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On manganese-induced reddening of florets from dyer's saffron capitula

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The effect of external conditions on permanganate-mediated carthamin formation was studied in floret suspensions under various experimental conditions. The reaction proceeded readily in acidic media (pH 3·4), where carthamin exhibits a stable red coloration. Temperature affected the reaction greatly: the red coloration in florets developed effectively at a lower temperature range (0 ± 1°C) rather than at a higher one (80 ± 1°C), showing that it follows first-order reaction kinetics. Antioxidants and metal chelators variously affected the permanganate catalysis. Attempts to promote the metal-induced floret reddening were found to be of little use.

INTRODUCTION

It has been suggested in a preceding study (Saito, 1991*a*) that $KMnO_4$ is an excellent reagent for reddening of florets from dyer's saffron capitula. A red product isolated from the processed florets was shown to be identical to carthamin, which is usually formed enzymatically in the matured floral tissues (Saito *et al.*, 1983*a*). Based on the carthamin-generating efficiency, the permanganate method seems to surpass previously applied procedures, where an endogenous enzyme reaction is used (Saito, 1990) or β-D-glucose oxidase is fed to the triturated floret suspension (Wada & Ota, 1986). With the permanganate method, a higher rate of carthamin productivity could be achieved, giving a yield of 0·031%; this value exceeded (by 2·6-fold) that of the native enzyme method and (by 1·2-fold) that of the glucose oxidase treatment (Saito, 1991*a*).

To establish this useful technique more firmly and to generalize it more widely, evidence for the affects of external conditions on the metal catalysis seems to be important. This communication will summarize data on the factors which are thought or expected to influence carthamin productivity through controlling manganese-mediated chemical reaction.

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MATERIALS AND METHODS

Materials

$KMnO_4$, hydroquinone, D-isoascorbic acid, EDTA, α, α'-dipyridyl, KCN, sodium azide, 8-hydroxyquinoline and *o*-phenylenediamine dihydrochloride were purchased from Wako Pure Chemical (Osaka, Japan). Avicel cellulose, other chemicals and dried florets of dyer's saffron were from the same sources as described in our preceding paper (Saito, 1991*a*).

Observation of external factors affecting manganese-mediated floret reddening

Model systems consisted of weighed materials matched to each experimental scheme. At the beginning of the study, bulk florets were portioned into small units neatly, the size required for a batch of the model system. Each portion, in a 100 ml flask, was wrapped tightly in sealon film. The same samples of the florets, Avicel and other chemicals were used throughout the study. They were stored in a closed container at room temperature. Some of the florets were prepared by freezing in liquid nitrogen, and then grinding with a porcelain pestle and mortar. Grinding was continued until the florets were triturated into fine pieces as judged visually. Individual tests were carried out following the experimental menu as described below.

1 $KMnO_4$ concentration
5–2000 μmol $KMnO_4$ was added to the floret (1 g) suspension in 50 ml of 50 mM citrate buffer, pH 3·4, if not stated otherwise, and stirred at room temperature (22 ± 2°C) for 30 min with a magnetic stirrer.

2 pH value
The relative carthamin productivity by added permanganate salts was examined at varied pH ranges from 2·5 to 7·5. Three buffer systems, phthalate/HCl, citrate and phosphate, were used at 50mM concentration. Incubation time and temperature were the same as described above.

3 Temperature
Carthamin production by $KMnO_4$ was studied at six different temperature ranges (0 ± 1, 10 ± 1, 20 ± 1, 40 ± 1, 60 ± 1, 80 ± 1°C) using floret (1 g) suspension in 50 ml of 50 mM citrate buffer, pH 3·4 containing 20 μmol $KMnO_4$. The incubation was carried out for 30 min.

4 Type of buffer
To examine the effects of buffers on the floret reddening, the following buffer systems were used at 50 mM and at pH 3·4: citrate, glycine/HCl, citrate/phosphate, citric acid/KH_2PO_4/H_3BO_3/diethylbarbituric acid/NaOH. Florets (1 g each) were floated in the test buffers and incubated under given conditions (30 min, 22 ± 2°C, stirring with a magnetic stirrer).

5 Chemicals
Two antioxidants (hydroquinone and D-isoascorbic acid) and six metal chelators (EDTA, KCN, α, α'-dipyridyl, sodium azide, *o*-phenylenediamine dihydrochloride and 8-hydroxyquinoline) were tested at 1 and 10 μmol levels. They were mixed separately into the incubation fluid and their effects on the rate of the colour production evaluated spectrophotometrically.

Test for promotion of floret reddening

1 Crushing of florets
Florets (1 g) were frozen in liquid nitrogen and crushed with a pestle and mortar. The finely powdered florets were thawed in 50 ml of 50 mM citrate buffer, pH 3·4 and left to stand at 22 ± 2°C for 30 min immediately after addition of 20 μmol $KMnO_4$.

2 Blasting of florets
Florets (10 g) were placed in a blasting oven (Nitto Koatsu, type 3867, 3868) and kept for 1 min at 20–24 atm. After the end of the operation, the maceration paste was transferred to a flask containing 20 μmol $KMnO_4$ in 500 ml of 50 mM citrate buffer, pH 3·4 and the mixture treated at 22 ± 2°C for successive 30-min periods.

3 Infiltration of $KMnO_4$
A weighed sample (1 g) was suspended in 50 ml of 50 mM citrate buffer, pH 3·4, containing 20 μmol $KMnO_4$ in a beaker (300 ml), which was placed in a suction funnel and sucked several times under reduced pressure, then it was left to stand for 30 min at 22 ± 2°C by stirring incessantly with a magnetic stirrer.

4 Bubbling of floret suspension
An aliquot of the sample (1 g) was transferred to a flask containing 50 ml of 20 μmol $KMnO_4$ in 50 mM citrate buffer, pH 3·4 and the suspension was bubbled vigorously with oxygen gas for 30 min at 22 ± 2°C.

5 Hypersonic oscillation of floret suspension
An aliquot of floret (1g) suspension containing 20 μmol $KMnO_4$ in 50 ml of 50 mM citrate buffer, pH 3·4 was kept in a ultrasonic oscillator (Bransonic, model B-1200) at 45 kHz for 30 min at 22°C. The rate of the red colour increase was determined after treatment of the test material through the process of extraction as described below.

Quantification of incubation product

Reddish-orange florets were floated on 20 ml of 0·5% (w/v) K_2CO_3 and stirred with a magnetic stirrer for 3 min at room temperature. The suspension was filtered on a Büchner funnel with suction and the residue treated twice more with 0·5% (w/v) aqueous K_2CO_3. All filtrates were combined and acidified by the addition of solid citric acid to become 1·7% of the final concentration. To this acid solution, 1 g Avicel cellulose was suspended and stirred quickly for 2–3 min at 22 ± 2°C. The supernatant was decanted off and the residue washed thoroughly with deionized/distilled water (300 ml × 5). The resulting reddish slurry was rinsed in 60% (v/v) acetone and the acetone extract reduced in volume (50 ml). UV spectra were measured with a Hitachi U-1100 spectrophotometer using the acetone eluate. The concentration of the incubation product was determined from the reading of the light-absorption peak at 521 nm (Saito, 1991*a*).

RESULTS

Effects of external factors on floret reddening

A delicate but clearly perceptible fine bathochromic change was found in the floral tissues when incubated in permanganate-containing solution under different conditions. The observed results were as follows.

1 Permanganate concentration
The effect of $KMnO_4$ is summarized in Fig. 1, which is obtained from testing with a variety of concentrations.

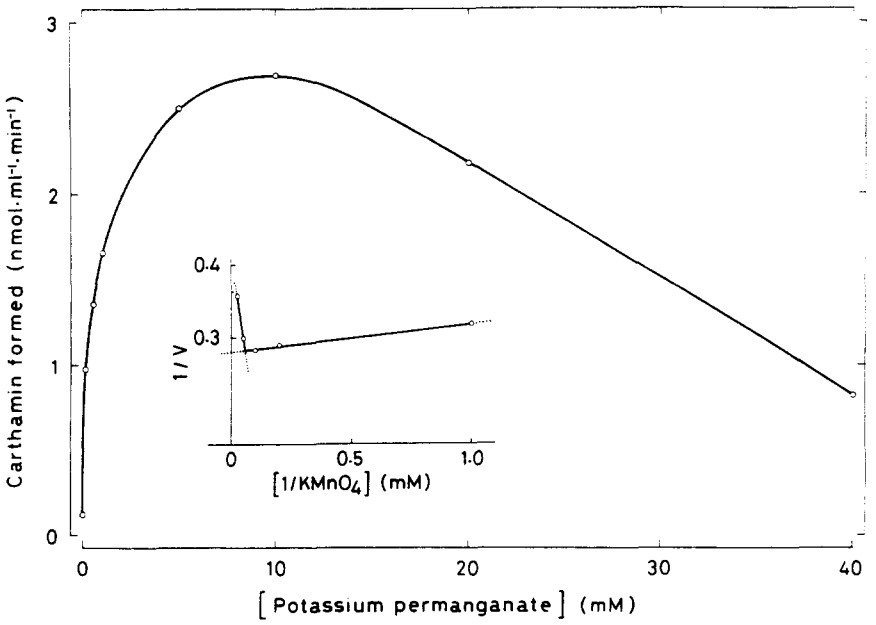

Fig. 1. Effect of $KMnO_4$ concentration on detached floret reddening. The inset shows the double-reciprocal plots. The composition of the mixture was 1 g dry florets, 0·1–40 μmol $KMnO_4$ and 50 mM citrate buffer, pH 3·4 in a total volume of 50 ml. The reaction was carried out for 30 min at 22 ± 2 °C.

The saturation curve of $KMnO_4$ versus carthamin content shows the reliability of this type of assay. $KMnO_4$ is effective at least up to about 8 μmol. Upon addition of higher concentrations of permanganate (over 10 μmol), carthamin productivity decreases promptly with simultaneous accompaniment of reddish brown or blackish brown precipitates. The apparent K_m value of 19·4 mM was calculated from the double-reciprocal plots (see an insertion in Fig. 1).

2 pH value

The permanganate-directed bathochromic reaction was examined over the pH range of 2·5–7·5 (Fig. 2). The optimum pH for the activity was pH 3·4, a value far lower than that of the carthamin-synthesizing enzyme which regulates the terminal step of carthamin formation from precarthamin in vegetative tissues of the herbal plant (Homma *et al.*, 1985). Both higher and lower pHs resulted in a decrease of the red colour development, presumably through changing catalytic environments such as reactant stability, ionic balance and manganese permeability, all of which can switch the productive conditions to unfavourable states and reduce the red colour content readily.

3 Temperature

When floret suspension was allowed to stand for 30 min at several temperature ranges, the rate of the red

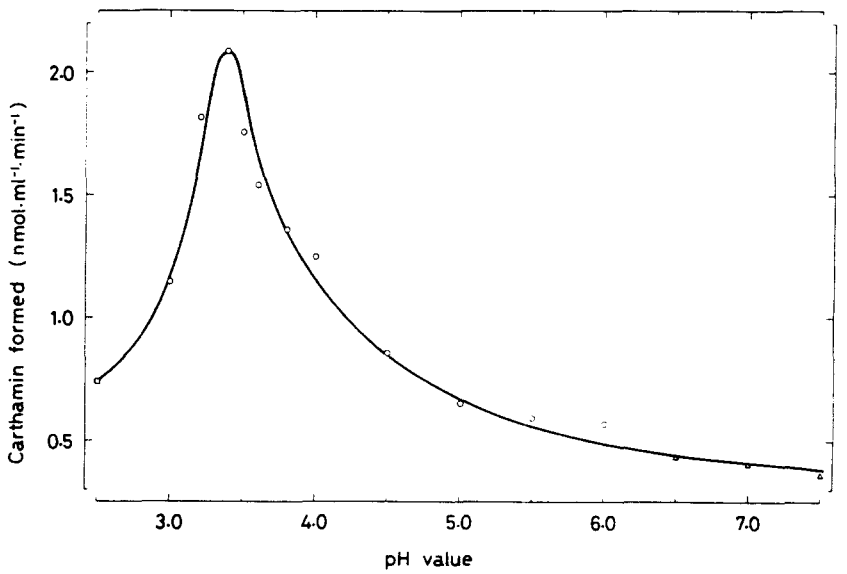

Fig. 2. Effect of pH on manganese-dependent floral reddening. Incubation was carried out as described in Materials and Methods, except that buffers used in the reaction were varied as shown; □, 50 mM phthalate/HCl; ○, 50 mM citrate; Δ, 50 mM phosphate.

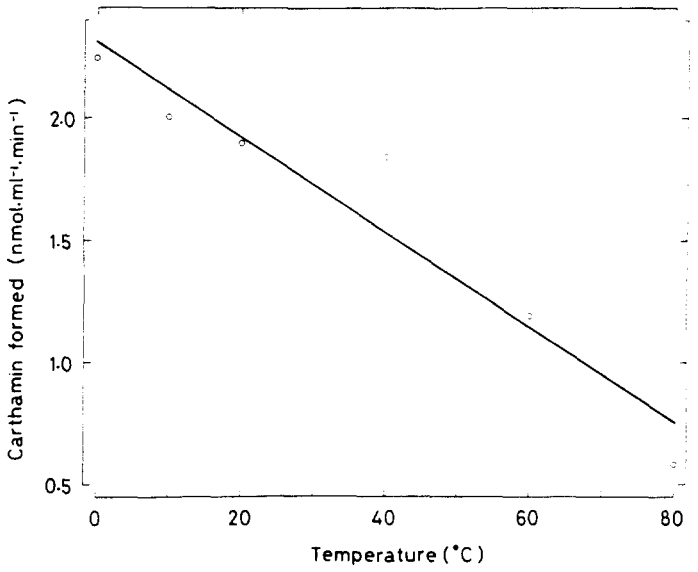

Fig. 3. Effect of temperature on manganese-dependent floret reddening. Incubation was performed as described in Materials and Methods, except that the reaction temperature was varied.

colour production was affected markedly. A typical result from the temperature test is presented in Fig. 3. As seen in the figure, the pigment production changes sensitively under varied temperature, indicating that the reaction is absolutely dependent upon the activation energy. The greatest rate was observed at 0 ± 1°C. At higher temperatures, apparent increments of the pigment content decrease greatly.

4 Buffer system

For floret suspension left in the air at 22 ± 2°C for 30 min, the buffer systems had a significant influence on the red colour presentation. This was confirmed by tests using 7 different buffers (50 mM, pH 3·4) in total (Table 1). Citrate was most promising. Citrate/phosphate came next and citric acid/KH_2PO_4/H_3BO_3/diethylbarbituric acid/NaOH followed this. Carthamin productivity was lowered further in phthalate/HCl and acetate. Both glycine/HCl and KCl/HCl acted on florets with far less effect, giving colours of only about one-third that of citrate. This indicates that the type of buffer and/or buffer composition is a determinative factor for preparing red florets.

Table 1. Effect of buffer systems on manganese-dependent floret reddening

Buffer system[a]	Carthamin formed (nmol carthamin/ml/min)
—[b]	0·66
A	2·11
B	0·85
C	1·21
D	1·23
E	0·84
F	1·85
G	1·63

20 μmol/$KMnO_4$ ml was used in this study.
[a] A, citrate; B, glycine/HCl; C, phthalate/HCl; D, acetate; E, KCl/HCl; F, citrate/phosphate; G, citric acid/KH_2PO_4/H_3BO_3/Diethylbarbituric acid/NaOH.
[b] Distilled water (pH 6·4 ± 2; free, pH 5·6; with 20 μmol/$KMnO_4$ ml). For further details, see Materials and Methods.

5 Chemicals

In the presence of 20 μmol $KMnO_4$, the effect of two antioxidants on the initial rate of red colour formation

Table 2. Effect of chemicals on manganese-dependent floret reddening

Chemicals[a]	Concentration (μmol)	Carthamin formed (nmol carthamin/ml/min)
A	1	2·09
	10	2·59
B	1	2·31
	10	2·59
C	1	2·23
	10	1·72
D	1	2·41
	10	2·63
E	1	2·33
	10	2·45
F	1	2·01
	10	2·21
G	1	2·05
	10	1·65
H	1	2·01
	10	2·92

20 μmol/$KMnO_4$ ml was used as a reactant. Specific value of the control was 2·00 nmol carthamin/ml/min.
[a] A, D-Isoascorbic acid; B, hydroquinone; C, KCN; D, 8-hydroxyquinoline; E, sodium azide; F, *o*-phenylenediamine dihydrochloride; G, α, α'-dipyridyl; H, EDTA. For further details, see Materials and Methods.

Table 3. Effect of preparative treatments on manganese-dependent floret reddening

Treatment	Carthamin formed (nmol/carthamin/ml/min)
—	2·03
Hypersonic oscillation	1·65
Infiltration	1·60
Oxygen bubbling	1·85

Each treatment was carried out continuously for 30 min at 22 ± 2 °C. For further details, see Materials and Methods.

was studied. Data obtained with two concentrations, where maximal effects were observed at 1 and 10 μmol, are shown in Table 2. They contribute clearly to the batho-shift. D-Isoascorbic acid is stronger than hydroquinone, judging from its activity.

The effect of sequestrants on the floret reddening was also investigated under aerobic conditions (Table 2). Below the 1 μmol level, size test sequestrants all act facilitatively, although some differences in their effects are seen among the compounds fed. With higher dosages (10-fold), a characteristic feature appears in the carthamin productivity. KCN and α, α'-dipyridyl exert clear inhibition, while EDTA expedites (far more strikingly) the red colour presentation. 8-Hydroquinoline and *o*-phenylenediamine dihydrochloride are also stimulative. Sodium azide promotes the reaction but to a much lesser extent.

Results from tests for promotion of $KMnO_4$-directed floret reddening

Various attempts were made to find effective procedures for promoting manganese-mediated floret reddening. The results, however, were not successful (Table 3), possibly because the pretreatments are propagated equally through the menbrances of the floret pieces in fine powders and coarse fragments. Some of these treatments are very effective for enzymatic red colour manifestation, as demonstrated in our previous communications (Saito *et al.*, 1983*a*; 1985*a*,*b*; Fukushima *et al.*, 1990; Saito, 1991*a*; Saito & Fukushima, 1991).

DISCUSSION

In a previous report from this laboratory, it has been demonstrated that permanganate-directed floret reddening is an excellent technique for obtaining raw materials of carthamin dye (Saito, 1991*a*). The utility of this technique was confirmed unambiguously by direct comparison with those of traditionally applied methods. In terms of pigment productivity, technical expediency and economic merits, the new technique is far superior to endogenous enzyme-dependent floret processing (Saito, 1990) or glucose oxidase-mediated oxidation (Wada & Ota, 1986).

This study has been undertaken to examine the effect of physico-chemical conditions through which the rate of endogenous precarthamin oxidation is regulated in dried florets of dyer's saffron during permanganate catalysis. As expected previously, the results here clearly show that the manganese-dependent reaction is affected variously by external factors tested. It can be seen from the figures and tables that temperature, pH, buffer systems, antioxidants and sequestrants are all decisive in red colour presentation in the detached floral tissues. The initiating reactants also cannot be disregarded. Treatment of floral suspension with 0·1–8 μmol $KMnO_4$/ml promoted accelerating red colour formation during 30 min incubation at 30°C, whereas higher dosages (10 μmol/ml or more) of the metal salts resulted in a decrease in the pigment production. With excess permanganate in the reaction media, dark-red florets appeared accompanied by substantial amounts of blackish-brown precipitates as seen previously (Saito, 1991*a*). The surplus $KMnO_4$ could perhaps be changed to insoluble hydrolysed manganese, which resulted, directly or indirectly, in a serious decrease in pigment production. Under the conditions of the present study, the apparent K_m value of 19·4 mM for permanganate was calculated from double-reciprocal plots (see an insertion presented in Fig. 1).

Temperature has often been suggested to be one of the strongest effectors of anabolic and/or catabolic process in *Carthamus* pigments (Saito & Fukushima, 1986, 1991; Kanehira *et al.*, 1990). Heating of floret suspensions in $KMnO_4$ solution resulted in a serious decrease of carthamin productivity, indicating that the metal-catalysed carthamin generation depends closely on heat activation energy. The reaction follows first-order reaction kinetics as shown in Fig. 3. At 0 ± 1°C, the half-value of the red colour formation was 1·12 nmol/ml/min, whereas, at 40 ± 1°C, the value was reduced by about 18% (0·92 nmol/ml/min). At 80 ± 1°C, the colour intensity in the extract was far less (74%: 0·29 nmol/ml/min) (see Fig. 3). This might be because carthamin is formed swiftly at higher temperatures and the pigment, once formed, is degraded during further prolonged heat treatment. Carthamin is very unstable in aqueous media, particularly when left free at higher temperature (Saito & Fukushima, 1986, 1991; Kanehira *et al.*, 1990). The results therefore suggest incubation at lower temperatures to improve pigment production. During treatment of the flower tissues in aqueous media, we usually encounter many difficulties. Amongst these, biological putrefaction is one of the most serious problems. Thus, the newly established method is very useful, because the reaction proceeds fast enough even at lower temperature ranges.

Our several investigations (Saito *et al.*, 1983*a*,*b*,

1985*b*; Saito & Fukushima, 1986; Kanehira *et al.*, 1990; Kanehira & Saito, 1990; Saito, 1991*a,b*) and others (Alexiev & Mutafchiev, 1983) have demonstrated that pH acts decisively in the process of red colour shift in aqueous mixtures. The effect of pH on the pigment generation was studied over the range of 2·5–7·5 using dried materials in three different buffer solutions. The optimum pH for carthamin induction by permanganate was 3·4. Alexiev & Mutafchiev (1983) reported a similar result in their experiments with manganese, i.e. that the reaction proceeds at lower pH, 4·6–4·9, in a ternary mixture. Both higher and lower pHs give total loss of carthamin, while the enzymatic process was intensified by increasing the pH value to 4·8–5·2 (Saito *et al.*, 1983*a,b*, 1985*a,b*). In any case, the lower optimum pH is helpful for preparing red materials without regard to microbial propagation.

Buffer systems control carthamin production by permanganate (Table 1). Direct participation of buffer systems in the transcoloration reaction has already been suggested in our previous papers (Saito & Takahashi, 1985; Saito & Fukushima, 1991). Citrate, citrate/phosphate and citric acid/KH_2PO_4/H_3BO_3/diethylbarbituric acid/NaOH are all positive effectors for carthamin formation. Phthalate/HCl and acetate are also promising; however, they are inferior to the former three. Glycine/HCl and KCl/HCl cannot be regarded as stimulators for permanganate induction of red carthamin. Among the buffers tested, citrate seems to act as a coeffector for permanganate oxidation. The mechanism by which the catalysis of permanganate is promoted in the presence of citrate remains unknown. The present data indicate that floret reddening is better carried out in citrate buffer rather than in water (see Table 1).

Since manganese-stimulated carthamin formation is thought to involve electron transfer reactions between manganese cations and precarthamin, it was of interest to study the influence of antioxidants and/or metal chelators on the red colour presentation in floating floral pieces. Antioxidants employed in this experiment were D-isoascorbic acid and hydroquinone. They were added at concentrations of 1 and 10 μmol/ml. Results of this study, however, showed that they contribute only to an increase in carthamin content. Quantitative data (Table 2) prove that D-isoascorbic acid is more effective than hydroquinone. This may have occurred, because the antioxidants acted as prooxidants. The prooxidant nature has been documented in the literature (Scarborough & Watts, 1949; Watts & Wong, 1951; Kelly & Watts, 1957; Kanner *et al.*, 1977). Oxidative reaction mechanisms are recognized as free radical chain reactions. One such naturally occurring antioxidant, which regulates free radical termination, is hydroquinone. If floret reddening occurred by free radical production, then addition of hydroquinone in solution should inhibit this reaction and, as a matter of fact, it does reduce carthamin content when compared to the control. However, it only contributes to the bathoshift; therefore, hydroquinone and isoascorbic acid may perhaps be involved in the prooxidative activity as shown by the earlier observations (Kanner *et al.*, 1977, 1978).

Previous investigations (Savolainen & Kuusi, 1978; Attoe & von Elbe, 1984) have demonstrated that oxidative reactions are affected sensitively in the presence of metal chelators. Commercially available sequestrants were added at two different levels. No decrease in the amount of carthamin was observed when 1 μmol of each of six compounds was used. The lack of effectiveness in preventing carthamin production was attributed to inadequate amounts of chemicals in the solution. Therefore, a higher concentration of each compound was examined further. The addition of 10 μmol of the sequestrants was expected to decrease the initial floret reddening and therefore cause a distinct change in the amount of carthamin extracted. Results of this experiment (Table 2), however, conflicted with the previous prediction. At this concentration, KCN and α, α'-dipyridyl acted as inhibitors as expected, while the other four did not. In particular, EDTA, 8-hydroxyquinoline and *o*-phenylenediamine dihydrochloride influenced the red colour shift in the floret-$KMnO_4$ system co-operatively. The mechanism by which they promote carthamin formation is not clear.

With the aim of finding out more effective methods for red colour development in permanganate-affected floating materials, we conducted preoperative treatments as mentioned above. However, almost all of these attempts were unsuccessful, although some were practically contributive to enzymatic red colour manifestation (Saito, 1990; Saito *et al.*, 1991). Permanganate fed to the floral tissues may readily penetrate and quickly reach the reaction site within a cellular matrix, where flame-coloured precarthamin is oxidized chemically to red carthamin.

In the current studies, we have examined a number of external factors which could influence carthamin formation in dried florets of dyer's saffron and revealed many factors, some of which were practically useful and others not. These findings will be of value in the preparation of carthamin dye for textiles, cosmetics, food colorants and other related products.

CONCLUSIONS

1. $KMnO_4$ is an effective inducer of carthamin in dried *Carthamus* florets. The apparent k_m value calculated for $KMnO_4$ was 19·4 mM.
2. Permanganate-dependent floret reddening proceeds readily in buffer solutions at pH 3·4.
3. For effective preparation of reddened florets, processing should be carried out at lower temperature ranges.

4. Buffer systems and/or buffer compositions could become one of the determinative factors for obtaining red materials for carthamin dye.
5. Preparative treatments for promoting the permanganate reaction need not be carried out.

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REFERENCES

Alexiev, A. A. & Mutafchiev, K. L. (1983). A new catalytic colorimetric reaction for the determination of submicrogram amounts of manganese. *Annal. Lett.*, **16**, 769–83.

Attoe, E. L. & von Elbe, J. H. (1984). Oxygen involvement in betanin degradation. Oxygen uptake and influence of metal ions. *Z. Lebensm. Unters. Forsch.* **179**, 232–6.

Fukushima, A., Takahashi, Y., Ashihara, H. & Saito, K. (1990). Relationship between floret elongation and pigment synthesis in the flowers of *Carthamus tinctorius*. *Ann. Bot.* **65**, 361–3.

Homma, T., Sawaguchi, M., Kawai, H., Takahashi, Y. & Saito, K. (1985). Preparative studies on the isolation of an enzyme associated with carthamin synthesis in *Carthamus tinctorius* L. *Acta Soc. Bot. Pol.*, **54**, 403–16.

Kanehira, T. & Saito, K. (1990). Decomposition of carthamin by peroxidases from *Carthamus tinctorius*. *Biochem. Physiol. Pfl.*, **186**, 179–87

Kanehira, T., Naruse, A., Fukushima, A. & Saito, K. (1990). Decomposition of carthamin in aqueous solutions: influence of temperature, pH, light, buffer systems, external gas phase, metal ions, and certain chemicals. *Z. Lebensm. Unters. Forsch.*, **190**, 299–305.

Kanner, J., Mendel, H. & Budowski, P. (1977). Prooxidant and antioxidant effects of ascorbic acid and metal salts in a β-carotene-linoleate model system. *J. Food, Sci.*, **42**, 60–4.

Kanner, J., Mendel, H. & Budowski, P. (1978). Carotene oxidizing factors in red pepper fruits (*Capsicum annum* L.): oleoresin-cellulose solid model. *J. Food Sci.*, **43**, 709–12.

Kelly, G. G. & Watts, B. M. (1957). Effect of copper chelating agents on the prooxidant activity of ascorbic acid with unsaturated fats. *Food Technol.*, **11**, 114–6.

Saito, K. (1990). Flower colour transition in capitula of *Carthamus tinctorius* L.: its mechanism. *Proc. Hokkaido Tokai Univ., Sci. Engin.*, **2**, 39–45.

Saito, K. (1991*a*). A new method for reddening dyer's saffron florets: evaluation of carthamin productivity. *Z. Lebensm. Unters. Forsch.*, **192**, 343–7.

Saito, K. (1991*b*) New diagnostic spray reagents for detecting precarthamin in aqueous extracts from dyer's saffron flowers. *Z. Lebensm. Unters. Forsch.*, 193, 134–5.

Saito, K. & Takahashi, Y. (1985). Studies on the formation of carthamin in buffer solutions containing precarthamin and oxidizing agents. *Acta Soc. Bot. Pol.*, **54**, 231–40.

Saito, K. & Fukushima, A. (1986). Effect of external conditions on the stability of enzymically synthesized carthamin. *Acta Soc. Bot. Pol.* **55**, 639–51.

Saito, K. & Fukushima, A. (1991). Thermal stability of carthamin bound to insoluble polymers—effect of pH and organic and inorganic compounds. *Int. J. Food Sci. Technol.*, **26**, 21–6.

Saito, K., Takahashi, Y. & Wada, M. (1983*a*). Enzymic synthesis of carthamin in safflower. *Biochim. Biophys. Acta*, **756**, 217–22.

Saito, K., Takahashi, Y. & Wada, M. (1983*b*). Distribution and variation of the catalytic activity of the enzyme responsible for carthamin synthesis in safflower seedlings. *Z. Naturforsch.* **38c**, 724–9.

Saito, K., Takahashi, Y. & Wada, M. (1985*a*). Formation of carthamin by a partially purified enzyme from safflower seedlings. *Experientia*, **41**, 59–61.

Saito, K., Takahashi, Y. & Wada, M. (1985*b*). Preparation of an enzyme associated with carthamin formation in *Carthamus tinctorius* L. *Z. Naturforsch.*, **40c**. 819–26

Saito, K., Kanehira, T. & Homma, T. (1991). Promotive effect of supersonic oscillation on carthamin bleaching induced by peroxidatic enzyme oxidation. *Food Chem.*, **41**, 285–92.

Savolainen, K. & Kuusi, T. (1978). The stability properties of golden beet and red beet pigments: influence of pH, temperature, and some stabilizers. *Z. Lebensm. Unters. Forsch.*, **166**, 19–22.

Scarborough, D. A. & Watts, B. M. (1949). The prooxidant effect of ascorbic acid and cysteine in aqueous fat system. *Food Technol.*, **3**, 152–5.

Wada, K. & Ota, S. (1986). *Japan Kokai Tokkyo Koho, J. P.*, **61**, 199, 798.

Watts, B. M. & Wong, R. (1951). Some factors affecting the antioxidant behavior of ascorbic acid with unsaturated fats. *Arch. Biochem.*, **30**, 110–20.