SUPERCRITICAL FLUID EXTRACTED CHESTNUT TANNIN AS NATURAL BIOCIDE FOR SOAKING BEAMHOUSE PROCESS OF LEATHER PRODUCTION

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ABSTRACT. Microorganism activity during soaking beamhouse process of leather production is highly dangerous and should be kept under control. Some chemical components are used for this purpose to protect the hides/skins against microbial activities. Because of the banned and restricted substances due to the environmental and health risks in the recent times, natural active ingredients are considered for industrial production as sustainability. Moreover, supercritical fluid extraction as an environmentally friendly technology is applied to isolate biologically active extracts and supercritical extracted natural compounds are reported as potential antioxidant and antibacterial agent for several applications. In this study, supercritical fluid extracted chestnut tannin was used in soaking process of leather production and was determined as potential natural biocide with very good effect against microorganism activity in the process floats.

KEY WORDS: leather, soaking, chestnut, biocide, bactericide, sustainability

UTILIZAREA TANINULUI DIN CASTAN EXTRAS ÎN FLUID SUPERCRITIC CA BIOCID NATURAL ÎN PROCESUL DE ÎNMUIERE A PIELII

REZUMAT. Activitatea microorganismelor în timpul procesului de înmuiere a pielii este foarte periculoasă și trebuie ținută sub control. Unele componente chimice sunt folosite în acest scop pentru a proteja pieile împotriva activității microbiene. Datorită substanțelor interzise și restricționate care prezintă riscuri pentru mediu și sănătate în ultima vreme, ingredientele active naturale sunt luate în considerare în producția industrială pentru sustenabilitate. Mai mult, extracția în fluide supercritice, fiind o tehnologie prietenoasă cu mediul, este aplicată pentru a izola extractele active din punct de vedere biologic, iar compușii naturali extrași în fluid supercritic sunt raportați ca potențiali agenți antioxidanți și antibacterieni pentru mai multe aplicații. În acest studiu, taninul din castan extras în fluid supercritic a fost utilizat în procesul de înmuiere a pielii și a fost considerat potențial biocid natural cu efect foarte bun împotriva activității microorganismelor în procesele umede.

CUVINTE CHEIE: piele, înmuiere, castan, biocid, bactericid, sustenabilitate

UTILISATION DU TANIN DE CHÂTAIGNIER EXTRAIT PAR UN FLUIDE SUPERCRITIQUE COMME BIOCIDE NATUREL DANS LE PROCESSUS DE TREMPAGE DE LA PEAU

RÉSUMÉ. L'activité des micro-organismes pendant le processus de trempage du cuir est très dangereuse et doit être maîtrisée. Certains composants chimiques sont utilisés à cette fin pour protéger les peaux contre l'activité microbienne. En raison des substances interdites et restreintes présentant des risques pour l'environnement et la santé ces derniers temps, les ingrédients actifs naturels sont envisagés dans la production industrielle pour la durabilité. De plus, l'extraction par fluide supercritique en tant que technologie respectueuse de l'environnement, est appliquée pour isoler des extraits biologiquement actifs, et des composés naturels extraits par fluide supercritique sont signalés comme des agents antioxydants et antibactériens potentiels pour plusieurs applications. Dans cette étude, le tanin de châtaignier extrait par un fluide supercritique a été utilisé dans le processus de trempage de la peau et a été considéré comme un biocide naturel potentiel avec un très bon effet contre l'activité des micro-organismes dans les processus humides.

MOTS CLÉS : cuir, trempage, châtaignier, biocide, bactéricide, durabilité

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INTRODUCTION

Leather production is an ancient technology that has been described as man's first manufacturing process. It comprises the transformation of raw putrescible animal hide/skin into useful materials [1, 2].

Soaking is the first and important beamhouse process of leather production and applied to gain water content to the hides/skins and soften them for preparation of the next operations. In soaking applications, several synthetic antimicrobial agents are mostly used to prevent the hides/skins against microbial activity and growth. On the other hand, considering the toxicity of these chemical components especially in the recent years, it has become an important focus of leather industry players. In the recent scientific studies, natural antimicrobial products were also reported for leather processing steps. Aloe vera was reported as antimicrobial agent for fatliquoring process of leather [3]. Quebracho, mimosa, gall-nut and chestnut vegetable tannins were used as biocide in soaking process of leather as well [4] owing to their antimicrobial properties [5, 6].

Vegetable tannins are complex and heterogeneous group of polyphenolic secondary metabolites of higher plants with molecular weights between 500 and 20,000 Da and soluble in water and polar organic solvents [7]. Although almost all plants contain tannins, only few species have sufficient amounts to be of commercial importance. Many of the commercially most significant tannin materials originate in tropical or sub-tropical climes [8].

Chestnut is a kind of fruit cultivated in Asia, Europe and America continents of the Northern Hemisphere and in South America partially. Asia is the most important region and China is the leader of Asia. Southern Europe and Turkey are the second important regions [9]. According to FAO statistics [10], the world's largest producers of chestnut are China (1,000,000 tons), South Korea (75,000 tons), Italy and Turkey (55,000 tons), and Japan (26,000 tons) [11].

Chestnut shells are the waste product from chestnut food processing and have not been fully utilised to date. The glace chestnut industry produces each year a huge amount of chestnut shells as waste [12]. The shell, which represents around 10% by weight of the chestnut, is removed in the peeling process. Chestnut wood tannins are also of the most common hydrolyzable tannins used in leather industry as vegetable tanning agent [13]. Chestnut bark is rich in tannic acid which is a specific form of tannin, a type of polyphenol [4, 14].

The extraction processes of tannins from natural matrix are currently performed by empirical methods [15, 16], especially for the leather industry. Extraction process is influenced by the chemical nature of the tannins and extraction methods have great influence on the yield, concentration and tanning phenolic content of the extract [17].

Supercritical fluid extraction (SFE) is a technique usually employed for the extraction of relatively high polar compounds by utilizing solvent extraction at elevated pressures and controlled temperatures [18-20]. It serves as a substitute to overcome the drawbacks encountered in other extraction methods such as long extraction time and high solvent consumption in conventional solvent extractions (CSE). SFE also provides advantages as increasing the mass transfer, lowering the costs and being an environmentally friendly solvent. It is called as green separation technology [21].

Supercritical extraction is a superior "green chemistry" technology which is used for the isolation of herbal extracts without the need for organic solvents. The most striking advantage is its low operating temperatures, making it possible to preserve thermo-degradable compounds and easily separate extracts. The superiority of this technology is also selective and reflected in the possibility of modeling the conditions for the production of standardized extracts with desirable attributes. It has been shown that some SFE herbal extracts have extraordinary antibacterial activity [22-25]. In this perpective, our study aims to put forward the effect of supercritical fluid extracted chestnut tannin as natural biocide in soaking process of leather.

MATERIALS AND METHODS

Materials

Chestnut shells as an industrial waste were supplied from a chestnut sweet producing food company in Turkey. Thick dry-salted domestic raw sheepskins were used as the leather material during soaking process.

Extraction of the Chestnut Tannin with SFE/Methanol and SFE/Water Method

Supercritical CO_2 extraction was carried out at 70°C under 70 bar during 60 min. (20% co-solvent: methanol) (SFE/methanol method). Water/CO_2 binary pressurized fluid system as SFE was applied with the parameters of S/F=100 with 100 bar during 3 h and at 80°C (SFE/water method) optimized in the previous study by Onem *et al*. (2015) [26] inside high pressure view cell as shown in Figure 1 below.

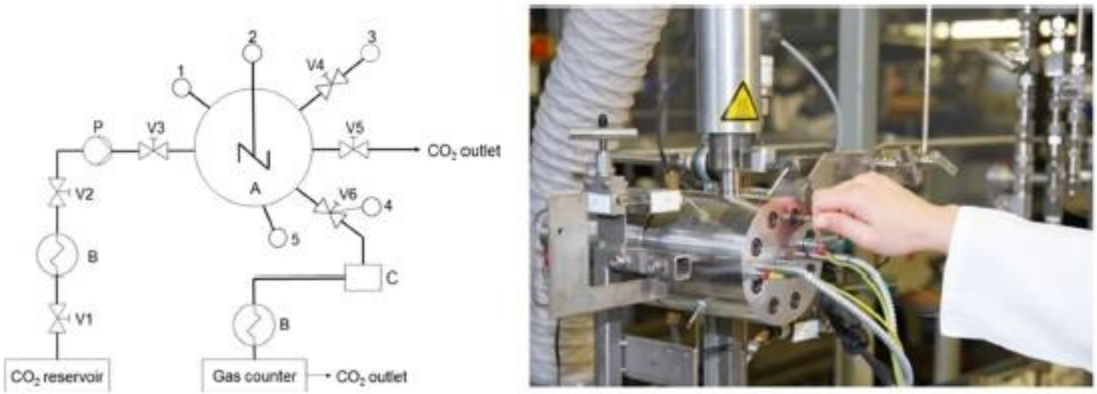

Figure 1. Experimental set-up – high-pressure view-cell for SFE operations

Extraction Yield

10 mL of samples were taken from the SFE extract solution into clean, dried and weighed glass dishes, in order to determine amount of carried solid matter into extraction medium. The extracts were dried in a hot-air oven (100±2°C) until all the water evaporated and only the solid matter was left. The dishes were then cooled in a desiccator and weighed, in this way, the amount of solid matter was determined.

Tannin Content Analysis (Hide-Powder Method)

The tanning phenolic contents of the produced chestnut extracts were determined with hide-powder method according to the official standards of SLC 114, 115, 116 and 117.

Pre-soaking and Soaking Process of Sheepskins with Natural Biocide

Pre-soaking process was applied just with water during 4 hours, then drained. The aim of the pre-soaking process is to allow the skins to take water and to remove the undesired contaminations without any protective additions to the float. The float was drained after pre-soaking, filled water again, then 1.5% SFE chestnut extract was added into the soaking bath for antimicrobial agent against bacteria. Table 1 provides the applied soaking process recipe for production.

Table 1: Applied pre- and soaking process recipe for production of the skins

Process	%	Chemicals	Temperature (°C)	Time (hour)	Remarks
Pre-soaking	500	Water	25	4	
					Drain
Soaking	500	Water	25		
	1.5	Chestnut extract			
	0.5	Soaking agent		48	
					Drain

In order to determine the bacterial growth in soaking, samples were taken from the float at the end of 4, 24 and 48 hours and the microorganisms in the float were counted.

Bacterial Counting Method in Soaking Float

1 mL soaking float sample was obtained from each process to determine the total aerobic bacteria numbers at the end of 4h, 24h and 48h. Floats were introduced into the analysis tubes having 9 mL physiological dilution water. Then, floats were diluted by 10^1 to 10^6. After serial dilutions, samples were prepared according to the pour plate method. The petri dishes were incubated during 48h in owen at 37°C. Calculations of bacterial counts were carried out in petri dishes containing 30-300 bacterial numbers. Following the 48h, total bacteria numbers in each soaking float were calculated from the colony numbers. After that, results were presented as colony forming units per millilitre (cfu/mL).

RESULTS AND DISCUSSION

According to the SFE green separation way applied to the chestnut barks, extraction yield and tannin contents of chestnut extracts produced in different SFE extraction conditions for the SFE/methanol and SFE/water methods were given in Table 2 by comparison.

Table 2: Extraction yield and tannin contents related to SFE methods

Extracts	Extraction yield (%)	Tannin content (%)
SFE/methanol method	35.23	60.10
SFE/water method	25.65	55.35

The maximum extraction yield from chestnut shell with the value of 35.23% was obtained in parameter of SFE/methanol method. Addition of methanol into supercritical CO_2 was increased the solubility and extraction yield was increased. SFE/Water method provided 25.65% extraction yield.
Tannin content of the extract was also higher in SFE/Methanol method compared to the SFE/Water method with 60.10% and 55.35% values. SFE/Methanol way was more advantageous according to the extraction and tannin yield.

Table 3 indicates the biocide application results obtained for 4 h, 24 h and 48 hours soaking time in float with SFE chestnut extract application.

Table 3: Biocide application results obtained for 4 h, 24 h and 48 hours soaking time in float

Biocide application	Colony numbers (cfu/mL), 4h (pre-soaking float)	Colony numbers (cfu/mL), 24h (soaking float)	Colony numbers (cfu/mL), 48h (soaking float)
Control float, without application	4.25×10^7	1.78×10^7	2.56×10^7
Chestnut extract (SFE/Methanol)	-	4.46×10^5	1.02×10^6
Chestnut extract (SFE/Water)	-	1.17×10^6	0.91×10^7

Added chestnut extract into the soaking bath had the superior effect on the microorganisms as biocidal application. Microorganismal activity decreased from 1.78×10^7 to 4.46×10^5 by adding the vegetable extract compared to the control soaking float at the end of 24 h by SFE/Methanol method. The beginning ratio was 4.25×10^7 during pre-soaking process after 4 h. At the end of 48 h, bacterial growth decreased from 2.56×10^7 to 1.02×10^6 by chestnut extract addition in the float compared to the control soaking process at the end of 48 h via SFE/Methanol method. Bacteria number was 1.17×10^6 and 0.91×10^7 during 24 and 48 h, relatively by SFE/Water extraction way. SFE/Methanol method was provided better effect on antibacterial activity than SFE/Water application according to the Table 3. On the other hand, both SFE techniques were determined as effective to block the bacterial growth for chestnut extract as natural biocide. This is because of the high content of the tannic acid in chestnut structure and extracted more active compounds via supercritical extraction way for chestnut compound. It is reported that chestnut bark is rich in tannic acid which is a specific form of tannin, a type of polyphenol and has a potential as biocidal effect [4, 14]. Moreover, supercritical fluid extraction method was reported in many studies that SFE provided more antibacterial and antioxidant effect to the extracted compounds compared to the conventional extraction ways [22, 27-30]. By this way, active ingredients are separated with environmentally friendly green separation way and have superior activity properties. Thus, waste materials have gained economical perspective for the industrial applications. There are also some studies on antibacterial activity of extracted tannins for textile industrial applications which is similar and close to the leather sector. Zhang *et al*. (2014) indicated the natural dye extracted from Chinese gall for the application of antibacterial activity on the wool fabrics [31].

Results proved the superior separation effect of SFE techniques for the both methanol and water ambient and effective results as potential natural biocidal agent of chestnut extracts produced.

CONCLUSIONS

In this study, a waste product, chestnut shells from food industry, was converted to a usable product with an economic value, a natural biocide used in soaking beamhouse process of leather production. 35.23% extraction and 60.10% tannin yield were obtained after SFE green separation way in carbon dioxide and methanol ambient. Environmentally friendly separation application was successfully applied for chestnut tannin. Moreover, the biocidal properties of the extracts in SFE were found acceptable and can be enhanced with different techniques for further studies. Considering that, in recent years there is a demand on usage of ecologic and natural products in the world, usage of environmentally friendly vegetable bactericidal agents and producing natural leathers can be more advantageous to gain the admiration and attention of consumers towards sustainable production, industry, life and world.

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