Effect of pH, Temperature, and Time on Inactivation of Tannins and Possible Implications in Detannification Studies

H. P. S. Makkar and K. Becker*

Institute for Animal Production in the Tropics and Subtropics (480), University of Hohenheim, D-70593 Stuttgart, Germany

The tannins from quebracho and leaves of *Acioa barteri* after purification, and commercially available tannic acid, were stored in buffers of different pH values (6−11) at 0, 20, and 37 °C. The recovery of tannins was monitored at different time intervals (up to 18 h) using Folin−Ciocalteu and butanol−HCl−Fe^{3+} reagents. The recovery of tannins decreased with increase in time of storage, increase in pH, and increase in temperature. The decrease in the recovery of tannins was accompanied by a decrease in their protein precipitation capacity, and also there was no defined peak for the assay medium following the butanol−HCl−Fe^{3+} method for the purified quebracho and *A. barteri* tannins when stored at pH 11. These observations suggested that the decrease in recovery of tannins was due to inactivation of tannins. *A. barteri* tannins were found to be most susceptible to pH-mediated inactivation. The stirring of quebracho tannins increased the rate of inactivation at pH 11. The results suggested that alkaline pH inactivates the tannins. The rate of inactivation is higher at higher temperatures, and it can be further increased by oxygen present in the air or possibly by any other oxidizing agent. These observations were exploited to detannify tannins in some agroindustrial byproducts by using hydrogen peroxide (a strong oxidizing agent) in the presence of sodium hydroxide. This treatment was found to be very effective; the decrease in tannins reached as high as 99% in some cases. The results of the present study also highlighted a problem related to quantification of tannins in samples treated with alkalis. During extraction of tannins from such materials, tannins were inactivated due to alkalinity in the extraction medium, thereby giving an underestimate of the actual tannin levels. The alkalinity in the extraction medium results from the residual alkali present in the treated materials. This problem was overcome by addition of hydrochloric acid in the extraction medium so that the pH during extraction did not rise above 7. The results of the present study also suggest that for studying the nutritional, physiological, and biological effects of tannins (i) the pH of the tannin-containing solution should never at any stage be alkaline, (ii) the solution should be kept at low temperature, and (iii) the exposure to oxygen or to any other oxidizing agent should be avoided.

Keywords: *Inactivation of tannins; pH; temperature; detannification; quantification of tannins*

INTRODUCTION

Tannins are chemically diverse polyphenolic compounds. Phenolic compounds are highly prone to oxidation—under the influence of enzymes or metal ions or autocatalytically in basic media (Appel, 1993; Haslam, 1993). For this reason, various workers (Hagerman and Klucher, 1986; Peng and Jay-Allemand, 1991; Scalbert et al., 1990; Makkar and Becker, 1994) have used antioxidants while extracting tannins from plant sources. Furthermore, alkali treatments have been used, particularly in sorghum, to decrease the contents of assayable tannins and to increase their nutritive value [for example, Price et al. (1979), Mukuru et al. (1992), and Waichungo and Holt (1995)]. This work originated from our earlier observations on detection of a gallic acid peak in the tannin hydrolysate according to the method of Charrier et al. (1992) for which the pH of the hydrolysate needed to be adjusted to between 5 and 6 before injection into the HPLC column (Makkar and Becker, 1994). An accidental increase in pH beyond 7 and the subsequent adjustment of the pH to between 5 and 6 did not reveal the gallic acid peak. This was later confirmed by giving a high pH shock to authentic gallic acid followed by injection after the pH was adjusted to between 5 and 6; the gallic acid peak was not observed (unpublished observations). Similarly, in another study (Makkar et al., 1995), the condensed tannins added to the buffer used in the *in vitro* gas method of Menke et al. (1979) were not recovered after 15 min. The pH of this medium was observed to be 8.4. The pH of the *in vitro* buffer used in the method of Menke et al. (1979) is between 6.6 and 6.9 under CO_2 or when kept in a closed vessel such as *in vitro* flasks or syringes once it is saturated with CO_2. The pH rises slowly to about 8.4 when taken out of the *in vitro* flasks/syringes and the contents are kept open. As little information is available on the stability of tannin preparations under different storage and working conditions, we studied the recovery of tannins at different time intervals when stored in buffers of different pH values and at different temperatures. A corollary of this could be that the lesser the recovery of tannins, the higher is the inactivation of tannins. The implications of the results are discussed.

* Address correspondence to this author at Institute for Animal Production (480), University of Hohenheim. D-70593 Stuttgart, Germany (e-mail kbecker@uni-hohenheim.de).

MATERIALS AND METHODS

Materials. Tannic acid was obtained from Merck Chemical Co. (Darmstadt, Germany), and spray-dried quebracho extract

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was a kind gift from Trask Chem Corp., Marietta, GA. The leaves of *Quercus incana* and seeds of *Acacia nilotica, Mangifera indica*, and *Tamarindus indica* were sun-dried before use.

Methods. Tannins from quebracho and leaves of *Acioa barteri* were purified by adsorption on Sepadex LH-20 (Makkar and Becker, 1994).

Effect of pH. The stock solutions of tannins were prepared in distilled water. A small aliquot from the stock solution was transferred into buffers (0.05 M) of different pH (6, 7, 8, 9, 10, and 11) values. The phosphate buffers were used for pH 6 and 7, and for pH 8, 9, and 10 the Tris-HCl buffer was used. For pH 11, the Tris solution was adjusted to pH 11 with sodium hydroxide. The final concentrations of tannins in the buffer solutions were 0.1 mg/mL for tannic acid, 0.4 mg/mL for quebracho tannins, and 0.2 mg/mL for *A. barteri* tannins. These tannin solutions were kept on ice, at room temperature (20 °C) and at 37 °C. Suitable aliquots were taken from these solutions at different time intervals for analysis of tannins.

Analysis of Tannins. The condensed tannins were determined in aliquots (0.25 mL) from the solutions of quebracho and *A. barteri* tannins according to the butanol−HCl−Fe^{3+} method of Porter et al. (1986) as described in Makkar and Becker (1994). Tannic acid is a hydrolyzable tannin and cannot be determined by using this method. Total phenolic content was determined by Folin−Ciocalteu reagent (Julkunen-Tiittoo, 1985) in suitable aliquots (100 and 30 μL, respectively) from the solutions of tannic acid and quebracho tannins. In brief, distilled water was added to the aliquot to make 0.1 mL, and then 0.25 mL of 1 N Folin−Ciocalteu reagent and 1.25 mL sodium carbonate reagent (20%) were added. The absorbance was recorded after 30 min at 725 nm using cuvettes of 1-cm light path. The qualitative assessment of precipitation of protein for quebracho and *A. barteri* tannins and the quantitative assessment for the tannic acid were carried out using the blue BSA method of Asquith and Butler (1985). The protein precipitation capacity for quebracho and *A. barteri* tannins could not be determined due to insufficient availability of purified quebracho and *A. barteri* tannins. For quebracho and *A. barteri* tannins, the solutions (0.75 mg/mL) in buffer of pH 11 maintained for 18 and 3 h, respectively, were brought to pH 5 (the pH of the assay; Asquith and Butler, 1985) using a minimum amount of concentrated HCl. One milliliter of this aliquot was added to 2 mL of the dye solution (Asquith and Butler, 1985). Similarly, aliquots (1 mL each) of these tannin solutions in buffer of pH 6 were brought to pH 5 as above and then added to 2 mL of the dye reagent. These were kept at approximately 4 °C for about 15 h and then centrifuged to observe the formation of blue pellet. For tannic acid solution (0.75 mg/mL) in buffer of pH 11 after 18 h of storage at room temperature and at 37 °C, the pH was adjusted to 5 and the protein precipitation capacity was determined using the dye reagent as described in Makkar and Becker (1993).

In another experiment to study the effect of stirring on the inactivation of tannins, two similar sets of quebracho tannins in pH 11 buffer were prepared. Both were kept at room temperature. One was stirred using a magnetic stirrer, and the other was kept without stirring. Aliquots were taken at different time intervals for determination of proanthocyanidins.

Detannification of Tannin-Rich Feedstuffs. *Oak Leaves.* One hundred milliliters of distilled water containing 4.7 g of sodium hydroxide was mixed thoroughly with 100 g of the leaves (95% dry matter). After 30 min, 9.1 mL of 30% hydrogen peroxide added to 50 mL of distilled water was mixed with the leaves containing the alkali. The contents were transferred to polyethylene bags. The openings of the bags were closed with a rubber band and kept at room temperature. After 24 h, the bag was opened and the contents were well aerated. The samples were dried using a lyophilizer.

Seeds of A. nilotica, M. indica, and T. indica. The procedure was similar to that for oak leaves except that the quantity of water used was half that used for oak leaves.

Tannin Quantification in Treated and Untreated Feedstuffs. Samples were finely ground to pass through a sieve of 80 mesh. Tannins were extracted from 200 mg of the ground samples using 10 mL of the two extraction media: (i) usual extraction medium (70% aqueous acetone) and (ii) modified extraction media [0.075 mL of concentrated HCl (37%) in 10 mL of 70% aqueous acetone]. The procedures for extraction of tannins and quantification of total phenols, tannins and condensed tannins were those described in Makkar et al. (1993). For untreated samples, the extraction medium without HCl (i, above) was used and for treated samples both extraction media, with and without HCl (i and ii; above), were used.

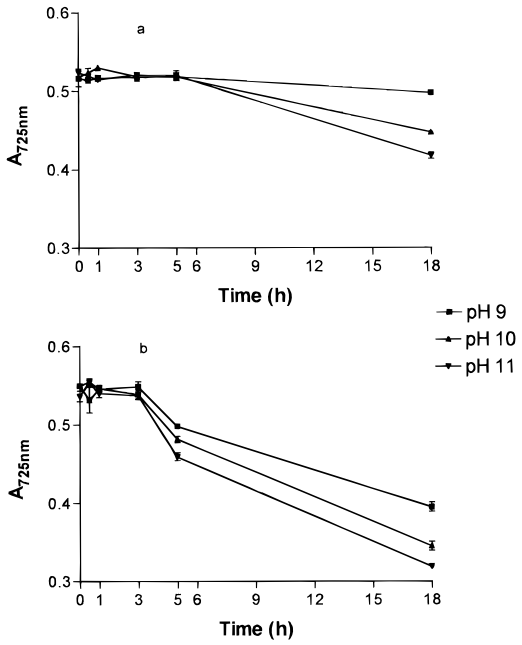

Figure 1. Effect of storage of tannic acid solutions of different pH values at room temperature (20 °C; a) and at 37 °C (b) for different lengths of time on assayable tannins using Folin−Ciocalteu assay.

Statistical Analysis. The significance of difference between the two means was compared using Student's *t*-test.

RESULTS AND DISCUSSION

Tannic Acid. At room temperature, the recovery of tannic acid as total phenols did not decrease up to 18 h of storage in buffers of pH 6, 7, and 8 (results not shown). At pH 9, 10, and 11 the recovery did not decrease up to 5 h, and at 18 h the recovery was found to be lower (Figure 1a). The decrease in recovery was higher at higher pH. After 18 h of storage at pH 11, the recovery of tannic acid as total phenols was approximately 20% lower. Although the recovery did not decrease up to 5 h of storage from pH 8 to 11 when measured as total phenols, there was a change in the color of the tannic acid solutions at these pH values, which were yellowish brown in color. The intensity of this color increased as the pH increased, suggesting that some changes take place in the tannic acid solutions at alkaline pH without a change in their total phenolic content. A better insight can be obtained by running these tannic acid solutions in HPLC (Hagerman et al., 1992). Under alkaline conditions, conversion of tannic acid to gallic acid and then disappearance of gallic acid has been observed (Osawa and Walsh, 1993).

At 0 °C, recovery of tannic acid did not decrease up to 18 h at all pH values studied, although the tannic acid solutions of pH 10 and 11 were slightly yellowish in color (results not shown). At 37 °C, recovery was the same up to 18 h at pH 6 and up to 5 h at pH 7 and 8. After 18 h of storage at pH 7 and 8, recovery was lower by 4% and 20%, respectively. At higher pH values (9−11), the recovery started decreasing at 5 h (Figure 1b).

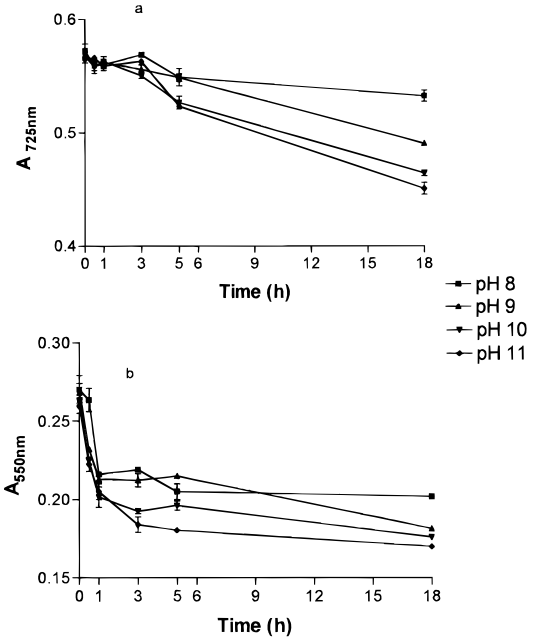

Figure 2. Effect of storage of quebracho tannin solution of different pH values at room temperature (a; using Folin−Ciocalteu assay, b; using butanol−HCl−iron reagent).

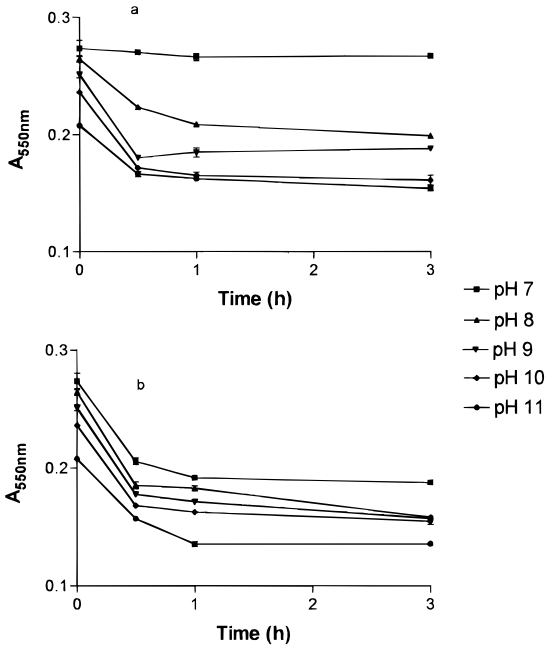

Figure 3. Effect of storage of *A. barteri* tannin solutions of different pH values at room temperature (20 °C; a) and at 37 °C (b) for different lengths of time on assayable tannins using butanol−HCl−iron reagent.

After storage of tannic acid at 37 °C for 1 h in buffers of alkaline pH, the solutions were yellowish brown in color (as observed at 0 °C and at room temperature), suggesting again some changes in tannic acid that were not reflected in absorbance measurements.

Quebracho Tannin. Using the Folin−Ciocalteu assay for total phenols, the recovery of quebracho tannins in buffers of all pH values studied did not decrease up to 3 h of storage at room temperature. At 5 and 18 h of storage, recoveries were lower at pH 9, 10, and 11 (Figure 2a). On the other hand, using butanol−HCl−Fe^{3+} reagent, the recoveries of quebracho tannins started decreasing at 30 min of storage at pH 8, 9, 10, and 11 at room tempature (Figure 2b). There was no change in recovery at pH 6 and 7 even up to 18 h of storage (results not shown). These results suggest that the butanol−HCl−Fe^{3+} method for proanthocyanidins is better than the Folin−Ciocalteu method for studying the transformation of condensed tannins. Therefore, in further studies only the butanol−HCl−Fe^{3+} method was used. At 37 °C, the results for quebracho tannins using the butanol−HCl−Fe^{3+} reagent were similar to those obtained at room temperature. The curves overlapped and therefore have not been presented. When quebracho tannin was stored on ice (0 °C), the recovery did not decrease up to 3 h at all pH values studied (results not shown).

***A. barteri* Tannin.** At room temperature, tannins of *A. barteri* at pH 6 and 7 were stable up to 3 h, whereas at alkaline pH tannin recovery decreased even at 30 min (Figure 3a). At 37 °C, recovery of *A. barteri* tannins was the same up to 3 h at pH 6, whereas the recovery started decreasing at 30 min when the tannin was stored at pH 7 or above. The decrease in recovery of *A. barteri* tannins was higher at 37 °C (Figure 3b). When this tannin was kept on ice, there was no inactivation at pH 6 and 7 up to 3 h, and at alkaline pH the inactivation started even at 30 min, although the rate of inactivation was lower than at room temperature or at 37 °C (results not shown). Among the three tannins studied, the tannin of *A. barteri* was found to be the most susceptible to inactivation by pH.

Table 1. Effect of Stirring Quebracho Tannin Solution in Buffer of pH 11 Maintained at Room Temperature (20 °C)

	absorbance at 550 nm[a]	
time (min)	unstirred	stirred
0.00	0.263 ± 0.008	0.263 ± 0.009
30.00	0.221 ± 0.005	0.218 ± 0.006
60.00	0.209 ± 0.008	0.185 ± 0.002*
180.00	0.189 ± 0.007	0.163 ± 0.004*

[a] Data are mean ± SD (n = 3). *Values in the same row are significantly different at $P < 0.01$.

Absorption Spectra. The absorption peak of the assay medium following the butanol−HCl−Fe^{3+} method was compared for quebracho tannin at 0 and 18 h. Similarly, the absorption peak for *A. barteri* tannin was compared at 0 and 3 h. Both of these tannins were maintained in a buffer of pH 11 at 37 °C before analysis with the butanol−HCl−Fe^{3+} reagent. For quebracho and *A. barteri* tannins, the absorption peaks were 529.5 and 558 nm, respectively, at 0 h, whereas no definite peak was observed between 400 and 600 nm for quebracho and *A. barteri* tannins stored for 18 and 3 h, respectively. These results suggested a transformation in tannins, a probable reduction in the phloroglucinol ring, and the appearance of carbonyl groups when stored at alkaline pH (Ohara, 1994).

Protein Precipitation Capacity. The blue pellet resulting from precipitation of the bovine serum albumin−blue dye was not formed for quebracho and *A. barteri* tannins stored under alkaline conditions, whereas the pellet was observed for these tannins stored at pH 6. Similarly for tannic acid, the protein precipitation capacity for samples stored at pH 11 for 18 h both at room temperature and at 37 °C, followed by adjustment of pH to 5, could not be detected. The protein precipitation capacity of tannic acid that had been stored at pH 6 for 18 h and then adjusted to pH 5 was 8.67 mg of bovine serum albumin/mg of tannic acid. Tannins stored under alkaline conditions lose their capability to bind proteins. These results suggested that the trans-

Table 2. Effect of Hydrogen Peroxide Treatment under Alkaline Condition on the Levels of Total Phenols, Tannins, and Condensed Tannins in Some Agroindustrial Byproducts[a]

tannin-rich feedstuff	total phenols (% tannic acid equiv)	tannins (% tannic acid equiv)	condensed tannins (% leucocyanidin equiv)
A. nilotica seeds			
untreated	15.30	13.40	3.10
treated	4.4 (71) [1.0]	3.6 (73) [0.2]	0.2 (94) [nd][b]
M. indica seed kernels			
untreated	13.80	12.40	0.24
treated	5.6 (59) [2.1]	4.9 (60) [1.2]	0.04 (83) [nd]
Q. incana leaves			
untreated	11.50	9.70	5.90
treated	1 (91) [nd]	0.4 (96) [nd]	0.03 (99) [nd]
T. indica decorticated seeds			
untreated	8.10	5.70	8.30
treated	1 (88) [nd]	0.7 (88) [nd]	0.4 (95) [nd]

[a] Values are on dry matter basis. Values in parentheses are the percent decrease calculated from the values obtained using the modified extracting medium (containing HCl) for the treated samples. Values in brackets are those observed by the usual extraction procedure (the extraction medium without HCl); the pH of the medium after extraction of tannins was >8. [b] nd, not detected.

formation of tannins under alkaline conditions leads to inactivation of tannins.

Effect of Stirring. The results of the effect of stirring quebracho tannins at pH 11 are presented in Table 1. Stirring resulted in a higher inactivation of the tannin, which could be attributed to a higher exposure of the tannin solution to oxygen as a result of stirring.

Implications. The above-mentioned results suggest that tannins can be inactivated by exposing tannin-containing solutions to oxidizing agents under alkaline conditions. An increase in treatment temperature can further increase the rate of inactivation. Tannins present in various byproducts and tree leaves limit their utilization as livestock feed. The removal or inactivation of these tannins can pave the way for better utilization of these feed resources. The implications of the present findings in detannification studies could be twofold: (i) inactivation of tannins in some agroindustrial byproducts using an oxidizing agent under alkaline conditions and (ii) measurements of remaining tannins in the treated material. The treatment for the inactivation of tannins can be carried out as mentioned under Materials and Methods or can be optimized using different concentrations of oxidizing agent (H_2O_2) and the alkali. However, the availability of a proper method is imperative for monitoring the efficiency of these treatments. The use of the conventional extraction medium (70% acetone or 50% methanol) is not sufficient to measure the remaining true tannins, as the sodium hydroxide present in the feedstuff after treatment would be present with the tannins in the extraction medium, leading to a higher pH during extraction (in the present study the pH was between 8 and 9), which will lead to reduction of the recovery of tannins. This is evident from the negligible levels of total phenols and tannins observed using the usual extraction medium (Table 2). This problem was overcome by adding concentrated HCl to the extraction medium (70% acetone), which neutralizes the alkali present. Therefore, in such studies, extraction of tannins from alkali-treated feeds/foods needs to be done in aqueous acetone or aqueous methanol containing enough HCl so that at no stage during extraction does the pH rise above 7 and the pH of the medium after extraction is almost similar to that obtained for the untreated sample. To achieve this, preliminary experiments need to be done by taking different quantities of HCl in the extraction medium. In the present study, the pH of the extraction medium after extraction for the untreated samples varied from 5.9 to 6.0. This pH for the treated samples was achieved by adding 0.075 mL of the concentrated HCl (37%) in 10 mL of 70% aqueous acetone under the extraction conditions mentioned under Materials and Methods. Using these conditions for extraction of tannins from the treated samples, the tannin levels were higher (Table 2). This represents the true tannins present in the treated materials. The use of hydrogen peroxide under alkaline conditions was found to be very effective in removal of tannins from some tannin-rich agroindustrial byproducts. This treatment can be optimized to reduce the cost of treatment. Furthermore, at high alkaline pH, H_2O_2 splits to give the hydroxyl free radical, which reacts with various resistant bonds present in the lignin molecule, thereby significantly increasing *in vitro* dry matter digestibility (Chaudhry and Miller, 1994).

Conclusions. The present study has shown that alkaline conditions and the presence of oxidizing agent lead to inactivation of tannins. This has been (see Table 2) and can be further exploited to detannify tannin-rich feedstuffs or to make effluents from the tanneries safer. However, the presence of alkali poses a problem in the determination of tannins, which can be overcome by adding a suitable quantity of HCl in the extraction medium. In the past, various studies were carried out on removal of tannins from tannin-rich feedstuffs using alkalis. Although there is ample evidence in the literature which suggests higher nutritional value of alkali-treated tannin-rich feeds, in particular sorghum grains [see reviews: Hahn et al. (1984), Kumar and Singh (1984), Butler et al. (1986), and Griffiths (1989)], the actual tannins present in the treated material might be higher than the levels assayed. This artifact is expected to be higher for nonvolatile alkalis such as NaOH, CaO, and K_2CO_3, as compared to NH_4OH which is volatile. In addition, this offers a possible explanation to the observation that available protein in the high-tannin grain following alkali treatment increased, but not to levels found in low-tannin grain, even though the residual assayable tannins were negligible (Price et al., 1979). Similarly, a reduction of 97% in assayable tannins after faba beans were treated with 4% NaOH but no significant increase in nutritive value [see Griffiths (1989)] could also be due to the presence of levels of tannins in the treated material higher than the levels assayed. Lately attention has been focused on the degradation of tannins by microbes (Bravo et al., 1994; Terril et al., 1994; Nguz et al., 1994; Makkar et al., 1995a). The pH and temperature mediated inacti-

vation of tannins can lead to misleading results. This problem can be serious with the buffer solution used in various *in vitro* rumen studies (Makkar et al., 1995b). For studying the nutritional, physiological, and biological effects of tannins (i) the pH of the tannin-containing solution should never at any stage be alkaline, (ii) the solution should be stored at low temperatures, and (iii) the exposure to oxygen or to any other oxidizing agent should be avoided; use of antioxidants such as ascorbic acid, bisulfite, and cysteine may be considered.

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LITERATURE CITED

Appel, H. M. Phenolics in ecological interactions: the importance of oxidation. *J. Chem. Ecol.* **1993**, *19*, 1521−1552.

Asquith, R. A.; Butler, L. G. Use of dye-labeled protein as spectrophotometric assay for protein precipitants such as tannins. *J. Chem. Ecol.* **1985**, *11*, 1535−1544.

Bravo, L.; Abia, R.; Eastwood, M. A.; Saura-Calixto, F. Degradation of polyphenols (catechin and tannic acid) in the rat intestinal tract. Effect on colonic fermentation and faecal output. *Br. J. Nutr.* **1994**, *71*, 933−946.

Butler, L. G.; Rogler, J. C.; Mehansho, H.; Carlson, D. M. Dietary effects of tannins. In *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships;* Liss: New York, 1986; pp 141−157.

Charrier, B.; Marques, M.; Haluk, J. P. HPLC analysis of gallic and ellagic acids in European oakwood (*Quercus robur* L.) and eucalyptus *(Eucalyptus globulus). Holzforschung* **1992**, *46*, 87−89.

Chaudhry, A. S.; Miller, E. L. In vitro digestibility of barley and wheat straws treated with hydrogen peroxide, sodium hydroxide and sodium peroxide under various conditions. *Anim. Feed Sci. Technol.* **1994**, *50*, 1−15.

Griffiths, D. W. Polyphenolics and their possible effect on nutritive value. *Aspects Appl. Biol.* **1989**, *19*, 93−103.

Hagerman, A. E.; Klucher, K. M. Tannin-protein interactions. In *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure Activity Relationships*; Cody, V., Middleton, E., Harborne, J., Eds.; Liss: New York, 1986; pp 67−76.

Hagerman, A. E.; Robbins, C. T.; Weerasuriya, Y.; Wilson, T. C.; McArthur, C. Tannin chemistry in relation to digestion. *J. Range Manage.* **1992**, *45*, 57−62.

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

Haslam, E. Polyphenol complexation. In *Polyphenolic Phenomena;* Scalbert, A., Ed.; Institut National de La Recherche Agronomique: Paris, 1993; pp 23−31.

Julkunen-Tiittoo, R. Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. *J. Agric. Food Chem.* **1985**, *33*, 213−217.

Kumar, R. S.; Singh, M. Tannins: their adverse role in ruminant nutrition. *J. Agric. Food Chem.* **1984**, *32*, 447−453.

Makkar, H. P. S.; Becker, K. Behaviour of tannic acid from various commercial sources towards redox, metal complexing and protein precipitation assays of tannins. *J. Sci. Food Agric.* **1993**, *62*, 295−299.

Makkar, H. P. S.; Becker, K. Isolation of tannins from leaves of some trees and shrubs and their properties. *J. Agric. Food Chem.* **1994**, *42*, 731−734.

Makkar, H. P. S.; Blümmel, M.; Borowy, N. K.; Becker, K. Gravimetric determination of tannins and their correlation with chemical and protein precipitation methods. *J. Sci. Food Agric.* **1993**, *1*, 161−165.

Makkar, H. P. S.; Becker, K.; Abel, Hj.; Szegletti, C. Degradation of condensed tannins by rumen microbes exposed to quebracho tannins (QT) in rumen simulation technique (RUSITEC) and effects of QT on fermentative processes in the RUSITEC. *J. Sci. Food Agric.* **1995a**, *69*, 495−500.

Makkar, H. P. S.; Blümmel, M.; Becker, K. In vitro effects and interactions between tannins and saponins and fate of tannins in rumen. *J. Sci. Food Agric.* **1995b**, *69*, 481−493.

Menke, K. H.; Salewski, A.; Steingass, H.; Fritz, D.; Schneider, W. The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from gas production when they are incubated with rumen liquor. *J. Agric. Sci.* **1979**, *93*, 217−222.

Mukuru, S. Z.; Butler, L. G.; Rogler, J. C.; Kirleis, A. W.; Ejeta, G.; Axtell, J. D.; Mertz, E. T. Traditional processing of high-tannin sorghum grain in Uganda and its effect on tannin, protein digestibility, and rat growth. *J. Agric. Food Chem.* **1992**, *40*, 1172−1175.

Nguz, K.; de Mey, L.; Huyghebaert, A. Evaluation of the degradation of sorghum condensed tannins by a *Penicillium sp* by determination of proanthocyanidins and flavan-3-ols. *Sci. Aliments* **1994**, *14*, 51−59.

Ohara, S. Chemistry and utilization of condensed tannins from tree barks. *JARQ* **1994**, *28*, 70−78.

Osawa, R.; Walsh, T. P. Effects of acidic and alkaline treatments on tannic acid and its binding property to protein. *J. Agric. Food Chem.* **1993**, *41*, 704−707.

Peng, S.; Jay-Allemand, C. Use of antioxidants in extraction of tannins from walnut plants. *J. Chem. Ecol.* **1991**, *17*, 887−896.

Porter, L. J.; Hrstich, L. N.; Chan, B. C. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* **1986**, *25*, 223−230.

Price, M. L.; Butler, L. G.; Rogler, J. C.; Featherston, W. R. Overcoming the nutritionally harmful effects of tannins in sorghum grain by treatment with inexpensive chemicals. *J. Agric. Food Chem.* **1979**, *27*, 441−445.

Scalbert, A.; Duval, L.; Peng, S.; Monties, B. Polyphenols of *Quercus robur* L. II. Preparative isolation by low-pressure and high-pressure liquid chromatography of heartwood ellagitannins. *J. Chromatogr.* **1990**, *502*, 107−119.

Terrill, T. H.; Waghorn, G. C.; Woolley, D. J.; McNabb, W. C.; Barry, T. N. Assay and digestion of ^{14}C-labelled condensed tannins in the gastrointestinal tract of sheep. *Br. J. Nutr.* **1994**, *72*, 467−477.

Waichungo, W. W.; Holt, D. L. Use of ammonium hydroxide to reduce the level of assayable tannin in high-tannin sorghum grain. *J. Agric. Food Chem.* **1995**, *43*, 728−732.

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