Chemical processing of raw florets of dyer's saffron and successful extraction of a resulting product (carthamin): an approach to improve conventional methods

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Abstract

Efficacy of KMnO_4 on processing red florets of dyer's saffron was examined in the presence of micromolar concentrations of the metal salts. Apparent value for KMnO_4 determined by double-reciprocal plots was $126 \mu\text{M}$, whose value corresponds to a 1/154-fold of dried florets. Successful extraction of red carthamin was performed by using the processed matters with various solvent systems. HCl/NaOH , acetic acid/ammonia water and pyridine/methanol were found to be effective. For purification of carthamin, the cellulose adsorption technique was very promising. The data are assessed to standarize the new technique.

Key words: dyer's saffron, raw floret, chemical processing

INTRODUCTION

The suitability of the procedures for processing raw material and the yield of the resulting products have always become determinative factors in the marketing process.

Dyer's saffron is known to produce a red dye, carthamin. In Japan, the tube flowers are picked every two or three days from much of the orange- or red-flowered capitula. After processing, they are used as a raw material for

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dye-stuff or of other purposes (Saito 1990). The method for processing raw flowers is very complicated and laborious, and, moreover, all manufacturing processes must be performed instantly within duration of the limited flowering season. The routinely applied processes involve following treatments: squashing, kneading, pressed out, washing and drying of the resulting paste. In consequence, every year, economic damage has unexpectedly overwhelmed many dye-manufacturers: some or all of the picked materials are seriously spoiled during the course of the inevitably prolonged preparatory operations. There is no wonder why processed flowers are sold at very high-price.

To improve these troublesome and hazardous methods, we have recently introduced a new technique to provide reddened flowers more effectively (Saito 1991a, Saito and Katsukura 1991). This is obviously superior to the conventional procedures in the following several points: (1) it is simple, (2) product yield is high, (3) the reaction proceeds swiftly, (4) it is completed even at lower temperature, (5) no affection can be observed by changing external factors including pH, gas phase and moisture, (6) the dexterities of the operators are negligible, if any, (7) the cost is inexpensive, (8) all process can put through a program of semi-industrial scale. However, for processing red materials, mangano-ions are, if possible, to be fed at far more reduced levels. Hence, accumulation of further indicative evidence on the topics seems to be indispensable for standarizing the newly devised technique.

The present work was designed to prepare reddened flowers at minimized mangano-ion levels. Below, we also centred our attention to the increment of carthamin yield.

MATERIALS AND METHODS

Dyer's saffron sample. The seeds of dyer's saffron (cv. Mogami Benibana), harvested in 1990, were purchased from a market (Yamagata, Japan). Uniform seeds which had been selected manually were sown on our field in May 25, 1991. All florets were harvested from the freshly opened flowering heads in August 5-28, 1991.

Chemicals. Chemicals used were as follows: Avicel cellulose (Asahi Kasei Kogyo, Tokyo, Japan); KMnO_4 , K_2CO_3 , citric acid, acetic acid, HCl , NaOH , ammonia water, pyridine and acetone (Wako Pure Chemical, Osaka, Japan). All these chemicals were of analytical grade of purity, which were used for the process of carthamin formation, subsequent extraction, partially purification and spectrophotometric estimation.

Instruments. Hitachi UV/VIS double-beam spectrophotometric apparatuses, model U-1100 and model U-3210 were used to measure a fixed absorption peak and long range of absorption bands, respectively.

Methods of processing red florets. Bright-orange florets (1 g fresh wt. each) were ground finely with a pestle and mortar. The paste was suspended in 10 cm³ of 5-250 μ M KMnO₄ in 50 mM citrate buffer, pH 3.5 and stood in open air for desired intervals at 26°C. The red paste thus obtained was transferred on a Büchner funnel and filtered by suction.

METHODS OF CARTHAMIN EXTRACTION

Potassium carbonate/citric acid method (Ladcliffe 1897). The semi-dried floret pieces were suspended in 20 cm³ of 0.5% (w/v) K₂CO₃ and the suspension was stirred with a magnetic stirrer for 3 min at room temperature, then it was filtered on Büchner funnel with an aspirator. The extraction was repeated once again under the same conditions as presented above. The combined filtrates were acidified with 1 g citric acid. The acid solution was poured into a beaker, in which 1 g Avicel cellulose had been placed, and stirred for successive few minutes. The resulting red slurry was washed exhaustively with distilled water (300 cm³ $\times$ 5) to remove water-soluble impurities. Carthamin was recovered from the cleaned Avicel with 60% (v/v) acetone and the acetone extract was messed up to a net volume, which was used to the estimation of the pigment content.

Pyridine/methanol method (Kuroda 1930). The damp flower paste was suspended in 20 cm³ pyridine/methanol (3:7, v/v) and stirred for 3 min at room temperature. The organic phase was retained and the residue extracted once again with the same composition of new pyridine/methanol. The pooled extracts were filtered and the filtrate was evaporated to dryness at less than 35°C, then the solvent free concentrate was resuspended in 50 cm³ distilled water and to this suspension 1 g Avicel was mixed stirring. Carthamin was extracted as described in above section.

Aqueous acetone method (Wada 1953). The KMnO₄-processed flower pieces were dipped in 20 cm³ of 80% (v/v) acetone at 26°C and extracted two times successively at the intervals of 3 min. Before subsequent extraction, the solvent was replaced by 20 cm³ of new one. The extraction was made with 80% (v/v) acetone. After being removed the solvent, carthamin was adsorbed by 1 g Avicel in 50 cm³ of distilled water and washed several times, then the reddish Avicel treated with a net 50 cm³ of 60% (v/v) acetone. Thus prepared acetone extract was applied to the process of the spectrophotometric measurement.

Other methods. The red paste was treated separately with a given concentration of methanol, HCl/NaOH, HCl/ammonia water or acetic acid/ammonia water (20 cm³ each). Carthamin thus extracted was entrapped by Avicel (1 g each) and the pigment content was determined spectrophotometrically after being eluted with a net volume of 60% (v/v) aqueous acetone.

PRELIMINARY TESTS FOR PROMOTING CARTHAMIN YIELD

Three methods, infiltration of KMnO_4 , maceration of floral tissues and non-treatment of florets, were tested here.

Infiltration of KMnO_4 . Fresh flowers (1 g) in a suction bottle (100 cm^3) containing 10 cm^3 of $100\text{ }\mu\text{M}$ KMnO_4 were sucked under reduced pressure for 10 min at 26°C . At the end of the treatment, flowers were transferred to a beaker and extracted with 20 cm^3 of 0.5% (w/v) K_2CO_3 followed by being acidified with 1 g citric acid. The acid filtrate was treated with Avicel (1 g) and the carthamin content in the acetone extract was determined spectrophotometrically.

Maceration of floral tissues. Another batch of flowers (1 g) were macerated carefully in a porcelain mortar with a pestle. To this slurry, 10 cm^3 of $100\text{ }\mu\text{M}$ KMnO_4 solution was poured and stirred quickly with a pestle. After standing for 10 min, the slurry was subjected to the extraction and estimation of carthamin content.

Non-treatment of flowers. An aliquot weight (1 g) of flowers was left for 10 min in 10 cm^3 of $100\text{ }\mu\text{M}$ KMnO_4 solution, then the test materials were applied to the process of the carthamin yield determination.

ESTIMATION OF CARTHAMIN CONTENT

The content of carthamin was determined by the reading of the absorption peak at 521 nm. The data from the spectral monitoring were referred to a standard curve and the apparent content of carthamin was determined finally.

For conducting another set of spectrophotometric studies, crude extracts obtained through several different extraction methods were evaporated to dryness and the residues were dissolved into a net 750 cm^3 of 60% (v/v) acetone, which were subjected directly to the process of the spectroscopic assay. The light absorption was followed in the long range from 320 to 600 nm.

RESULTS

 KMnO_4 CONCENTRATION VERSUS CARTHAMIN YIELD

Various concentrations of KMnO_4 were fed to the triturated floral tissues of dyer's saffron to assess the effect of pentavalent manganese on the carthamin yield. For the double-reciprocal plots of KMnO_4 concentration and carthamin yield showed a linear relationship (Fig. 1). At very low concentrations of KMnO_4 , the reaction rate rose sharply with increasing the metal cations. At intermediate concentrations (5 to $20\text{ }\mu\text{M}$), the rate still rose with KMnO_4 concentration, but the effect on the carthamin yield became apparent. A concentration above $80\text{ }\mu\text{M}$ KMnO_4 , the reaction rate decreased. The apparent value for

KMnO_4 was $126 \mu\text{M}$ ($V_{\max}$ $63 \mu\text{M}$) (see Fig. 1 and an inset in the figure). This value corresponds to 1/154-fold of methanol-denatured and dried flowers (Saito and Katsukura 1991).

Fig. 1. Effect of the concentration of KMnO_4 on the rate of carthamin production. An inset shows Lineweaver-Burk plots of reaction velocity versus KMnO_4 concentration. For details of the assay conditions, see Materials and Methods

ASSESSMENT OF THE METHODS FOR CARTHAMIN EXTRACTION

In this studies, various techniques based ont only on currently designed procedures, but also on often applied or on once attempted methods (L a d c - l i f f e 1879, K u r o d a 1930, W a d a 1953) were assessed experimentally. The data from the UV/VIS absorption spectra of crude extracts registered in the long scale renges are illustrated in Fig. 2A and B. It is evident that all test solvents have their characteristic effects on carthamin recovery. This is supported by the quantitative data summarized in Table 1. Among the solvents tested. HCl/NaOH is most effective (1.3-fold of K_2CO_3 /citric acid, which has often been applied as a standard solvent). Acetic acid/ammonia water comes net (1.2-fold). Pyridine/methanol follows this (1.1-fold). Other solvents seem not to be so effective as the former three. Carthamin extracted by these solvents can be purified easily through the cellulose powder adsorption method (S a i t o et al. 1983), because the pigment is entrapped and stabilized by this glucose polymer (S a i t o and F u k u s h i m a 1988). With this property, water-soluble impurities

Fig. 2. UV/VIS-spectra of crude extracts obtained by using various solvents. A — polar solvents: citric acid/ K_2CO_3 (1), HCl/NaOH (2), $\text{HCl}/\text{ammonia}$ water (3), acetic acid/ NaOH (4), acetic acid/ ammonia water (5). Each 90 mM acid was mixed with each 35 mM alkali. B — non-polar solvents: partially purified carthamin (1), pyridine/methanol (3:7, v/v) (2), acetone/water (8:2, v/v) (3), methanol/water (8:2, v/v) (4). For details of experimental conditions see Materials and Methods

Table 1
Efficiency of solvent systems on carthamin extraction

Solvent system	Carthamin content $\mu\text{g}\cdot\text{cm}^{-3}$	Ratio*
HCl/NaOH	43.0	1.3
HCl/Ammonia water	22.8	0.7
Acetic acid/NaOH	30.1	0.9
Acetic acid/Ammonia water	38.9	1.2
K_2CO_3 /Citric acid	33.0	1.0
Pyridine/Methanol	36.4	1.1
Acetone	27.9	0.9
Methanol	29.6	0.9

* The value was defined as the ratio to K_2CO_3 /citric acid (1.0). Compositions of each solvent system used, see Fig. 2.

can be removed almost completely (see Fig. 2B). Hence, one may not be concerned about the contaminated mangano-ions and other minor substances.

TESTS FOR ACCELERATING CARTHAMIN ACCUMULATION

Technical acceleration of carthamin synthesis was planned by applying three different methods: infiltration of KMnO_4 , maceration of flower tissues and no treatment of flowers. The results are listed in Table 2. It is obvious that

Table 2
Effect of technical treatments on carthamin production

Treatment*	Carthamin content $\mu\text{g}\cdot\text{cm}^{-3}$
I	2.0
II	2.4
III	40.8

* I — non-treated flowers (1 g fresh wt) were immersed in 10 cm³ of 100 μM KMnO_4 for 10 min at 26°C, II — flowers (1 g fresh wt) were placed in a suction bottle containing 10 cm³ of 100 μM KMnO_4 and sucked for 10 min at 26°C, III — flowers (1 g fresh wt) were macerated with a pestle and mortar in 10 cm³ of 100 μM KMnO_4 for 10 min at 26°C. For further details of carthamin extraction, spectrophotometric estimation or others, see text.

maceration process is very effective to speed up carthamin production (20.1-fold of non-treated flowers), while the infiltration technique is not so contributive as expected usually, although this has often been used as an useful method particularly in the studies on radio-isotope feeding.

DISCUSSION

In this study, a chemical method for inducing red colour in dried *Carthamus* flowers (Saito 1991a) was expanded to the fresh materials and its utility was evaluated by examining the rate of the product yield. On the basis of the experimental data, it is clear that the currently applied protocol is very useful to process red flowers, which are applicable as starting materials for cathamin preparation. Supporting this, a reciprocal correlation is suggested on the saturation curve of requirement for KMnO_4 versus velocity of cathamin yield (Fig. 1). KMnO_4 acts on bright-orange flowers effectively at least up to about $80\text{ }\mu\text{M}$. On excess dose of the metal salts over $80\text{ }\mu\text{M}$, cathamin yield falls by degrees. It is worth-while to note that apparent value for KMnO_4 obtained here is far lower than that observed with dried flowers (1/154-fold) (Saito and Katsukura 1991). The mechanism by which Mn (VII) acts on fresh materials so sensitively is still unclear, although the interesting problem is to be studied in time. Similar results have already been reported in our preceeding paper (Saito 1991b), indicating that the efficacy of multivalent manganese on the floret reddening process may be controlled by enzyme(s) or enzyme system(s) which hitherto been characterized in *Carthamus* species. In any way, co-existence of the metal cations makes much for our advantage to prepare red flower florets.

For getting informative evidence about the increment of cathamin yield, several solvents were tested during the course of the pigment extraction and the resulting data compared with those of the methods which are often used (Ladcliffe 1897) or once applied earlier (Kuroda 1930, Wada 1953). In this study, various solvent systems were used at five different combinations as follows: (1) strong acid/strong alkali, (2) strong acid/weak alkali, (3) weak acid/strong alkali, (4) weak acid/weak alkali and (5) organic solvent. Some new evidence could be acquired from these tests, indicating that cathamin yield is possible to elevate much higher if acids and alkalies are mixed applying at appropriate compositions. Based on our current observations, it can be safely said that strong acid/strong alkali and weak acid/weak alkali are most promising. Pyridine/methanol seems to be effective, although during this experiment we have encountered somewhat difficulties to remove traced pyridine in the extract. The difficulties, however, may perhaps be overwhelmed by using cellulose adsorption technique (Saito et al. 1983).

Technical pre-treatment of raw materials are familiar to biomass engineers and biotechnologists. They shorten frequently the intervals required for obtaining useful metabolites. The results of our preliminary operation on fresh *Carthamus* florets were indicative of the fact that maceration process is very useful to prepare red materials, though this process had little effect on the dried flowers even in the presence of milimolar KMnO_4 (Saito and Katsukura 1991). Again this seems to support our previous assumption that certain biocatalysts may control the chemical reaction within the living tissues (Saito 1991b).

We can now list up additional merits of the manganese method in the following points: (1) the procedure is applicable not only to dried matters, but to fresh flowers; (2) in preparation of red materials with fresh florets, required metals can be reduced to far lower levels; (3) maceration of floral tissues is essential process for accelerating carthamin production by manganese; (4) carthamin yield is possible to be leveled up if acids and alkalies are applied at appropriate combinations; (5) for purifying crude carthamin, cellulose adsorption methods is promising.

Red carthamin thus prepared is applicable, of course, as a dye-stuff of textile fabrics, cosmetic rouges and of other purposes. Food technologists considering this dye as a colouring agent can also utilize safely to processed foods, coloured tablets and soft beverages after purification through appropriate techniques (Saito 1990, 1991a), as carthamin has recently been reported to be non-toxic (Saito and Fukushima 1991). In practice, the contaminated mangano-ions and other water-soluble impurities can be aside of the question because carthamin adsorbent (mostly Avicel cellulose or cellulose powder) is washed thoroughly with sufficient amounts of water during the purification steps as mentioned above.

In conclusion, the present results, together with those reported earlier (Saito 1991a, Saito and Katsukura 1991), lead to some practical hints on the floret processing conditions required for high-quality carthamin dye. Our results may be of relevance to the technology of biocolorants for textiles, cosmetics, medicinal goods and food products before long.

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Obróbka chemiczna surowca z płatków szafranu oraz udana ekstrakcja produktu końcowego (kartaminy): dalsze doskonalenie powszechnie stosowanych metod

Streszczenie

Badano wpływ KMnO_4 na obróbkę czerwonych płatków szafranu, stosując mikromolarne stężenia soli metalu. Widoczny efekt KMnO_4 otrzymano przy stężeniu $126 \mu\text{M}$, co jest zbliżone do 1/154-krotności wysuszonych płatków. Udaną ekstrakcję czerwonej kartaminy przeprowadzono stosując obróbkę z użyciem różnych systemów rozpuszczalników. Skuteczne okazały się: HCl/NaOH , kwas octowy/woda amoniakalna i pirydyna/metanol. Obiecujące wyniki oczyszczania kartaminy otrzymano stosując metodę adsorpcji na celulozie. Wyniki posłużyły do standaryzacji nowej metody.