Analysis of Release of Free Formaldehyde Originated from THP Salt Tannages in Leather by High Performance Liquid Chromatography: *Origanum onites* Essential Oil as Free Formaldehyde Scavenger

by

Eser Eke Bayramoglu,* Ali Yorgancioglu and Ersin Onem

Ege University, Faculty of Engineering Department of Leather Engineering

35100 Bornova, Izmir, Turkey

Abstract

This study was about the release of free formaldehyde from tetrakis (hydroxymethyl) phosphonium (THP) salts and various syntan leather products, and the antioxidative blocking defense mechanisms of various essential oils. For that purpose, *Citrus reticulata* L., *Melaleuca alternifolia*, *Origanum onites* and *Thymus vulgaris* essential oils of 2% were used during fatliquoring. Formaldehyde analyses in finished leathers were performed according to TS EN ISO 17226-1 international standard by high performance liquid chromatography (HPLC) test method. To make a comparison on the analyses, some leather samples as a control group were processed without any essential oil. The results were evaluated statistically by using One-Way ANOVA and Duncan tests with the SPSS 15 statistic program. The results showed that *Origanum onites* essential oil used in fatliquoring process was significantly determined to eliminate the release of free formaldehyde in leather.

Introduction

Today, chrome tanning causes environmental pollution even though it ranks first among the tanning methods applied most in the world. Besides, chromium (III) salts used in the process may oxidize to Cr^{+6} as a result of the fact that the leathers tanned with chrome age after a while and they create big disadvantage in terms of human health if antioxidants compounds are not present in leather.[1] Because of these reasons, studies with regard to the fact that chromium salts are used as little as possible or are not used in the processing of leather have increased and new, more environmentally friendly systems are being introduced. In addition, the regulations are becoming stricter and more complex in the fields of environmental protection and ecotoxicology and thus promote the scrutiny of new alternatives.[2,3]

Tetrakis hydroxymethyl phosphonium sulphate (THPS) has been used for a long time as biocide and as flame retardant in textiles, and is one of the chemicals used instead of chrome[4] in tanning. The hydroxymethyl groups in THP salts may react with the amino groups in collagen. These constitute short and strong cross-bonds by exhibiting a combined tanning property. The reactive formula that THPS created with leather was schematized in Figure 1.

*Corresponding author e-mails; eser.eke@ege.edu.tr, eserekebay@gmail.com; Tel: +90 232 311 2644; fax: +90 232 342 5376.

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Phosphonium tanning agent can be used in combination with other tanning substances in leather production. Phosphonium tanning agent has advantages such as low toxicity, high biodegradation and lack of metals.[6] Thanks to phosphonium tanning, leather acquires the properties such as hydrothermal stability (T_s=80°C), ease in dyeing, a gain in surface area, durability against mechanical effects, and high sensitivity of light.[2,4] In addition, phosphonium salts are extremely suitable for the production of wet-white and wet-pink leathers.[7] However, apart from all of these positive properties, formaldehyde formation in the leathers tanned with THPS cannot be ignored.[7,8] The reaction of formaldehyde, which occurs when tanning is performed with tetrakis hydroxymethyl phosphonium chloride (THPC), is presented in Figure 2.

Formaldehyde content in leather is one of the primary harmful chemicals and whose analysis is required in finished product. Formaldehyde was included by the E.U. in the list of Category 3, known to be cancerogenic.[10] The formation of such harmful contents in leathers causes all of the leather products that may or may not come into direct contact with the body to seriously affect the human health.[11] Formaldehyde, also known as methanal, is an organic compound which is used in many industries and whose chemical formula is expressed as CH_2O. The common symptoms that occur, when it exists above 0,3-0,4 ppm concentrations in the air, are the irritations in the eyes, nose and throat. As a result of swallowing or inhaling large amounts of formaldehyde, acute pain, vomiting, coma and death may occur.[12,13]

The fact that formaldehyde is utilized in the production and condensation of whitening syntans, melamine resins, phenolic resins and urea-formaldehyde often used in the leather industry causes the finished leather to contain formaldehyde, while it is not directly used in leather production.[14,15] The fact that the reaction of reactive with formaldehyde occurs quickly and moderately and that the production techniques of the products produced in this way are simple and cheap are the reasons for preference in the production of leather chemicals as it happens in many industrial branches.[16] However, the fact that the interest in ecological products increases and that a number of limitations have been introduced as regards some products that menace human health leads to some restrictions in export in the leather industry in recent times. Because syntans and resins that gains positive characteristics to leather are among the products not easy to give up in terms of performance properties expected from the finished products, it is not easy to completely remove these products from the leather processes.[17] The above-mentioned syntans are often used particularly with phosphonium tanning materials and in the production of white leather. Then, it can be thought practical to eliminate the disadvantages of these products instead of removing them from the recipes in the first stage. The production of syntans not containing formaldehyde is an important subject on which an emphasis is placed.[18]

Essential oils of plants have been used for a long time for various purposes, particularly in many scientific and commercial fields. Essential oils have different biological activities; their antibacterial,[19] antifungal[20] and antioxidant[21] properties are some of these properties. While it is known that phenolic and terpenoit compounds included in the essential oils have a certain antioxidant activity due to their structures,[22] their antioxidant activities show differences because of the differences in their structures.[23]

In this study, the leathers are processed in accordance with a recipe applied by leather factory and built on phosphonium tanning. In the formula are phosphonium salt and synthetic tannins expected to form free formaldehyde in leather. In order to eliminate the free formaldehyde to occur in leather, on the other hand, the essential oils of *Citrus reticulata* L.,

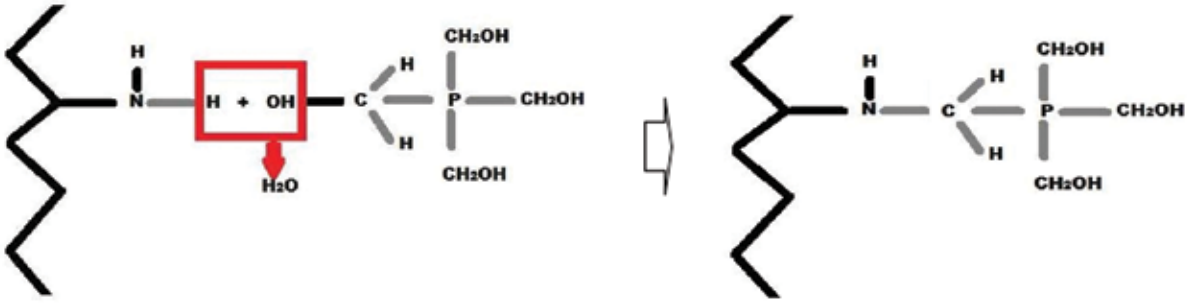

Figure 1. Reaction mechanism of THPS with amines.

$$P(CH_2OH)_4Cl \rightleftharpoons P(CH_2OH)_3 + HCHO + HCl$$

Figure 2. The reaction of formaldehyde formation in the tanning process performed with THPC.[8,9]

Melaleuca alternifolia, *Origanum onites* and *Thymus vulgaris*, being emulsified with natural oils and synthetic oils, were used. Thus, the advantages of phosphonium tanning, which are the alternative of chrome tanning, were realized and at the same time, the disadvantages were to be eliminated. In the analyses, some leather samples as control group were made into finished products without being treated with any essential oils so as to make comparison. In the finished product leathers, formaldehyde analyses were made spectrophotometrically with high performance liquid chromatography (HPLC), and the results were statistically comparatively evaluated according to the samples of control group.

Materials and Methods

Materials

In the study, 15 pieces of commercial pickled sheep skins were used. As phosphonium tanning material, a commercial preparation called Maratan TSF from (Turquaz Chemical Company, Izmir, Turkey) was used. The essential oil of *Origanum onites* used in the research was obtained from Turer Agriculture Co., operating in Turkey, and the other essential oils used in the study were provided by Sigma-Aldrich in Turkey.

Methods

Leather Manufacturing Processes

The processing of leathers for phosphonium tanning was made in accordance with a production process applied commercially at a leather factory. The production formula applied was provided in Table 1.

The leathers of control group were produced with the same production process without using any essential oils.

Formaldehyde Analysis

Formaldehyde analyses were carried out in compliance with TS EN ISO 17226-1 test method.[24] First of all, grinding process of finished leathers was made and 2±0.01g was weighed from the ground leather samples. Then, the samples were extracted within a period of 60±2 minutes at 40°C±0.5 in an erlenmeyer, by shaking them. After the process of shaking, the solutions in the erlenmeyers were subjected to filtration using a membrane filter (polyamide 0.45µm). 5 ml was taken from the prepared sample solution and then the solution, into which 4 ml acetonitrile solution and 0.5 ml DNPH solution were added, was covered to 10 ml with water. The prepared solutions sat for 60 minutes. The amounts of these solutions obtained, by analyzing them them with HPLC at 350 nm, were determined. HPLC conditions were provided in Table 2.

The amount of formaldehyde found in the leather samples was calculated as follows:

$$C_F = C_s * F / E_w$$

C_F: Formaldehyde concentration in the leather sample (mg/kg)

C_s: Formaldehyde concentration derived from calibration graphics (µg/10ml)

F: Dilution coefficient

E_w: Weight of leather sample (g)

Statistical Analysis

The results were evaluated statistically by using One-Way ANOVA, descriptive statistical test and Duncan test at SPSS 15.0 statistical software package. All data were represented as mean for three independent measurements. Comparison of means was analyzed by Duncan test and differences were considered significant when $p<0.05$.

Results and Discussion

In the private demands of some premium brands and specifications of the products with eco-label, the limitations regarding the level of free formaldehyde in leather are introduced for the protection of the consumer. Concerning the formaldehyde limit value in leather, there are some standards and regulations such as Austrian and German Legislation, EC ECO Label for Footwear, SG Mark, Oeko-Tex Standard 100. Phosphonium tanning has in recent years come into prominence as an alternative to chrome tanning. However, phosphonium tanning and the syntans used along with them have some disadvantages such as causing formaldehyde in leather, as well as having some advantages. In our study, the amounts of formaldehyde formation in the leather products that were finished in compliance with phosphonium tanning process and the influence of various essential oils used in the process on free formaldehyde were examined statistically (SPSS 15.0). Table 3 analogically presents the reducing effects of the essential oils of *Citrus reticulata* L., *Melaleuca alternifolia*, *Thymus vulgaris* and *Origanum onites* on the formation of formaldehyde arising from phosphonium tanning.

In Figure 3, amounts of formaldehyde in the finished leathers are shown in graphics.

In our study, it was established that the essential oil of *Origanum onites* used in the process of leather statistically reduced the amount of free formaldehyde in leather in a significant way. The effects of other essential oils apart from

TABLE I
Recipe for the production of leathers.

Process	%	Chemicals	Temperature (°C)	Time (min.)	Remarks
Depickle	200	Water 7Bé	28		
	0.5	Degreasing agent		20	
	3	Sodium formate		3x20+40	pH:4.0
	1	Sodium bicarbonate		45	pH:4.8, drain
Washing	200	Water 7Bé	28	10	Drain
Fleshing					
Degreasing	5	Degreasing agent		60	
	50	Water	28		
	2	Salt		90	Run overnight, drain
Washing	200	Water	30		
	6	Salt		30	Drain
Washing	200	Water	30	30	Drain
Washing	200	Water	30	30	Drain
Washing	200	Water	30	30	Drain
Pickle	80	Water	30		
	12	Salt		20	6 Bé
	0.5	Formic acid			
	1	Synthetic oils and esters		45	pH:3.9
Phosphonium tanning	2	Phosphonium salt		90	Check for penetration
	0.5	Formic acid		45	pH:3.4
	1	Synthetic oils and esters			
	1	Synthetic fatliquoring agent		20	pH:3.5
	5	Phenolic syntan		20	
	5	Phenolic syntan			
	1	Synthetic fatliquoring agent		60	
	0.3	Formic acid		45	pH:3.4

Table I continues on following page.

Table I continued.

	7	Whitening syntan		60	Run overnight
	1	Sodium acetate		45	pH:3.6
	0.5	Sodium bicarbonate		60	pH:3.9, drain
Neutralization	150	Water	35		
	2	Neutral syntan		30	pH:4.8
	1	Sodium bicarbonate		30	pH:5.0, drain
Washing	200	Water	30	10	Drain
Fatliquoring	100	Water	50		
	6	Natural fatliquoring agent			
	4	Synthetic fatliquoring agent			
	2	Essential oil*		90	
	1	Formic acid		3x15+15	Drain
Horsing-Drying					

* *Citrus reticulata* L., *Melaleuca alternifolia*, *Origanum onites* and *Thymus vulgaris* essential oils.

TABLE II
HPLC conditions.

Flow rate	1.0 ml min-1
Mobile phase	Acetonitrile/water, 60:40
Column	C 18
UV detection wavelength	350 nm
Injection volume	20 µl

Origanum onites were not found to be significant statistically. The essential oil of *Origanum onites* produced the most effective result.

As it is known, chrome tanning has been the most common tanning method for a long time due to both the properties it brings in the leather and its ease in use. However, due to the environmental consciousness that was introduced in the 21st century, alternative methods have been developed in recent years to tanning without chrome or with less amount of chrome. Phosphonium salts, also developed as an alternative to chrome tanning, is an organic tannage and has become an important tanning material for producing white leather, which has been in great demand in recent years in the leather industry.[7] In addition, phosphonium salts show extremely positive results both in the leather properties they bring and its effect on the waste load.[2,4] In spite of all of their positive properties, it is required that in the tanning made by phosphonium salts, great care should be taken with respect to the free formaldehyde which may develop during tanning. In particular, the formaldehyde that has been observed in this way in the finished products in recent times puts the leather exporters in a difficult position.

In a study conducted with regards to formaldehyde formation in the phosphonium tanning, it was determined that as the pH was increased from 4 to 6 during the tanning, the amount of free formaldehyde substantially increased.[4,8] In another study, it was demonstrated that while working with phosphonium salts, the amount of formaldehyde considerably decreased in the washings with sodium perborate and hydrogen peroxide present in aqueous media and in addition, thanks to the use of herbal tannins together with phosphonium, phenolic groups of the tannins play an important role in inhibiting formaldehyde formation.[7]

TABLE III
The effects of essential oils on free formaldehyde in leather.

	Formaldehyde (ppm)			Reduction (%)
Essential oils	Min.	Max.	Mean±Standard Deviation	
Control	101.54	115.31	107.42±7.10[a]	-
Citrus reticulata L.	100.29	104.55	102.69±2.18[a,b]	4.4
Melaleuca alternifolia	92.10	101.32	98.22±5.30[a,b]	8.56
Thymus vulgaris	94.91	98.12	96.89±1.73[a,b]	9.8
Origanum onites	58.55	70.17	62.99±6.28[b]	41.36

[a,b] values in the same column with different superscript letters are significantly different ($p<0.05$).

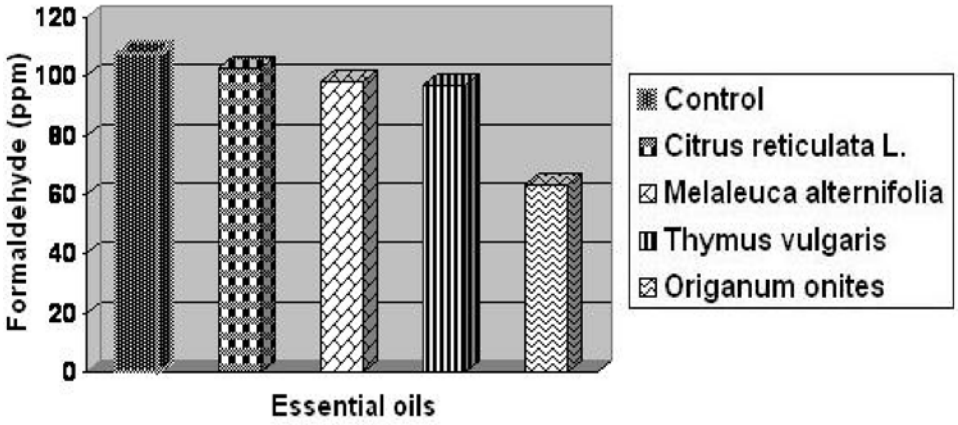

Figure 3. Amount of formaldehyde in the finished leathers.

In this study, the formaldehyde formation caused by phosphonium salt used in the white leather production was attempted to be eliminated by using essential oils in the fatliquoring process, and it was established that the essential oil of *Origanum onites* is extremely successful in the elimination of formaldehyde formation.

In the literature, there are different studies that are intended for the use of essential oils in the leather industry. In the process of leather production, in which the pH's are suitable for fungal growth such as pickle and tanning, the use of essential oil of *Origanum minutiflorum*'s as a fungicide was investigated. In the microbial tests, a number of studies were carried on the trouble-causing fungi such as *Aspergillus niger*, *Alternaria alternata*, *Penicillium rubrum* and *Trichoderma viride*; as s result of the tests, it was determined that this essential oil has anti-fungal effect and the antifungal effect increased as the concentration of essential oil increased.[25] In another study, three different kinds of *Origanum* were compared with a commercial bactericide commonly used in the leather process and it was found that *Origanum* species have much more strong bactericidal effect than the commercial bactericide.[26] *Liquidambar orientalis* Mill. var. *Orientalis* and *Origanum onites* essential oils were used in soaking process against bactericidal activity.[27] Again, there has been much research carried out, with regards to the anti-fungal and antibacterial properties of the essential oils.[28-33]

In this current research based on information from the previous studies, it was seen that the essential oil of *Origanum onites* blocks the free formaldehyde formation in the leather thanks to its antioxidative property. It was concluded that the other essential oils used in the study also inhibited; to some degree, the free formaldehyde formation in the leather, yet this

decrease was statistically not so significant. As a matter of fact, it is indicated in the literature that the essential oils in whose content high amount of carvacrol exists have antioxidant effect at high amounts.[34]

Aeschbach et al. (1994), in the study they carried out, put forward that thymol, carvacrol and 6-ginger oil as having extremely important antioxidant effects and that they are natural anti-oxidant substances which can be used instead of synthetic antioxidant additives.[34] Loziene et al. (2004) found that the extracts derived from the species of *Thymus pulegiodies* are effective in destroying free radicals.[35] Tepe et al. (2005) reported that carvacrol has strong antioxidant activity both in DPPH and in test systems of B-carotene/linoleic acid.[36] It is reported that the essential oils containing carvacrol at high levels like 78.6% have much stronger antioxidant effect than the essential oils containing carvacrol at low level.[37]

When the previous studies were carried out with regards the elimination of formaldehyde formation in leather products, the amount of free formaldehyde, which developed in leather, thanks to *Vinca rosea* and *Camellia sinensis* extracts, were decreased. Formaldehyde was eliminated at different ratios with 2 plant extracts used and *Camellia sinensis* extract exhibited better effect.[38] It was determined that waste tea powder extract decreases the free formaldehyde in leather.[39] Xuechuan et al. (2009) used collagen hydrolyzate they derived from shaving waste for formaldehyde elimination by modifying it and they prevented free formaldehyde formation.[40] Also, successful results were obtained in a number of studies conducted on the prevention of free formaldehyde formation in leather thanks to some plant extracts with antioxidant properties.[41,42] Blocking of free radicals by phenolic components is shown in Figure 4.

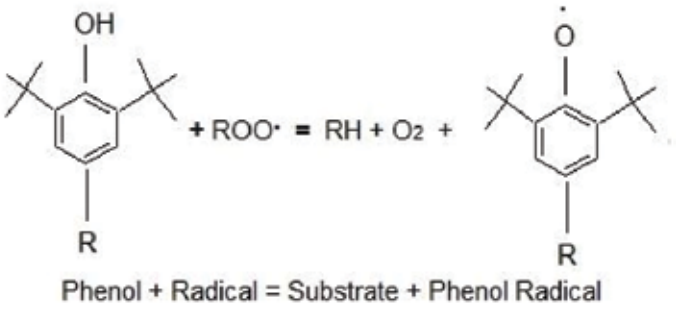

Figure 4. Reaction with primary antioxidants.[43]

It is thought that the essential oil of *Origanum onites* used in this study considerably eliminated the free formaldehyde formation in leather due to its high carvacrol content, thus its high antioxidant property. However, other essential oils besides *Origanum onites* were inefficient in blocking formaldehyde. With our research, a disadvantage of phosphonium tanning process, which is gradually becoming more accepted, was eliminated. This ecological process uses the essential oil of *Origanum onites*, containing 75.66% carvacrol;[44] the oil also has natural antioxidant properties.

Conclusion

The concept of sustainability has become important in the leather industry, as has been the case in the other branches of industry in recent times. The issues of taking precautions prior to production and solutions of the problems instead of the environmental pollution to arise in the wake of the production have come into prominence. Predicting and preventing the problems is cheaper and more effective than trying to fix them.

In the present study, the problem of formaldehyde formation resulting from phosphonium salts and the use of syntans in leather production was shown to be correctable by the use of essential oils. The use of *Origanum onites* at a rate of 2% in the fatliquoring process substantially reduced the free formaldehyde. This offers a solution of potential importance to the leather industry and likely to be the first of more studies.

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