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Tannin elimination and improvement of the digestibility of protein sorghum grains

R,A, Agudelo.[1], *G. Fliedel.*[2] *and O.M. Alarcón.*[3]

SUMMARY. Three hybrids of sorghum grains [*Sorghum bicolor* (L) Moench] containing 3.8, 3.0 and 0.2% of tannins were treated. Abrasive dehulling and storage of moist grains were tested separately and in combination to reduce the tannin content and to improve the nutritional quality of grains. The moisture content of the grains was increased from 12 to 30% by humidifying them with water, acetic acid, sodium bicarbonate or sodium hypochlorite solutions. Abrasive dehulling of the grains to a yield between 75 and 80%, humidifying the grains with acetic acid (1% v/v) and storing them during 7 days at 20 °C proved to be the most effective procedure. In this way tannin can be totally reduced and the in vitro digestibility of protein can be increased to 87.5%.
Keywords: sorghum, cereal, tannin, polyphenols, protein digestibility.

RESUMEN. Eliminación de taninos y aumento de la digestibilidad proteica del sorgo. Tres híbridos de sorgo [*Sorghum bicolor* (L) Moench] que contenian 3.8, 3.0 y 0.2% de taninos fueron tratados. El pelado por abrasión y el almacenamiento en medio húmedo fueron utilizados solos o en combinación para reducir el contenido de tanino de los granos y aumentar su digestibilidad proteica. El contenido de humedad de los granos se incrementó desde 12 hasta 30%, humedeciéndolos con agua, o soluciones de ácido acético, bicarbonato de sodio o hipoclorito de sodio.
Pelar por abrasión los granos a rendimento de 75-80%, humedecerlos con una solución de ácido acético (1% v/v) y almacenarlos durante 7 días a 20 °C fue el procedimento más efectivo. De este modo los taninos fueron practicamente eliminados en su totalidad y la digestibilidad proteica in vitro del grano fue aumentada hasta 87.5%.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L) Moench] is the fifth cereal in importance in the world and second leading cereal crop in Venezuelan production (1). It constitutes an important source of energy and protein for millions of inhabitants in Africa and Asia. Sorghum may be consumed in several forms: decorticated and cooked, porridge, couscous, crackers, alcoholic and non-alcoholic beverages. Venezuelan sorghums with high levels of tannin are frequently used for animal nutrition. The presence of polyphenols, or condensed tannins, reduces its nutritional quality and in certain varieties of sorghum they form complexes with proteins and enzymes of the grain (2). On the other hand, the tannins give sorghum a resistance to bird, insect and fungi depredation (3-6), and prevent germination of the grain before harvest (3).

Many workers favor the elimination and deactivation of the sorghum tannins in grains to increase its nutritional quality. This can be achieved in different ways: by direct removeal of the pericarp and testa (7-10), by cooking the grains in water or in alkaline medium (11-13), by germination and fermentation (14-18), by chemical treatment (7,19-23), and finally bu storing the moist grain (24-27).

The present study was designed to evaluate the effects of abrasive dehulling and storage of the moist grain (separated or combined) on the deactivation of tannins, and on the nutritional quality of the grain, measured by the in vitro protein digestibility. All this aimed to improve the commercial utilization of tannin-rich cultures which could become economically signficant in certain countries where the genotype of high tannin content gives good yields.

MATERIAL AND METHODS

Grains: Three sorghum hybrids: Chaguaramas II (Cha.III), Chaguaramas VII (Cha. VII) and Prosorgo 6 (Pro 6), produced at the Experimental Station of Chaguaramas (Guarico State, Venezuela) by the firm Protinal C.A., were tested. All grains were carefully cleaned after elimination of the glumes.

Tannin determination: The vanillin/HCl method was used according to a modified procedure of Price et al (28). The grains were previously milled (Ciclotec mill), and particles of less than 0.4 mm were obtained. The tannin was extracted successively with pure methanol and with methanol acidified with 1% HCl (v/v). In this method, the reaction of vanillin with leucoantocyanidins (catechins) and proantocyanidins (tannins) present in the extract produces a reddish colour. The absorbance was measured at 500 nm, and the tannin concentration was calculated from a standard curve using catechin.

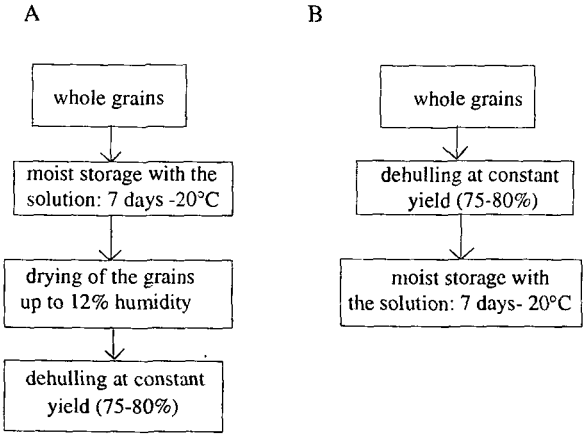
In vitro protein digestibility: In vitro protein digestibility (IVPD) was determined by the method of Mertz et al (29) modified by Rom et al (30). The grains, treated or not, were finely milled to obtain particles of less than 0.4 mm in diameter. After protein digestion by pepsin action in 0.1 M potassium phosphate buffer (pH 2.0), at 37 °C, during 2 hours, the remaining nitrogen content in the precipitate obtained after centrifugation at 4800 g, for 15 mn at 4 °C, was determined by the Kjeldahl method. IVPD was expressed as percentage of digested nitrogen in relation to the total nitrogen content of the sample.

1 Lab. Tecnología de Alimentos, Escuela de Nutrición, Universidad de Los Andes. Mérida, Venezuela.
2 Lab. Technologie des Céréales, Maison de la Technologie, CIRAD-CA. Montpellier, France.
3 Lab. Bioquímica Nutricional, Escuela de Nutrición. Universidad de Los Andes. Mérida, Venezuela

Procedure for elimination of tannins: «Storage of moist grains» and «abrasive dehulling» procedures were used separately and combined.

- Storage of moist grains: The moisture content of each of the hybrids was increased from 12% to 30% by humidifying the grains with different solutions: distilled water, acetic acid (1% v/v), sodium bicarbonate (1% w/v) or sodium hypochlorite (1% v/v). The proportion of the humidifying solution was 25% (v/w) related to the total grain weight. Once humidified with one of the various solutions, the grains were kept in closed containers during 7 days at room temperature (20° ± 3 °C). The tannin content was determined on 0,1, 2, 3, 4 and 7 days. IVPD was determined at the initiation and the end of the storage period.
- Abrasive dehulling: The three hybrids were dehulled in a device called TADD (tangential abrasive dehulling device) up to a constant yield between 75 and 80%. The effect of the abrasive dehulling on the tannin content and IVPD were determined.
- Combination of the abrasive dehulling with storage of moist grains (Schemes A and B, Fig. 1): This experiment was performed on the hybirid Prosorgo 6 only. Considering previous experiences with the combined abrasive dehulling and stored moist grains procedures, we used as humidifying agents only acetic acid (1% v/v) or sodium bicarbonate solution (1% w/v).

FIGURE 1
Combination of the abrasive dehulling with the moist storage



In Scheme «A» (Fig. 1), the whole grains were humidified with acetic acid or sodium bicarbonate and were stored during 7 days, at 20 ± 3 °C, in air-tight glass containers. At the end of this period, the grains were smoothly dried (37 °C) up to 12% humidity, and those dehulled by abrasion in the TADD up to a constant yield of 75 to 80%. According to Scheme «B» (Fig. 1), the grains were previously dehulled (yield between 75 and 80%) and the moisture content was increased to 30% with acetic acid (1% v/v) or sodium bicarbonate (1% w/v), and stored in air-thigt containers during 7 days at 20 ± 3 °C. The tannin content and IVPD of the grains were determined in the following steps using scheme «A»: whole grains, after storage, and dehulled grains, and according scheme «B»: whole grains, dehulled grains, and after storage.

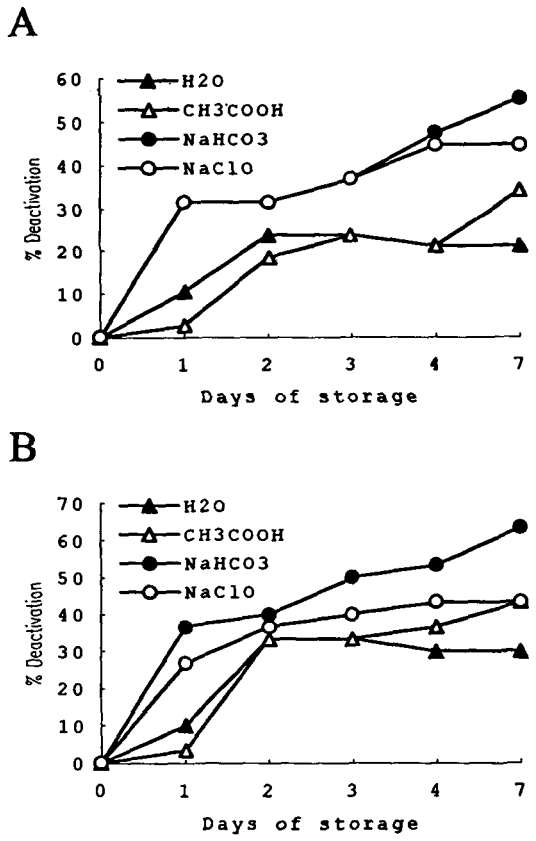
Statistical analysis: Each determination was carried out in triplicate, and the average ± SD was calculated. The statiscal analysis was carried out by one-way analysis of variance (31) and the post ad hoc Duncan multiple range test at a confidence level of 5% (32).

RESULTS AND DISCUSSION

Elimination of condensed tannins: Among the grains analyzed two tannin-rich hybrids were found Pro. 6 (3.8% tannin as catechin equivalent) and Cha. VII (3.0% tannin as catechin equivalent). A tannin-free hybrid Cha. III was also found, as shown in Table 1.

Treatment consisted in humidifying the whole grain with water and storing 7 days (Fig. 2) was the less efficient in eliminating tannins. In spite of this, at the end of the second day the percentage of deactivation was 23.7% in the hybrid Pro. 6 and 33.3% in the hybrid Cha. VII. From the second day onwards, there was no increase in the deactivation of tannins. After seven days of storage, the total percentage of deactivation was 21.1 and 30.0% (Pro. 6 and Cha. VII, respectively). After one week storage, fungi started to develop in the grains.

FIGURE 2
Deactivation of tannins during the moist storage of (A) hybrid Prosorgo 6, (B) hybrid Chaguaramas VII



Treatment with acetic solution to humidify the whole grains was moderately effective: 34.2% (Pro. 6) and 43.3% (Cha. VII) deactivation were achieved in a 7 days storage. Tannin deactivation similar to the values obtained in the previous treatment was observed when sodium hypochlorite (1% v/v) solution was used to increase the humidity of the grains. On the 7th storage day, the tannin content was reduced to 44.7 and 43.3% (Pro. 6 and Cha. VII, respectively). Treatment using a sodium bicarbonate solution (1% v/v) to humidify the whole grains was the most effective ($P<0.05$) in the tannis elimination: 55.3% (Pro.6) and 63.3% (Cha. VII) reduction at the end of the 7th day of storage. During the first two days, the curve of tannin deactivation showed a slope higher than that obtained at the rest of the period. The process of tannin deactivation by action of bicarbonate continued up to the 7th day, but immediately afterwards fungi started to develop.

Reicher et al (24) succeeded in reducing the content of tannin to 39%, 83% and 97% by soaking the sorghum grains in water, 0.8 N HCl and 0.8 N NaOH, respectively and storing them for only 2 days at 25 °C, in a CO2 rich atmosphere. These authors (24) obtained higher results in eliminating tannin when they increased the storage temperature and using complete anaerobic conditions. Similar results were reported by Mitaru et al (25), who extended the storage time to 20 days, but they incorporated a mixture of acetic and propionic acid to control the development of fungi. Under these conditions, they could eliminate the tannis completely.

According to Mitaru et al (13), the tannin deactivation during the storage of moist grains was due to repolymerization of the tannins, and this makes them less detectable by classic analytical methods, and less soluble and active (due to a greater molecular size) to form complexes with proteins.

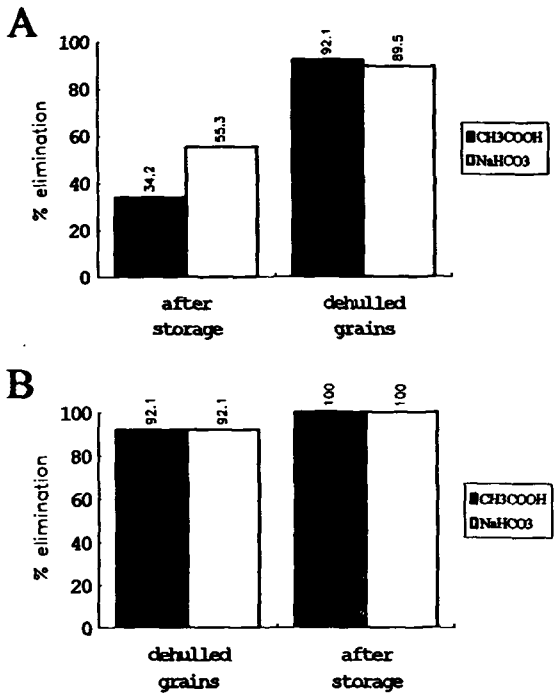
Results of abrasive dehulling are shown in Table 2. Elimination of the outer layers of the grains by abrasive dehulling (yield between 75 and 80%) resulted in the elimination of 92.1% (Pro. 6) and 90.0% (Cha. VII) of tannins, which was much more effective than that observed in the moist storage. This represents a valid alternative for eliminating the tannins, if the grains resist the mechanical action and do not break during dehulling. Chibber et al (8) reduced tannins to 64% by dehulling the sorghum grains up to a yield of 75.8%. In this experience, in order to eliminate the tannins to a 95% was necessary to adjust the dehulling to a yield of 63%.

Due to the elongated shape of the grains under study the elimination of the testa layer, where the condensed tannins are concentrated, by the action of abrasive dehulling was more effective in the frontal part of the grain (the side of the germ) than in the dorsal portion (opposite to the germ).

Results of the combined abrasive dehulling with moist grain storage of hybrid Pro. 6 are shown in Fig. 3 Similar results for tannin elimination was achieved during dehulling of the grains, with or without previous storage in a humid medium, as shown in Scheme A. The storage in humid medium (sodium bicarbonate solution) followed by abrasive dehulling resulted in tannin elimination of 89.5% while the storage in humid acetic acid solution followed by dehulling of the grains reduced tannin to 92.1%.

The procedure described in Scheme B was the most effective in eliminating the tannin. It can be noted that 92.1% of the tannin is eliminated by simple dehulling. Once the dehulled grains were stored in a humid medium, in a solution of acetic acid or soldium bicarbonate, the tannin detected by the method used was practically nil.

FIGURE 3
Tannin elimination of hybrid Prosorgo 6 through the combination of the abrasive dehulling with the moist storage: (A) Scheme A. (B) Scheme B.



- Improvement of the in vitro digestibility of the proteins:

The digestibility of protein in tannin-rich grains (Pro. 6 and Cha. VII) was significantly lower than that of the hybrid without tannin (Cha. III), with values of 26.0%, 29.4 % and 61.9%, respectively. All storage treatments of whole grains in a humid medium improved the in vitro protein digestibility but in a different degree, according to the type of solution used (Table 1). Treatment with distilled water to humidify the grains increases the in vitro digestibility of the proteins to 34.4% (Pro.6) and 47.3% (Cha. VII), respectively. On the other hand, the hybrid without tannin (Cha.III) shows a little variation in its protein digestibility 64.4% at the endd of the storage period. Similar increases in protein digestibility were observed when solutions of sodium bicarbonate and sodium hypochlorite were used to humidify the grains. The improvement of digestibility was significantly greater ($P<0.05$) when acetic acid solution was used. In the tannin-rich hybrids, the percentage of digestibility of proteins was 45.5% (Pro.6) and 59.5% (Cha.VII), while for the tannin-free sorghum (Cha.III) the digestibility was 71.3%. Storage of moist grains when the acetic acid is used apparently helped the bacterial growth which is responseble of grain fermentation. Thus, Au and Fields (16) reported that fermentation increases the proteolitic activity as result of decreasing of pH. This increase in protelytic activity favors the digestion by pepsin used for IVPD determination in this work.

TABLE 1
Effect of moist storage on the tannin elimination and digestibility of protein[c]

	Prosorgo 6		Chaguaramas VII		Chaguaramas III	
	Tannin[a]	IVPD	Tannin[a]	IVPD[b]	Tannin[a]	IVPD[b]
Before storage	3.8 ± 0.1	26.0 ± 0.1d	3.0 ± 0.1	29.4 ± 0.2c	0.1 ± 0	61.9 ± 0.2d
After storage						
Water	3.0 ± 0.1	34.4 ± 2.1c	2.1 ± 0	47.3 ± 1.6c	0	64.4 ± 0.9c
Acetic acid	2.5 ± 0.1	45.5 ± 1.2a	1.7 ± 0.1	59.5 ± 0.2a	0	71.3 ± 0.5a
Sodium bicarbonate	1.7 ± 0	39.0 ± 1.4b	1.1 ± 0	39.0 ± 1.4d	0	62.0 ± 0.7d
Sodium hypochlorite	2.1 ± 0.1	39.5 ± 0.3b	1.7 ± 0	56.3± 1.2b	0	69.2 ± 0.4 b

a Tannins as catechine equivalent
b *In vitro protein digestibility*
c Means followed by different letter within the same column IVPD are statiscally different (P<0.05)

In relation to abrasive dehulling (Table 2), the elimination of tannin was also accompanied by an increase in the in vitro digestibility of the proteins, as the values obtained after dehulling were 38.3% (Pro.6) and 45.8% (Cha.VII). These values can still be considered low, on comparing with sorghum without tannin Cha.III. Chibber et al (8) found a considerable increase in the in vitro protein digestibility after grains were dehulling up to a yield of 63%. These authors indicate that the protein digestibility was 22% and 71% before and after dehulling, respectively.

TABLE 2
Effect of abrasive dehulling on the tannin elimination and digestibility of protein[c]

	Prosorgo 6		Chaguaramas VII		Chaguaramas III	
	Tannin[a]	IVPD	Tannin[a]	IVPD[b]	Tannin[a]	IVPD[b]
Before dehulling	3.8 ± 0.1	26.0 ± 0.1	3.0 ± 0.1	29.4 ± 0.2	0.1 ± 0	61.9 ± 0.2
After dehulling	0.3 ± 0	38.3 ± 0.1	0.3 ± 0.1	45.8 ± 0	0	61.1 ± 0.3

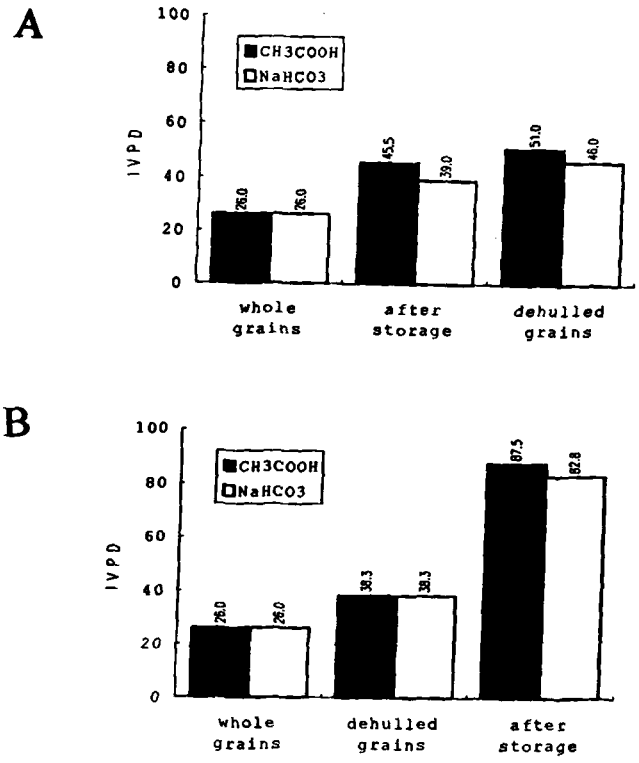
a Tannins as catechine equivalent b In vitro protein digestibility

In Figure 4, moist storage followed by abrasive dehulling (according to Scheme A) increased the grain protein digestibility. This increase is more evident in the storage phase, especially when acetic acid was used to humidify the grains. Once the grains stored in a humid medium were dehulled no significant increase (P<0.05) is noted in the protein digestibility, which is 46% (storage with sodium bicarbonate followed by dehulling) and 51% (storage with acetic acid followed by dehulling), respectively.

It is important to note that the procedure described in Scheme B is the most effective in increasing the in vitro protein digestibility of grains. For example, in moist storage (with acetic acid) of the grains previously dehulled, a protein digestibility, which is 46% (storage with sodium bicarbonate followed by dehulling) and 51% (storage with acetic acid followed by dehulling), respectively.

It is important to note that the procedure described in Scheme B is the most effective in increasing the in vitro portein digestibility of grains. For examples, in moist storage (with acetic acid) of the grains previously dehulled, a protein digestibility of 87.5% is observed (Fig. 4). That represents a considerable protein digestibility for sorghum rich in tannin. This value is higher than that of the in vitro protein digestibility (pepsin method) reported for sorghum without tannin, and it is equivalent to the value of corn protein digestibility. On the other hand, this digestibility represents almost twice the value observed when whole grains are stored with acetic acid solution, which may suggest that a previous dehulling process has a more favourable effect on the pepsin action on protein of milled grains.

FIGURE 4
In vitro digestibility of protein IVPD of hybrid Pro. 6 through the combination of the abrasive dehulling with the moist storage: (A) Scheme A, (B) Scheme B.



CONCLUSIONS

Moist storage and abrasive dehulling used separately or in combination resulted effective in the tannin elimination and in improving the in vitro protein digestibility of sorghum grains. Abrasive dehulling of grains up to yield 75 to 80% and humidifying them with acetic acid (1% v/v), and storing 7 days at 20 °C is the most effective procedure. Thus, the tannin detected by this method is practically traces and the in vitro protein digestibility of sorghum can be increased to values comparable to those of other cereals.

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