Study on the Degreasing of Sheep Skin Using Subcritical Fluid Extraction

by

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Abstract

Degreasing is one of the important processes for the production of leather and fur. This study aimed to develop a degreasing method using subcritical n-pentane. Sheep skin was chosen as raw skin for this investigation. The best possible combination of degreasing parameters was found using single factor experiment and response surface methodology. The effects of temperature and pressure on the degreasing efficiency were evaluated further with histological analysis. The results showed that the optimum degreasing parameters were a degreasing time of 58 min, pressure of 0.45 MPa, temperature of 41.0°C and thus yielded a degreasing rate of 52.46% theoretically and 51.46% experimentally. Histological sections showed that the degreasing effect of subcritical n-pentane was quite significant and the lipid droplets around the hair follicles were dramatically reduced. It has been proven that subcritical n-pentane degreasing is an effective technique for sheep skin degreasing.

Introduction

Wool sheep skins have a fat layer in the grain-corium junction. The structure is in the form of large lipocytes, closely associated in a mass, which can act as a barrier to the penetration of aqueous reagents. Also, there is sebaceous grease in the wool follicles. The natural fat content of different animals is variable, breed-dependent, and may range from 3 to 50% of the skin weight.[1] In the processing of lambskin or sheepskin into fur, it is significant that the grease should be removed from the sheepskin to allow chemical penetration and uniform reactions through the skin cross section and to avoid odor problems if the fat turns rancid.[2] Organic solvent degreasing has remarkable degreasing effect, but this approach produces a considerable increase of environmental pollution, by emission of volatile and semi-volatile organic compounds, and may present problems in the biological treatment plants for waste water.[3] In the traditional degreasing process in aqueous medium, fats are emulsified in the presence of an anionic or nonionic surfactant and solvent (light petroleum rich in hexane). A single stage is not sufficient to obtain high efficiency and consequently this operation is time demanding. Another disadvantage of the process lies in the pollution of the environment. Effluents contain high pollutant loads that are only partially, or not at all, recycled. The extracted fat is not recovered.[4] The use of enzymes for degreasing poses several advantages such as elimination of organic solvents and surfactants, improved fat dispersion, production of waterproof and low-fogging leathers and possible recovery of valuable by-products. However, the disadvantages are that the lipase does not remove all types of fats in the same way that solvents do and the process cost is slightly escalated.[5] Therefore, it is extremely urgent to seek new degreasing methods which are efficient, environmentally-friendly, energy-saving and clean. Subcritical fluid extraction (SFE), also called pressurized low polarity fluid extraction, is one of the most popular techniques and widely used in the extraction of oil and natural products.[6-8] While subcritical extraction was utilized in the lipid extraction industry, it has the advantages of a mild operational temperature which is as low as 30°C-45°C, products extracted free from solvent residues, relatively lower operation pressure, lower operating cost and lower equipment investment, shorter extraction time, environmental compatibility, good selectivity, one step from the extraction to the separation and avoidance of residual solvents compared with supercritical extraction.[9,10] SFE is rapidly emerging as a powerful means of extraction of solid samples, especially seed oil. It can be considered a technological revolution in the extraction industry.[11] In addition, when degreasing of sheep skin for fur-making following the soaking procedure, the rehydrated sheepskin is vulnerable and its denaturation temperature is 60°C,[1] the temperature of degreasing should be controlled lower than the denaturation temperature. So, it is possible to apply subcritical fluid extraction system to sheep skin degreasing under a mild temperature as low as 35°C-45°C. Till now, the application of subcritical solvents in fur processing for degreasing has is not been reported. Different subcritical fluids have been used in SFE, such as CO_2,[12] n-butane,[13] n-propane[14] and *n*-hexane.[15] Among them, n-pentane is used as the subcritical fluid mainly because it needs lower critical pressures and temperatures and it has excellent dissolving power for lipophilic compound. Also, this extractant has a low boiling point, is inexpensive, colorless, and a clean solvent that leaves no residue in the product. In summary, subcritical fluid is widely used in oil extraction and has the advantages of environmental protection and no solvent residue. The purpose of degreasing is to remove fat in sheep skin, so subcritical fluid extraction is theoretically an effective method for the degreasing of sheep skin. This study has sought to develop a degreasing method with subcritical n-pentane.

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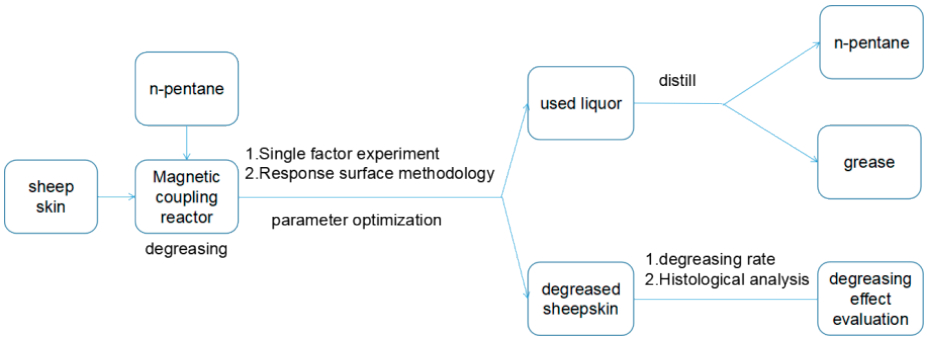

Figure 1. The sheep skin degreasing procedure with subcritical n-pentane.

Experimental

Materials & Instruments

N-Pentane was purchased from Jinshan Chemical Reagent Co., Ltd (Chengdu, China). Sheep skin is native of Chengdu. Magnetic coupling reactor was purchased from Weihai Huixin Chemical Machinery Co., Ltd. LEICA CM1950 freezing microtome and CX41 RF microscopes were purchased from Leica- (Germany).

Experiment Procedure

The subcritical n-pentane degreasing procedure for the sheepskin is illustrated in Figure 1.

Sample Preparation

After the salted fresh sheepskins had been soaked and then fleshed, they were cut into pieces in the size about 4cm ×4cm for degreasing treatment further. 100g pelt samples were weighed for each experiment.

Degreasing with Subcritical n-Pentane Extraction

Subcritical n-pentane extraction degreasing was carried out using a laboratory scale unit named Magnetic coupling reactor. For each degreasing, prepared skin samples were added into the reactor, and n-pentane was added. The ratios of skin sample to n-pentane varied according to the experimental design described in Table I and Table II. The degreasing was performed at different temperatures, pressures, and times, as shown in Table I and Table II. The factors effecting degreasing were optimized with single factor experiment and response surface methodology (RSM). At the end of degreasing, the subcritical n-pentane containing the lipid was transferred to a separator, the solvent was collected by reduction vaporization, the degreased oil was collected for further study. The degreased skin was collected for the degreasing effect evaluation and thermal stability analysis.

Single Factor Experiment Design of Subcritical n-Pentane Degreasing Process

The main factors affecting the degreasing effect of subcritical n-pentane include treatment temperature, time, pressure and solid-liquid ratio. In order to study the influence of these technological parameters on the degreasing effect, this study was conducted according to the single-factor experimental design (Table I and Table II).

Table II shows the variation range of factor A, B, C and D.

Table I

Single factor experiment scheme

Experimental scheme	Reaction time /min	Degreasing temperature /°C	Degreasing pressure /MPa	Solid-liquid ratio /(g/ml)
1	A	39.5	0.5	1:7
2	60	B	0.5	1:7
3	60	40.5	C	1:7
4	60	40.5	0.4	D

Table II

Factor table for single factor experiment

Experimental factor	A: Reaction time /min	B: Degreasing temperature /°C	C: Degreasing pressure /MPa	D: Solid-liquid ratio /(g/ml)
Variable range	30	38.5	0.2	1:3
	40	39.5	0.3	1:5
	50	40.5	0.4	1:7
	60	41.5	0.5	1:9
	70	42.5	0.6	1:11

Table III

Response surface experiment level and coding

Level NO.	Factors		
	Degreasing time X_1/min	Degreasing temperature X_2/°C	Degreasing pressure X_3/MPa
-1	50	39.5	0.3
0	60	40.5	0.4
+1	70	41.5	0.5

Optimization Design of Subcritical n-Pentane Degreasing Process by RSM

The single factor experiment found that the degreasing temperature, degreasing time and degreasing pressure have significant influence on the degreasing effect. The process was optimized according to Box-Behnken design.[16] The designed response surface experimental scheme was shown in the Table III.

The second-order polynomial equation[17] was used to establish a predictive model of the degreasing ratio:

$$Y = \beta_0 + \sum_{t=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j$$

where Y represents the degreasing ratio: β_0, β_i, β_{ii} and β_{ij} are the constant term, linear term, quadratic term and interaction term coefficients of the fitting formula respectively; X_i and X_j represent the coded values of each factor level, namely, reaction temperature, time and pressure.

Characterization of Degreasing Effect

Degreasing Rate

The oil content in the sheep skin before and after being degreased was determined by Soxhlet extraction and the test process was carried out in accordance with the ISO 4048:2018 (Determination of matter soluble in dichloromethane and free fatty acid content).[18]

The degreasing rate was calculated by the following formula:

$$Y = \frac{X_0 - X_1}{X_0} \times 100\%$$

Where Y is degreasing rate, X_0 and X_1 respectively represents the oil content of sheepskin determined before and after degreasing.

Histological Analysis of Degreased Skin

In order to characterize the degreasing effect, the sheepskins before and after being degreased were treated with the section and stain method described in the published paper[19] then observed with bio-optical microscope.

Results & Discussion

Effect of Degreasing

The results of single factor experiments show that degreasing pressure, degreasing time and degreasing temperature have significant influence on the degreasing effect of subcritical n-pentane. The optimal degreasing temperature is 40.5°C, degreasing time 60 min and degreasing pressure 0.40 MPa. The effect of the ratio of solid to liquid on the degreasing effect is not obvious which results about 0.8% difference of degreasing rate.

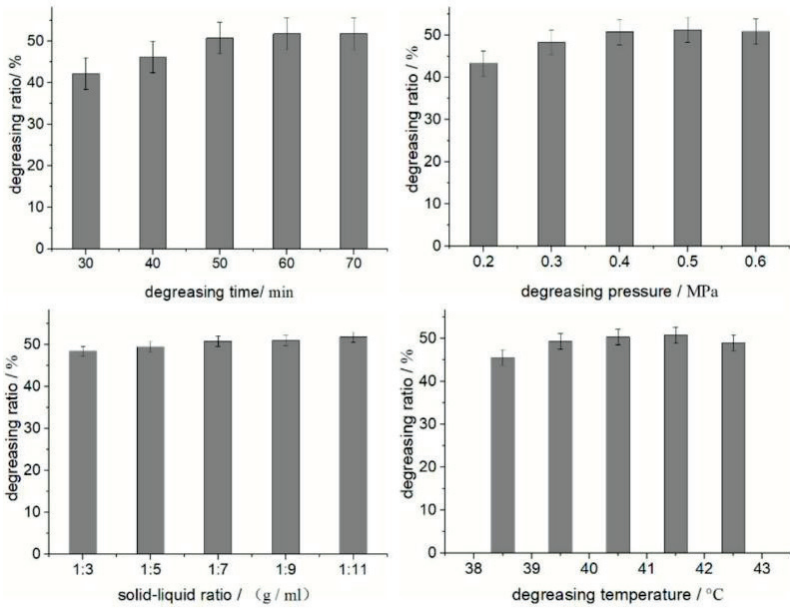

Figure 2. Single factor results

Table IV

Experimental scheme of response surface methodology and response value of degreasing rate

Level NO.	Independent variable factor			Y degreasing rate (%)	
	Time X_1(min)	Temperature X_2(°C)	Pressure X_3(MPa)	Experimental value	Predicted value
1	-1	1	0	52.8075	52.28
2	0	-1	-1	51.5114	51.28
3	1	-1	0	49.9567	50.49
4	1	1	0	52.3995	52.08
5	1	0	1	49.9050	49.99
6	0	0	0	52.0733	51.81
7	0	1	-1	49.7490	50.36
8	-1	-1	0	50.8181	51.14
9	0	-1	1	50.2329	49.62
10	0	0	0	51.7019	51.81
11	0	0	0	51.8267	51.81
12	0	0	0	51.8075	51.81
13	0	0	0	51.6568	51.81
14	-1	0	1	50.6343	50.93
15	-1	0	-1	49.8857	49.80
16	1	0	-1	50.1800	49.88
17	0	1	1	53.0314	53.26

Optimization of Subcritical N-Pentane Degreasing Process by RSM

Multiple regression analysis is performed on the experimental data in Table IV to obtain the fitting regression equation:

$$Y=51.81-0.21X_1+0.68X_2+0.31X_3+0.11X1X2-0.26X_1X_3+1.14X_2X_3-0.65X_1^2+0.33X_2^2-1.01X_3^2$$

Where Y represents defatted ratio: X_1, X_2 and X_3 is degreasing temperature, degreasing time and degreasing pressure respectively.

Table V

Analysis of variance of regression model of subcritical n-pentane degreasing rate

Source of variance	Coefficient	Sum of squares	Df	Mean square	F value	Prob>F
Model		17.03	9	1.89	6.88	0.0094
X_1- time	-0.21	0.36	1	0.36	1.32	0.2884
X_2- temperature	0.68	3.74	1	3.74	13.58	0.0078
X_3- pressure	0.31	0.77	1	0.77	2.79	0.1389
X_1X_2	0.11	0.051	1	0.051	0.19	0.6786
X_1X_3	-0.26	0.26	1	0.26	0.95	0.3617
X_2X_3	1.14	5.20	1	5.20	18.90	0.0034
X_1^2	-0.65	1.77	1	1.77	6.44	0.0388
X_2^2	0.33	0.46	1	0.46	1.68	0.2364
X_3^2	-1.01	4.32	1	4.32	15.71	0.0054
Residual		1.93	7	0.28		
Lack of fit		1.82	3	0.61	23.19	0.0054
Pure error		0.10	4	0.026		
Correlation total		18.95	16			
R-Square	0.8984					
C.V.%	1.02					

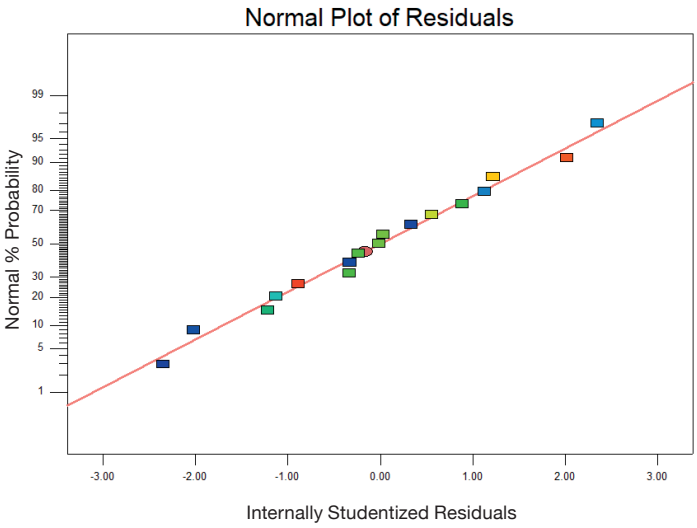

Figure 3. Residuals

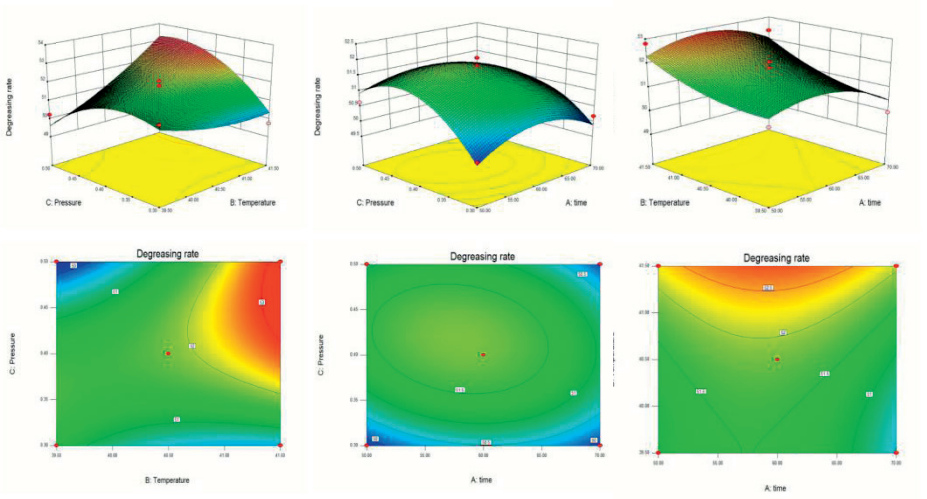

Figure 4. Response surface graph and contour map

From the variance data, it can be seen that the regression model equation F=6.88 for the subcritical n-pentane degreasing rate, Prob > F <0.05, the residual distribution probability is roughly in a straight line, indicating that the fitting model has high fitting accuracy and good fitness. The determination coefficient (R^2) of this model is 0.8984, very close to 1, and the coefficient of variation (C.V.) is 1.02% < 5%, indicating that the better the correlation between the experimental value and the predicted value,[20] the response surface equation model is used for subsequent process optimization. The F value of X_2, the primary term of degreasing temperature, is 13.58; the F value of X_3^2, the secondary term of degreasing pressure, is 15.71; and Prob>F<0.01, indicating that degreasing temperature and degreasing pressure have a significant impact on the degreasing effect of subcritical n-pentane.[21]

The interaction between degreasing time and pressure has a significant effect on the degreasing efficiency of subcritical n-pentane. The design-expert 10 software (made by Stat-Ease lnc. which is located in Minneapolis, USA) is used for calculation and analysis, and the optimal process parameters are obtained as follows: degreasing time 58 min, degreasing temperature 41°C, degreasing pressure 0.45MPa. The degreasing rate of subcritical n-pentane under this process condition is 51.46±1.90%. Therefore, the response surface model has a good fitting degree and the optimized parameters for the subcritical degreasing process are reliable.

The Effect of Degreasing Temperature and Pressure on the Grease left in the Subcritical N-Pentane Degreased Skin

Figure 5 shows histological micrographs of blank specimens and degreased skins treated with n-pentane at different temperatures. Similarly, the reddish brown particles are lipid droplets and the pink structures are fibrous tissue such as fibrin. The histological sections in Figure 5-a shows a slight decrease in granular lipid droplets in the two groups compared with the blank histological sections, and only a small part of the lipid glands became empty. When the degreasing temperature rises to 40.5°C and 42.5°C, more lipid glands become empty in the histological sections of the skin, and the lipid droplets in the lipid glands become less. Therefore, when the degreasing temperature of subcritical n-pentane rises above 40.5°C, the lipid droplets around the hair follicles decrease significantly.

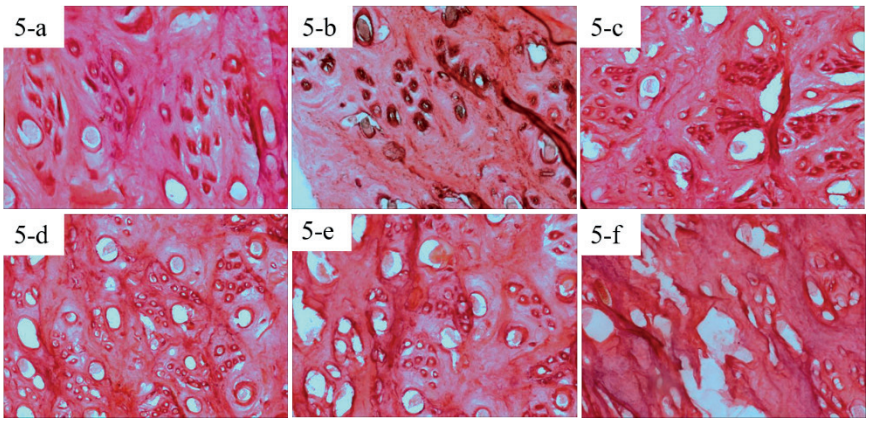

Figure 5. Histological photomicrographs of degreased skin under different temperature

(a, b, c, d, e, and f indicate blank samples and degreasing temperatures of 38.5°, 39.5°, 40.5°, 41.5°, and 42.5°C, respectively)

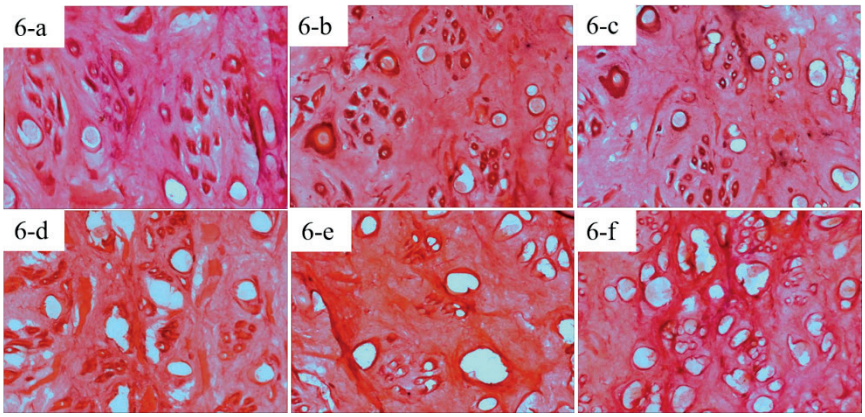

Figure 6. Histological photomicrographs of degreasing skin under different pressure

(a, b, c, d, e, and f indicate blank samples and degreasing pressures of 0.2, 0.3, 0.4, 0.5, and 0.6 MPa, respectively)

Figure 6 shows histological micrographs of blank specimens and degreased sheep skin treated with n-pentane at different pressures. Reddish brown granular material is stained lipid cells, and fibrous tissue structures such as collagen fibers are stained red or pink. As can be seen from the blank histological sections shown in Figure 6, there are many groups of granular lipid droplets around the hair follicles in the skin before being degreased. When the degreasing pressure is 0.2MPa and 0.3MPa, after subcritical n-pentane treatment, the granular lipid droplets in the skin showed a slight decrease. From the histological section diagrams of 0.4, 0.5 and 0.6MPa, it can be seen that the gradual increase of the degreasing pressure, the granular lipid droplets in the degreased skin showed a significant decrease. It indicates that when the degreasing pressure rises above 0.4MPa, the degreasing effect of subcritical n-pentane extraction is very significant.

Conclusion

A degreasing method using subcritical n-pentane extraction has been developed. The optimized degreasing parameters were obtained as follows: time 58 min, temperature 41°C, pressure 0.45 MPa according to single factor experiment and response surface methodology. The degreasing rate was 52.46% theoretically and 51.46% experimentally under the optimal degreasing parameters. Histological micrographs showed that the lipid droplets around the hair follicle were significantly reduced in the degreased sheep skin. The result indicated that degreasing of sheep skin with subcritical n-pentane is efficiency, and the operation condition is mild.

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