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Studies on the Extraction of Natural Compounds from Plants and Microorganisms

Part 2. Extraction of Orange-yellow Pigment from Defatted Gardenia

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生物體로부터 天然化合物 抽出에 관한 硏究

第Ⅱ報：脂肪을 제거한 치자로부터 치자色 色素 抽出에 관한 硏究

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要 約

微碎한 치자粉末에서 Orange-Yellow 色素를 抽出하면 짧은 時間에서 $\log t^{1.15}$에 比例하였고 最適 抽出時間은 30分이 選定되었으며 溫度가 上昇함에 따라 $\log T^{3.73}$에 比例하였고 最適 抽出溫度는 40°C가 選定 되었으며 試料 0.1g當 溶媒量의 增加에는 $\log S^{3.7}$의 色素 抽出量을 나타냈고, 脂肪을 完全히 除去한 試料는 전혀 除去하지 않은 試料보다 0.6(O.D 448 mμ)만큼의 좋은 抽出效果를 보였다. 이들을 綜合하여,

$$P_{total} \fallingdotseq 1.15 \log t + 3.73 \log T + 3.7 \log S - 4X - 6.4$$

P_{total}: total amounts of extracted orange-yellow pigment from Gardenia.
t: extraction time (0.5~60 min)
T: extraction temperature (5~50°C)
S: volume of solvent (5~50 ml)
X: fat contents (0~15%)

와 같은 修正된 實驗式을 유도했다.

INTRODUCTION

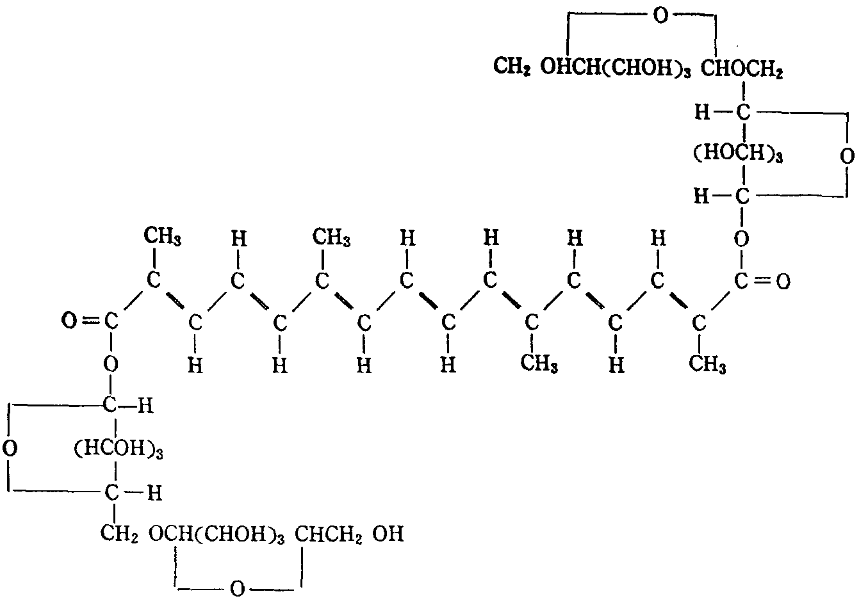
Edible pigments have some functions such as colouring, additives, antiseptics, and so on. Also, they increase one's appetite.

At present, instead of natural pigments that have contributed to improve the diet of mankind, many kinds of synthetic pigments have been developed. But some of these synthetic pigments involve virulences and often make public censure.

Therefore, all countries of the world have enacted of laws of food sanitation and have made efforts to investigate the safety of food from adding pigments.

By Antatso Kogi (1970),[1] although the amount of natural pigments extracted from plants is small in quantity, they have original taste, smell, and have little virulences in comparison with synthetic pigments. On the whole, natural pigments are unstable in light, heat, acid and base solution.

In the 1920's, P. Karrer (1927~1930)[2] [6] who had been studying Safranpigments discovered that Crocin, the digentiobiose ester of Crocetin, exists in Safran and Gardenia, and its colour is orange-yellow. Kozo Miki and P. Karrer (1929)[8] determined the formula of Crocin to be the following;



The maximal wave lengh of Crocin in methanol was known to 434 mμ, 464 mμ, but its available characteristics had not been studied as yet. Donald F. Othmer and Jagdish. C. Agarwal(1955)[7] studied the theory and mechanism for the extraction of soybeans. Sang Choe et el (1970)[8] reported the extraction of leaf protein concentrates of some plants growing in Korea. In a previous report (1974)[9] the pigment-extraction from the natural Gardenia, which was not defatted, was studied and a general empirical equation to the amounts of extracted-pigment was derived.

This research was attempted; i) to determine the extraction mode of orange-yellow pigment from defatted Gardenia, ii) to find a laboratory technique which would give the data of optimal conditions for plant design, iii) and to improve the empirical equation that was derived by Yu and Hong (1974).[9]

METERIALS AND METHODS

Materials

Sample that was supplied for the experiment came from the Nam Hae island in Korea. The average weight of a Gardenia fruit is 1.2002 g, containing about 14.9% (wt/wt) of fat contents, and involves about 5.541% (wt/wt) of moisture contents.

Fully dried samples including cuticle were ground with mortar to pass 24 mesh screen(Tailor style:dia: 0.701 mm).

Methods

a) Measuring the amounts of orange-yellow pigment and fat contents.

The amounts of pigment was estimated by measuring the optical density of extracted-solution at 448 mμ with UV-spectrophotometer HITACHI model 101 by following the ways of previous report (1974)[9] and T.V. Ramakrishnan (1973).[10] The amounts of fat contents was measured with Soxhelt equipment.

b) Method of pigment-extraction

Extraction conditions were based on the following; 0.1 g of defatted Gardenia was accurately weighed, adding 20 ml of water, orange-yellow pigment was extracted at room temperature (18°C) for 10 minutes.

c) Derivation of empirical equation

The total amounts of the extracted pigment(P_{total}) was effected by the change of extraction time (t), extraction temperature (T), volume of solvent (S), and fat contents (X).

Therefore, $P_{total}=f(t.T.S.X)$

Now that, to determine the optimal conditions of operating the experiments, we studied the effect of one variable while others fixed.

Differenciating the above equation,

$$dp_{total} = \left(\frac{\partial P_{total}}{\partial t}\right)_{T\cdot S\cdot X} dt + \left(\frac{\partial P_{total}}{\partial T}\right)_{T\cdot S\cdot X} dT + \left(\frac{\partial P_{total}}{\partial S}\right)_{tT\cdot X} dS + \left(\frac{\partial P_{total}}{\partial X}\right)_{t\cdot T\cdot S} dX$$

and linearizing the above equation by purtubation technique,

$$P_{total} = \left(\frac{\partial P_{total}}{\partial t}\right)_{T\cdot S\cdot X} t + \left(\frac{\partial P_{total}}{\partial T}\right)_{t\cdot S\cdot X} T + \left(\frac{\partial P_{total}}{\partial S}\right)_{t\cdot T\cdot X} S + \left(\frac{\partial P_{total}}{\partial X}\right)_{t\cdot T\cdot S} X$$

So, P_{total} was determined by the sum of four independent variables of the right in above equation.

RESULTS AND DISCUSSION

1) Solvent and analytical wave length

To determine the proper solvent for extraction of orange-yellow pigment from defatted Gardenia, various solvents were assayed and the results were illustrated in Table 1. It shows that the amounts of the extracted-pigment was more productive for the defatted sample than that of the natural sample. And it seems that methanol is more useful than any other solvents, but water was used in this experiment.

Table 1. The amounts of extracted orange-yellow pigment from Gardenia by using of various solvents

	Solvent	Water	Methanol	Ethanol	Acetone	Benzene	Ethyl-ether
Optical density at 448mμ	Natural Gardenia	4.51	6.10	1.02	0.178	0.027	0.032
	Defatted Gardenia	5.10	8.80	1.40	0.240	0.015	…

Sample 0.1g, solvent 20 ml, extraction time 60 min., extraction temperature 18°

When water was employde as the solvent, the absorption curve of extracted solution at each wave length was measured by Beckmann Grating spectrophotometer DB-G and shown in Fig. 1. But the extraction of Crocin was not our aim, we only wanted to extract mixed orange-yellow pigment from a crude sample. So, 448 mμ was chosen as the basis to measure the o. tical density of orange-yellow pigment.

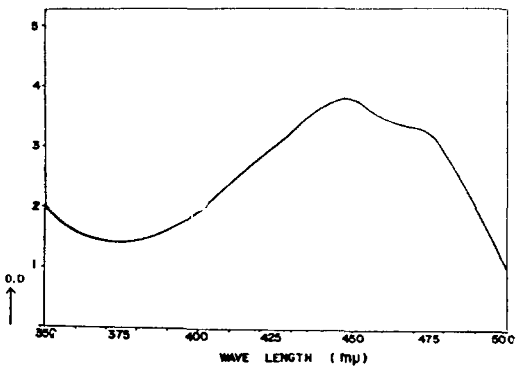


Fig. 1. UV-absorption spectrum of the extracted orange-yellow pigment from Gardenia. solvent:water

2) Heat-stability of the extracted orange-yellow pigment in solution.

To investigate the heat-stability of pigment for increasing the temperature of solution of extracted-pigment, 20 ml of water was added to the 0.1 g of

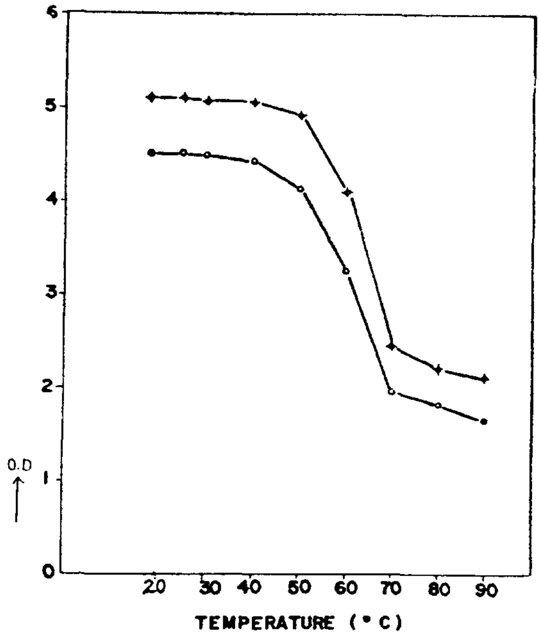


Fig. 2. Heat-stability curve of the extracted orange-yellow pigment from Gardena. Exposure of sample solution for 10 min., at each temperature.

—○—: Natural sample solution

-⊙- : Defatted sample solution

sample and orange-yellow pigment was extracted at 18°C for 1 hr. Then, the optical density of the solution was measure d at 448 mμ after setting it in the ther mos tat for 10 minutes at each temperature (Fig. 2). The pigment-solution was relatively stable under the temperature of 40°C, but the optical density of solution was fairly decreased above 50°C because of the oxidation of Crocin which has double bonds in the structure.

3) **Effect of time on the extraction of orange-yellow pigment from defatted Gardenia.**

Defatted sample, adding 20 ml of water per 0.1 g ground Gardenia, was used. The optical density at 448 mμ of extracted-solution, which was measured with the variety of extraction time at each temperature, was illustrated in Fig. 3.

As a whole, the extraction was progressed in a short time and the amounts of extracted-pigment from defatted sample was more productive than that of natural sample which was described in the previous report about 0.6 (O.D 448 mμ) for each extraction time.

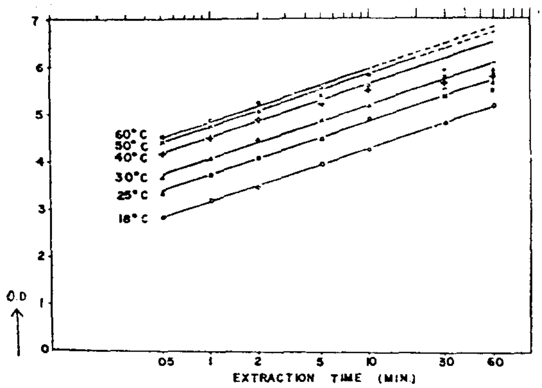


Fig. 3. Effect of time on the extraction of orange-yellow pigment from defatted Gardenia. Sample 0.1g, solvent 20 ml.

In Fig. 3, the straight lines, one for each temperature, are parallel with a constant slope of 1.15. This indicates a general equation;

$P_t \simeq 1.15 \log t + m$ ··················(1)

In this equation

t is the time of extraction in minutes(0.5~60 min.),

P_t is the amounts of extracted orange-yellow pigment(O.D 448 mμ), and m is the constant for each temperature, which may be evaluated from Fig. 3 by taking the value of the intercept with the time crdinate for 1 min (log 1=0).

But, the values of P_t were not suitable to the equation (1) with the increasing of extraction time over 60 minutes. Therefore, about 30 min. was chosen as the optimal extraction time.

4) **Effect of temperature on the extraction of orange-yellow pigment from defatted Gardenia.**

The optical density of extracted-solution at 448 mμ, which was measured with a variety of extraction temperature for 10 minutes at each volume of solvent on defatted sample, was illustrated in Fig. 4. They also showed that the amounts of extracted orange-yellow pigment was proportional to the logarithm of extraction temperature with a constant slope of 3.73. This was indicated as the equation (2).

$P^T \simeq m \simeq 3.73 \log T + m'$ ···············(2)

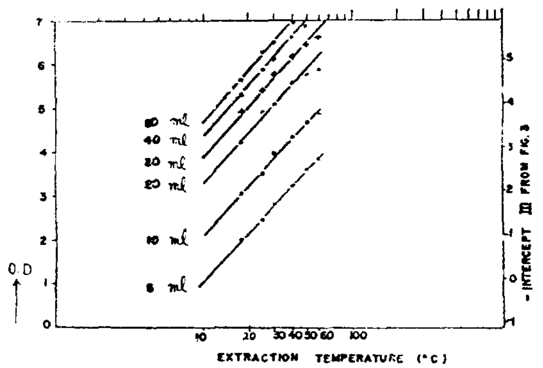


Fig. 4. Effect of temperature on the extraction of orange-yellow pigment from defatetd Gardenia. Sample 0.1g, extraction time 10 minutes.

In this equation,

T is the temperature of extraction in centigrades (5~6°C), P^T is the amount of extracted orange-yellow pigment (O.D 448 mμ), and m′ is the constant for each volume of solvent, which may be evaluated from Fig. 4 by taking the value of intercept with the temperature ordinate for 1°C (log 1=0).

Also, the amounts of extracted-pigment was higher than that of natural sample about 0.6 (O.D 448 mμ) for each extraction temperature. But the values of P_T were not adequate to the equation (2) above the temperature 50°C. It means that the amounts of extracted-pigment was limited to the temperature and the orange-yellow pigment was destroyed at the high temperature. Therefore, the optimal extraction temperature was supposed to be 40°C.

5) Effect of volume of solvent on the extraction of orange.yellow pigment from defatted Gardenia.

The optical donsity of extracted-solution at 448 mμ, which was measured with the variety of volume of solvent per 0.1 g of defatted sample at 18°C, for 10 minutes, was illustrated in Fig. 5. The amounts of extracted orange-yellow pigment was proportional to the logarithm of increasing the volume with a constant slope 3.7.

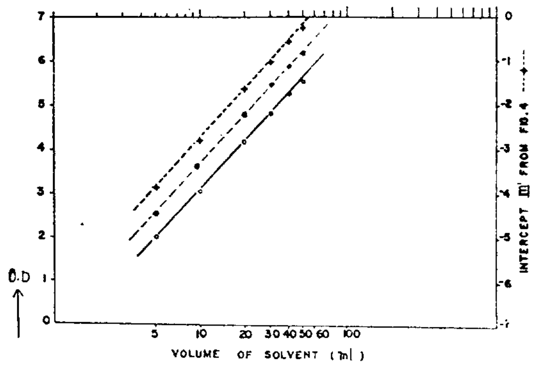


Fig. 5. Effect of volume solvent of on the extraction of orange-yellow pigment from defatted Gardenia. Sample 0.1 g, extraction time 10 min., extraction temperature 18°C.
○……○ : natural Gardenia
●……● : defatted Gardenia

As a result, equation (3) was derived.

$P_S \simeq 3.7 \log S + K$ ………………(3)

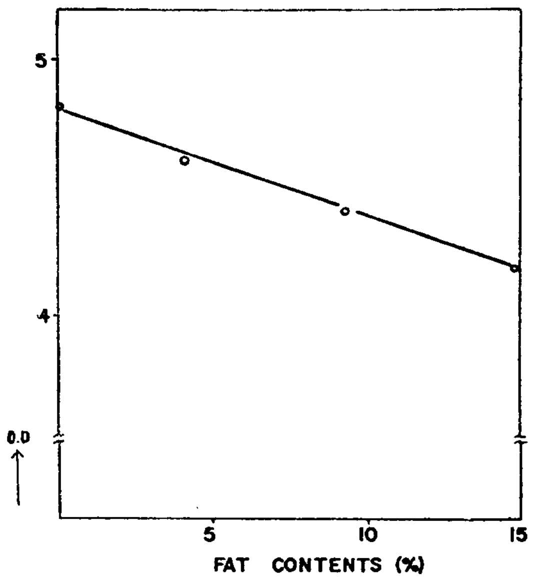


Fig. 6. Effect of fat contents on the extraction of orange-yellow pigment from Gardenia. Sample 0.1 g, solvent 20 ml, for 30 min., at 18°C.

In this equation,

S is the volume of solvent per 0.1 g of sample in milli-liters (5~50 ml).

P_s is the amounts of extracted orange-yellow pigment (O.D 448 mμ), and k is the constant that indicates the value of the intercept with the volume ordinate for 1 ml, and it also involves the meaning of values between natural and defatted samples.

However, the amounts of extracted-pigment was not increased in pace with the equation (3) more than 50 ml of solvent per 0.1 g of sample. Also, it becomes hard to separate the pigment from solution for much volume of solvent per 0.1 g of sample. The muitiple extraction process was more useful than using batch process, because the amount of pigment which was extracted three times with 20 ml of water per 0.1 g of sample was more than that extracted one time with 60 ml of water per 0.1 g of sample.

6) Effect of fat contents of sample on the extracton of orange-yellow pigment from Gardenia..

Obviously, the fat contents effected the amounts of extracted-pigment as the observations of previous data and the average amounts of extracted-pigment from defatted sample is 0.6 (O.D 448 mμ) more than that of natural sample.

k, the intercept of equation (3), varied in the amount of fat contents of sample. Therefore, the optical density of extracted-solution at 448 mμ, which was measured with the variety of fat contents, adding 20 ml of solvent per 0.1 g of sample at 18°C for 30 minutes, was illustrated in Fig. 6. As the figure shows, the amounts of extracted-pigment was decreased in proportion to the constant slope of -4 while increas -ing the fat contents of sample. This indicates a general equation;

$k \simeq -4X + 4.8$ ………………………(4)

In equation (4)

X is the fraction of fat contents in sample(0~0.15),

k is the amount of extracted pigment that varies within the range of 0.6 (O.D 448 mμ) between natural and defatted sample.

Now, the above equations that were derived from the change of extraction time (t), extracion temperature (T), volume of solvent(S), and fat contents of sample (X), should be united in one equation by concentrating on the origin. (log t=0, log T=0, log

S=0, X=0). As the result of concentrating them on the origin, the values of m′, the intercepts of the equation (2), were parallelled as the amounts of −1.15 (O.D 448 mμ) and indicated at the right ordinate of Fig. 4. Also, the values of k, the intercep-ts of equation (3), were parallelled as the amounts of −4.68 (O.D 448 mμ) after moving the amounts of −1.15 (O.D 448 mμ). ......(see the right ordinate of Fig. 5).

Therefore, the values of k were determined with the range of between −6.4 (O.D 448 mμ) and −7.0 (O.D 448 mμ) that where shown in Fig. 5 as a values of intercepts.

Consequently, the improved empirical equation was derived.

$$P_{total} \simeq 1.15 \log t + 3.73 \log T + 3.7 \log S - 4X - 6.4 \quad \cdots\cdots(5)$$

Fig. 7, Fig. 8 were the results of comparison between experimental values and computational values. Their deviation range is only ±1.750%.

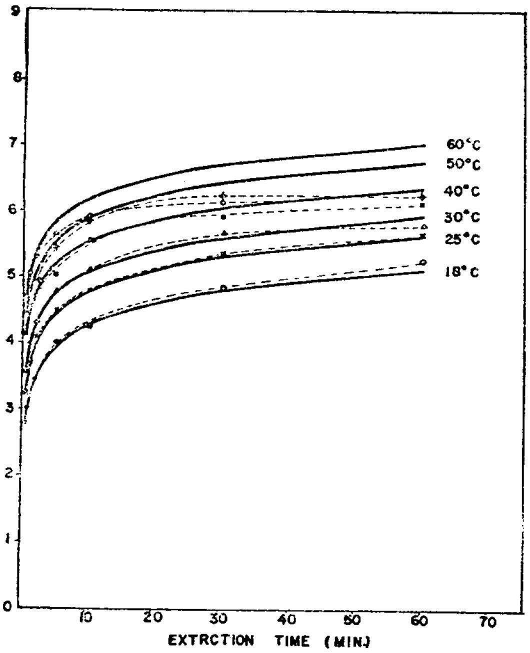


Fig. 7. Comparison of data between the computational curve and experimental curve on time of the extracted orange-yellow pigment from defatted Gardenia
——; Computational curve
......; Experimental curve

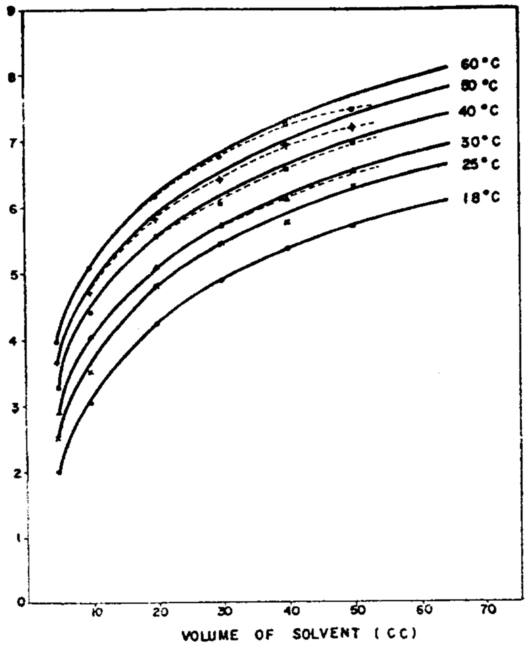


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Fig. 8. Comparison of data between the complutational curve and the experimental curve on time of the extracted orange-yellow pigment from defatted Gardenia.
——; Computational curve
......; Experimental curve

ABSTRACT

The extraction efficiency of orange-yellow pigment from the Gardenia was greatly depended upon the extraction time, extraction temperature, volume of solvent used and fat contents of the Gardenia.

Fom the experimental results, the amounts of extracted pigment (P) was proportional to the log $t^{1.15}$ of extraction time(t; 0~60 min.), the log $T^{3.73}$ of extraction temperature(T; 5~60°C), the log $S^{3.7}$ of volume of solvent(S; 5~50 ml), and the −4X of fat contents of sample (X; 0~0.15) at 18°C for 10 minutes.

Finally, the modified empirical equation was derived as follow;

$$P \simeq 1.15 \log t + 3.73 \log T + 3.7 \log S - 4X - 6.4$$

In addition to that, the most optimum conditions of pigment extraction were determined as 30 minutes of operation time, 40°C of temperature. Deffated Gardenia

was more productive than natural Gardenia in the pigment extraction.

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