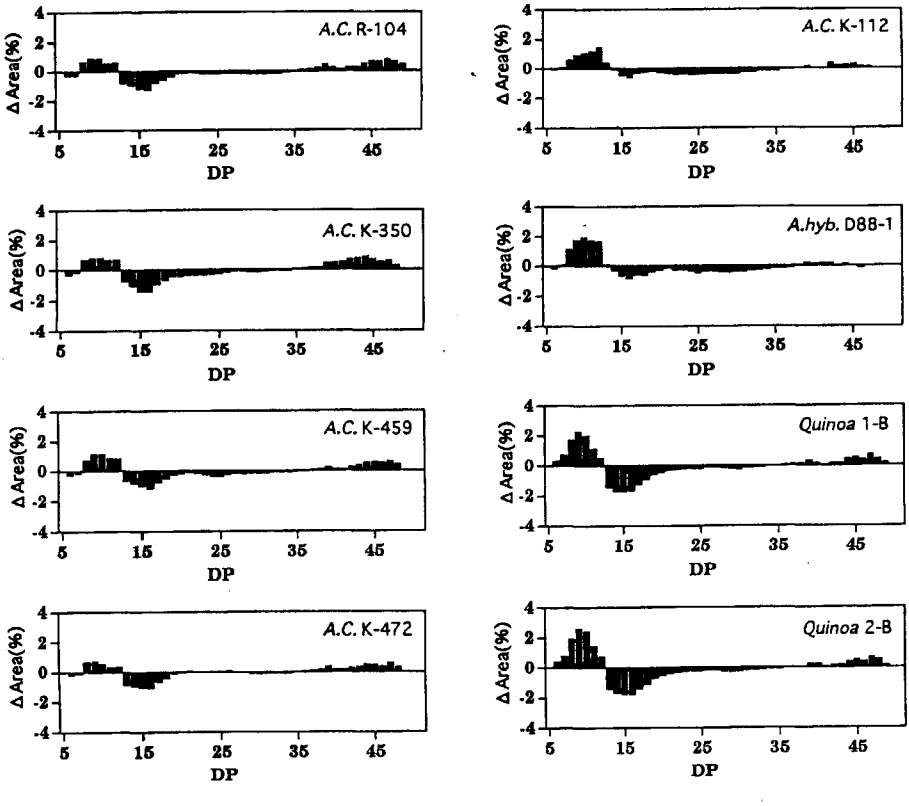

Fig. 2. Chain-length distributions of debranched amylopectins of rice plants grown under different temperature conditions after antesis. (a) group I-5; and (b) group II-5.

DSC characteristics of amaranth and quinoa starches

Amaranth starches had slightly higher temperatures for gelatinization (To, Tp, and Tc) and smaller heats of gelatinization (ΔH) comparing with the normal maize starches (Table 4). Quinoa starches showed lower To, Tp, and Tc and smaller ΔH comparing with the normal maize starches (Table 4).

Table 4

DSC characteristics of starches obtained from maize, amaranth, and quinoa

Starch sample	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)
Normal maize	65.3 ± 0.3	69.9 ± 0.2	75.2 ± 0.4	14.8 ± 0.1
A. hypocondriacus K343	63.2 ± 0.5	72.4 ± 0.1	80. ±1 0.5	10.0 ± 0.0
A. cruentus R104 ('96)	70.0 ± 0.2	76.1 ± 0.1	82.2 ± 0.3	11.1 ± 0.2
A. cruentus R104 ('97)	65.9 ± 0.6	74.6 ± 0.7	80.7 ± 0.8	10.9 ± 0.4
A. cruentus K112 ('96)	65.8 ± 0.5	71.2 ± 0.3	77.9 ± 0.9	9.5 ± 0.0
A. cruentus K112 ('97)	67.7 ± 0.5	74.8 ± 0.2	82.8 ± 0.2	13.5 ± 0.3
A. cruentus K350 ('97)	71.6 ± 0.4	76.9 ± 0.5	83.9 ± 0.7	12.4 ± 0.8
A. cruentus K459 ('97)	67.4 ± 0.1	75.2 ± 0.0	81.7 ± 0.0	12.3 ± 1.1
A. cruentus K472 ('97)	66.3 ± 0.7	73.6 ± 0.1	80.7 ± 0.1	11.9 ± 0.9
A. hybridus D88-1 ('97)	65.3 ± 0.1	72.8 ± 0.1	79.4 ± 0.2	12.6 ± 0.7
C. quinoa Qinua Real	52.8 ± 0.7	60.2 ± 0.1	67.8 ± 0.3	8.1 ± 0.8
C. quinoa	57.4 ± 0.4	61.9 ± 0.3	67.6 ± 0.5	7.3 ± 0.1
C. quinoa 1-B	52.2 ± 0.2	58.9 ± 0.2	68.5 ± 0.5	10.3 ± 0.1
C. quinoa 2-B	46.1 ± 0.4	54.2 ± 0.1	66.2 ± 0.5	10.5 ± 0.3

*To, Tp, and Tc are onset, peak, and conclusion temperatures for gelatinization and ΔH is heat of gelatinization, respctively.

Digestibility of amaranth and quinoa starch granules

Starch granules of amaranth and quinoa were digested by a mixture of glucoamylase and α-amylase faster than those of the normal maize (Tables 5 and 6). The main reason may be the smaller sizes of these two kinds of starch granules than those of the normal maize.

Table 5

Degradation of starch granules (% degradation) obtained from maize, amaranth, and quinoa by amylase* – 1

Starch sample	Duration of enzyme reaction (hr)			
	1	3	6	24
Normal maize	21.7	54.9	83.6	99.1
A. hypocondriacus K343	57.5	90.5	97.7	99.3
A. cruentus R104 ('96)	59.2	89.7	93.9	94.5
A. cruentus K112 ('96)	65.8	90.3	89.4	100.6
C. quinoa Qinua Real	77.7	100.4	100.4	101.3
C. quinoa	70.7	96.8	99.5	102.2

*Commercial preparation composed of a mixture of α-amylase and glucoamylase obtained from Aspergillus sp. K- 27.

Table 6

Degradation of starch granules (% degradation) obtained from maize, amaranth, and quinoa by amylase* – 2

Starch sample	Duration of enzyme reaction (hr)			
	1	3	6	24
Normal maize	17.0 2.3	50.9 2.6	79.6 4.8	92.2 5.5
A. cruentus R104 ('97)	59.8 0.5	87.2 0.3	99.7 0.1	96.7 7.5
A. cruentus K112 ('97)	55.7 2.4	90.5 0.2	92.8 2.3	91.1 0.0
A. cruentus K350 ('97)	49.4 4.5	91.1 1.2	94.4 3.7	100 0.0
A. cruentus K459 ('97)	57.2 0.1	82.9 3.5	97.7 4.9	97.3 2.4
A. cruentus K472 ('97)	53.1 4.4	93.3 2.1	86.8 5.6	89.1 4.0
A. hybridus D88-1 ('97)	57.3 3.1	90.7 3.8	91.8 2.9	91.2 1.2
C. quinoa 1-B	46.6 3.5	90.2 1.7	85.1 7.5	86.1 2.4
C. quinoa 2-B	55.4 1.2	94.5 1.6	94.1 1.5	89.8 4.5

*Commercial preparation composed of a mixture of α-amylase and glucoamylase obtained from Aspergillus sp. K- 27.

Conclusions

We found that amaranth and quinoa amylopectins had unique short chain length distributions. Namely they had increased amounts of long chains and decreased amounts of short chains, however, they had increased amounts of chains with DP from 6 to 12 comparing with the waxy maize amylopectin. Moreover there were wide variations in the amylose content of amaranth starch.

The textural contribution of starch to food and non-food industrial products varies with size, proportion, and degree of branching of the starch molecules present in them, in addition to their granular size and structure. There have been several investigations for application of amaranth and quinoa starches [18-22] These investigations are, however, immature. I hope our studies offer useful information for food and other industrial uses of these starches.

REFERENCES

[1] Sugimoto Y., Yamada Y., Sakamoto S., Fuwa H.: Starch/Stärke, **33**, 1981, 112-116.
[2] Tomita Y., Sugimoto Y., Sakamoto S., Fuwa H.: J. Nutr. Sci. Vitaminol., **27**, 1981, 471-484.
[3] Konishi Y., Nojima H., Okuno K., Asaoka M., Fuwa H.: Agric. Biol. Chem., **49**, 1985, 1965-1971.
[4] Sugimoto Y., Yamashita Y., Kunishige N., Fuwa H.: Oyo Toshitsu Kagaku (in Japanese), **42**, 1995, 223-229.
[5] Sugimoto Y., Nishihara K., Fuwa H.: Denpun Kagaku (in Japanese), **33**, 1986, 169-176.

[6] Sugimoto Y., Yamamoto Y., Abe K., Fujita S., Fuwa H.: Denpun Kagaku (in Japanese), **34**, 1987, 1-10.
[7] Ranhotra G.S., Gelroth J.A., Glasser B.K., Lorenz K.J., John D.L.: Cereal Chem., **70**, 1993, 303-308.
[8] Lopez M.G., Bello-Perez L.A., Paredes-Lopez O.: [in] "Amaranth: Biology, Chemistry, and Technology", ed. by Paredes-Lopez O., CRC Press, Boca Raton, 1994, 110-140.
[9] Coulter L., Lorenz K.: Lebensm.-Wiss. u. -Technol., **23**, 1990, 203-207.
[10] Schoch T.J.: [in] "Method in Enzymol:', III, ed. by Colowick S.P., Kaplan N.O., Academic Press, New York, 1954, 5.
[11] Ando H., Inouchi N., Asaoka M., Okuno K., Fuwa H.: Żywność. Technologia. Jakość., **2**, 1996, 9-15.
[12] Inouchi N., Glover D.V., Fuwa H.: Starch/Stärke, **39**, 1987, 259-266.
[13] Inouchi N., Glover D.V., Sugimoto Y., Fuwa H.: Starch/Stärke, **36**, 1987, 8-12.
[14] Inouchi N., Glover D.V., Fuwa H.: Starch/Stärke, **47**, 1994, 421-426.
[15] Fuwa H., Nakajima M., Hamada A., Glover D.V.: Cereal Chem., **53**, 1977, 355-364.
[16] Inouchi N., Glover D.V., Sugimoto Y., Fuwa H.: Starch/Stärke, **43**, 1991, 468-472.
[17] To K.-G., Shimizu M., Toshida T., Watanabe K., Mitsunaga T.: 36th Meeting for Kinki Branch of Japanese Society for Food and Nutrition, Abstracts, 1997, 37.
[18] Zhao J., Whistler R.L.: Food Technol., **48**, 1994, 104-109.
[19] Ahamed N.T., Singhal R.S., Kulkarni P.R., Kale D.D., Pal M.: Carbohydr. Polymers, **31**, 1996, 157-160.
[20] Teli M.D., Shanbag V., Kulkarni P.R., Singhal R.S.: Carbohydr. Polymers, **31**, 1996, 119-122.
[21] Ahamed N.T., Singhal R.S., Kulkarni P.R., Pal M.: Carbohydr. Polymers, **31**, 1996, 99-103.
[22] Walkowski A., Fornal J., Lewandowiez G., Sadowska J.: Polish J. Food Nutr. Sci., **6/47**, 1997, 11-22.

CHARAKTERYSTYKA SKROBI O MAŁYCH GAŁECZKACH – SKROBIA Z AMARANTUSA I QUINOA

Streszczenie

Gałeczki skrobiowe wydzielono z dojrzałych ziaren 9 próbek amarantusa i 4 próbek *Chenopodium quinoa.* Za pomocą zwykłej chromatografii żelowej (GPC) skrobi pozbawionej odgałęzień za pomocą izoamylazy z *Pseudomonas* stwierdzono, że skrobia amarantusowa zawiera od 0 do 28% amylozy. W skrobi z quinoa znaleziono 25 do 27% amylozy. Stosunek liczby łańcuchów krótkich do łańcuchów długich w amylopektynie wynosił dla tych skrobi od 2,2 do 3,3 i był nieco niższy niż dla zwykłej skrobi kukurydzianej. Materiał pozbawiony odgałęzień za pomocą izoamylazy rozdzielono za pomocą wysokosprawnej chromatografii cieczowej (HPLC) z refraktometrem różnicowym (RI) i niskokątowym fotometrem laserowym światła rozproszonego (LALLS) jako detektorami oraz za pomocą wysokorozdzielczej chromatografii jonowymiennej z amperometrycznym detektorem pulsacyjnym (HPAEC-PAD). Stwierdziliśmy, że amylopektyny z amarantusa i z quinoa miały więcej długich łańcuchów B i mniej krótkich łańcuchów aniżeli amylopektyna ze skrobi kukurydzianej woskowej. Jednakże, miały one więcej łańcuchów krótkich o stopniu polimeryzacji (DP) od 6 do 12. W porównaniu ze zwykłą skrobią kukurydzianą skrobie amarantusowe miały nieco wyższą temperaturę kleikowania (T_o, T_p i T_c) i mniejsze ciepła kleikowania (ΔH) zmierzone różnicowym kalorymetrem skanningowym (DSC). Skrobia z quinoa miała niższe T_o, T_p i T_c i mniejsze ΔH. Gałeczki skrobi z amarantusa i z quinoa były trawione przez amylazę szybciej niż gałeczki zwykłej skrobi kukurydzianej.