ELIMINATION OF FREE FORMALDEHYDE IN LEATHER BY *VINCA ROSEA* AND *CAMELLIA SINENSIS* EXTRACTS*

by

ESER EKE BAYRAMOĞLU**, AYŞEGÜL KORGAN, DENIZ KALENDER, GÜRBÜZ GÜLÜMSER, BUĞRA OKCU AND EYLEM KILIC***

Ege University, Faculty of Engineering, Department of Leather Engineering

35100 BORNOVA - İZMİR - TÜRKİYE

****Usak University, Leather Research Development and Application Centre*

64200 USAK - TÜRKIYE

ABSTRACT

Even though modern technologies are applied, many chemicals that are hazardous to the environment and human health are used during the leather processing. Formaldehyde is one of the chemicals known to exhibit carcinogenicity. In this research, elimination of free formaldehyde in leather by the application *Vinca rosea* and *Camellia sinensis* extracts was investigated. Formaldehyde was quantified with two methods; Draft IUC 19 (HPLC) method and AATCC test method 112. When measured by each method *Camellia sinensis* extract provided the lowest formaldehyde content in leathers.

RESUMEN

Aunque se apliquen las tecnologías modernas, muchos productos químicos que son peligrosos al medio ambiente y a la salud humana se utilizan durante el proceso del cuero. El formaldehído es uno de los productos químicos conocidos por exhibir propiedades carcinogénicas. En esta investigación, la eliminación del formaldehído libre en cuero por la aplicación de los extractos de Vinca Rosea y de Camelia Sinensis, fue investigada. El formaldehído libre fue cuantificado con dos métodos: el método IUC 19 (HPLC) y el método de prueba 112 AATCC. Cuando es medido por cada método, el extracto de Camelia Sinensis, proporcionó el contenido más bajo de formaldehído libre en el cuero.

INTRODUCTION

The chemical compound formaldehyde (also known as methanal), is a gas with a strong pungent smell. It is the simplest aldehyde and its chemical formula is H_2CO. It has a boiling point of -21°C (262 K)[1]. Under mild alkaline conditions formaldehyde reacts readily with protein and collagen and this has been used in the past formaldehyde tannages of leather.

Ecological leather production has become more important in recent years because the customers are increasingly specifying their own limits of chemicals used in leather production process. The problems posed by PCP and benzidine prompted a great deal of discussion in the early 1990s, and much attention is currently being paid to issue of formaldehyde in leather. Formaldehyde and products based on formaldehyde are employed at the tanning and retanning stages, and they are an established part of the leather production process. In the production and modification of chemicals like synthetic tanning agents, resins, naphthalene based products, oxazolidine derivatives, phenol and sulphone compounds, dyes, finishing chemicals and casein-based finishes, formaldehyde is used.[2]

Although it is used in many applications as well as leather industry, formaldehyde is not easy to handle. It has a penetrating odour and it irritates the eyes and mucous membranes, and it has been found to have an allergenic potential. For instance formaldehyde resin allergy is more common than has been previously recognized.[3] Moreover formaldehyde has been classified by the European Union as a suspected carcinogen in Category 3 and this has certainly speeded up the effort to tighten the limits on formaldehyde in leather.[2] The trend towards more stringent limits makes it even more important to identify the sources of formaldehyde in leather.

It is not so simple to cross off these products from leather processing recipes, owing to their providing desired properties for leather. At least they should be used until substituted with another product. In this study formaldehyde which is released especially during applying these formaldehyde containing products, is tried to be eliminated by antioxidant substances. For this purpose *Vinca rosea* and *Camellia sinensis* plants were extracted, the specifications of these extracts were analyzed and by applying different leather processing recipes free formaldehyde in leather is tried to be eliminated.

*A **Technical Note** based on a visual presentation at the XXIX IULTCS Congress and103nd annual meeting of the American Leather Chemists Association at the JW Marriott Hotel, Washington, DC, on June 23, 2007
**Corresponding autoress: Email: eser.eke@ege.edu.tr
Study received March 30, 2007 and accepted for publication September 5, 2007

EXPERIMENTAL

Materials and Methods

Green Tea (Camellia sinensis)

Fresh green tea leaves were supplied from Rize (Türkiye) and dried in a cool place before grinding. When completely dried, it was ground. Ground material was extracted for 3 hours. Extracts were filtered and filtrates were concentrated.

Tea *(Camellia sinensis)* contains large amount of tannins or phenolic substances (5-27%) consisting of catechin (flavanol) and gallic acid units. In general, fresh green tea leaves contain 36 % polyphenols, among which catechins prevail. Pharmacological properties of tea are due primarily to its alkaloids (caffeine) and catechins, which are divided into four primary compounds, epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG).[4] All of these components have antioxidant properties but EGCG has been described as being a more active antioxidant than the other components. EGCG is the predominant catechin present in green tea leaves (48-55% of total polyphenols).[5] Numerous studies have found EGCG to be effective in preventing and inhibiting cancer growth. In laboratory studies using animals, catechins scavenged oxidants before cell damage occurred, reduced the number and size of tumors, and inhibited the growth of cancer cells.[6,7]

Vinca rosea (Catharanthus roseus (L.) G. Don)

Vinca rosea – Catharanthus roseus (L.) G. Don., an important medicinal plant, belongs to the family Apocynaceae and is cultivated mainly for its alkaloids.[8] *Vinca rosea* produces widely used alkaloids such as the anticancer drugs vinblastine and vincristine, as well as the antihypertensive compounds ajmalicine and serpentine. Catharantine, tabersonine, lochnericine and horhammericine are other indol alkaloids found in *Vinca rosea.*[9] *Vinca rosea* flowers were picked up with their stems from Izmir Municipality park areas. *Vinca rosea* was extracted both from the leaves and the stems of the plant. After *Vinca rosea* flowers were picked up and washed, their stems and leaves were separated and dried in a cool place. When completely dried, stems and leaves were ground separately. Ground material was extracted for 3 hours. Extracts were filtered and filtrates were concentrated.

Apparatus

High pressure liquid chromatographic analyses were carried out on a HPLC system consisting of a Jasco PU980 intelligent pump and a VWD Agilent UV detector. The analyses were performed with a Supelco C_{18} column ODS-3 (150x 4.6 mm i.d.).
The photometric measurements were carried out using Shimadzu 1601 UV 1601 spectrophotometer.

An Elga Pure Water System and reverse osmosis system was used in all experiments.

Reagents

Formaldehyde (approximately 37% w/v stabilized with 10 methanol), HPLC grade acetonitrile, o-phosphoric acid iodine, sodium hydroxide, sulphuric acid, sodium thiosulphate, and starch were obtained from Merck. HPLC grade 2,4 dinitrophenyl hydrazine was purchased from Fluca. Otherwise stated, all reagents used were analytical grade.

Procedures

HPLC determination

Determination of formaldehyde in leathers according to reference method IUC 19 requires High performance liquid chromatography (HPLC). This HPLC method is selective.

Formaldehyde is being separated and quantified as a derivative from other aldehydes and ketones by liquid chromatography. Free formaldehyde and formaldehyde is detected which is hydrolyzed during extraction to yield free-formaldehyde. The leather sample is eluated and mixed with 2,4 dinitrophenylhydrazine, whereby aldehydes and ketones react to give the respective hydrazines. Therefore derivatization of the carbonyl compounds

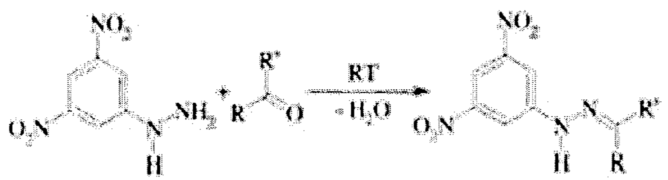

Figure 1: Derivatization of carbonyl compounds using 2,4-dinitrophenylhydrazine (R,R'.H,alkyl,aryl)

Table I Specification of HPLC analyzing conditions

Injection volume	20 µm
Flow rate	1,0mLmin^{-1}
Mobile phase	acetonitrile/water, 60:40
UV detection wavelength	350n

TABLE II
Comparison of Analysis Methods

MODEL Reagent	PROCEDURE	RESULTS
AATCC 112 (Acetylacetone)	Extraction: 20 h 50°C	Free formaldehyde + formaldehyde from the hydrolysis
IUC 19 (HPLC) (DNPH)	Extraction: 1 h 40°C	Free formaldehyde + a little formaldehyde from the hydrolysis

TABLE III
The Results of the Analyses

Sample Type	AA TCC 112 formaldehyde ppm	Draft IUC 19 HPLC formaldehyde ppm
Control(37%F)	722.96	47.57
C.sinensis(37%F)	306.96	34,01
V.rosea (37%F)	609.07	42.39
Mixture (37%F)	430.22	40.71
Control (syntans+resin)	418.85	54.23
C.sinensis (syntans+resin)	309.61	37.39
V.rosea (syntans+resin)	390.33	41.68
Mixture (syntans+resin)	308.73	39.72

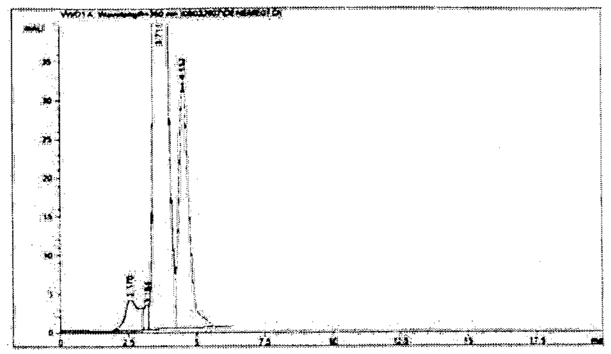

Figure 2: The comparison of formaldehyde content of syntan and resin retanned leathers treated with 4% Vegetal Extracts (AATTCC 112 method)

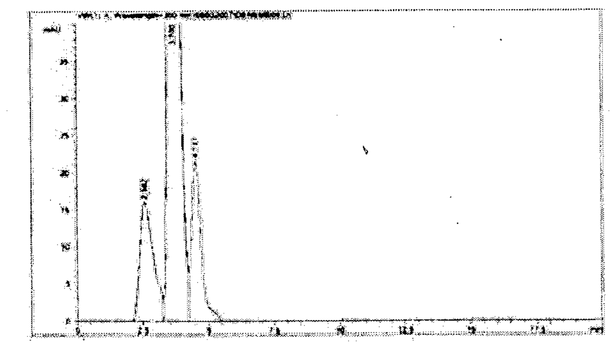

Figure 3: The comparison of formaldehyde content of syntan and resin retanned leathers treated with 4% Vegetal Extracts (AATTCC 112 method)

with 2,4-dinitrophenylhyrdrazine (DNPH) followed by HPLC_UV/DAD- analysis is widespread (Fig. 1).[10] The solution is separated by means of HPLC-reverse phase method, detected at 350nm and quantified. Analyses were performed in accordance with IUC 19-HPLC method for the formaldehyde tanned and both syntan and resin retanned leathers.[11]

Spectrophotometric determination

In spectrophotometric method besides free formaldehyde, formaldehyde from the hydrolysis was also determined. The comparison of each method is presented in Table II. In accordance

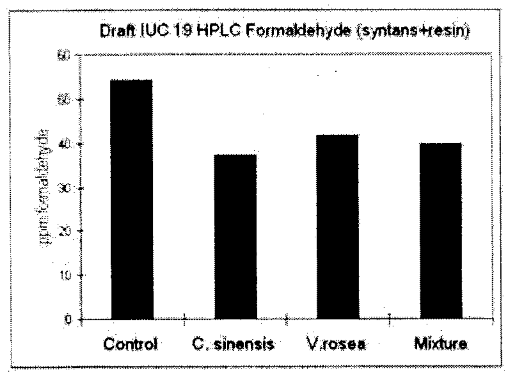

Figure 4: The comparison of formaldehyde content of syntan and resin retanned leathers treated with 4% Vegetal Extracts (AATTCC 112 method)

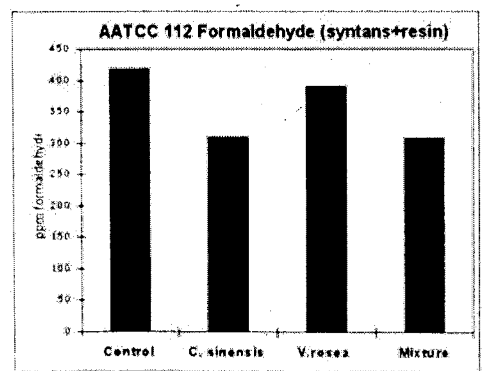

Figure 5: The comparison of formaldehyde content of syntan and resin retanned leathers treated with 4% Vegetal Extracts (Draft IUC 19 method)

with AATCC 112 method formaldehyde released in a sealed jar at 50°C for 20 hours is determined spectrophotometrically with Nash reagent at 412nm wavelength.[12,13]

Raw skin

For trials domestic wet salted sheep skins were used.

Formaldehyde

HPLC grade formaldehyde (37%, Merck) was used for this study.

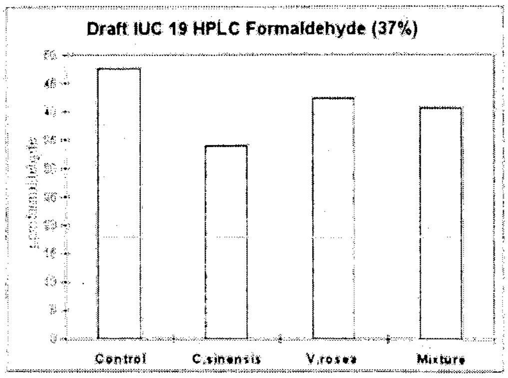

Figure 6: The comparison of formaldehyde content of formaldehyde tanned leathers treated with 4% Vegetal Extracts (Draft IUC 19 method)

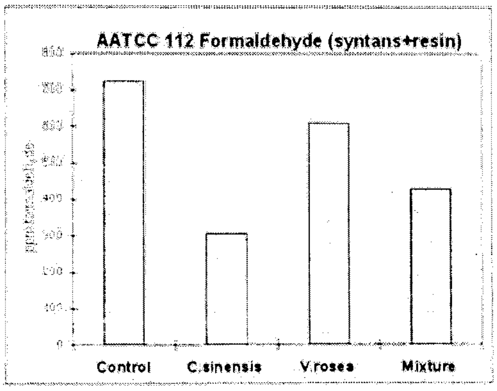

Figure 7: The comparison of formaldehyde content of formaldehyde tanned leathers treated with 4% Vegetal Extracts (AATCC 112 method).

Processing of leathers

Wet salted sheep skins which were used for the trials were pickled with a conventional garment recipe. Afterwards the pelts in the first group were pre-tanned with 3% formaldehyde (37%). Then the leathers were chrome-tanned and processed separately with a conventional garment reçipe using 4% vegetal extracts.

In the second group, pickled pelts were chrome-tanned and at the second chrome-retanning process wet-blues were treated with 2% chrome syntan + 2% syntan + 2% chrome. At the retanning process 3% methylene–linked condensation product from dicyandiamide resin was given. Afterwards 4% of vegetal extracts were given.

RESULTS AND DISCUSSION

The results of this study are presented in Table III. Free formaldehyde was measured by gas phase method (AATCC 112) and reversibly bound formaldehyde was measured by extraction method (Draft IUC 19). The results obtained by each method showed that *Camellia sinensis* extract provided the lowest formaldehyde content in leathers.

Even formaldehyde was not used directly in leather processes, the release of substantial amounts of free formaldehyde and formaldehyde from the hydrolysis that arises from using syntan and resin, was determined by each method.

Various approaches can be taken to eliminate formaldehyde from leather or reducing its contents like incorporation of formaldehyde scavengers, modifying mechanical processes and modification of products that release formaldehyde. This is an original study which was performed in order to avoid released formaldehyde by using antioxidant substances in retanning process. In this research it was found that both extracts were effective in eliminating formaldehyde but *Camellia sinesis* showed better effect. This effect can be improved by employing different extraction techniques or by increasing the amount of the extract used in processes. The method is expected to find a significant application area in leather industry in near future.

REFERENCES

1. Hart, H., Hart, D.J., Craine, L.E., Organic Chemistry, Hougton Mifflin Company, 1995.
2. Wolf, G.; Formaldehyde in Leather: Causes and Avoidance, *World Leather* February / March ,46,2002
3. Fowler Jr., J.F., Skinner, S.M., Belsito, D.V.; Allergic contact dermatitis from formaldehyde resins in permanent press clothing: An underdiagnosed cause of generalized dermatitis, *Journal of the American Academy of Dermatology*, **27**, 962, 1992.
4. Uzunalic, A.P., Skerget, M., Knez, Z., Weinreich, B., Otto, F., Grüner, S.; Extraction of Active İngredients from Green Tea (*Camellia sinensis*):Extraction Efficiency of Major Catechins and Caffeine, *Food Chemistry* **96**, 597, 2005
5. Ho,C.W., Chen, U.N. Wanasundara and F. Shahidi; Natural antioxidants from tea in natural antioxidants 213-223,AOCS Press, Champaign,IL,1997
6. Dufresne CJ, Farnworth ER. ;A review of latest research findings on the health promotion properties of tea. *J. Nutri Biochem* **12**, 404, 2001
7. Hakim IA, Harris RB.; Joint effects of citrus peel use and black tea intake on risk of squamous cell carcinoma of the skin. *BMC Derm.*,**1** (3). 2001
8. Data S.C.; Cultivation of *Catharanthus roseus* in West Bengal. In: C.K. Atal and B.M. Kapur, Editors, Cultivation and Utilization of Medicinal Plants, CSIR, Jammu Tawi pp. 279–283,1980
9. Tikhomiroff,C.,Jolicoeur,M.; Screening of Catharanthus roseus secondary metabolites by high-performance liquid chromatograpy, *Journal of Chromatograpy* **A**, 955,87-93,2002
10. Sandner,F.,Dott,W.,Hollender,J.; Sensitive Indoor Air Monitoring of Formaldehyde and Other Carbonyl Compounds Using the 2,4-Dinitrophenylhydrazine Method, Int.J.Hyg.Environ. Health 203,275, 2001
11. IUC 19, Determination of Formaldehyde Content in Leather
12. AATCC Test Method 112-1984, 1989, Formaldehyde Odor İn Resin Treated Fabric, Determination of: Sealed Jar Method, AATCC Technical Manual
13. Gülümser, G.; Ege Bölgesi Deri İşletmelerinde Üretilen Giysilik Derilerde Formaldehit Miktarları Üzerinde Bir Araştırma, Ege Üniv. Ziraat Fak. Derg., **38**(2-3),151, 2001