Utilization of Chromium-tanned Leather Solid Wastes in Microencapsulation

by

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Abstract

In this research, solid collagen-based protein hydrolysate was isolated from chromium-tanned leather wastes and its chemical properties were determined. After that, the use of collagen hydrolysate (CH) was investigated as a polymeric wall material in the microencapsulation process. The effects of variations in concentrations of CH, lavender oil (LO) and glutaraldehyde (GA), which were used during the microencapsulation process, on the oil load of microcapsules, oil content, encapsulation efficiency and release rate of oil were determined. The morphological structure of the microcapsules was investigated using optical and scanning electron microscopes (SEM). It was determined by FTIR studies that there was no evidence for any significant interaction between CH and LO.

Resumen

En esta investigación, un hidrolizado colágenico sólido se aisló de desechos de cuero al cromo y sus propiedades químicas fueron determinadas. Luego, el uso del hidrolizado colágenico (CH) fue investigado como un material polimérico para utilizarse como componente estructural en los procesos de micro-encapsulación. Los efectos atribuibles a la variaciones en concentraciones de CH, aceite [esencial] de Lavanda (LO) y glutaraldehído (GA) que se utilizaron en el proceso de micro-encapsulación, sobre la carga aceitosa presente en las micro-cápsulas, el contenido de aceite, la eficiencia en encapsulación, y la velocidad de descarga del aceite fueron determinadas. La estructura morfológica de las micro-cápsulas se investigó por medio de microscopia electrónica por barrido (SEM). Se determinó también por medio de estudios FTIR que no hubo apreciable evidencia de interacción entre CH y LO.

Introduction

Chromium was discovered near the end of the 18th century. Its use spread quickly and it is now one of the most important tanning materials in the world as it imparts near perfect properties to leathers at a low price.[1] Chromium-tanned leather accounts for over 90% of world leather production.[2] Treatment of chromium-tanned leathers by mechanical processes such as shaving, buffing, splitting and trimming results in chromium-tanned leather solid wastes (so-called shavings).[3] Every year, over 500 000 metric tons of shavings are generated in the world.[4] These collagen-based solid leather wastes contain 3-6% chromium III and require special attention because chromium III can oxidize to toxic chromium VI which may endanger ecological life and human health.[5,6,7] The wastes are discharged to landfills, which is legal but very expensive.[8] In addition, shavings have become an important problem for the leather industry in the last few years because of the limited and decreasing availability of landfills in terms of size and number.[7] However, the marketability of a product of tannery wastes with a potential application could encourage the industry to recycle them instead of dumping them into landfills.[9]

There are many studies concerning modified or alternative leather processing techniques or processes to reduce the solid wastes from the leather industry.[10,11] However, these studies have not provided a viable solution because they are not economical and cannot achieve the desired properties for leather in the same way as chromium, and for this reason chromium tanning still keeps its place in the leather industry.[12] For these reasons, chromium and protein isolated from shavings have recently been studied by researchers in many parts of the world to evaluate their potential uses in different areas.[13] However, these collagen-based protein products have not seen adoption in many potential areas such as the food, medical and cosmetic industries because of the presence of the bound chromium. Hence researchers have focused on the re-usability of these protein products in leather processing or other areas of industry that do not put human health at risk.

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Studies on lavender essential oil have been reported due to its applicability in odor industry. In addition it can be used as a pesticide and an antimicrobial agent.[14] Essential oil, like LO, are instable compounds. They can suffer volatilization and environmental damages. Microencapsulation of essential oils is a feasible alternative to increase the stability of these compounds for improving their efficiency. The studies regard the microencapsulation of volatile essential oils used for the purpose of durable aroma finishing in the textile industry especially.[15] Cabeza et al. demonstrated the potential application in microencapsulation of gelatine isolated from chromium-containing solid tannery waste.[9] However, we have found no reports in the literature describing the production of microencapsulation using CH obtained from shavings. In this study, we investigated the possibility of using CH obtained from shavings, as a polymeric wall material to microencapsulate LO using the technique of coacervation, which has increased in importance in the last few years as the technology developed.[16] We chose LO as a core material due to its eco-friendly and biodegradable nature and its great usefulness and application as an essential oil.[17]

MATERIALS AND METHODS

Materials

Magnesium oxide (Merck, Germany), Rodazym ML (Rohm, Darmstadt), glutaraldehyde 25% w/v (Merck, Germany), anhydrous sodium sulphate (Merck, Germany), silicone antifoam 30% w/v (Sigma-Aldrich, USA), Tween 80 (Sigma-Aldrich, USA), lavender oil (Sigma-Aldrich, USA), acetic acid (Merck, Germany) were used as received, without further purification. Shavings were obtained from a local tannery in Izmir, Turkey. Besides this, other reagents used (Sigma-Aldrich, USA), were of analytical grade. Ultra-pure water was used throughout the study.

Methods

Preparation of collagen hydrolysate

Chromium-tanned leather wastes were soaked in water five times their weight and treated with 4% MgO at 65°C for 30 min. In the following step, the enzyme Rodazym ML was added to this solution at a concentration of 1% (w/v) and the mixture was digested for four hours. This mixture was then filtered hot through Whatman # 1 filter paper to separate the chrome cake, and the liquid CH was dried to powder form in a spray drier (Niro Atomizer brand).[12]

Phase separation behaviour of collagen hydrolysate

In order to determine the phase separation temperature reached with the addition of the anhydrous sodium sulphate, several experiments were performed with CH. To that end, an Erlenmeyer flask containing a known quantity of CH was immersed in a temperature-adjusted water bath kept at 5°C. Anhydrous sodium sulphate (10% w/v) was added under continuous mixing gradually. The temperature of the water bath was then increased by 1ºC per minute, and the temperature at which the phase separation began and the proportions of sodium sulphate solution to CH were recorded. The minimum temperature and ratio of CH to sodium sulphate at which phase separation occurred were 40°C and 1:6.

Preparation of microcapsules

The microcapsules were produced by slightly modifying the method in Maji et al.[18] After inserting 50 ml CH solution (6-10% w/v) into the reaction vessel (250 ml working volume) placed in a temperature-controlled water bath, the internal temperature of the reaction vessel was adjusted to 30°C. The CH solution in the reaction vessel was stirred at 1200 rpm with the help of a mechanical stirrer. One drop of silicon-based antifoaming agent was added, and the internal temperature of the reaction vessel was raised to 40°C. Then, the LO (9-17 ml) used as the active substance was added into the reaction vessel. Coacervation was achieved by gradual addition of aqueous sodium sulphate solution (10% w/v) for about 90 min.

The internal temperature of the reaction vessel was kept at 40°C for 30 minutes more, after which it was decreased to 12°C. The collagen-hydrolysate-walled microcapsules were crosslinked through the slow addition of 16.67% methanol, 5% acetic acid, 0.17% sulphuric acid, and GA solution containing 25% GA at the stirring speed of 800 rpm with the mechanical stirrer and the internal temperature of the reaction vessel was raised to 40°C again, and after continuous stirring for 6-8 hours, was decreased to room temperature. Then, the microcapsules were filtered and rinsed in 0.1% Tween 80 solution.

The rinsed microcapsules were transferred to stainless steel trays and frozen at -30ºC for 24 hours, after which they were dried for 24 hours in a freeze dryer (Christ ALPHA 1-2 LD) at -30°C with 100 mTorr vacuum, and stored at 4°C in an amber-coloured glass bottle.[19]

Determination of some chemical characteristics of collagen hydrolysate

In order to evaluate the quality of CH, the following processes were performed: determination of nitrogen and hide substance (protein) (IUC 10), determination of sulphated total ash and sulphated water insoluble ash (IUC 7), determination of chromic oxide content (IUC/8:4) and determination of volatile matter (moisture) (IUC 5).[20] The chrome content of the CH was measured with Perkin Elmer Optima 2100 DV ICP-OES.

Calibration curve of lavender oil

A known concentration of essential oil in distilled water containing 0.3% Tween 80 was scanned in the range of 200–800 nm by using a UV-VIS spectrophotometer. The sharp

peaks caused by the sequential concentrations of 0.05–5 g/100 ml LO in 0.3% Tween 80 at 341 nm (λ_{max}) were recorded. Unknown concentration of LO was determined by extrapolating the absorbance values from the calibration curve prepared by plotting the known LO concentration versus its corresponding absorbance values.

Determination of the oil load, oil content and encapsulation efficiency

After crushing the microcapsules completely in a porcelain mortar, 1.0 ± 0.001 g of them was placed in a volumetric flask. 100 ml of 0.3% Tween 80 (in distilled water) was poured into the volumetric flask, and the flask was shaken all night in a shaker. The encapsulation efficiency (%), oil content (%), and the oil load (%) were determined using the calibration curve and the following formulae.[18, 21, 22]

Oil load (%) = w_2/w_3 x 100

Oil content (%) = w_1/w x 100

Encapsulation efficiency (%) = w_1/w_2 x 100,

where w=weight of microcapsules; w_1=actual amount of oil encapsulated in a known amount of microcapsules; w_2=amount of oil introduced in the same amount of microcapsules; w_3=total amount of polymer used including crosslinker.

Oil release studies

The rate of LO released from the microcapsules was determined using a UV-VIS spectrophotometer (UV 1601 Shimadzu). A known quantity of microcapsules was placed in the Erlenmeyer flask with 0.3% Tween 80 and shaken at room temperature. After filtering the 5 ml microcapsule-Tween 80 mixture, oil release rate at 341 nm (λ_{max}) was measured. In order to keep the volume of the microcapsule-Tween 80 mixture constant, the 5 ml 0.3% Tween 80 solution was transferred back into the Erlenmeyer flask after the UV measurements.[18, 21, 22] The experiments were repeated three times.

Optical and scanning electron microscopy studies

Optical images of the crosslinked microcapsules were acquired using an Olympus BX 50 U-SDO optical microscope connected to a digital camera, and the electron microscope images of the gold-palladium coated microcapsules were obtained with a Philips XL-30S FEG scanning electron microscope.

FTIR study

FTIR spectra at a wavelength range of 4000-400 cm^{-1} of the CH, LO and microcapsules used in the microencapsulation process were acquired in a Perkin-Elmer Spectrum BX device that had ATR attachment.

RESULTS AND DISCUSSION

Some chemical characteristics of collagen hydrolysate

Table I shows the chemical analysis results of the CH. The most important parameters affecting the assessment areas of the CH are the amounts of protein, ash, chromium, and moisture.[23] In our research, the amounts of protein and ash in the CH were found to be 88 ± 0.57 % and 12 ± 0.1 % respectively. These results are similar to the previous research results.[4, 24, 25] The chromium content of the CH, on the other hand, was found to be 4.58 ± 0.01 ppm. This value is negligible in terms of the assessment of CH in areas not related to the human health.[7, 13, 26, 27] The moisture content of the spray-dried CH was found to be 8.2 ± 0.14 %, and this value is comparable to values acquired in previous studies.[12, 13, 24]

TABLE I
Chemical characteristics of collagen hydrolysate

Chemical Analyses	Mean±SD
Hide substance (%)	88.0 ± 0.57
Ash (%)	12.00 ± 0.09
Chromium (ppm)	4.58 ± 0.01
Moisture (%)	8.22 ± 0.14

The effect on microcapsules of variations in oil concentration

The effect of variations in the concentration of the LO used in microencapsulation on the microencapsulation parameters and release rate are given in Table II and Figure 1. These reveal that the encapsulation efficiency decreased with the increase in concentration of the LO used in the microencapsulation process while the oil content (%), oil load (%), and the oil release rates (%) of the microcapsules increased. A possible reason for the decrease in encapsulation efficiency may be an increase in oil loss during encapsulation of the oil, resulting from the amount of oil used in excess could result to high oil lost.[18, 28]

The increase in the oil release rate may be the result of a decrease in the wall thickness of the microcapsules caused by the quantity of the CH used as wall substance being kept constant while the LO amount was increased (Figure 1). Also, the dispersion power of the stirrer decreases along with an increase in the oil concentration, and the wall substance can isolate bigger oil droplets. The decrease in microcapsule wall thickness caused by this may shorten the diffusion path of the oil and thus increase the oil release rate.[18, 21, 28, 29] A similar type of results are reported in the literature.[30, 31, 32]

TABLE II
Effects of variation of oil loading, collagen hydrolysate and glutaraldehyde concentration on the behaviour of microcapsules*

Sample particulars			Oil load (%)	Oil content (%)	Encapsulation efficiency (%)
CH (g)	GA (ml)	LO (ml)	Mean	Mean ± SD	Mean ± SD
5	15	9	100.03	44.71 ± 1.19	86.78 ± 2.25
5	15	11	131.99	45.23 ± 0.63	80.46 ± 1.17
5	15	13	175.10	47.33 ± 0.94	77.21 ± 1.78
5	15	15	208.74	57.86 ± 1.12	72.54 ± 1.92
5	15	17	242.37	64.65 ± 0.71	71.86 ± 1.43
3	7	9	269.00	51.43 ± 0.68	72.87 ± 0.99
4	7	9	133.48	49.11 ± 0.58	90.64 ± 1.12
5	7	9	75.15	27.51 ± 0.59	86.71 ± 1.90
4	2	9	182.19	37.42 ± 0.80	54.96 ± 1.20
4	3	9	161.07	39.46 ± 0.92	60.75 ± 1.41
4	4	9	157.53	42.62 ± 0.95	64.44 ± 1.50

* CH: (3-5 g); GA: (2-15 ml); LO: (9-17 ml); water: 50 ml; temperature: 30°C.

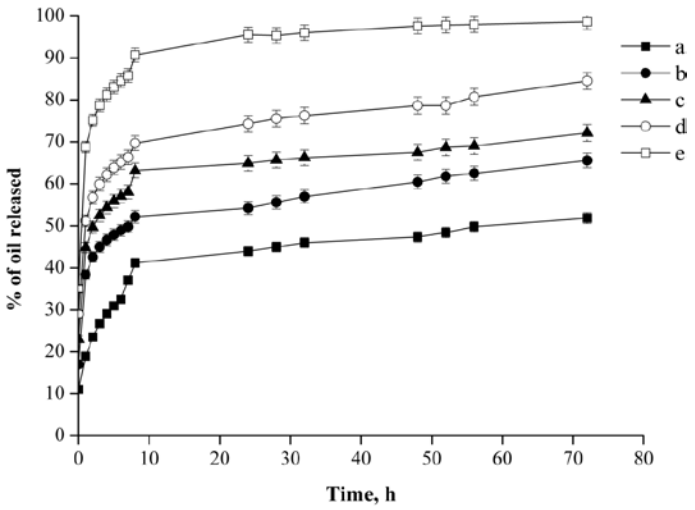

Figure 1. The effects of variation of oil loading on the release rates: (a) CH 5 g, GA 15 ml, LO 9 ml; (b) CH 5 g, GA 15 ml, LO 11 ml; (c) CH 5 g, GA 15 ml, LO 13 ml; (d) CH 5 g, GA 15 ml, LO 15 ml; (e) CH 5 g, GA 15 ml, LO 17 ml.

Effects of variation of hydrolysate concentration

It was found that the oil load (%) and oil content (%) values of the microcapsules decreased as the CH concentration rose (Table II). The findings of the present study concerning the effects of changes in concentration of the wall substance on the oil load and oil content are similar to the findings of other researchers.[21, 28, 33]

It was observed that, as the CH concentration increases, the encapsulation values of the microcapsules accordingly increase first and then fell (Table II). As the concentration of the wall substance was increased during the microencapsulation process, it was possible to encapsulate all oil droplets added to the system. Excess CH remaining in the system, on the other hand, can make the walls of the microcapsules thicker and causing the encapsulation efficiency to decrease after peaking at a certain concentration.[18]

As seen in Figure 2, the higher the CH concentration, the lower the rate of oil release from the microcapsules. The reason for this decrease may be due to the increase in the concentration of the polymeric wall substance used in the microencapsulation process and an increase in the wall thickness of the microcapsules.[34, 35] Our findings concerning the effect of the increase in the CH concentration on the oil release from the microcapsules were comparable to the other researchers' findings.[18, 21, 34, 35]

The effect of variations in glutaraldehyde concentration

The results relating to variations in GA concentration are given in Table II and Figure 3. An examination of the values obtained reveals that, as the GA concentration increased, the oil content increased while the oil load values of the microcapsules decreased. In addition, it was observed that the increase in the GA concentration led to an increase in encapsulation efficiency values (Table II). Concerning a

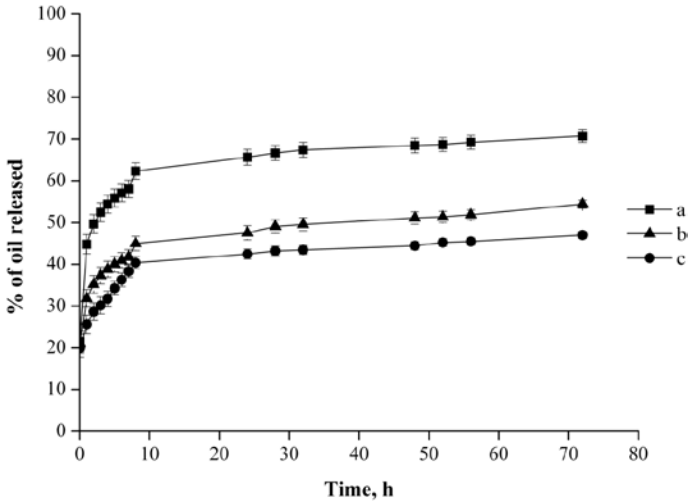

Figure 2. The effect of variation of CH concentration on release rate: (a) CH 3 g, GA 7 ml, LO 9 ml; (b) CH 4 g, GA 7 ml, LO 9 ml; (c) CH 5 g, GA 7 ml, LO 9 ml.

reason for this increasing encapsulation efficiency, (in %), Maji et al. pointed out that the increase in the oil retention capacity of the microcapsules may be due to the reaction of the wall substance to the crosslinker.[18] Similar types of observation are reported in the literature.[21, 32]

In our research, it was found that the release of the LO from the microcapsules obtained decreased as the GA concentration increased (Figure 3). This behaviour of the microcapsules arises from the fact that the wall becomes denser as the crosslinking grade increases.[18] It can be seen that the effect of the GA variations on the rate of oil release from the microcapsules is similar to findings in the literature.[29, 35, 36, 37]

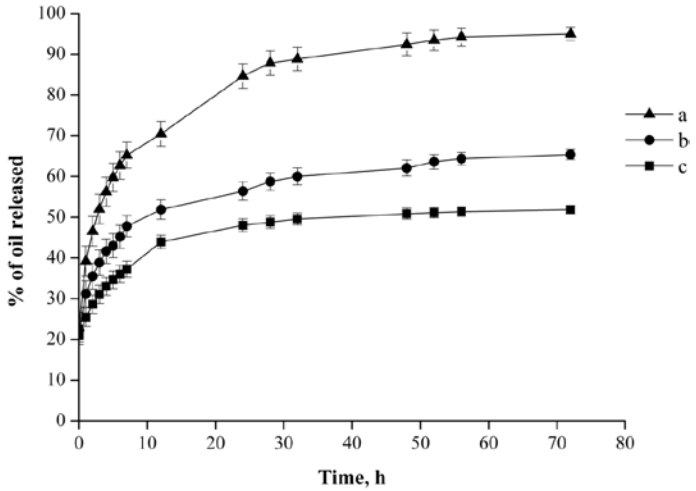

Figure 3. The effect of variations in crosslinker on the release rate: (a) CH 4 g, GA 2 ml, LO 9 ml; (b) CH 4 g, GA 3 ml, LO 9 ml; (c) CH 4 g, GA 4 ml, LO 9 ml.

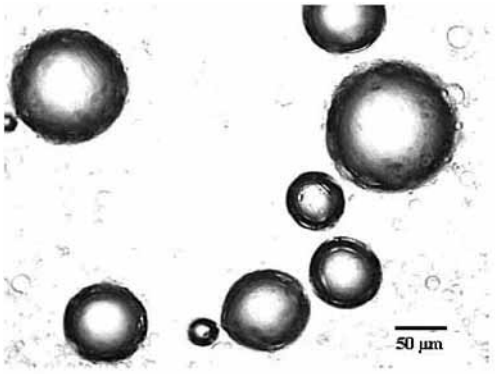

Figure 4. Optical microphotograph of microcapsules (100X).

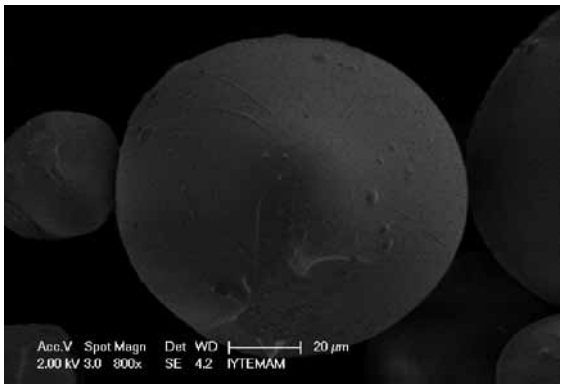

Figure 5. Scanning electron microphotograph of microcapsules.

Optical and scanning electron microscopic studies

An optical microphotograph of the microcapsules crosslinked with GA is shown in Figure 4, and an SEM microphotograph in Figure 5. The optical microscope and SEM photographs reveal that the microcapsules have a spherical form. These results are consistent with reports from other researchers.[18, 34, 35, 37, 38]

Fourier Transform Infrared (FTIR) study

The basic components of the LO are linalyl acetate, linalool, lavandulol, and alcohol isobutyrate. These components constituting the LO include characteristic molecule groups such as –COOR and C=O, and their characteristic absorption peaks lead to vibration in the infrared spectrum at 1740-1755 cm^{-1}.[33] In our study, it was found that the strong absorption peak of the LO was at the 1753 cm^{-1} wavelength in the FTIR spectrum (Figure 6). And it was also found that the absorption peaks of the CH were at 3300, 1647, and 1537 cm^{-1} (Figure 6). It was confirmed that the FTIR absorption peak values in our research were close to those found in the literature.[39]

When we examine the FTIR spectrum (c) of the CH microcapsules containing LO as shown in Figure 6, we see that the carbon stretching bands of the LO remain unchanged at a wavelength of 1753 cm^{-1}. Therefore, it was concluded that the LO was encapsulated successfully by the CH and thus the

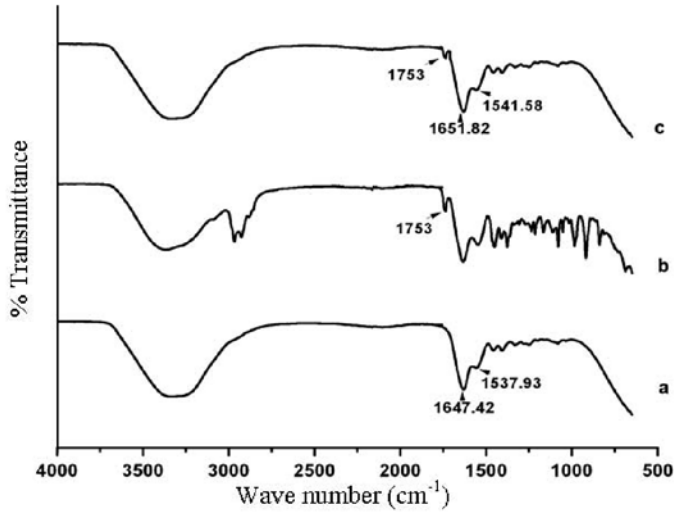

Figure 6. FTIR spectra of CH (a); LO (b) and microcapsules (c)

CH microcapsules containing LO were obtained. It was also found that there was no interaction evident between the LO and the CH polymer.

CONCLUSION

It was possible to encapsulate LO successfully in a CH wall with the help of GA. The release rate was dependent on oil content, crosslinking density and encapsulating polymer concentration. Consequently, the use of CH obtained from solid wastes of chromium-tanned leather in the production of microcapsules containing LO has transformed a substance into a substrate meeting a different ecological use.

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