Determination of Antioxidant Properties of Commonly Used Vegetable Tannins and Their Effects on Prevention of Cr(VI) Formation

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Abstract

Even though Cr(VI) is not used in any step of leather making, the presence of Cr(VI) in leather has become a concern in the leather industry. Free radicals usually play an important role in formation of Cr(VI) in leather. The effectiveness of antioxidant materials on prevention of free radical formation is well known. The tannins as phenolic materials are also known to have antioxidant properties. However, the antioxidant effectiveness shows variation regarding to the type and structure of tannins. In the present study, the vegetable tannins which are commonly used in leather industry (mimosa, quebracho, sumac, tara, valonea and chestnut) were selected and their antioxidant powers were determined by FRAP (The Ferric Reducing Ability of Plasma) and TEAC/ABTS (Trolox-Equivalent Antioxidant Capacity/2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) methods. Furthermore, these vegetable tannins were used in leather processing and their effect on prevention of Cr(VI) formation was examined. From the results; gallotannins within the hydrolysable tannins have taken the first places in ranking with superior antioxidant activities and compatible with their antioxidant powers they were found to be the most effective Cr(VI) formation preventing tannin types.

Keywords: tannin, Cr(VI), antioxidant power, leather

1. Introduction

Today approximately 80% of total leather tanned is chromium based. Although many alternatives have been studied and do exist, chrome tanning offers significant economic and technical advantages, which cannot be ignored and still make it the preferred way of tanning hides.[1]

With regard to chrome tannage, the most important technical issues concerning ecology and consumer health are: avoiding chromium (VI) and avoiding formaldehyde release.[2] Normally chrome tanning merely involves Cr(III), however, there have been many reports, Cr(VI) has been abnormally detected in some leather samples, this has been widely debated.[3-5]

In time, the chemicals used in production and other environmental factors like heat, light and etc. may cause aging and some changes and deformations in leather products e.g.: oxidation of Cr(III) to Cr(VI), decrease in shrinkage temperature, fading or yellowing, loss of physical resistance, odor problems due to oxidations of fatliquors or formaldehyde release. These deformations

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generally occur due to the oxidant groups, which are called free radicals, arise depending on these factors.

Hindering formation of free radicals prevents occurrence of the many cases mentioned above. Antioxidants are used for hindering free radical formation. Plant raw materials are known to be a very rich source of natural antioxidants such as phenolic compounds[6,7] carotenoids[8] and tocopherols[9]. The most effective are polyphenolics compounds. Among them one can mention, in decreasing order of antiradical activity: green tea phenols such as epigallocatechin gallate (very syrong antioxidants)[10]; tannins (also strong antioxidants)[11]; phenolic acids (the antioxidant activity strongly depends on the number of hydroxyl groups in the molecule and the position of these groups)[12]; and flavonoids (flavonols seems to be the strongest among them, and their activity strongly depends on the number and position of hydroxyl group in the B ring)[13-15].

There exist many vegetable materials (tannins) having polyphenolic characteristics used in tanning and retanning processes in leather making. Mimosa, quebracho, sumac, tara, valonea and chestnut are commercially the most significant tannins used for this purpose.

Although Zalacain et al.[11] investigated antiradical efficiency of different vegetable extracts by DPPH (2,2′-diphenyl-1-picrylhydrazyl) method and demonstrated quebracho and sumac extracts' efficiency against Cr(VI) formation in the leathers exposed to light and tara and mimosa's Cr(VI) preventing effects were investigated by Palop et al.,[16] the relation between total phenolic contents and antioxidant powers of commonly used vegetable tannins and their Cr(VI) preventing effects were not discussed in an article. For this reason, the objective of the present study was to investigate total phenolic contents (by Folin method) and antioxidant powers of commonly used vegetable tannins (by FRAP and TEAC/ABTS methods) and try to establish a relation with their Cr(VI) formation preventing effects.

2. Material and Method

2.1. Materials

The vegetable tannins used for the study were industrially produced, commercially available products (except sumac): two condensed tannins: quebracho (72% tannin, Silvachimica S.r.l. Cuneo, Italy) and mimosa (72% tannin, Silvachimica) and four hydrolysable tannins: tara (min. 48% tannin, Silvachimica), chestnut (72% tannin, Silvachimica) valonea (68% tannin, Ar-Tu Kimya San. ve Tic. A.Ş. Manisa, Turkey) and sumac powder (20.8% tannin). These are well-known vegetable tannins used in the Leather Industry and supplied in the form of spray-dried powders, except sumac and tara.

For total phenolic content and antioxidant power analysis analytical grade sodium acetate trihydrate (Merck), glacial acetic acid (Merck), hydrochloric acid (Merck), gallic acid (Sigma), TPTZ [2,4,6 tris (2-pridyl-s-triazine)] (Sigma), iron (III) chloride ($FeCl_3.6H_2O$) (Merck), ABTS [2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) ammonium salt] (Sigma), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma), potassium peroxodisulphate (J.T.Baker), dipotassium hydrogen phosphate (Merck), phosphoric acid (Merck), 1.5-diphenylcarbazide ($CO(NHNHC_6H_5)_2$) (Merck), acetone and methanol (Merck) were used.

In the study, chrome-tanned wet-blue goat skins were used as the leather material.

2.2. Methods

For the total phenol and antioxidant analysis, 0.1 g of tannin was dissolved in 100ml of solvent. For hydrolysable tannins acetone:water (1:1) mixture, for condensed tannins methanol:water (1:1) mixture was used as solvent. Whilst the tannins which were in extracted form were dissolved before the analysis, the grinded ones (tara and sumac) were leaved one night in solvent for extraction. The solutions were diluted appropriately and their antioxidant powers were calculated according to equivalents. All analysis was done as two repetitions.

2.2.1. Determination of Total Phenols

Total phenolics content was determined by the Folin–Ciocalteu method, which was adapted from Swain and Hillis[17]. The solutions used in the Folin assay of polyphenolics were Folin Ciocalteu reagent (1:10 diluted with water) and 20% (w/v) Na_2CO_3 solution. The 300 μL of extract, 2400 μL of ultra-pure water, and 150 μL of 0.25 M Folin–Ciocalteu reagent were added in a flask and then mixed well using a vortex. After 3 min, 300 μL of 20% Na_2CO_3 solution was added and the mixture was incubated in the dark for 2 h shaking. The absorbance was measured at 725 nm. The same procedure was repeated to all standard gallic acid and the results were expressed in gallic acid equivalents[18].

2.2.2. Determination of Antioxidant Powers of Vegetable Materials

Evaluation of antioxidant and antiradical activities of plant products cannot be carried out accurately by any single universal method. There are numereous methods for evaluating antioxidant activity[19-25]. Among these we decided to use FRAP (Iron (III) Reducing Antioxidant Power) and TEAC/ABTS (Trolox Equivalent Antioxidant Capacity) methods in order to reliably investigate antioxidant powers of vegetable tannins decided to be used.

The FRAP method was conducted according to Benzie and Strain[26], Ozgen[27]. The FRAP method measures antioxidant power by using ferric reducing ability of plasma; the ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) by a reductant at low pH. Fe(II)-TPTZ has an intensive blue color and can be monitored at 593 nm.

In analysis; TPTZ, 10×10^{-3}M was prepared in 40×10^{-3}M HCl. 20×10^{-3}M $FeCl_3$ was prepared by dissolving $FeCl_3.6H_2O$. TPTZ and ferric chloride solutions were diluted in 300 mM sodium acetate buffer (pH 3.6) at a ratio of 1:1:10. Standards or extracts (both 150μL) were added to 2850μL of the FRAP solution and the absorbances were determined after assay samples were allowed to react for 30 min.

The Trolox Equivalent Antioxidant Capacity method which is called TEAC/ABTS was developed by Miller et al. for determination of antioxidants. This method is based on inhibition of chromogen radical cation’s absorbance by hydrogen donating antioxidants. By using reduction in absorbance the total antioxidant capacity can be determined as trolox equivalent. Antioxidant ranking was established based on their reactivity relative to a 1.0 mmol/l trolox standard[28].

In analysis; Trolox, $1x10^{-3}$M was prepared in 96% ethanol. The chromogenic radical reagent $ABTS^{+\cdot}$, at $7x10^{-3}$M concentration was prepared by dissolving $2.45x10^{-3}$M potassium persulfate. The resulting ABTS radical cation solution was left to mature at room temperature in the dark for 12–16 h and was diluted 90 times with acetate buffer (pH=4.5) and was named as working solution throughout the work for TEAC (Trolox-equivalent antioxidant capacity assays). 50μL trolox (varied between 0 and $2x10^{-3}$M) or extract solutions were added to 2950μL of the working solution and the absorbance at 734 nm were read at the end of tenth minutes.

2.2.3. Usage of Vegetable Materials in Leather Production

The croupon areas of the leathers were divided into pieces in size of 20x20 cm as experiment samples. The leather samples processed according to recipe in Table I. 2 pieces were processed without any vegetable material as blank samples according to the same recipe. All vegetable materials were used in 1%, 2.5% and 5% proportions with two replicates in processes.

Table I. The recipe of process

Process	**%**	**Product**	**°C**	**Min**	
Washing	200	Water	35		
	0.2	HCOOH		30	
Neutralization	150	Water	35		
	2.5	Neutralising syntan		15	
	0.5	$NaHCO_3$		45	pH:5.0-5.5
Washing	200	Water	35	20	
Retanning	150	Water	40		
	x	Vegetable material		15	
	2.0	Condensed naphtalene sulphone acid		15	
	2.0	Phenol sulphone condensation product		15	
	2.0	Melamine-Urea formaldehyde resine		15	
Fatliquoring			45		Float heated
	5.0	Sulphited mix of natural fatliquors			
	2.0	Synthetic fatliquor			
	2.0	Synthetic fatliquor		45	
	1.0	HCOOH		30	
	0.5	HCOOH		30	pH: 3.8
Washing	200	Water	35	20	

2.2.4. Artificial Aging of Leather Samples and Cr(VI) Analysis

Cr(VI) contents of the leather samples were determined according to IUC 18 standard test methods[29]. In many researches it is stated that formation of Cr(VI) was triggered or increased by certain factors e.g. high pH values and temperatures and UV light etc[30]. Exposing the leathers to UV light, initially which do not contain Cr(VI) may cause gradual occurrence of this element[29]. Font et al.[31] and Candar et al.[32] kept the leather samples at 80ºC for 24 hours in order to determine whether Cr(VI) will occur or not.

In this part of the research we aimed to investigate possible effects of vegetable materials on Cr(VI) formation under drastic conditions. For this purpose after determining the initial Cr(VI) contents of the leather samples, they exposed to UV light (360nm) and kept at 80ºC for 7 days. Thus, it is tried to investigate effects of vegetable materials on Cr(VI) formation under drastic conditions.

3. Results and Discussion

3.1. Total Phenolic Contents and Antioxidant Powers of Plant Extracts

Considering the total phenolic contents of the vegetable tannins; quebracho was found to be containing the highest amounts of phenolics, followed by chestnut, tara, mimosa, valonea and sumac.

From the analysis applied to investigate antioxidant powers of the vegetable tannins, they were sorted by descending order according to FRAP, as valonea > tara > mimosa > chestnut > quebracho > sumac and according to ABTS, as tara > chestnut > valonea > quebracho > mimosa > sumac (Table II).

Table II. Total phenolic contents and antioxidant powers of plant extracts

Vegetable Material	Total Phenolic Content (mgGAE/g)	According to FRAP (mmol/L)	According to ABTS (mmol/L)
Quebracho	972.1	25.1	5.5
Mimosa	803.6	25.5	4.8
Chestnut	896.3	25.3	6.2
Tara	843.2	26.5	6.6
Valonea	780.2	33.3	5.8
Sumac	487.9	11.1	2.7

However, the concentrations (active matters) of the vegetable materials are very important in such properties. Considering the big differences between active matters of the vegetable materials selected to be used in analysis, their total phenolic contents and antioxidant powers were recalculated by taking in account their active matters (Table III).

Table III. Total phenolic contents and antioxidant powers of plant extracts according to their active matters

Vegetable Material	Total Phenolic Content	According to FRAP	According to ABTS

	(mgGAE/g)	(mmol/L)	(mmol/L)
Quebracho	1350.1	34.9	7.6
Mimosa	1116.1	35.4	6.7
Chestnut	1244.9	35.1	8.6
Tara	1756.7	55.2	13.8
Valonea	1147.4	48.9	8.5
Sumac	2139.9	48.7	11.8

From the recalculation of the analysis results according to equal active matters (100 unit), the total phenolic contents of the vegetable materials were found to be sorting by descending order as **sumac > tara > chestnut > quebracho > valonea > mimosa** (Table III). Similar recalculation according to active matters rearranged the antioxidant power sequence by descending order as; **tara > valonea > sumac > chestnut > mimosa > quebracho** and according to FRAP and as; **tara > sumac > chestnut > valonea > quebracho > mimosa** according to ABTS methods.

The results obtained in our study by FRAP and ABTS antioxidant analysis were in accordance by the results obtained from the studies carried out by Zalacain et al.[11] on antiradical efficiency of different vegetable extracts. They also pointed out that hydrolysable tannins (sumac, tara, chestnut, and myrabolans) were more powerful antioxidants than condensed tannins (quebracho, mimosa). The origin of these differences was presumably attributed to molecules' shape, conformation and solvation properties by them. While the condensed tannins are helical chains, hydrolysable tannins are flat, disc like molecules with phenolic groups on the outside of the disc.

3.2. Cr(VI) Contents of Leather Samples

Before aging treatment, Cr(VI) was not detected neither in blank nor in any of the vegetable tannin treated leather samples. But, after artificial aging treatment, in average 13.1 ppm Cr(VI) was detected in blank leather samples which were not treated with any vegetable material. That means an important amount of Cr(III) oxidized to Cr(VI) due to the applied drastic conditions (Table IV).

Table IV. Comparison of Cr(VI) levels (mg kg^{-1}) for leather samples tanned with different vegetable materials

Tannin type	Amount of tannin %	The content of Cr(VI) before ageing (mg kg^{-1})	The content of Cr(VI) after ageing (mg kg^{-1})
Blank	-	0.7	13.1
Mimosa	1.0	0.4	4.0
	2.5	0.4	0.8
	5.0	0.3	0.7
Quebracho	1.0	0.1	3.0
	2.5	0.3	0.7
	5.0	1.4	0.4
Tara	1.0	0.5	0.7
	2.5	0.5	0.4

	5.0	0.6	0.1
Sumac	1.0 2.5 5.0	0.3 0.2 0.3	2.0 1.9 0.8
Valonea	1.0 2.5 5.0	0.4 0.2 0.2	2.9 1.3 0.1
Chestnut	1.0 2.5 5.0	0.6 0.2 0.5	3.5 0.8 0.7

From the investigation of the Cr(VI) contents of the artificially aged leather samples treated with vegetable tannins; it was seen that use of 2.5 and 5.0% of any type of vegetable tannin prevented Cr(VI) formation in all leather samples quite effectively. But, in case of 1.0% vegetable tannin use, it was pointed out that Cr(VI) contents of the leather samples differed and some of the leathers' Cr(VI) contents were higher than 3.0 ppm. While the leather samples treated with 1% mimosa, quebracho and chestnut were containing 3.0 ppm and higher amounts of Cr(VI), Cr(VI) contents of the leather samples treated with same amounts of tara, sumac and valonea were below 3.0 ppm. At this point it was interesting that all these tannins were hydrolysable and the best results were obtained by tara and sumac which are belong to hydrolysable tannins' subgroup gallotannins.

Recalling the antioxidant powers sequence, we realize that these tannins were found to be the tannins with highest antioxidant powers among the rest used in trials. Considering the antioxidant powers and Cr(VI) contents of the leathers treated with these vegetable tannins, here it is possible to say that tannins perform in accordance with their antioxidant powers in Cr(VI) formation preventing effects in leathers.

4. Conclusions

In the present study, total phenolic contents and antioxidant powers of commonly used vegetable tannins were determined. Then, they were used in leather processing in various amounts in order to investigate their Cr(VI) formation preventing effects comparatively.

Considering the analysis results, among the vegetable tannins commonly used in leather industry, hydrolysable ones were found to be having higher antioxidant powers than condensed ones. Within the hydrolysable tannins, gallotannins (tara and sumac) have the highest antioxidant powers and correspondingly among other tannins they exhibited the most effective Cr(VI) formation preventing effects in leathers.

The findings in the present study revealed that not only the total phenolic contents of the vegetable tannins but also their antioxidant powers determine their Cr(VI) preventing effect in chromium containing leathers. The vegetable tannins commonly used in leather industry perform

Cr(VI) preventing effects in accordance with their antioxidant powers and their antioxidant efficiency differs due to their molecular structure, conformation.

5. References

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