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Study on Extraction and Stability of Pigment in the *Cordyceps militaris* Medium

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Cordyceps militaris **were taken as raw material, studied of *Cordyceps militaris* pigment extraction and physicochemical properties of pigment. The results shown that, *Cordyceps militaris* pigment dissolved easily in 80% ethanol solvent, The maximum absorption wavelength of 452 nm, The optimum extraction process was extraction temperature 30℃, extraction time 3 h, pH value 5, solid-liquid ratio 1:20, in which the temperature was remarkable influencing factor. Alkaline conditions had a strong hyperchromic effect, In certain conditions light, redox agent, Zn^{2+}, Mg^{2+}, Cu^{2+}, Mg^{2+}, K^{+}, Na^{+} instability had unstable characteristics. Fe^{3+} could make it fade, Food additives had irregular effect.**

Key words: Cordyceps militaris medium, Pigment, Extraction, Stability.

Food coloring is an important part of the food additive. It is not only widely used in the food industry to improve food gloss, but also are widely used in the medicine and cosmetics. The food coloring is usually divided into two categories: The edible natural pigment and edible synthetic pigment[1]. The edible natural pigment is the most widely used class of food coloring. Compared with synthetic pigment is more safe and good for safe and environmental protection. Tone is also more natural. It is mainly present in the plants, animals and microorganisms. Cordyceps militaris (Fr.) Lin, also known as North Cordyceps, which is similar with Cordyceps sinensis in medicine and pharmacology .It is a tonic function of medicines and functional food[2]. As a kind of important Chinese traditional medicine, they are widely used as raw materials and folk nourishing food in the East Asia[3]. Medicinal mushrooms are sources of useful natural products which contain a variety of biological activity[4], It is not only a kind of precious drugs in the treatment of many diseases, such as cough, lung and kidney disease, also a popular and nourishing food. According to recent reports it has an anti-aging effect[5]. The research of Cordyceps is limited to investigation, classification, biological activity screening and comparison of the chemical components of Cordyceps sinensis. The research of chemical constituents are more concentrated in the nucleoside, Cordyceps polysaccharide, Cordyceps acid, sterol compounds[6]. The research of pigment *Cordyceps militaris* is involved, is main about pigment extraction and structure research .But the research of *Cordyceps militaris* medium extraction and stability has yet been involved. *Cordyceps militaris* also contains many effective components[7]. Yellow pigment is one of effective components of *Cordyceps militaris* , which is soluble in water, ethanol, methanol and other more polar solvent. It has strong antioxidant activity and is important constituents of Cordyceps health food processing .This paper aims to investigate the nature of *Cordyceps militaris* pigment and stability, lays the theoretical foundation for its

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development of *Cordyceps militaris* pigment as an natural edible pigment.

MATERIALS AND METHODS

Chemicals

Anhydrous ethanol, methanol, ether, ethyl- acetate, Sodium bisulfite, hydrogen peroxide, Ferric chloride, Sodium chloride, potassium chloride, calcium chloride, zinc sulfate, copper sulfate, aluminum, magnesium chloride were analyzed for pure.

Material

From the Greater Khingan Range Jin song town to expand North Cordyceps growing base of solid fermentation cultured fruiting bodies of culture medium.

Method

The extraction of natural pigment from *Cordyceps militaris* ,process: raw material, cleaning, drying, Smash, solvent extraction, centrifugation, pigment solution[8].

Pigment extraction agent selection

Weigh crushing *Cordyceps militaris* powder, place in taper bottle , add different polar solvent (water, 30% ethanol, 50% ethanol, 80% ethanol, methanol, ether, ethyl acetate), in bath in 50° extraction 1H, observe the extract color, determination of absorbance at the wavelength 452 nm.

Drawing absorption spectrum

The extract was properly diluted with 0.5 cm absorption pool, distilled water as reference, in 350 nm 600 nm wavelength range scanning determination of absorbance, drawing the absorption spectrum.

Militaris pigment extraction orthogonal test

Accurately weigh Cordyceps militaris powder, according to single factor experiment method, investigated the material liquid ratio, temperature, time, pH on extraction rate influence, according to the experimental results of screening of influencing factors, orthogonal experiment, determine the optimum conditions for [9].

The effect on the stability of pigment

In different conditions to test pH, temperature, light, redox agent, metal ion, food additives on stability of pigment.

RESULTS

From the Fig. 1, we know that absorption peak wavelength of Cordyceps pigment is 452nm. It has a complementary relation to its performance of yellow.

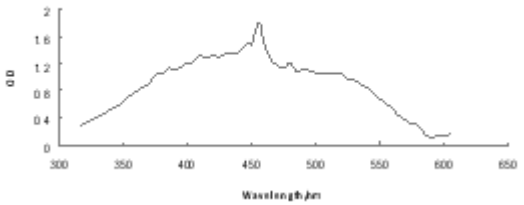

Fig. 1. Ultraviolet spectra of pigment in Cordyceps militaris

Table 1. Effects of different extractants on extraction of pigment

Solvent	Ethyl ether	Ethyl acetatr	Metha nol	Water	30% ethanol	50% ethanol	80% ethanol
Extract color	Colorless	Colorless	Light yellow	Deep yellow turbid	Yellow	Light yellow	Yellow
A_{454nm}	0.001	0.000	0.42 4	1.18 6	1.06 8	1.03 7	1.10 8

From table we known different extraction effect are different. *Cordyceps militaris* pigment is soluble in water, ethanol, methanol and other polar solvents, Insoluble in ethyl acetate, ethyl ether and other non-polar solvents. Compared with extraction effect of 3 kinds of polarity solvent, water absorbance is the biggest, but appears cloudy. It shows that the extraction of pigments and proteins, sugars and water-soluble macromolecules are presented together. After Comparing extraction effect of methanol and ethanol, we choose 80% ethanol as extraction agent.

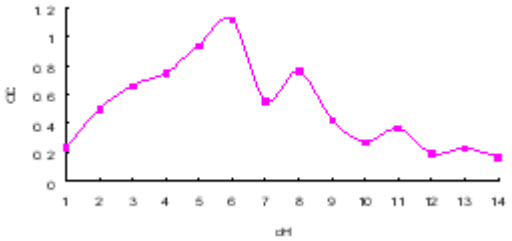

Fig. 2. Absorbance of the pigment extract in different pH

The results showed, the pH value increased from 1 to 6, the light absorption value increases ceaselessly, the increasing volume of pigment extraction . pH from 5 to 6 increased obviously, pH=6, militaris pigment solution absorption reaches the maximum value, 1.118. pH from 6 to 7 the decline was most evident. In alkaline conditions of pigment solution absorption value was not rule changes. Therefore, selection of extraction of acidic condition, pH6 is plus.

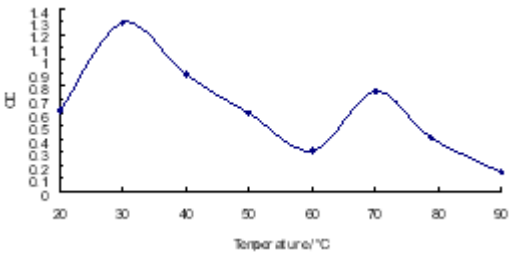

Fig. 3. Absorbance of the pigment extract in different temperatures

From the Fig.3 ,we can see the extraction efficiency decreases with increasing temperature and decreasing trend, 30 !absorbance reaches a maximum value. When the temperature rises but the absorbance decreased, possibly due to pigment poor thermal stability or other impurities, so militaris pigment in low temperature storage.

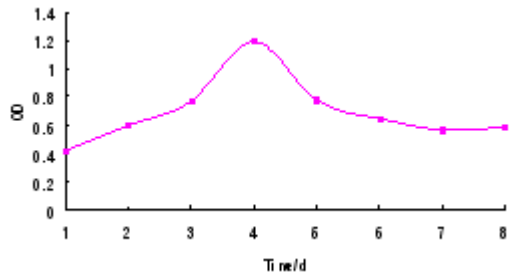

Fig. 4. Absorbance of the pigment extract in different time

Figure 4 shows the pigment solution absorbance with extraction time increased first and then decreased, within 4 hours of pigment extraction volume reached the maximum, then the solution absorbance decrease. Absorbance decrease may be caused by pigmented component degradation. So the extraction time of not more than 4 hours.

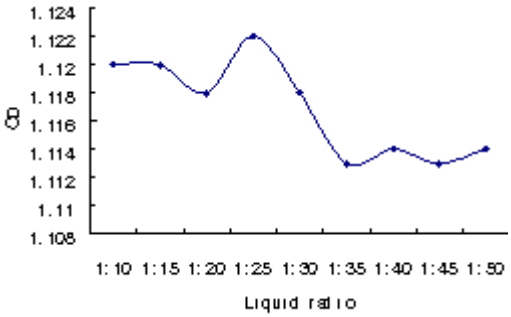

Fig. 5. Effect of Liquid ratio on stability of pigment in Cordyceps militaris

Figure 5 shows liquid ratio of 1:25 extraction efficiency, the pigment solution absorption value is 1.122, With the material liquid ratio increases the absorption value drops quickly, solid-liquid ratio was more than 1:35 absorption value increased, but increased to a lesser extent.

Table 2. Cordyceps militaris medium test results of extraction conditions

Test No	A (°C) Temp.	B (h) Time	C pH	D Ratio	OD_{452}
1	30	3	5	1:20	1.113
2	30	4	6	1:25	1.088
3	30	5	7	1:30	1.096
4	40	3	6	1:30	1.081
5	40	4	7	1:20	1.079
6	40	5	5	1:25	1.071
7	50	3	7	1:25	1.086
8	50	4	5	1:30	1.064
9	50	5	6	1:20	1.058
K_1	3.297	3.279	3.249	3.249	
K_2	3.231	3.231	3.228	3.246	
K_3	3.207	3.225	3.261	3.24	
k_1	1.099	1.093	1.083	1.083	
k_2	1.077	1.077	1.076	1.082	
k_3	1.069	1.075	1.087	1.080	
R	0.030	0.018	0.011	0.003	

According to table 2 comprehensive factors of different levels of the experimental results, the extraction militaris pigment best level combination of A1B1C1D1, extraction temperature 30 !, extraction time 3 h, pH value 5, solid-liquid ratio of 1:20 was the best extraction process combination. Range analysis show that, the factors affecting the order is A>B>C>D, The temperature is the main factor of extraction effect, Next is the time, ratio of material to solvent effects of minimum

From the Fig.6 we can see, direct sunlight on the pigment stability influence, in the 5D time, pigment solution absorption value from 1.118 to 0.898, pigment retention rate of 80.3%. In dark condition, the stability of the pigment is better, 5D, pigment retention rate was 90.8%. Therefore, Cordyceps pigment should avoid sun light irradiation, appropriate in the light of preservation condition.

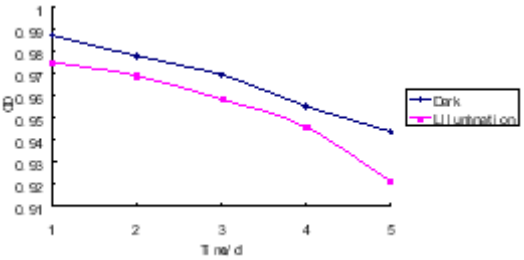

Fig. 6. Effect of light on stability of pigment in Cordyceps militaris

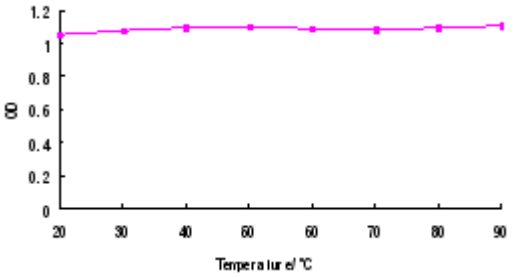

Fig. 7. Effect of tempriture on stability of pigment in Cordyceps militaris

Table 3. Color of the pigment under different pH

pH	2	3	4	5	6	7	8	9
Color	Light yellow	Yellow	Yellow	Yellow	Yellow	Deep Yellow	Deep Yellow	Bright yellow

Figure 7 shows, In 30!pigment extraction absorbance is almost the same with initial absorbance of ambient temperature ,with temperature increasing, pigment absorbance is increased slowly, but steadily . *Cordyceps militaris* pigment are stable on high temperature .

Table 3 shows: Cordyceps pigment are yellow even in different pH conditions . It's colour doesn't change along with the changes of acidity . In acidic conditions its color is light yellow. With the increase of pH value, the pigment liquid color deepens gradually, until a pH of 9 or more it turns bright yellow, which indicats that the alkaline conditions have a hyperchromic effect. Figure 8 shows absorbance doesn,t change greatly along with the increase of pH .

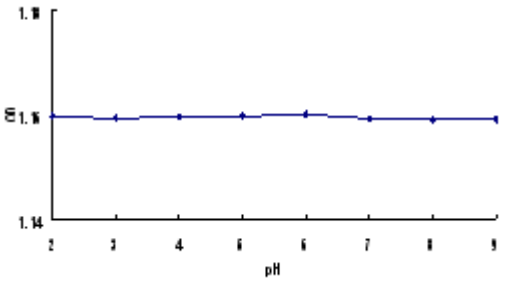

Fig. 8. Color of pigment of Cordyceps militaris with different pH

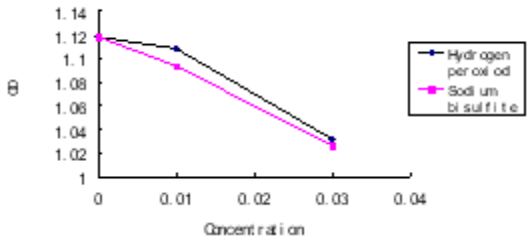

Fig. 9. Effect of $H_2O_2$and $NaHSO_3$ on stability of pigment in Cordyceps militaris

From the graph 9 we can see, The existence of a reducing agent has great influence on the absorbance . As the concentration increases absorbance significantly decreased. Oxidizing agent H_2O_2 has a relatively small effect on the stability of pigment Cordyceps solution . The *Cordyceps militaris* pigment reduction resistance ability is poor, when used not suitable to add reducing agent.

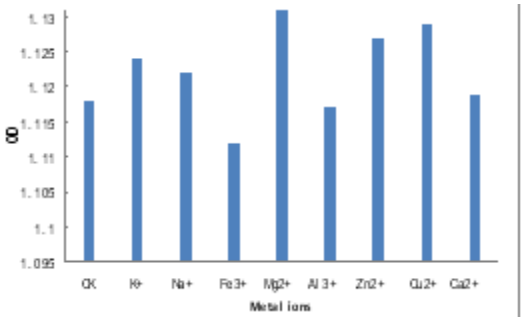

Fig. 10. Effects of metal ions on pigment stability

The test shows that Na^+, K^+, Zn^{2+}, Cu^{2+}, Mg^{2+} plasma could increase the absorbance of Cordyceps pigment in 452nm significantly, which has synergistic effect on pigment 's colours. Except that Fe^{3+} has a greater impact on the pigment, the remaining ions such as Ca^{2+}, Al^{3+} have little effect on the stability of pigment .

From the graph 11 we can see: citric acid has an hyperchromic effect on the pigment

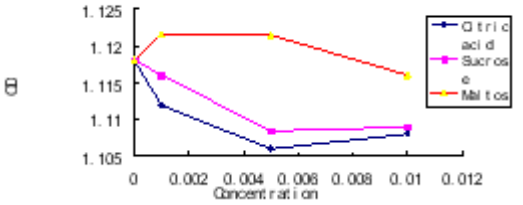

Fig.11. Effects of food additives to pigment stability

solution, which may be associated with the pigment itself that is stable under acidic conditions to a certain extent, but the effects of the concentration is irregular. Sucrose and maltose have obvious effects on the stability of pigment solution, Therefore we should be very careful to choose it in actual use.

DISCUSSION

Cordyceps militars is a kind of water soluble pigment , which can be extracted with 80% ethanol. Under the condition that pH and temperature is stable, we studied the process conditions of Cordyceps medium pigment, and found the effect of various factors on extraction sequence from large to small were extraction temperature, extraction time, pH, solid to liquid ratio, in which the extraction time is remarkable influencing factor. The best technology condition from optimization of extraction conditions is the temperature 30 °, The extraction lasts for 3 hours,and its pH is 5, ratio for solid to liquid is 1:20. In the alkaline solution it has an hyperchromic effect. Because of it was sensitive to light, it should be stored away from light. The maximum absorption wavelength is 452 nm. What is more it is not stable in $NaHSO_3$ and H_2O_2 solution . Metal ion Fe^{3+} will make it fade. these features are the same with ketone pigment . The further test for exact chemical structure is still needed.

ACKNOWLEDGEMENTS

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