The Separation, Purification of Tea Saponin and its Application in the Soaking Enzymes

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Abstract: Tea saponin, a natural non-ionic surfactant, is widely used in chemical industry. In this paper, the orthogonal experiment was used to optimize the technology parameters of the tea saponin extraction from the tea seeds firstly, and then the macroreticular resin separation method was used to obtain tea saponin with purity up to 95%. The tea saponin was applied in the soaking enzymes to study the effect on the enzyme activity and on the leather immersion technology. The results showed that with the increase of the tea saponin, the activity of protease increased firstly, and then reduced with further increase of the tea saponin. When the tea saponin content was 2.5g / L, the water content of rawhide after the immersion was highest, and the effect of returning on the fresh was good.

Key words: tea saponin; the activity of soaking enzymes; soaking

1 Introduction

Tea sapogenin, a natural Non-ionic surfactant, not only has a good emulsifying, separating and dispersing capability but is also a good foaming and foam stabilizer with a great and cleaning capacity with a hydrophile-lipophilic balance value of 16[1]. Thus it can be used as foam stabilizer for the building concrete [2], pesticide synergist [3] soil amendment [4], antioxidants [5, 6], Pesticides and surfactants, so it is widely used in daily chemical industries, building materials, food industries and agriculture. There is 10-13% tea sapogenin in tea seed, which makes it an ideal resource for the extraction of tea sapogenin. Usually tea sapogenin is extracted by using organic solvent like water, ethanol and methanol. Among these, water will have produce a high percentage of impurity, which makes separation and purification difficult, and methanol is too poisonous and brings serious pollution to the environment. Accordingly this experiment will use ground tea seed meal as raw material and ethanol as the solvent and orthogonal experimental method. Meanwhile the impact brought about by different ultrasonic frequencies [7, 8], ethanol of different concentrations, various extraction durations and different solid -liquid ratios will be studied. Along with this, macroporous resin purification technique is used to select the best concentration of the solvent. It is discovered that if eluted by 95% of ethanol, then after concentration and the drying process, tea sapogenin can be obtained, and if this very kind of tea sapogenin is used in the leather-soaking process [9, 10], it shows that with the increase of the amount of tea sapogenin, the activity of the soaking enzyme will first increase and then decrease, specifically when the amount of tea sapogenin reaches 2.5g/L, the moisture in the leather will be the most, and the back to fresh of leather is the best, and will has the least damage after the tanning process.

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2 Experimental

2.1 Material and Main Equipment

Pressed tea seed meal, from Biological Pharmacy Enterprise, Yuxi City, Yun Nan Province; ethanol, vanillin, sulphuric acid, N-butanol, glacial acetic acid, purchased from reagent companies in Xi'an; HZ-841 macroporous resin purchased from Mosu Science Equipment Co., Ltd in Shanghai; JP-1 protease, self-made; Wet salted cowhide, from Friendship Leather-making Factory in Xi'an.

A multi-frequency sonochemical reactor (SC-III), Jiu Zhou Mechanical and Engineering Reserch Center, Chengdu; UV Spectrophotometer (Spr756), Shanghai Spectrum Instruments Co., Ltd; Rotary Evaporator (R201BL), Shiyuan Science Equipment Co., Ltd, Shanghai; Medicinal Herb Grinders (WKX-130), Jingcheng Medical Equipment Manufacture Co., Ltd, Qingzhou Shandong Province; 30×100 SG13 thin-layer chromatography silica gel plate, Qingdao Makall Group; Spectrophotometer (722), Shanghai Precision and Scientific Instrument Co., Ltd.

2.2 Experiment Methods

2.2.1 Pretreatment of the Tea seed Meal

Take 80g of pressed tea-seed meal with a particle size of 60 meshes, 250mL of acetone, and put them both into a Soxhlet extractor, after this heat it and reflux till there is no fat and take it out to be used.

Take pretreated and not treated tee-seed powder each 100g, and when the ultrasonic frequency is 25.80 KHz, the ethanol volume fraction is 75%, and the solid-liquid ratio is 1:5, extract them both for 40 minutes, filter and record the volume of the filtrate. Then take 1mL of the filtrate, weigh after drying it, after this take 1mL of the filtrate, put it into a 100mL volumetric flask and measure the absorption value, then compute the mass fraction of the tea sapogenin.

Mass of the tea sapogenin= the volume of the extraction liquid ×dry weight of 1mL extract ×the mass fraction of the tea sapogenin

2.2.2 The extraction of Tea sapogenin

Take 100g of pretreated pressed tea-seed powder with a 250mL flask and add a certain volume of ethanol and then fix the flask onto the reaction frame, activate the ultrasonic pulverizer. Then use the extraction time, the volume of ethanol, solid-liquid ratio and ultrasonic frequency as factors to do an orthogonal experiment, after the reaction, reclaim the ethanol and then dry to get raw tea sapogenin.

2.2.3 Purrifaction of the Tea sapogenin

Take 200g of HZ-841 macroporous protease and put it in a 500mL flask and add proper amount of ethanol to soak it for 2 hours, and then elute it with ethanol till the liquid coming out is no longer turbid, after this get rid of the ethanol with water and get it ready by soaking it with water.

Take 200mL of the extraction liquid with a mass fraction of 35mg/mL and then slowly put into a separation column, after which wait for 10 hours, then elute it with NaOH and ethanol solution of different concentrations to select the best solution fort he elution.

2.2.4 The Applcation of Tea sapogenin in the Soaking of Skin

2.2.4.1 The Relative Activity of JP-1 protease in relation to Tea Sapogenin

Add a certain amount of tea sapogenin to the buffer system of the combine of JP-1 protease and Borax-NaOH, and then compute the relative activity of JP-1 protease.

Relative Activity= X1/X2×100% [11] (equation 1)

(X1: the activity of the soaking enzyme after the tea sapogenin and the enzyme has integrated, U/g;

X2: refers to the activity when distilled water is used instead of the tea sapogenin (blank sample).

2.2.4.2 The Moisture Content in the raw soaked leather

Take 2g-6g of representative sample from the wet salted cowhide and then put it into a protease solution of tea sapogenin of different concentrations and then stir it and soak it for two hours then drain dry it. After this take down the mass before and after the draining process, then use equation 2 to get the variations of the mass fraction of the moisture in the raw soaked skin.

$\Delta w = (m_2 - m_1) / m_1 \times 100\%$ (equation 2)

(Δw: the variation of the mass fraction of the soaked raw skin; m_2:the mass of the soaked raw skin, g; m_1: the mass of the raw skin before the soaking process, g)

3 Results and Discussions

3.1 The Pretreatment of the tea seed meal and its Effect on the Extraction of Tea sapogenin

Do the experiment according to 1.2.1 and compare with the results without pretreatment, the testing results are shown in Table1.

Tab. 1 Different Treatment Methods and Their Individual Effects on Tea sapogenin Extraction

Treatment Methods	Desiccation Situation	Characters of the Dry Substance	The Concentration of Tea sapogenin in the Residual Extraction liquid /mg/mL	The Mass of Tea sapogenin /g
Pretreated	easy	off-white powder	0.02390	11.9340
Not treated	difficult	reddish brown thick solid	0.02079	10.3950

Note: The mass of the tea sapogenin refers to the quality of mass sapogenin extracted from 100g of tea seeds.

It is shown in table1 the each of criteria of the tea sapogenin acquired when the tea-seeds are pretreated is far better than that got when the seeds are not pretreated. The main reason lies in the fact that the tea seed meal still has about 10% of tea seed oil, and this will have negative effects on the extraction of tea sapogenin, so the tea seed powder should better be pretreated to get rid of as much of the fat as possible when used to extract tea sapogenin.

3.2 Selection of the Best Condition for the Extraction of Tea sapogenin

The factors in the orthogonal experiment and their levels are shown in Table 2and the results are shown in Table 3.

Tab. 2 the Factors and in the Orthogonal l Experiment for the Extraction of Tea sapogenin and their Levels

Ultrasonic frequency/KHz	The Volume Fraction of Ethanol /v/v	Extraction Time/min	Solid -Liquid Ratio
14.52	65	20	1：3
25.80	75	30	1：4
35.74	85	40	1：5

Tab. 3 Experiment Results

	Ultrasonic frequency/KHz	The Volume Fraction of ethanol /v/v	Extraction Time/min	Solid and Liquid Ratio	Tea sapogenin. /g
1	14.52	65	20	1：3	10.93
2	14.52	75	30	1：4	11.51
3	14.52	85	40	1：5	11.84
4	25.80	65	30	1：5	11.75
5	25.80	75	40	1：3	11.81
6	25.80	85	20	1：4	11.79
7	35.7	65	40	1：4	11.48
8	35.7	75	20	1：5	11.34
9	35.7	85	30	1：3	11.55
K_1	22.44	34.16	34.06	34.29	
K_2	35.35	35.18	34.81	34.78	
K_3	34.37	34.66	35.13	34.93	
R	12.91	1.02	1.05	0.64	

As shown in Table3, the factors that influence the extraction of tea sapogenin are put in the order of ultrasonic frequency, extraction time, the volume fraction of ethanol and solid-liquid ratio, and the best combination is when the ultrasonic frequency is 25.80 KHz, the volume fraction of ethanol is 75% and the solid-liquid ratio is 1:5 with an extraction time of 40 min. Under these conditions, the experiment will bring an extraction rate of 11.91%.

3.3 The Purification of Tea sapogenin

3.3.1 The Influence NaOH of Different Concentrations has on the Mass of the Tea Sapogenin

After the tea sapogenin in the residual extraction liquid is absorbed by the macroporous resin, and then eluted with NaOH of different concentrations, the experiment shows that the mass and purity of Tea Sapogenin is closely connected with the concentration alternations of NaOH. The testing results are shown in Table 4.

Tab. 4 Effects Different NaOH Concentrations has on the Mass of Tea-Sapogenin

NaOH Concentrations /%	0.1	0.2	0.3	0.4	0.5
The mass of tea sapogenin/g	11.65	10.19	9.46	7.28	4.37
The color and luster of the Tea sapogenin	light yellow	light yellow	off-white	off-white	off-white
Purity quotient of the tea sapogenin/%	80	90	96	96	96

It can be seen from table4 that with the increase of the concentration of NaOH, the purity of the tea sapogenin increases too, and the color goes from light yellow to off-white, but the mass of the tea sapogenin decreases. What's more when the concentration of the NaOH is 0.3% , the eluent will elute the impurity and pigment the best; when the concentration drops to 0.1%or 0.2%, the result will not be so good and the purity of the tea sapogenin will not be as high; when the concentration reaches 0.4% or 0.5%, and then the liquid will be so alkaline that the some of the tea sapogenin will turn into dissolvable

salt, and these salt and impurity and pigment will all be eluted, and this will lead to a loss of tea-sapogenin, so the production rate will be low. Thus the best concentration of NaOH should be 0.3%.

3.3.2 The Influence that Ethanol of Different Concentrations has on the TLC

If elute with NaOH and then washed to neutral with water, after which elute again with ethanol of different concentrations, and if developer made up of n-butanol, acetic acid and water in the ratio of (4:1:5), color developing reagent made of ethanol with 1% $AlCl_3$ and then 10% H_2SO_4 is used and a TLC test is carried out, then in the end when the eluting liquid is collected, the experiment result will show as in Table 5.

Tab. 5 TLC Results in accordance with Ethanol of Different Concentrations

The Volume Fractor of Ethanol/v/v	20	30	40	50	95
$AlCl_3$	bight Red	bright Yellow	bright Red	bright Red	bright Red
H_2SO_4	off-white	off white	black	black	black
Conclusion	Flavone, Tea sapogenin	Flavones, Tea Saponin	tea sapogenin	tea sapogenin	tea sapogenin

Note: “flavone, tea sapogenin” refers to the coloration that has both flavone and tea sapogenin when the eluting solvent is tested through TCL; “tea sapogenin” refers to the situation when only tea sapogenin is present when the solvent is tested through TCL.

It can be seen from the above table that ethanol of 20% or 30% both mainly has flavone with little tea sapogenin, and when the eluting solvent is of 40%, 50% and 95 % , there will only be tea sapogenin. Further studies have shown that with the concentration of ethanol increases, the elution volume of the tea sapogenin will also increase because the higher the concentration of the ethanol gets, the closer its polarity gets to that of the tea sapogenin, so it is best to use ethanol of 95% will be used as the eluting solvent.

In addition, when ethanol of 95% is vacuumed condensed and cooled till it dry styliæs, then the tea sapogenin in crystal form will be acquired.

3.4 The Influence Tea sapogenin has on the Soaking of Leather

3.4.1 The Influence Tea sapogenin has on the Activity of JP-I Protease

The influence tea sapogenin has the activity of JP-I protease (See figure1).

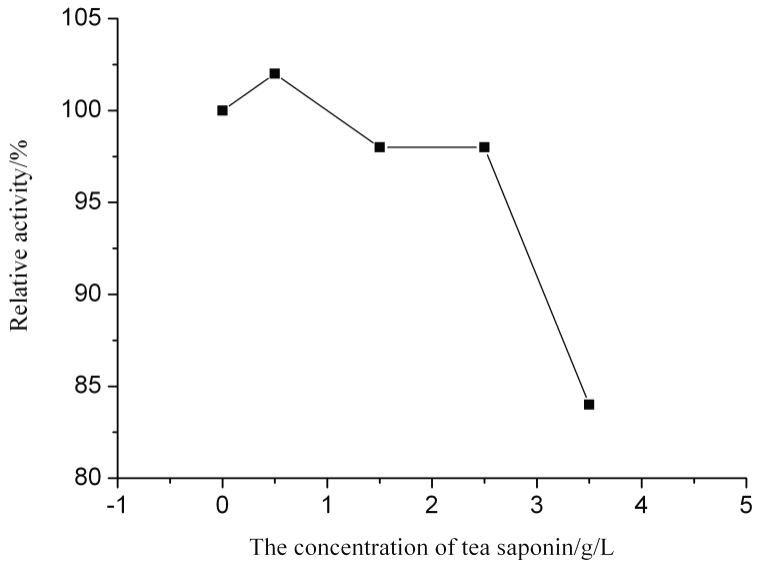

Fig. 1 The influence tea sapogenin has the activity of JP-I protease

It can be seen from Fig.1 that with the increase of the concentration of tea saponin, the relative activity of the JP-1 protease will first rise but then decrease, and when the concentration rate of the saponin gets 0.5g/L, the activity of the enzyme will also reach the highest.

3.4.2 The Influence that Tea sapogenin has on the Moisture Content of the Soaked Raw Skin

The Influence that tea sapogenin has on the moisture content of the soaked raw skin (See figure 2).

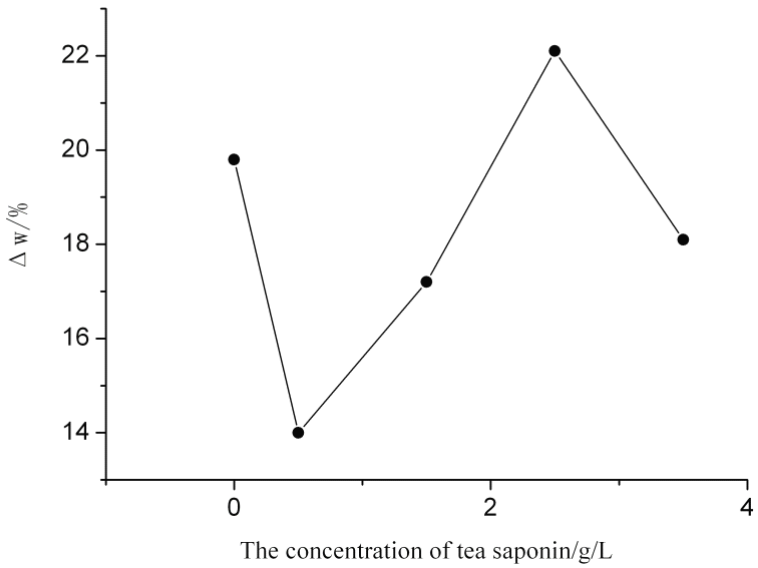

Fig. 2 The Influence that tea sapogenin has on the moisture content of the soaked raw skin

It can be seen from above that with the increase of tea sapogenin, the moisture content in the skin tends to rise first and then decrease. The reason for this is that with increase of tea sapogenin, the activity of JP-1 protease will also increase, which will have a hydrolyzing effect on the surface of the raw skin during the soaking process, and because of which the skin's ability to retain water will decrease and when the condensation of the tea sapogenin reaches 2.5g/L, the protease will on the contrary decrease. During this time due to the miniaturization and penetration of the surfactant, the protease is

urged to enter the leather skin and partially hydrolyze the protein inside, thus the skin's ability to retain water is increased.

4 Conclusions

If mechanically produced tea seed meal is used as the raw material and the orthogonal method is adopted, the extraction technique of tea sapogenin can be optimized, meanwhile if the macroporous resin absorption technique is used to purify the tea sapogenin. The result shows that when eluted with 95%e ethanol, 96% of tea sapogenin can be collected. And if the tea sapogenin got from the above two steps is used in the soaking process of skin, and when the concentration of the sapongenin is 2.5g/L, the leather has the best ability retain water and good effect of back to the fresh with the least damage to the surface.

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References

[1] H.Guo. Study of Technologies on Refining High Quality Tea Seed Oil and Comprehensivly Utilizing Tea Seed, Hunan Agricultural University, 2007.

[2] B. Y. Pu; Y. B. Zhu. Natural Product Research and Development, 1999, 11(5): 55-59.

[3] Y. M. Li; K. J. He; X. Y. Wang; et al. Hunan Agricultural Science, 2005, 4: 55-57.

[4] K. J. Hong; S. Tokunaga. Chemosphere, 2000, 41: 345-352.

[5] M. R. Venukumar; M. S. Latha. Indian Journal of Clinical Biochemistry, 2002, 17(2): 80-87.

[6] S. E. Lee; E. M. Ju; J. H. Kim. Experimental and Molecular Medicine, 2003, 34(2): 100-106.

[7] E. Riera; Y. Golas, A. Blanco, et al. Ultrasound Sonochemistry, 2006, 13: 471-479.

[8] H. Z. Li; L. Pordesimo; J. Weiss. Food Resin, 2004, 37(7): 731-738.

[9] J. Z. Ma; B. Lv; Z. M. Xue. Leather science and engineering, 2006(6): 20-26.

[10] D. Ivanova; L. Costadinnova; R. Todorova. Journal of the Society of Leather Technologists and Chemists, 2004, 88: 161-163.

[11] X. Y. Yang; J. Z. Ma; D. G. Gao. China Surfactant Detergent & Cosmetic, 2009, 30(2): 76-90.