Abrasive Dehulling of Quinoa (*Chenopodium quinoa*): Effect on Saponin Content as Determined by an Adapted Hemolytic Assay[1]

R. D. REICHERT,[2] J. T. TATARYNOVICH,[3] and R. T. TYLER[2]

ABSTRACT

A hemolytic method was optimized and standardized against a commercial saponin preparation to provide a sensitive assay of the saponin content of quinoa. Increasing the extraction temperature and time (beyond 1 hr) and storage (30° C) of ground quinoa flour decreased the hemolytic activity of a crude extract of the grain. For 17 cultivars of quinoa, saponin content ranged from 0.14 to 0.73%, the 1,000-seed weight from 1.99 to 5.08 g, and abrasive hardness index from 40.3 to 80.3 sec. The 1,000-seed weight correlated significantly with both saponin content ($r = 0.72$, $P<0.01$) and

the abrasive hardness index ($r = 0.79$, $P<0.01$) indicating that larger seeds contained more saponin and were harder. Abrasive dehulling to a flour extraction ranging from 85.2 to 98.8% reduced the saponin content to a low level. Generally, the higher the initial saponin content of the cultivar, the greater the loss of bran required during dehulling. Concentrations of oil and protein were similar in dehulled (flour extraction >90%) and whole grain, whereas ash content was reduced slightly by dehulling. Cultivars with a low level of saponin provide optimum yields with abrasive dehulling.

Quinoa (*Chenopodium quinoa* Willd.) has been grown in South America since ancient times (Weber 1978). The main agronomic advantages of quinoa are its ability to grow at high elevations under semiarid conditions and on poor soils (Carmen 1984). The protein quality of the grain is very good, averaging 14% in protein and 6% in lysine (Weber 1978). An agronomic trial of quinoa as a salt-tolerant forage crop in Saskatchewan was promising (Kernan et al 1983).

A significant problem with quinoa grain is the high saponin content present in some varieties. These compounds cause a bitter, soapy taste, and possibly have an antinutritive effect (Cardoza and Tapia 1979). A number of techniques have been used to determine saponin content of quinoa based on quantitative isolation of saponins (De Bruin 1964, Machicao 1965), foaming capacity of the grain (Rios et al 1978), high-performance liquid chromatography (Burnouf-Radosevich and Delfel 1984), gas-liquid chromatography (Ruiz 1979, Burnouf-Radosevich et al 1985), and hemolysis of red blood cells by saponins (Aguilar et al 1979). In the last method, the saponin concentration required to hemolyze 50% of the cells was determined spectrophotometrically and compared to the response of a standard saponin. A hemolytic method based on the diameter of a clearing zone produced by hemolysis of red blood cells in solidified agar was used by Burnouf-Radosevich and Paupardin (1983).

Saponins are located in the outer layers of quinoa grain (Aguilar et al 1979, Simmonds 1965). These layers include the perianth, pericarp, a seed coat layer, and a cuticle-like structure (Varriano-Marston and De Francisco 1984). Saponins have traditionally been removed by washing the grain in alkaline water, and this principle was used in an effort to develop a commercial process for saponin removal (Briceno 1972, Junge 1973). Dry scouring of the seed in a laboratory rice scourer (flour extraction, 70%) was used by Junge (1973) to remove saponins. A machine equipped with brushes to scour the surface of the grain has been tested in Peru (Tapia 1979), and roller-milling has also been attempted (Amaya-Farfan et al 1978).

Recently, efficient, abrasive-type dehullers have been developed (Reichert 1982, Reichert et al 1984). The small-sample Tangential Abrasive Dehulling Device (TADD) was developed to simulate the abrasive action of the larger dehullers (Reichert et al 1986). The objective of the present study was to investigate the abrasive dehulling characteristics of several quinoa varieties with the TADD to determine if this technology could be used to eliminate

saponins from the grain, and to define those seed characteristics associated with efficient saponin removal. To determine saponin content, a visual hemolytic assay based on the method of Jones and Elliot (1969) for alfalfa saponins was optimized for quinoa and standardized.

MATERIALS AND METHODS

Quinoa Samples

Samples (40–50 g) of 20 cultivars of quinoa grain ranging from sweet to bitter were obtained from Peru (Cuzco and Puno) and Bolivia in 1980; the grain was not treated before shipment. The samples were representative of the more commonly grown cultivars. Larger quantities of the cultivars Real and Kancolla Rosanna were obtained from Peru. All grain samples were cleaned by aspiration to remove dust and chaff. The moisture content of all samples was similar (9.8%, SD 0.4%).

Analytical Methods

A hemolytic assay based on the method of Jones and Elliot (1969) was optimized and standardized to determine saponin content. Erythrocytes were obtained by centrifuging heparinized human blood (prepared fresh daily from the same donor) at 1,686 × g for 15 min and washing four times with physiological saline (pH 7.4). The latter was prepared by combining 5.95 g of $Na_2HPO_4 \cdot 7H_2O$, 0.76 g of KH_2PO_4, 7.2 g of NaCl, and enough distilled water to make a total of 1,000 g. A 2% erythrocyte suspension was prepared (2 ml of erythrocytes in 100 ml of physiological saline). Crude saponin extracts were obtained by rotating 3 g of ground seed in 14 ml of physiological saline in test tubes (22° C) for 1 hr and centrifuging at 14,500 × g for 20 min. Supernatant (10 ml) was transferred immediately to a 30-ml beaker and used the same day. A dilution series of the crude extract was prepared in 10-ml tubes. After transferring 1 ml of crude extract to tube 1, the remaining 9 ml was diluted by adding 1 ml of physiological saline. One milliliter of this mixture was transferred to tube 2, and the remaining 9 ml was again diluted with saline. This procedure was repeated until the desired number of dilutions was reached, usually not more than 15. With each dilution, the concentration of crude extract decreased by a factor of 0.9. One milliliter of 2% erythrocyte suspension was added to each tube while mixing on a test tube agitator. The tubes were immediately placed in a water bath at 37° C for 30 min and subsequently allowed to stand undisturbed at room temperature for 15 min before reading. The lowest concentration of plant extract causing complete hemolysis was termed the "titer" concentration. Complete hemolysis was defined as the absence of erythrocyte sedimentation at the bottom of the test tube. Hemolytic activity was defined as:
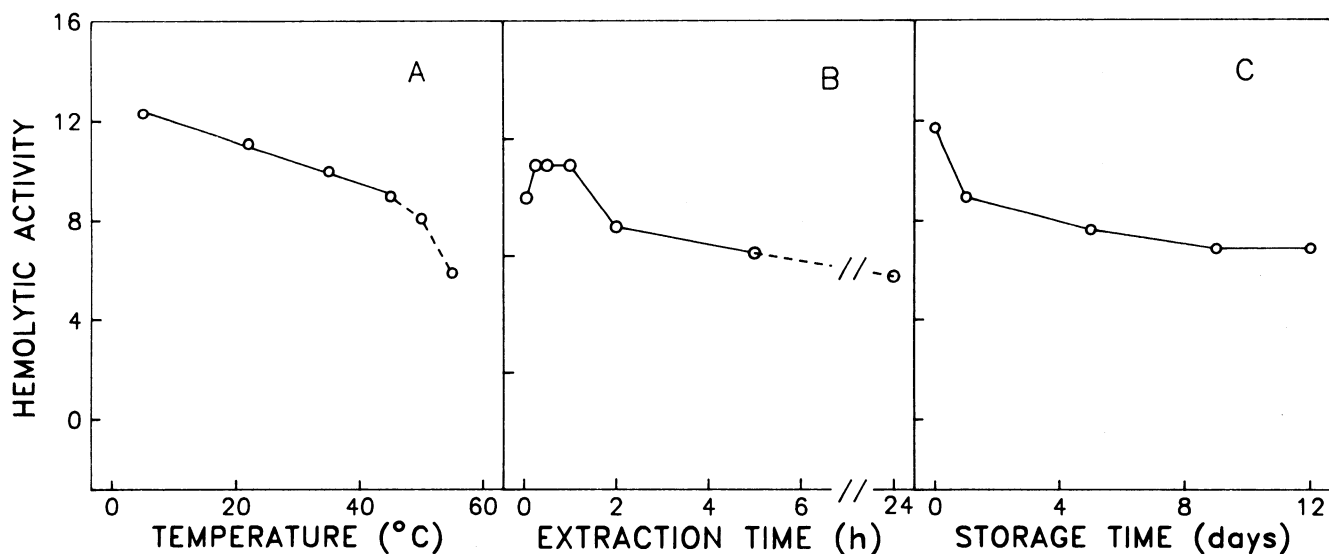
$$\frac{1}{\text{Volume of undiluted extract at the titer concentration}}$$

Fig. 1. Major variables affecting the hemolytic activity of a crude extract of quinoa grain (cv. Real): **A,** Effect of the temperature of extraction (1 hr); **B,** Effect of the duration of extraction (22°C); and **C,** Effect of the duration of storage (30°C) of the flour before extraction.

Fig. 2. Hemolytic activity of commercial saponin standard (Fisher Scientific Co.) added to Sajama (hemolytic activity <1.00) quinoa flour in dry form, and extracted and analyzed in the regular manner.

Hemolytic activity increased incrementally (1.000, 1.111, 1.234 ⋯ 3.934, 4.371, 4.857), the minimum measurable hemolytic activity being 1.0 (i.e., the titer concentration being the crude extract concentration). Where the hemolytic activity (HA) was expected to be greater than four, a predilution of the crude extract was used to reduce the number of tubes required. For whole grains, results of the hemolytic assay were reported in terms of a standard saponin preparation obtained from the Fisher Scientific Co., Pittsburgh, PA. Commercial saponin was added (0.1, 0.15, 0.2, 0.3, 0.4, 0.6, 0.8%) to Sajama quinoa flour (HA <1.00) in dry form and well mixed. The spiked flours were extracted with saline and analyzed in the regular manner. A calibration curve of saponin (%) versus HA was prepared and used to obtain saponin content from hemolytic activities.

Protein (N × 6.25) was determined by the automated Kjel-Foss method (method 46-08, AACC 1983). Ash and moisture contents were determined by AOAC methods 14.006 and 14.002,

respectively (AOAC 1980), and oil by the Butt-type extraction procedure using petroleum ether (method BA-3-38, AOCS 1984). The 1,000-seed weight was calculated from the average weight of four replicates of 100 seeds.

Dehulling of Grain

Samples (5 g) of quinoa grain were dehulled for various times (1–13 min) in the TADD described by Reichert et al (1986) using the 12-cup plate and the ground face of an A36Q6VBE grinding wheel rotating at 1,750 rpm. The dehulled grain was removed from the sample cups using an aspirating device (Oomah et al 1981) that collects the grain and simultaneously removes any residual bran. The dehulled grain was weighed and the yield expressed as a percentage of the initial weight. The percentage of kernel removed was calculated as 100 minus the yield of dehulled grain. Whole and dehulled samples were ground in a Udy Cyclo-Tec mill equipped with a 0.4-mm screen. An abrasive hardness index (AHI) was calculated for each quinoa variety from the regression (excluding the origin) of the dehulling time (x) in minutes versus the percentage of kernel removed (y), where AHI is the inverse of the slope of the regression line multiplied by 60 and is defined as the time in seconds to remove 1% of the kernel as fines (Oomah et al 1981). The cultivar Kancolla Rosanna was progressively dehulled at 1,200 rpm for a total of 135 sec in a larger scale abrasive-type dehuller (PRL mini dehuller) described by Reichert et al (1984). Wooden baffles were mounted on the shaft to reduce the volume of the dehulling chamber. At 20–30-sec intervals, the grain and bran were removed from the dehuller and separated by aspiration. A grain sample was taken for analysis and the remainder returned to the dehuller for further processing.

RESULTS AND DISCUSSION

Hemolytic Assay, Seed Size, and Hardness of 17 Quinoa Cultivars

The HA of quinoa grain (cultivar Real) as determined with the hemolytic assay was markedly affected by extraction temperature and time, as well as the storage conditions of quinoa flour before extraction (Fig. 1). As the extraction temperature was increased from 5 to 45°C, a linear decrease ($r = -0.997$; $P < 0.01$) in hemolytic activity was observed (Fig. 1A). The suspension of quinoa flour began to solidify at about 50°C, becoming an unmanageable gel at 60°C. Although an extraction temperature of 5°C would have given somewhat higher hemolytic activities, for convenience, extractions were routinely performed at room temperature (22°C). Extraction times of 15 min to 1 hr gave the highest hemolytic activities (Fig. 1B) and an extraction time of 1 hr was chosen for routine analysis. Hemolytic activities decreased as extraction time was increased from 1 to 24 hr. When quinoa flour

was stored (1–12 days) at 30°C (Fig. 1C), hemolytic activity decreased; the rate of decline was especially rapid during the first few days. For routine analysis, quinoa grain was analyzed immediately after grinding, or frozen and subsequently analyzed within 24 hr. The marked effect of extraction and storage conditions on hemolytic activity of quinoa extracts indicated that highly standardized conditions must be used in the assay to obtain reliable results. The reaction of quinoa saponins with other flour constituents was apparently accelerated by an increase in the temperature or contact time.

Standardization of the hemolytic assay using purified saponins would yield absolute values for saponin content. Because quinoa saponins were not available, however, a commercial saponin preparation was used instead. Three commercial saponin preparations obtained from the Fisher Scientific Co., Matheson, Coleman and Bell Co., and J. T. Baker Chemical Co. were compared. Concentrations of the respective saponins required for complete hemolysis of 1 ml of erythrocyte suspension were 24.4, 24.4, and 36.4 μg/L. Because the saponin preparation from the J. T. Baker Chemical Co. was lower in hemolytic power than that from either the Matheson, Coleman and Bell Co. or the Fisher Scientific Co., which were equivalent, the latter was used to prepare the calibration curve (Fig. 2). Saponin content of the grain was calculated from its hemolytic activity by using the equation:

$$\% \text{ Saponin} = 0.00675 + 0.0551 \text{ HA} - 0.00121 \text{ HA}^2 + 0.000009 \text{ HA}^3$$

($R = 0.998$, standard error $= 0.019$).

The HA and saponin content of 17 cultivars of quinoa ranged from 2.6 to 22.3, and 0.14 to 0.73%, respectively (Table I). Samples that gave a hemolytic activity of less than 2.0 were not included in this study; these included Sajama (<1.00), Puno-7 (1.24), Puno-12 (1.24), Ccoito (1.52), and Witulla (<1.00). The 1,000-seed weight and AHI of the 17 samples ranged from 1.99 to 5.08 g, and 40.3 to 80.0 sec, respectively.

The 1,000-seed weight was correlated with the saponin content ($r = 0.72$, $P <0.01$), indicating that cultivars with larger seeds generally contained more saponin; when the three cultivars with HA between one and two (Puno-7, Puno-12, and Ccoito) were included, the correlation ($r = 0.724$) was fractionally improved.

The 1,000-seed weight was also correlated with the abrasive hardness index ($r = 0.79$, $P <0.01$) indicating that cultivars with larger seeds also tended to be harder. There was no significant correlation between saponin content and the abrasive hardness index.

There is considerable discrepancy in the literature regarding the saponin content of quinoa grain. De Bruin (1964) extracted saponins with water, precipitated them with tannic acid, and purified them by several precipitations in a methanolic solution with an excess of ether. Saponin content was reported to range from 3.4 to 3.9% for three quinoa cultivars. Machicao (1965) also used gravimetric procedures to isolate saponin from quinoa and reported levels ranging from 1.5 to 2.8%. Rios et al (1978) measured the height of foam produced by four quinoa varieties and compared it to the height of foam produced by digitonin. Saponin content was reported to range from 1.7 to 2.8%. Aguilar et al (1979) used a sensitive hemolytic assay for saponin content, basing their quantitation on saponin obtained from J. T. Baker Chemical Co. The respective saponin contents of Kancolla grown in 1975 and 1976 were reported to be 0.333 and 0.400%, whereas Blanca de Juli and Blanca de Junin were reported to contain 0.304 and 0.077% saponin, respectively. Using our hemolytic assay, we determined the saponin contents of samples of these three varieties to be 0.23, 0.15, and 0.16%, respectively. Burnouf-Radosevich and Delfel (1984) quantitated the saponin aglycones of quinoa (cultivar Real) and found a total of 0.44% of oleanolic acid and hederagenin, which are the major triterpenes in the grain. Using a hemolytic assay and a standard obtained from Coultronics Co., Burnouf-Radosevich and Paupardin (1983) found that Real contained 0.81% of saponin. The saponin content of Real in our study was 0.50%. It appears that gravimetric procedures and the foam method have overestimated the saponin content of quinoa. The hemolytic assay described in this paper gives results that are generally within the range reported by Aguilar et al (1979), Burnouf-Radosevich and Delfel (1984), and Burnouf-Radosevich and Paupardin (1983).

Saponin Reduction by Abrasive Dehulling

The saponin content of all 17 quinoa cultivars was reduced by abrasive dehulling, and the results for five of the cultivars are shown in Figure 3. The HA of Real and Pasancalla were reduced in a similar manner by dehulling, whereas saponin was removed more
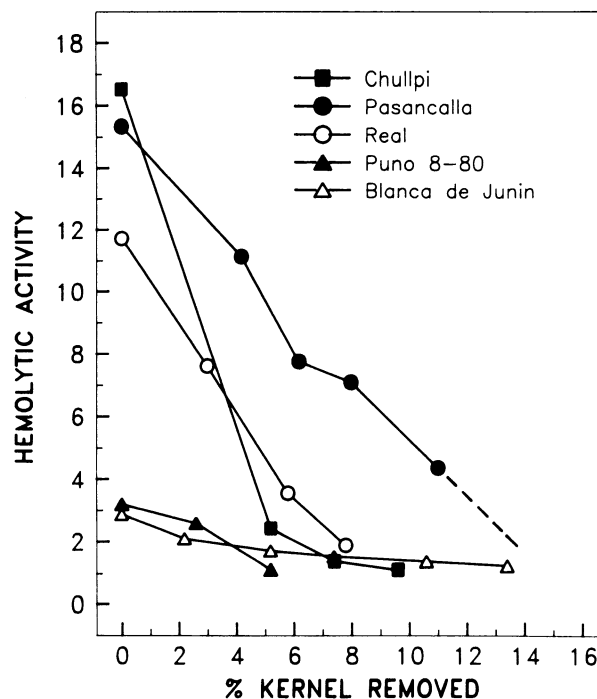
TABLE I
Hemolytic Activity, Saponin Content, 1,000-Seed Weight, and Abrasive Hardness Index of 17 Quinoa Cultivars[a]

Cultivar	Hemolytic Activity[b] (HA/3g)	Saponin[c] Content (%)	1,000-Seed Weight (g)	Abrasive Hardness Index (sec)
Oca Suca	2.6 ± 0.0	0.14	2.42	51.4
Blanca de Juli	2.8 ± 0.4	0.15	2.60	50.8
Blanca de Junin	2.9 ± 0.0	0.16	1.99	46.7
Puno 8-80	3.2 ± 0.0	0.17	2.46	60.2
Puno-15	3.7 ± 0.5	0.19	2.08	48.1
Kancolla	4.4 ± 0.5	0.23	2.31	45.9
Cheweca	4.8 ± 0.3	0.26	2.39	40.3
Kancolla Rosanna	9.9 ± 0.5	0.46	2.55	55.9
Real	11.7 ± 0.6	0.50	4.04	56.9
Kaslala	12.7 ± 0.3	0.53	4.71	65.2
Wila Coymini	13.0 ± 0.7	0.54	4.50	66.7
Janku	14.1 ± 0.4	0.57	4.82	60.6
Kellu	14.2 ± 1.9	0.57	5.08	80.0
Puca	14.5 ± 0.8	0.58	3.45	46.2
Pasancalla	15.3 ± 1.6	0.60	2.65	43.6
Chullpi	16.5 ± 0.4	0.63	3.19	54.5
Amarilla de Junin	22.3 ± 3.5	0.73	3.87	55.4

[a] Hemolytic activity and saponin content are based on duplicate determinations, whereas 1,000-seed weight is based on quadruplicate analyses. Abrasive hardness index is based on the slope of a line with 5 or 6 points. All analyses are on a dry weight basis.
[b] Mean and standard error.
[c] Calculated from hemolytic activity on the basis of a saponin standard obtained from the Fisher Scientific Co.

Fig. 3. Effect of abrasive dehulling on the hemolytic activity of the crude extract of five quinoa cultivars.

efficiently from Chullpi than from any of the other 16 cultivars. The superior performance of Chullpi did not appear to be related to its seed size or hardness. However, the seeds did appear to be more spherically shaped, which would likely make them more suitable for abrasive dehulling.

For comparative purposes the percentage of kernel removed to reduce the HA to 2.0 was determined for each cultivar; removal of from 1.2 to 14.8% of the kernel was required (Fig. 4). In general, the higher the HA of the whole grain, the greater the loss of bran required to reduce it to 2.0 ($r = 0.85$, $P < 0.01$). Cultivars with an HA of 5.08 or less could be effectively processed with a flour extraction of 95% or greater. All cultivars could be processed with a flour extraction of at least 85%. These flour extraction levels are considerably higher than the figure (70%) reported by Junge (1973) for processing of quinoa in a rice scourer; they are similar to flour extraction levels (88–90%) he reported for a process whereby a flotation cell was used to remove saponins from quinoa. Abrasive dehulling has advantages over the flotation process, as there is no effluent problem and seeds do not require drying.

To determine the effect of abrasive dehulling on other chemical constituents in quinoa, the cultivars Real and Kancolla Rosanna were dehulled in the TADD and analyzed for oil, protein, and ash

TABLE II
Effect of Abrasive Dehulling on the Oil, Protein, and Ash Content of Quinoa Grain[a]

Cultivar	% Kernel Removed	Oil (%)	Protein[b] (%)	Ash (%)
Real	0	6.72	13.4	3.37
	3.1	6.87	13.5	2.93
	6.7	6.96	13.7	2.62
	8.5	6.76	13.3	2.40
Kancolla Rosanna	0	6.03	15.9	3.07
	4.0	6.14	15.8	2.67
	6.7	6.31	16.0	2.61
	9.5	6.39	16.0	2.60

[a] Results are an average of duplicate determinations and are reported on a dry weight basis.
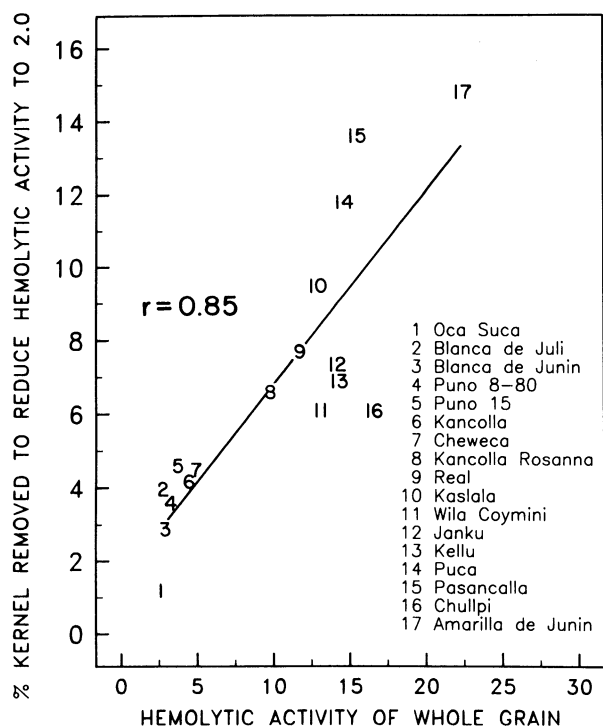[b] N × 6.25.

Fig. 4. Relationship between the hemolytic activity of whole quinoa grain and the percent kernel removed by abrasive dehulling to reduce the hemolytic activity to 2.0.

content (Table II). The oil and protein contents of dehulled grain (with up to 10% of the kernel removed) were similar to the concentrations of these constituents in the whole grain, whereas ash content was reduced slightly by dehulling. Overprocessing of Real to remove 18.2% of the kernel reduced the oil, protein, and ash contents to 5.50, 11.6, and 2.00%, respectively, suggesting that it would be advisable to remove the minimum acceptable amount of the kernel during processing. The flour color of both cultivars improved by dehulling.

Quinoa grain (cultivar Kancolla Rosanna) was dehulled progressively in a larger scale abrasive-type dehuller (PRL mini dehuller) (Reichert et al 1984). To reduce the HA of the grain from 10.4 to 2.0, it was necessary to remove 7.6% of the kernel with the PRL mini dehuller compared to 6.6% with the TADD, suggesting that abrasive dehulling of quinoa could be scaled up effectively. Relatively fine-grit grinding wheels are recommended for optimal abrasive dehulling of quinoa.

CONCLUSIONS

This investigation described a visual, calibrated hemolytic assay that does not require any spectrophotometry or solidified agar, which were used previously for determination of saponins in quinoa (Aguilar et al 1979, Burnouf-Radosevich and Paupardin 1983). The assay was used to demonstrate that the saponin content of quinoa grain could be effectively reduced by minimal abrasive dehulling. Higher flour extraction levels were obtained with cultivars containing a lower level of saponin. Selection of such cultivars may result in a reduction in seed size. Selection of cultivars with spherically shaped seeds (e.g., Chullpi) may also increase extraction rates.

LITERATURE CITED

AGUILAR, R. H., GUEVARA, L., and ALVAREZ, J. O. 1979. A new procedure for the quantitative determination of saponins and its application to several types of Peruvian quinoa (Span.). Acta Cient. Venez. 30:167.

AMAYA-FARFAN, J., CIACCO, C. F., AGAPITO, F. J. L., and AUGUSTO-RUIZ, W. 1978. Removal of saponins from quinoa (Chenopodium quinoa, Willd.) grain by milling. 5th Int. Congr. Food Sci. Technol. (Abstr.) Sept. 17–22, Kyoto, Japan.

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC. Method 46-08, approved October 1975. The Association: St. Paul, MN.

AMERICAN OIL CHEMISTS SOCIETY. 1984. Official and Tentative Methods. Method BA 3-38, approved 1973. The Society: Champaign, IL.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1980. Official Methods of Analysis. The Association: Washington, DC.

BRICENO, O. 1972. Desarrollo de un metodo de lavada par agitacion y turbulencia de la grana de quinoa (Chenopodium quinoa) (Span.). Programa Multinacional de Technologia de Alimentos. OEA-Univ. Nac. Agrar.: Lima, Peru.

BURNOUF-RADOSEVICH, M., and DELFEL, N. E. 1984. High-performance liquid chromatography of oleanane-type triterpenes. J. Chromatogr. 292:403.

BURNOUF-RADOSEVICH, M., and PAUPARDIN, C. 1983. Triterpenoid saponins from plant organs and in vitro tissue cultures of Chenopodium quinoa Willd. (French). C. R. Acad. Sci. Paris 296:429.

BURNOUF-RADOSEVICH, M., DELFEL, N. E., and ENGLAND, R. 1985. Gas chromatography-mass spectrometry of oleanane- and ursane-type triterpenes—application to Chenopodium quinoa triterpenes. Phytochem. 24:2063.

CARDOZA, A., and TAPIA, M. 1979. Nutritive value. Page 149 in: Quinoa and Kaniwa Andean Crops (Span.). M. Tapia, H. Gandarillas, S. Alandia, A. Cardoza, and A. Mujica, eds. Cent. Int. Invest. Desarrollo: Bogota, Columbia.

CARMEN, M. L. 1984. Acclimatization of quinoa (Chenopodium quinoa Willd.) and canihua (Chenopodium pallidicaule, Aellen) to Finland. Ann. Agric. Fenniae 23:135.

DE BRUIN, A. 1964. Investigation of the food value of quinoa and canihua seed. J. Food Sci. 29:872.

JONES, M., and ELLIOT, F. C. 1969. Two rapid assays for saponin in individual alfalfa plants. Crop Sci. 9:668.

JUNGE, I. 1973. Lupine and Quinoa. Research and Development in Chile. An. Escuela Ing., Publ. no. 1. Univ. Concepcion: Chile.

KERNAN, J. A., DARRACH, W., JAMES, D., COXWORTH, E. C., CROWLE, W. L., KNIPFEL, J. E., and GREEN, D. 1983. Assessment of the potential of growing unconventional energy crops or multi-use crops on saline or marginal land, phase 1, year 2. SRC Publ. no. R-711-6-E-83. Saskatchewan Res. Council: Saskatoon, Sask., Canada.

MACHICAO, E. 1965. The saponins of quinoa. (Span.) Sayana (Bolivia) 4:24.

OOMAH, B. D., REICHERT, R. D., and YOUNGS, C. G. 1981. A novel multi-sample, tangential abrasive dehulling device (TADD). Cereal Chem. 58:392.

REICHERT, R. D. 1982. Page 547 in: Sorghum in the Eighties: Proc. Int. Symp. Sorghum, 2–7 November, 1981. L. R. House, L. K. Mughogho, and J. M. Peacock, eds. Int. Crops Res. Inst. Semi-Arid Tropics (ICRISAT): Patancheru, India.

REICHERT, R. D., OOMAH, B. D., and YOUNGS, C. G. 1984. Factors affecting the efficiency of abrasive-type dehulling of grain legumes investigated with a new intermediate-sized, batch dehuller. J. Food Sci.

49:267.

REICHERT, R. D., TYLER, R. T., YORK, A. E., SCHWAB, D. J., TATARYNOVICH, J. E., and MWASARU, M. A. 1986. Description of a production model of the Tangential Abrasive Dehulling Device and its application to breeders' samples. Cereal Chem. 63:201.

RIOS, M. L. T., SGARBIERI, V. C., and AMAYA, J. 1978. Chemical and biological evaluation of quinoa (*Chenopodium quinoa* Willd.). Effect of extracting the saponins by heat treatment (Span.). Archivos Latinamericanos de Nutricion 28:253.

RUIZ, A. W. 1979. Determination of oleanolic acid in quinoa by gas-liquid chromatography (*Chenopodium quinoa* Willd. cv. Kancolla) (Span.). Boletin Sociedad Quimica Peru 45:266.

SIMMONDS, N. W. 1965. The grain Chenopods of the tropical American highlands. Econ. Botany 19:223.

TAPIA, M. 1979. Industrialization. Page 193 in: Quinoa and Kaniwa Andean Crops (Span.). M. Tapia, H. Gandarillas, S. Alandia, A. Cardoza, and A. Mujica, eds. Centro Int. Invest. Desarrolo: Bogota, Columbia.

VARRIANO-MARSTON, E., and DE FRANCISCO, A. 1984. Ultrastructure of quinoa fruit (*Chenopodium quinoa* Willd.). Food Microstruct. 3:165.

WEBER, E. J. 1978. The Inca's ancient answer to food shortage. Nature 272:486.