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EFFECT OF CHEMICAL TREATMENT AND TORTILLA PROCESSING ON TANNIN CONTENT AND PROTEIN DIGESTIBILITY OF TWO EGYPTIAN SORGHUM CULTIVARS

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ABSTRACT: sorghum cultivar (Assiut 14) and a tunnin-free sorghum cultivar (Dorado) Tannin-containing were used for this study. Tannin contents of untreated sorghum grains were 0.84% for low-tannin sorghum grains and 2.46% for high-tannin sorghum grains. In-*vitro* protein digestibilities (IVPD) were 84.31% and 82.35% for the two cultivars, respectively. Soaking of sorghim grains in water at 30°C for 24 hrs. improved IVPD and reduced tannin content to 0.60% and 1.36% for Dorndo and Assiut 14 cultivars, respectively . Cooking in water at 100°C for 30 min gave similar results approximately, but in a short time. Soaking of high-tannin sorghum at 30°C in 0.01 and 0.05 M sodium hydroxyde removed 67-80% of the tannins in 24 hrs. Under similar conditions, 0.01 and 0.05 M Calcium hydroxide removed 65 and 75% of tannins content, respectively. Moreover, the results indicated that extraction of tannins in 0.01 and 0.05 M alkaline solution at 30°C for 24 hours, or 100°C for 30 min improved in *Vitro* protein digestibility of high-tannin grains as compared to untreated seeds. Percent of tannin removed and percent in vitro protein digestibility were increased with increase in time or alkali concentration, while application of high temperature reduced the time of incubation required. On the other hand, cooking of sorghum grains in 0.05 M calcium hydroxide during tortilla processing eliminated 50 and 70% of tannins after cooking for Dorado and Assiut 14 cultivars, respectively. Whilst, slightly decrease of tannin was observed after baking process.

INTRODUCTION

The phenolic compounds in sorghum grains play an important agronomic role by reducing grain damage and bird predation (Hahn et al., 1984). They can be divided into phenolic acids, flavonoids and condensed tannins. The condensed tannins in sorghum grain bind and precipitate proteins causing a reduction in nutritional value (Nyachoti et al., 1997). Tannins effect the availability of amino acids (Rostango, 1972) and utilization of protein (Eggum and Christansen, 1974). Ghali et al. (1987) found higher negative correlation coefficient between protein digestibility and tannins content (r=0.74) in sorghum grains. Also, tannins bind with amylases in the malt, making them unavailable for starch degradation (Daiber, 1975). It is therefore important that tannin-containing sorghum grains are properly treated befor they can be used for food. Decortication by a brasive action could remove the auter pericarp and seed coat layers of the grain where most of the tannins are located (Hahn and Rooney, 1986). Chemical detoxification has also been used to treat polyphenol-rich sorghum. Several alkalis, including ammonium hydroxide, sodium or potassium hydroxide, potassium or sodium carbonate, sodium bicarbonate and calcium oxide, have been used to detoxify high-tannin sorghum grains (Banda-Nyirenda and Vohra, 1990). Price et al. (1978) reported that most alkaline conditions in general are responsible for improved nutritional quality and reduced level of chemically assayable tannin in high tannin grains, but not to levels found in low tannin grain sorghum. Recently, sorghum has been considered as an alternative for tortilla production due to its drought resistance and lower price. However, sorghum use for tortilla is limited, because it produces off-colour tortillas and social and psycological factors limit its acceptance. The use of white genotypes or decorticated sorghum has partly overcome the problem due to improved appearance and overall acceptability (Khan et al., 1980 and Choto et al., 1985).

The purpose of this investigation was to compare the effects of various chemical treatments and tortillas processing on the tannin content and in vitro protein digestibility of the low and high tannin sorghum cultivars.

MATERIALS AND METHODS

Sorghum grains: Seeds of two sorghum (*Sorghum bicolor* (L) Moench) cultivars, Dorado (low tannin) and Assiut 14 (high tannin) were obtained as a pure impred lines (2001 season) from Shandweel Research Station (Sohag), Agriculture Rosearch Center.

Sample preparation: All the samples were carefully cleaned and freed from dirt, stones, chips, and other extraneous grains or grits. For tannin analysis and moisture determination, grains were ground to pass a 40 mesh screen. For protein digestibility both treated and untreated seeds were ground to pass a 16 mesh screen.(Babiker and El-Tinay, 1992).

Soaking and cooking processes: For experiments of soaking treatments, the cleaned grains were soaked in 4-5 volumes of water or alkali solution(0.01and 0.05M NaOH or $Ca(OH)_2$) at 30°C for 6-24 hrs under ambient laboratory conditions. In the experiment on cooking, the grains were cooked in water or alkali solutions (0.01 and 0.05 M NaOH or Ca $(OH)_2$ at 100° C for 10-30 minutes.

Tortilla processing:

The processing of tortilla bread from sorghum was carried out as decribed by Serna-Saldivar et al. (1987a). Sorghum grains were treated with alkali solution [0.05M Ca $(OH)_2$] at 100°C for 30 min, then grains were thoroughly washed. The cooked sorghum grains was then drained to get nixtamal. The cooked grains or nixtamal were ground in a stone grinder and the masa was sheated (2 mm thick) and baked on a hot oven (280°C) for 1.5 min on each side. Nixtamal and tortilla were dried at 60-65°C for 48 hours in an air oven. Grains and dried processed products were ground in a hummer mill to pass through 40 mesh screen. (Serna-Saldivar et al.(1988) The samples were stored in polyethylen bages in deep freezer until analysis.

Chemical Analysis:

Moisture, Protein, Crude fat, Crude fiber and ash were determined according to the methods described in A.O.A.C. (1990).

Tannins determination:

Tannins was determined by the method modified by Price et al. (1978) as described by Babiker and El-Tinay (1992), 200 mg of Sample was extracted with 10 ml 1% conc. HCl in methanol for 10

min in capped rotating test tubes. 5 ml of vanillin reagent (0.5%) was added to 1 ml aliquose and the absorbance of the colour developed after 20 min (30°C) was read at 500 nm. A standerd curve was prepared using catechin equivalents (CE) after correcting for blank.

In-vitro protein digestibility (IVPD): The IVPD of proteins of various samples of seeds was obtained by using digestive enzymes, pepsin and trypsin, by the method of Saunder et al. (1973). The rate of digestion was expressed in terms of μ mol of amino acids (L-leucine) released by the disgestive enzymes/gram of fat free powdered sample in 4 hours when corrected to zero time control. The percent IVPD was also determined by pepsin hydrolysis (A.O.A.C. 1990). The. IVPD was calculated from the difference in N content of sample ..before and after pepsin hydrolysis. The casein as a reference, was digested under the same conditions.

RESULTS AND DISCUSSION

Chemical composition of sorghum grains: Results in Table (1) shows the gross chemical composition of low and high-tannin sorghum grains. Percent of Ash, crude fat and crude fiber were higher in (Assiut 14 variety) than low-tannin sorghum cultivar (Dorado variety). Consequently, high-tannin sorghum contained the lowest amounts of protein and nitrogen-free extract. These results are in the line with those reported by Chibber et al. (1978). Ghali et al. (1987) found that the chemical composition of sorghum grains ranged from 9.25-12.94%, 2.55-3.00, 2.98 - 3.77%, 1.53 - 1.90% and 60.1 - 71.8% for protein content, crude fiber, hexan extract, ash and total carbohydratos, respectively.

Effect of soaking in water of sorghum grains on tannin content and IVPD. The soaking of sorghum grains in distilled water at 30°C are shown in Fig. (1). For Dorado variety, tannin content and in-vitro protein digestibility of untreated seeds were 0.84% and 84.31%, respectively. Soaking in distilled water at 30°C reduced tannin content to 0.60% and improved in-vitro protein digestibility to 86.80 % after 24 hr. For high-tannin sorghum cultivar (Dorado), tannin content and IVPD of untreated seeds were 2.46% and 82.35 %, respectively.

Table 1 : Chemical composition of sorghum grains (on dry weight basis)

Constituents	Dorado cultivar	Assiut 14 cultivar
Ash %	1.76	2.24
Protein %	12.31	9.85
Crude fat %	3.45	3.86
Crude fiber %	3.21	3.83
Total carbohydrates* %	78.43	77.76
Tannins** %	0.84	2.46

* Carbohydrate calculated by difference, ** Tannin expressed as g catechin/100 g sample

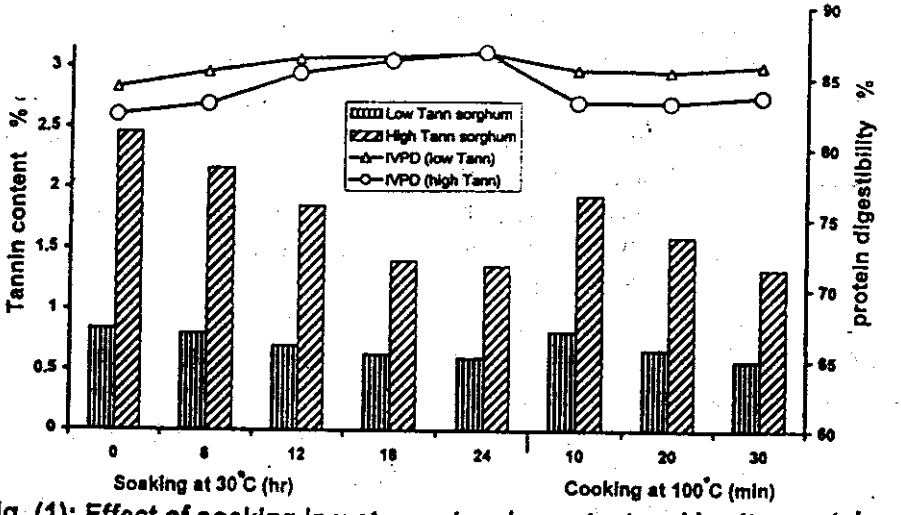

Fig. (1): Effect of soaking in water on tannin content and in vitro protein digestibility of two sorghum cultivars.

When soaking of seeds in distilled water at 30°C for 24 hours, the reduction of tannin content was about 45% of its initial content in raw grains. Also, soaking in water at 30°C for 24 hours improved the nutritional quality of high-tannin sorghum. Cooking of sorghum grains (Dorado and Assiut 14) at 100°C for 30 min gave similar results approximately, but in a shorter time (Fig.1). These results are in good agreement with those

reported by Chavan et al. (1979) and Beta et al. (2000).

Effect of alkali treatment of sorghum grains on tannin content and IVPD. Fig. (2) indicate the tannin content and in-vitro protein digestibility in low and high-tannin sorghum grains as affected by soaking in 0.01 M NaOH. For Doradow cultivar, soaking in 0.01 M NaOH at 30°C for 24 hours resulted in a reduction of tannin content and improvement in IVPD (0.42% and 86.31%, respectively). Treatment with 0.01 M NaOH at higher temperature (100°C) reduced tannin content to 0.30% after 30 min., causing slightly improvement in IVPD to 85.30% after 20 min and 85.6% after 30 min. For Assiut 14 (high-tannin) cultivar, soaking in 0.01 M NaOH at 30°C for 24 hours resulted in reduced tannin content to 0.80% and improved IVPD to 87.0%. When the extraction was made at 100°C, the time required to remove the same amount of tannin was reduced to 20 min (Fig. 2). Thus, the extraction at high temperature enhanced the rate of removal of tannins and reduced the time (Beta et al., 2000). For a given time and temperature, it was found that, as alkali concentration was increased (0.05 M), percent of tannin removed was also increased up to 70 and 80% after 24 hr of soakig at 0°C for Dorade and Assiut 14 cultivars, respectively (Fig. 3). Cooking at 100°C gave smilar results, but in a shorter period. Brewster and McEwen (1971) revealed that, phenols readily in dilute sodium hydroxide and form sodium phenate. Simillar findings on the effectivenes of NaOH in tannin reduction were reported by Reichert et al. (1980). The action of NaOH on tannins possibly involves oxidation of phenolic groups under the moist, alkaline conditions. Alkalin conditions promote oxidative polymerization of condensed tannins (Porter, 1992).

The results shown in Fig. (4 and 5) revealed that, soaking of sorghum grains in 0.01 M and 0.05 M $Ca(OH)_2$ at 30°C resulted in a decrease of tannin content and improvement of protein digstibility. At a given temperature and Ca $(OH)_2$ concentration, increase in time caused an increase in percent tannin extracted from sorghum grains. For high-tannin sorghum (Assiut 14), soaking in 0.01 M and 0.05 M Ca $(OH)_2$ at 30 °C eliminated 65 and 75% of tannin content after 24 hrs, respectively (Fig. 4 and 5).

Moreover, treatment of high-tannin cultivar with 0.01 and 0.05 M Ca $(OH)_2$ at 30°C for 24 hrs improved IVPD to 85.24 and 87.61%, respectively. When soaking was done at 100°C, it removed about the same a mount of tannin in 30 min. Furthermore, cooking in 0.05 M Ca $(OH)_2$ at 100°C improved IVPD to 85.24% for Dorado cultivar and 83.87% for Assiut 14 (high tannin) cultivar.

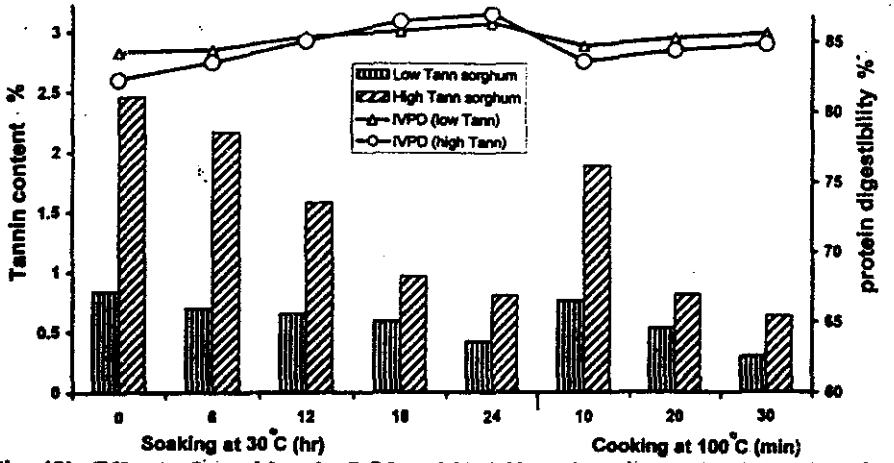

Fig. (2): Effect of soaking in 0.01mol NaOH on tannin content and in vitro protein digestibility of two sorghum cultivars.

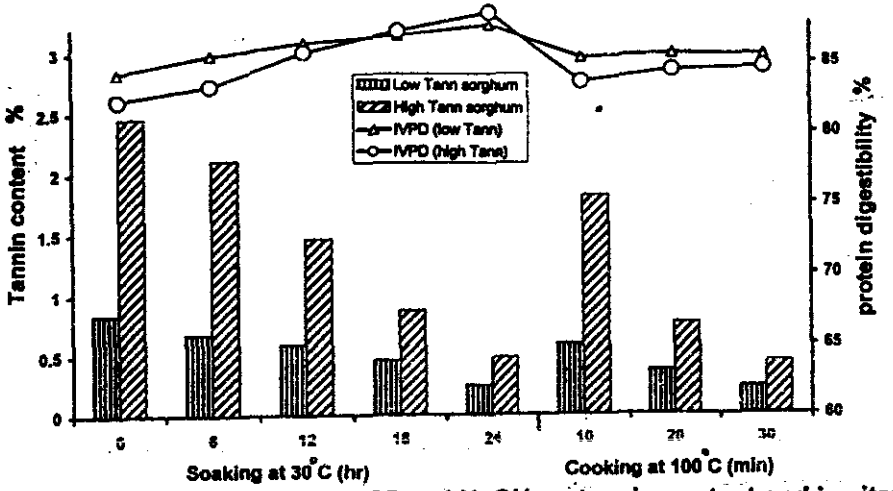

Fig. (3): Effect of soaking in 0.05 mol NaOH on tannin content and in vitro protein digestibility of two sorghum cultivars.

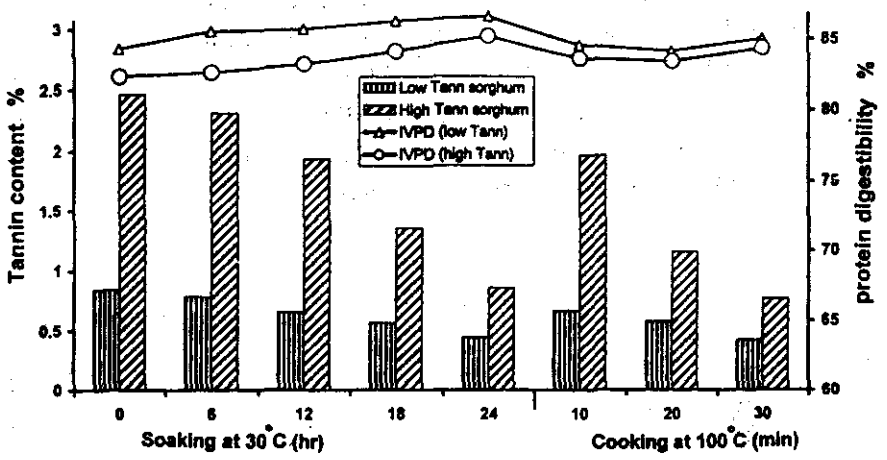

Fig. (4): Effect of soaking in 0.01 mol $Ca(OH)_2$ on tannin content and in vitro protein digestibility of two sorghum cultivars

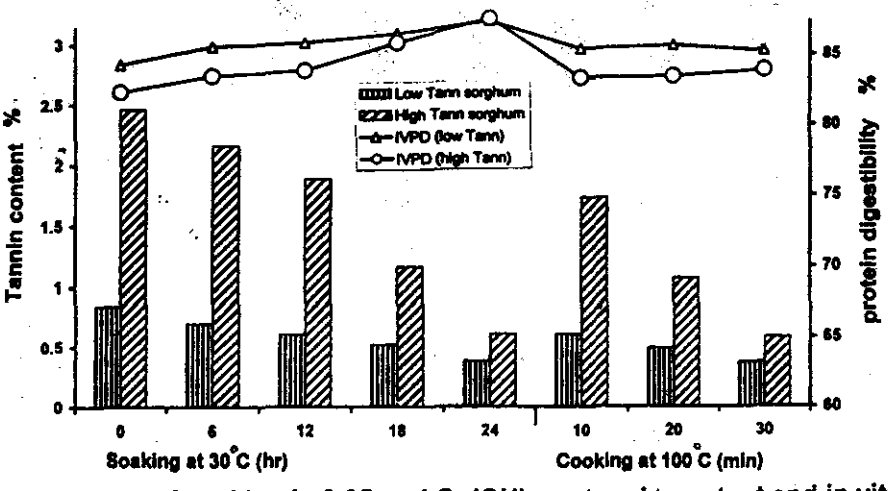

Fig. (5): Effect of soaking in 0.05 mol $Ca(OH)_2$ on tannin content and in vitro protein digestibility of two sorghum cultivars.

The results indicated that, tannin content of treated seeds were markedly decrease with increase in time or alkali concentration. This indicated that higher concentration of alkali enhanced the rate of reaction between alkali and polyphenols to form soluble compiexes (Babiker and El-Tinay, 1992). On the other

hand, the previous results illustrated that the low tannin cultivar showed a higher protein digestibility value than the high tannin cultivar. In sorghum, most of the tannins are present in the pericarp layer; the alkali-treated seeds exhibited increase in IVPD as compared to untreated seeds (Chavan et al., 1979). Alkaline treatment at 100°C has slightly improved in vitro protein digestibility of high tannin sorghum. Robbins et al. (1980) reported that, alkali cooking has a detrimental effect on protein quality because of a mino acid degradation, racemization and formation of lysinoalanine and lanthionine cross-links which render proteins insoluble.

Effect of processing on tannin content and protein digestibility. The results in Table (2) show the effect of tortilla processing on tannins content and in vitro protein digestibility. The obtained results indicated that the decreased of tannins as percentage was 46 and 70% after nixtamal in Dorado and Assiut 14 cultivars, respectively. The decrease of tannins was greater in Assiut 14 (high-tannin sorghum) than Dorado (low-tannin sorghum).

The decrement of tannins could be due to the use of Ca $(OH)_2$ in cooking process. The results also indicated that, a slightly decrease of tannins was observed during baking process. These results are in full agreement with Chavan et al. (1979) and Babiker & El-Tinay, 1992). Youssef et al. (1990) found that the extrusion process of sorghum reduced the polyphenals

Table 2 : Effect of processing on tannins content and in-vitro protein digestibility.*

Processing Stage	Dorado cultivar		Assiut 14 cultivar	
	Tannin %	IVPD** %	Tannin %	IVPD %
Before processing	0.84	84.31	2.46	82.35
After nixtamal	0.45	85.30	0.72	83.40
After baking	0.43	85.70	0.70	83.20

***Average of three replicates., **In-vitro protein digestibility**

(tannins) in the range of 55 to 78 %.

On the other hand, lime-cooking process of sorghum grains resulted a slightly improvement of protein digestibility for high tannin cultivar. The low tannin cultivar showed a higher protein digestibility values than the high tannin cultivar. The tortilla bread processed from low tannin cultivar showed higher protein digestibility values than the bread from high tannin cultivar. Serna-Saldivar et al. (1987) fed raw grains, nixtamal or lime-cooked grains and tortilla to rats and also found that nixtamals and tortillas had lower protein digestibilities than their respective raw grains. However, Beta et al. (2000) revealed that, alkali treatment was associated with improvement in sorghum nutritional value by causing a significance reduction in tannin content.

REFERENCES

A.O.A.C. (1990): Official Methods of Analysis (15th ed.) Association of official Analytical Chemists. Washington , D.C.

Babiker, E.E. and El-Tinay, A.H. (1992): Effect of alkali on tannin content and in vitro protein digestibility of sorghum cultivars. Food Chem. 45: 55.

Banda-Nyirenda, D.B.C. and Vohra, P. (1990): Nutritional improvement of tannin-containing sorghum (*Sorghum bicolor*) by sodium bicarbonate. Cereal Chem. 67: 533-537.

Beta, T.; Rooney, L.W.; Marovastanga, L.T. and Taylor, R.N. (2000): Effect of chemical treatments on polyphenols and malti quality in sorghum. J. Coreal Science 31: 295-302.

Brewster, R.Q. and McEwen, W. (1971): "Organic Chemistry", 3rded, P. 601. Prentice-Hall of India Ltd, New Delhi.

Chavan ,J.K.;Kadam,S.S.;Ghonsik,C .P. and Salunke, D.K (1979). Removal of tannins and improvement of in vitro protein digestibility of sorgum seeds by soaking in alkali. J.Food Sci. 44,1319-1321

Chibber, B.A.; Mertz, E.T. and Axtell, J.D. (1978): Effect of dehulling on tannin content, protein distribution and quality of high and low-tannine

Sorghum. J. Agric. Food Chem. Vol. Eb, 3: 679.

Choto, C.E., Morad, M.M. and Rooney, L.W. (1985): The quality of tortillas containing whole sorghum and pearled sorghum alone and in blends with yellow maize. Cereal Chem. 62: 51.

Daiber, K.H. (1975) Enzyme inhibition by poly phenols of Sorghum grain and malti. J. Of the Science of food and Agriculture 26: 1399-1411.

Eggum B.O. and Christansen, K.d. (1974): In breeding for seed protein improvement using nuclear techniques. IAEA, Vienna P, 135.

Ghali, Y.; Abdel-Samed, A.; Basyony, A.E.; Ibrahim, N. and Mahmoud, A.H. (1987): Tannins in sorghum milling fractions in relation to protein digestibility. Egyptian. J. Agron. 12: 139.

Ghali Y.; Abdel-Samed, A.; Basyony, A.E.; Ibrahim, N. and Mahmoud, A.H. (1987): Tannin in sorghum milling fractions in relation to protein digestibility. Egypt. J. Agron. 12: 139.

Hahn, D.H; Rooney, L.W. and Earp, C.F. (1984): Tannins and phenols of sorghum. Cereal Foods World. 29: 776-779.

Hahn, D.H., and Rooney, L.W. (1986): Effect of genotype on tannins and phenols of sorghum. Cereal Chem. 63: 4-8.

Khan, M.N.; Rooney, L.W.; Rosenow, D.T.and iller, F.R. (1980). Sorghum with improved tortilla making characteristics. J. Food Sci. 45: 720.

Nyachoti, C.M.; Arkinson, J.L. and Leeson, S. (1997): Sorghum tannin; a review. World's Poultry Science Journal 53: 5-21.

Porter, L.J. (1992): Structure and chemical properties of the condensed tannins. In "polyphenols" (R.w. Hemingway and P.E. Laks, eds), plenum press, New York, PP, 245-258.

Price, M.L.; Scogoc V.S. and Butler, L.G. (1978): A critical evalution of the vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem. 26: 1214.

Prike, M.L.; Butler, L.G.; Rogler, J.C. and Featherston, W.r. (1979): Overcoming the nutritionally harmful effects of tannin in sorghum grain by

treatment with inexpensive chemicals. J. of Agric and Food Chem. 27: 441-445.

Reichert, R.D.; Fleming, S.E. and Schwat, D.J. (1980): Tannin deactivation and nutritional improvement of sorghum by anaerobic storage of H_2O-Hcl-, or NaOH-Treated grain. J. Agric. and Food Chem. 28: 824-829.

Robbins, K.R.; Baker, D.H. and Finley, J.W. (1980): Studies on the utilization of lysinoalanine and lanth onine. J. Nutr. 10: 907.

Rostango H.S. (1972): Nutritive evalution of sorghum grains in chicks. Ph. D. Thesis purdue University, West Lafayette, I.N.

Saunder, R.M.; Conner, M.A.; Booth, A.M.; Bickoff, E.M. and Kohler, G.O. (1973): Measurement of digestibility of affalfa protein concentrates by in vivo and in-vitro methods. J. Nutr. 103: 530.

Serna-Saldivar, S.O.; Knabe, D.A.; Rooney, L.W., and Tanksley, T.D. (1987). Effect of lime-cooking on nutrient digestibilities of maize and sorghum. Cereal Chem. 64: 247.

Serna-Saldivar,S.O.;Knabe,D.A.; Rooney,L.W.; Tanksley, T.D. and Sproule,A.M. (1988). Nutritional value of sorghum and maize tortilla. J.Cereal Sci. 7: 83-94.

Youssef, M.M.; Moharam, Y.G. and Moustafa, E.K. (1990): Newextruded products from sorghum. Food Chem. 37: 189.

تأثير المعاملة الكيميائية وتصنيع خبز التورتيلا على محتوى التانينات وهضم البروتين لصنفين من الذرة الرفيعة المنزرعة فى مصر

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استخدم فى هذا البحث صنفين من كل من الذرة الرفيعة المنخفضة فى محتواها من التانينات (دورادو) والمرتفعة فى محتواها من التانينات (أسيوط ١٤) ولقد أظهرت الدراسة أن محتوى البذور غير المعاملة من التانينات كان ٠,٨٤ ، ٢,٤٦% فى كل من الذرة المنخفضة والمرتفعة فى محتواها من التانينات ، على التوالى وكانت القيمة الهضميه للبروتين ٨٤,٣١ ، ٨٢,٣٥% فى كلا الصنفين على التوالى وعند نقع حبوب الذرة فى الماء على درجة حرارة ٣٠ درجة مئوية لمدة ٢٤ ساعة تحسنت جودة البروتين وانخفض محتوى التانينات الى ٠,٦٠ ، ١,٣٦% لكل من الصنف دورادو ، أسيوط ١٤ على التوالى. كما أدت عملية الطبخ فى الماء على درجة ١٠٠ درجة مئوية لمدة ٣٠ دقيقة إلى إعطاء نتائج مشابهة تقريباً ولكن فى وقت أقصر .وأدى نقع حبوب الذرة الرفيعة فى محلول ٠,٠١ ، ٠,٠٥ مول من هيدروكسيد الصوديوم على درجة حرارة ٣٠ درجة مئوية الى إزالة ٦٧ -٨٠% من التانينات بعد ٢٤ ساعة من عملية النقع، وتحت نفس الظروف عند نقع حبوب الذرة فى محلول ٠,٠١ ، ٠,٠٥ مول هيدروكسيد كالسيوم أزيل ٦٥-٧٥% من التانينات الموجودة فى البذور غير المعاملة. وعلاوة على ذلك أظهرت الدراسة أن استخلاص التانينات فى محلول قلوى ٠,٠١، ٠,٠٥ مول على درجة حرارة ٣٠ درجة مئوية ولمدة ٢٤ ساعة أو الطبخ على ١٠٠ درجة مئوية أدى ذلك الى تحسن فى جودة البروتين للحبوب عالية التانين عند مقارنتها بالحبوب غير المعاملة. ومن ناحية أخرى أدت عملية الطبخ فى القلوى (هيدروكسيد الكالسيوم) ٠,٠٥ مول فى صناعة خبز التورتيلا إلى إزالة ٧٠% من محتوى التانينات بعد عملية الطبخ Nixtamal بينما لوحظ انخفاض طفيف فى محتوى التانينات بعد عملية الخبيز.