Study on the determination methods of free fatty acids in Leather

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Abstract: The content of free fatty acids was researched by qualitative and quantitative methods. The cable-extraction method was used to test six different types of leather sample in which the dichloromethane was as extraction agent. After the extraction was methylesterified, it was extracted again by petroleum ether. Then the color and titration method were used to quantitatively analyze the extraction. Meantime, the effect of different decolorization ways on the quantitative analysis was studied. The properties of extraction products were characterized by GC-MS instrument. The results showed that the main components of free fatty acids in leather were oleic acid, palmitic acid, stearic acid and a few linoleic acids. If the w (ethanol) was 80% to 85% of the solution was as depigmenting agent, the effect would be better.

Key words: Leather products, free fatty acid, content, determination method

1 Introduction

The vast majority of natural oils and fats in raw hides have been removed in tanning process. In order to increase lubrication and mobility between collagen fibers, improve the fullness and softness of leather, increase wear resistance and waterproofing of leather and so on, a certain amount of grease should be added. However, in the storage and use of leather goods, some grease will have a series of chemical changes by the effect of light, heat, micro-organisms. The change is called lipid oxidation. The free fatty acids, ketones, other aldehydes and peroxides will be produced during the oxidation process of oils and fats. As a result, some environmental problems, such as the spew, color changing, smell, hexavalent chromium will appear, and it is unbeneficial for human body.[1-5] The content of free fatty acid is an important parameter for reflecting lipid oxidation. Therefore, in order to control the quality of fatliquor and leather products, timely adjust the leather processing technology, an accurate and convenient determination method of free fatty acid content in leather products has become a problem requires an urgent solution.

The determination methods of free fatty acid include titration, colorimetry, chromatography and infrared method.[6-12] And the most common and convenient method is titration, but there are some problems during determining the free fatty acid in leather products, such as accurately and completely extracting free fatty acids and eliminating interference of leather colors. A series of pilot studies on eliminating the color interference are carried out. The purpose is to seek the best way to eliminating color interference. As a result, a reliable method to accurately and conveniently determine the content of free fatty acid in leather products is to provide.

2 Experimental

2.1 Materials and apparatus

R-202 rotary evaporator was provided by Shengshen biological technological Co., Ltd. in

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Shanghai; XK95-B vortex mixer was supplied by Kangtai medical equipment factory in Jiangyan City; GC 7890A/5975MS GC-MS was offered by An Jielun in America.

All reagents were analytical reagents and provided by Ke Miou reagent Factory. No.1 sample was brown pig skin split and its oil cream was serious; No.2 sample was black cowskin split and had moderate degree of oil cream; No.3 sample was light gray pig skin split and had serious greasiness and smell; No.4 sample was pink pig skin; No.5 sample was pickled pig skin; No.6 sample was black sheep skin and had no oil cream.

2.2 Methods

2.2.1 Preparation of reagents

(1) Alcohol sulfate：230mL anhydrous methanol was added into a dry flask, and then 2.0mL concentrated sulfuric acid was also placed into it. When mixed together, they were placed in a brown bottle.

(2) Triethanolamine－cuprum solution：12.0785g Copper nitrate trihydrate was weighted and prepared to be 0.1mol/L copper nitrate solution. 14.919g triethanolamine was prepared to be 0.2mol/L triethanolamine solution. During using the two solutions was mixed together with the same volume.

⑶ 0.1mol/L sodium hydroxide was prepared and calibrated according to the standard method.

(4) 0.1mol/L magnesium sulfate solution：12.4235g magnesium sulfate heptahydrate was solved in 500mL volume flask.

(5) 0.01mol/L and 0.05mol/L sulfuric acid solution.

(6) Ethanol solution with different concentration：5mL, 10mL, 15mL, 20mL and 25mL water was added in volume flask，and then increased by ethanol.

2.2.2 Extraction of Dichloromethane extract

The lipid was extracted according to reference 13, and then it was solved in 50mL volume flask.

2.2.3 Chromatography determination of free fatty acids

GC conditions: sampling system: diffluence, diffluence ratio was 100:1; carrier gas: helium; sample load: 1μL; capillary column: DB-5ms, 30m×0.25mm (inner diameter)×1μm (thickness); gas flow: 1.0mL/min; injection temperature: 280℃; GC-MS interface temperature: 250℃; column temperature: 100℃, keeping for 1min, and then raising temperature to 280℃ at the speed of 10℃/min, keeping for 10min.

MS conditions: ionization mode: EI; ionization energy: 70eV; detection mode: completely scan; quality scanning range: (50-400)amu; ion source temperature: 25 ℃; Quadrupoles temperature: 200℃; Solvent delay: 5min.

2.2.4 Depigment method

（1）Depigmented by water and sulfuric acid: ①10mL dichloromethane extract liquid was placed in separating funnel, and then 30mL water was added into. When they separated, the dichloromethane extract liquid was dropped into another separating funnel. After washed with water, the color change was observed. ②Three 20mL dichloromethane extract were removed dichloromethane by rotary evaporation, and then 20mL hexane, petroleum ether, isooctane were respectively added into above three separating funnel. When all were dissolved, they were transferred to separating funnels. The color change was observed. ③ For above operations in which the washed color were unchanged, they were washed by 0.01mol/L sulfuric acid in place of water. The color change was observed.

（2）Depigmented by magnesium sulfate: 20mL dichloromethane extract were removed dichloromethane by rotary evaporation, and then 30mL 0.1mol/L magnesium sulfate was added. When they were mixed together, they were transferred to separating funnels. 10mL sulfuric acid with the

concentration of 0.05mol/L and 20mL hexane were added into oil phase. Shaking up and removing water in lower layer. Then 30mL water was used to remove acid.

（3）Depigmented by ethanol and methanol: Eight 20mL dichloromethane extract were removed dichloromethane by rotary evaporation, and then 30mL petroleum ether respectively were added. When all were dissolved, they were transferred to separating funnels. 10mL ethanol with the concentration of 50%, 70%, 75%, 80%, 85% and 90%, 10mL methanol with the concentration of 50% and 70% were respectively added into above eight separating funnels. The color change was observed.

2.2.5 Determination of free fatty acid

The content of free fatty acid was determined according to GB T5530—1998.

2.2.6 Determination of recovery rate

20mL mixture of fatty acids and dichloromethane was removed dichloromethane by rotary evaporation, and then 40mL ether and ethanol mixture with equal volume was used to dissolve above mixture. The titration indicator was phenolphthalein and the consumption volume of sodium hydroxide was record. The best depigment program was selected and its recovery rate was calculated.

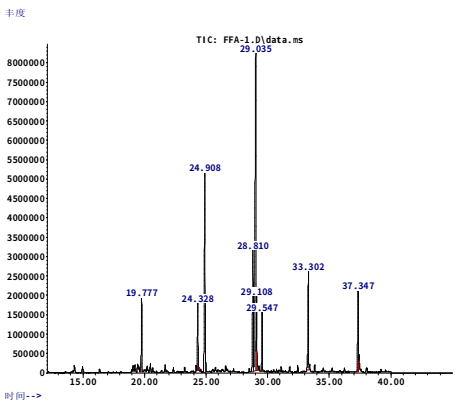
$$\textbf{Recovery rate} = \frac{V}{V_0} \times 100\%$$

In formula, V and V_0 were the consumption volume of sodium hydroxide before and after depigment, respectively.

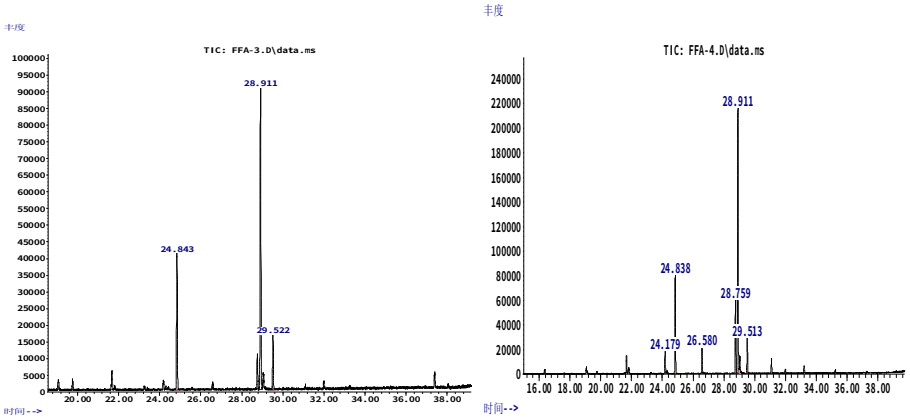
3 Results and discussion

3.1 Qualitative analysis of free fatty acids in leather products

The fatty acid could influence the property of lipid, so after the dichloromethane extracts were esterified by methyl ester, the composition of extracts was determined with GC-MS instrument. The Gas chromatography analysis results f six samples were shown in Fig.1.

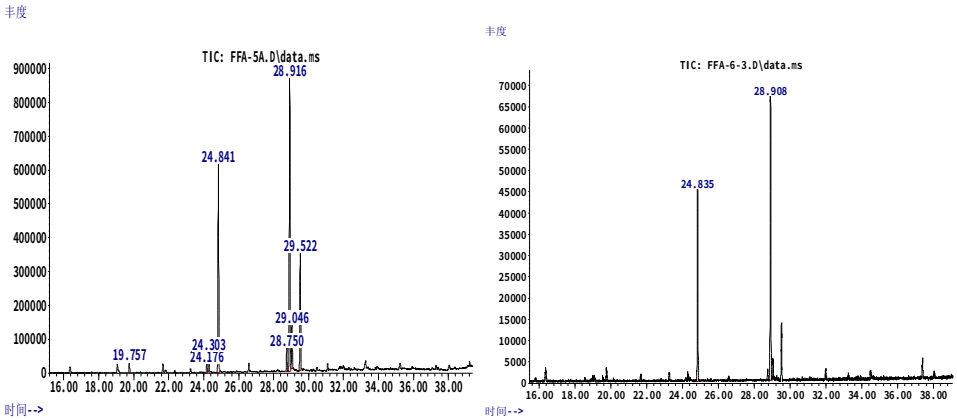


(a) Extract of sample 1#　　(b) Extract of sample 2#



(c) Extract of sample 3# (d) Extract of sample 4#

As shown in Fig.1, the distribution of peak in every figures were multi, Show that the samples contain a variety of fatty acids. There were characteristic peaks at 24.9min, 28.8min、29.0min and 29.5min, which showed that in extracts there were palmitic acid, stearic acid, oleic acid and linoleic acid. Moreover, the contrast between characteristics ion in mass spectrum further confirmed the existence of four fatty acids in the extracts.



(e) Extract of sample 5# (f) Extract of sample 6#

Fig.1 GC chromatography chart of six samples

In order to quantitatively reflect the relative content of palmitic acid, stearic acid, oleic acid and linoleic acid in extracts, basing on the content of stearic acid, the content of fatty acids were calculated. The results were shown in Tab.1.

Tab.1 The relative content of fatty acids in extracts of six samples(g/L)

Fatty acids	Content of fatty acids in different samples					
	1#	2#	3#	4#	5#	6#
stearic acid (29.548min)	1.00	1.00	1.00	1.00	1.00	1.00
palmitic acid (24.907min)	4.07	3.15	28.76	2.94	1.74	2.68
linoleic acid (28.811min)	2.61	1.57	0.65	2.05	0.18	0.01
oleic acid (29.033min)	8.88	3.04	42.93	8.57	2.76	5.96

Remarks: The calculation process was based on the area of fatty acids. The calibration was carried out by using the concentration of four fatty acids and its area of chromatographic peak.

As shown in Tab.1, the main free fatty acid in extract was oleic acid. The reason was that oleic acid was composed of triglycerides. Furthermore, the extracts all contained a certain amount of palmitic acid and the content of palmitic acid was higher than stearic acid. Compared with the content of palmitic acid, the content of linoleic acid was relatively lower. In No.6 sample, the linoleic acid almost was not detected.

Many literatures reported that higher content of stearic acid and palmitic acid caused oil cream appear. The experiment also confirmed the results. The reason for the emergence of oil cream was diverse, such as light, heat and micro-organisms. Until now it was difficult to quantify the content of stearic acid and palmitic acid in leather. Therefore, only through the data in Tab.1, the result which higher contents of certain fatty acids in leather caused oil cream. The conclusions of the research also required a large amount of data.

3.2 Quantitative analysis of free fatty acids in leather

The standard determination method of free fatty acid content was titration. method of determination of finished leather, when free fatty acid content, because the majority of finished leather were dyeing leather, the extract of colored leather samples were colorful and the extracts were unable to identify the titration by indicator. Aiming at the problem, a series of decolorization experiments for extracts were carried out, as a result, the bleaching agent which could be used in finished leather was selected.

3.2.1 The selection of decolorization method

As the solubility of liquid in different solvents was different, the dichloromethane, petroleum ether, hexane and isooctane with good solubility for liquid respectively were selected as solvents. In decolorization by water and sulfuric acid, because fatty acids and magnesium salt could form magnesium soap, the color would be off by removing the lower aqueous solution. Therefore, the magnesium sulfate was chosen as decolorant. The methanol and ethanol could dissolve organic substances and the solution could be layered with petroleum ether, so petroleum ether was selected as solvents. The decolorization experiments showed that in program (1) the adsorption capacity for organic dye was relatively strong, water and acid could not remove the color and the stratification was slow, time was too long. In program (2), magnesium soaps could not clean the color of fatty acid. In program (3), methanol and ethanol had a significant decolorization effect. For aqueous solution of methanol and ethanol with the same concentration, the decolorization effect of ethanol was better than that of methanol. For different concentrations of methanol and ethanol solution, the higher of the concentration, the better of the decolorization. The above results showed that the program (1) and (2) were not suitable to determine the content of free fatty acids in finished leather. In program (3), ethanol solution could be used as depigmenting agent in the determination of free fatty acids.

3.2.2 The decolorization effect of ethanol solution with different concentrations

The decolorization effect of ethanol solution was the best, and the greater the concentration of ethanol solution, the better decolorization effect. So the ethanol solution with the concentration of 75%, 80%, 85%, 90% and 95% was used to wash the extracts three times. The mixture of oleic acid, palmitic acid, stearic acid and linoleic acid was as standard acid and the recovery rate was determined.

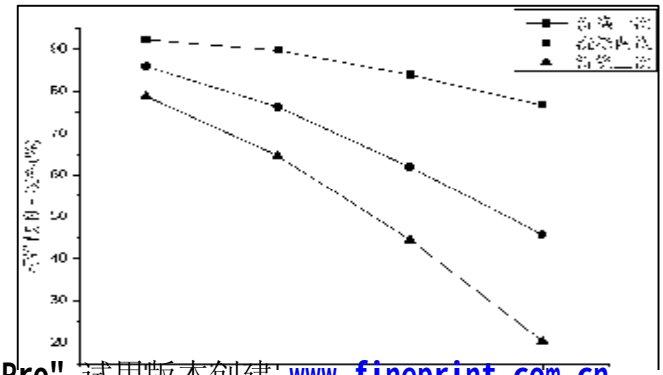


Fig.2 The recovery rate of mixed acid by ethanol with different concentrations

As indicated in Fig.2, there was no result about ethanol solution with the concentration of 95%. The reason was that after adding ethanol solution with the concentration of 95% to the mixture of free fatty acids and petroleum ether, the solubility of organic solvent in 95% ethanol solution was good and the solution was non-hierarchical. With the increase of the concentration of ethanol solution, the recovery rate gradually decreased; and with the increase of washing times, the recovery rate was also lower, but there was no significant difference in decolorization. Therefore, washing once by usint 80%~85% ethanol solution could guarantee decolorization effect and prevent experimental measurement error caused by decolorization.

3.2.3 Method improving titration precision

When the color of decolored samples was still deep, it was difficult to determine the end, so the following methods could be tried. （1）Basic blue 6B or barry phenolphthalein was as indicator. （2）The phenolphthalein was as external indicator. （3）Reducing the dosage of titration sample or increasing the amount of solvent. （4）Using potential difference titration.（5）Adding phenolphthalein indicator to samples which were dissolved in mixed solvent, and then adding appropriate saturated salt water, the titration was carried out. The titration end was determined by the color of salt water solution.

4 Conclusions

The qualitative analysis results showed that the free fatty acids in leather samples were oleic acid, palmitic acid and stearic acid, in which oleic acid and palmitic acid were the most. In decolorization, the effect of ethanol solution was the best, and the higher of the concentration, the better of the decolorization effect. However, with the increase of the concentration of ethanol, the recovery reduced. Therefore, the decolorization effect was carried out once by using 80% ethanol solution was excellent. As well as, the decolorization was done by using ethanol solution with lower concentration to ensure a high recovery rate.

Acknowledgments

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