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The influence of thermal treatments on the stability of safflor yellow B

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The orange-yellow coloration of safflor yellow B was investigated in aqueous solutions and in solid crystalline powders which were treated at a high temperature and pressure. On heating the solutions in a retort for given intervals at 121°C and 1·2 kgf/cm^2, about 21–46% of the coloration was lost, indicating that temperature (and pressure) is a definitive factor in the stability of safflor yellow B in media. Solid safflor yellow B resisted any serious bleaching by the heat treatment (decrease rate: 1·2%). The results are evaluated briefly in the context of applying safflor yellow B as a food colorant.

INTRODUCTION

Many bio-dyes with orange-yellow coloration are now obtainable from various materials, namely moulds, vascular plants and animals, in which flavines, quinones, carotenoids and flavonoids are all involved. Safflor yellow B (Fig. 1) is a flavonoid with a novel structure: it is a poly-oxy dimeric chalcoquinoid compound with two *C*-glucopyranosyl and one *C*-glucitol moieties. It is produced in the flower florets of dyer's saffron (*Carthamus tinctorius* L.) at the stage of inflorescence (Saito & Fukushima, 1989). Notwithstanding its inherently fine coloration, little or no safflor yellow B is used, because almost all of it is washed off the floral tissues during the course of carthamin preparation. From an economic point of view, this is very wasteful, because the herbal dye is usable as a dyestuff for colouring textiles and food products, giving an attractive orange-yellow colour.

The main theme of the present study is to accumulate the basic data for utilizing safflor yellow B as a food colorant. The stability of the coloration is investigated here after treating the pigment solution at high temperature (and pressure) in an airtight retort.

MATERIALS AND METHODS

Materials

Safflor yellow B, used in this work, was prepared from the freshly collected florets of dyer's saffron, which was grown in an experimental field (the collection was carried out during July–August 1992). Extraction and purification of the pigment were basically the same as those of Saito *et al.* (in press).

Preparation of safflor yellow B solution

Safflor yellow B (150 μg/ml) was dissolved, if not specified otherwise, in 50mM citrate/phosphate buffer, pH 5·0, and stored in a refrigerator at 3–4°C in the dark. Aliquots of the stock solution were diluted to 1/10 with citrate/phosphate buffer, pH 5·0 and used for the heat treatment.

Heating of safflor yellow B solution

Prior to conducting the heat treatment, UV/VIS absorbance (403 nm or 220–500 nm) was measured using a Hitachi spectrophotometer, model U-3210 (0 time). The pigment solutions in test tubes were each capped with aluminium foil and placed in a retort (Sanyo, Labo Autoclave, model MLSA-3000, Tokyo). Heating was carried out at 121°C and at 1·2 kgf/cm^2 for desired intervals and, after cooling, the colour in the solution was monitored spectrophotometrically. Solid samples of safflor yellow B in Thunberg tubes were capped tightly and heated in the retort under the same conditions as those described above and the colour change was recorded with a spectrophotometer.

RESULTS AND DISCUSSION

The specific orange-yellow colour of safflor yellow B is sensitive to temperature: it fades easily when treated at high temperature (and pressure) in a retort. The rates

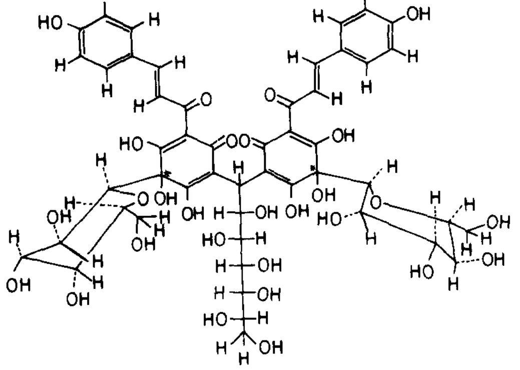

Fig. 1. Structure of safflor yellow B (Takahashi *et al.*, 1984). The original formula is redrawn.

of colour loss calculated at 10, 30 and 60 min were 21·4, 36·8, 45·5 (given as a percentage of the control). A typical profile of colour bleaching vs. heating interval is given in Fig. 2. It is clear from the figure that the VIS absorbance peak at 403 nm decreases, depending on the length of the heat treatment. The heat-sensitive nature of the pigment is characterized by pH values (Fig. 3). Safflor yellow B is relatively stable at pH 5·0, whereas the heat instability is accentuated at pH 3·0 and 7·0, indicating that the reaction proceeds chemically under pH control. Solid powders of safflor yellow B resisted any serious bleaching by the heat treatment. The rate of the discoloration (i.e. the rate of decrease in content or amount of safflor yellow B) was 1·15% of the control. UV/VIS spectra measured with or without heating of the solid samples are presented in Fig. 4.

Many metal ions are found in food systems as trace contaminants. Some of these have been known to act on food colorants as cooxidants, producing undesirable effects on the colour stability (Kanehira *et al.*, 1990; Saito *et al.*, in press). Here, typical metal cations were tested at a constant (0·3 mM) level. UV/VIS absorption profiles of pigment solutions are comparable with that of a non-metal ion-containing control (Fig. 5). The original UV/VIS spectrum is changed conspicuously by being in contact with external metals: all test metals influenced the coloration to some extent (Table 1). In particular Mn^{2+} and Mn^{3+} showed the strongest colour bleaching activities (94 and 88%). Cu^{2+}, Fe^{2+} and Fe^{3+} also facilitate the colour loss prominently (83, 82 and 80%, respectively). Zn^{2+} and Mg^{2+} are both weak activators (36 and 28%). The mechanism and process by which the specific orange-yellow colour fades away is not known at present, though these important problems are to be studied systematically and precisely in more detail.

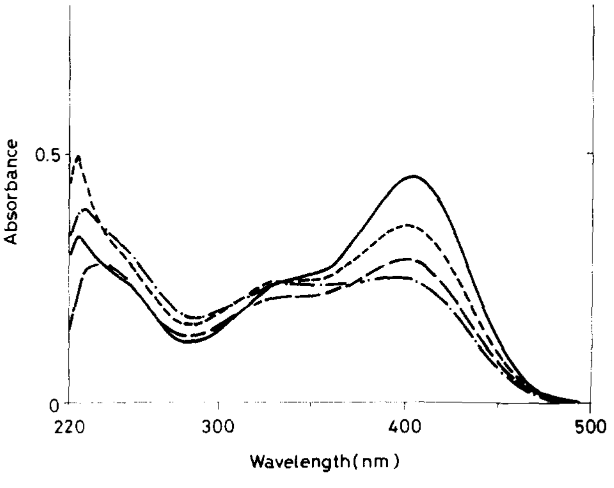

Fig. 2. UV/VIS absorption profiles of safflor yellow B recorded after treating at high temperature and pressure. Safflor yellow B sample (155 μg) in 10 ml 50mM citrate/phosphate buffer, pH 5·0 was heated, in a retort at 121°C and 1·2 kgf/cm^2 for desired intervals as indicated in the figure. ———, Control; - - -, 10 min; — — —, 30 min; — · — 60 min.

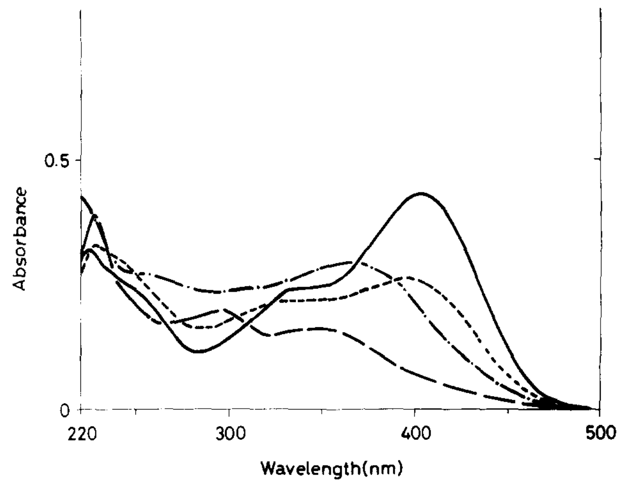

Fig. 3. pH profiles of safflor yellow B recorded after treating at high temperature and pressure. The safflor yellow B sample (155 μg) in 10 ml 50mM citrate/phosphate buffer was heated at different pH levels for 30 min at 121°C and 1·2 kgf/cm^2. ———, Control; — — —, pH 3·0; - - -, pH 5·0; — · — pH 7·0.

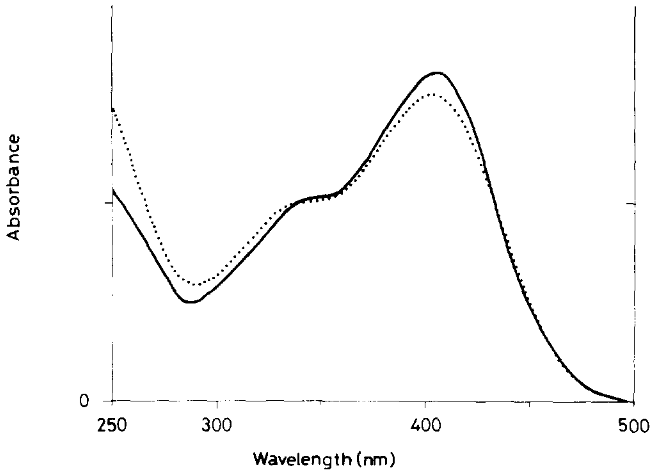

Fig. 4. UV/VIS pattern of solid safflor yellow B sample with or without treatment in a steam sterilizer. A 155 μg sample of safflor yellow B was used for the heat treatment. Before heating, UV/VIS absorbance (250–500 nm) of the sample was followed spectrophotometrically (0 time). The same sample, dried completely with a high-vacuum pump, was placed in a Thunberg tube, capped tightly, de-airated and then heated in a vacuum sterilizer for 60 min at 121°C and 1·2 kgf/cm^2. The heat-treated safflor yellow B was subjected to the spectrophotometric measurement under the same conditions as used at 0 time. ———, Control; ····, heat-treated sample.

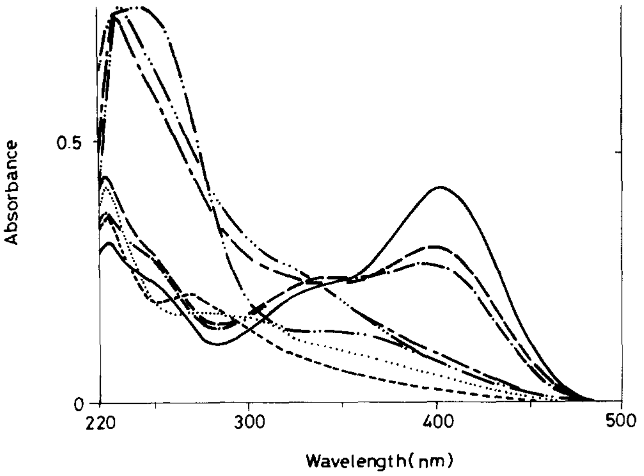

Fig. 5. Effect of metal ions on the heat stability of safflor yellow B. In the presence of each 0·3mM metal ions, the safflor yellow B sample (155 μg) was heated at 121°C and 1·2 kgf/cm^2 for 30 min in a retort. ---, Mn^{2+}; ····, Mn^{3+}; —···—, Fe^{2+}; —-—, Fe^{3+}; —··—, Cu^{2+}; —·—, Zn^{2+}; ———, Mg^{2+}; ———, control.

In the case of carthamin bleaching under normal conditions, external additives affect the coloration in various ways (Kanehira *et al.*, 1990). With a view to consideration of safflor yellow B as a food colorant, typical oxidizing agents, antioxidants and sugars were tested in this study. Table 2 presents the results from heating safflor yellow B for 30 min in the presence of various sugars. At a net 5mM concentration, they act as protectors against colour loss, as is obvious from the low rates of colour decrease (% as averages): pentoses (18·8), hexoses (17·4), disaccharides (19·9). This is a very convenient effect, for sugars can be used as sweetening agents as well as colour preservers for safflor yellow B when applied to food systems processed at high temperature and pressure. Oxidants and antioxidants are both positive promoters of safflor yellow B bleaching in a steam sterillizer. Table 3 illustrates this (% as averages): oxidants (78·8), antioxidants (14·9).

The above results suggest that the orange-yellow pigment is usable under limited conditions as an edible colorant for foods that are processed at low temperature or by instantaneous heating. Sugar-mediated conditions are desirable for producing coloured products by heating. Trace contamination by metal ions oxidants and antioxidants should be avoided as much as possible to avoid the original coloration suffering serious heat-denaturation. Adulteration of other noxious substances must be checked carefully. Together with earlier findings from this laboratory (Kanehira *et al.*, Saito *et al.*, in press), these new results will give colorant engineers some thought for producing coloured foods with safflor yellow B.

Table 1. Effect of metal ions on the heat stability of safflor yellow B

Metal ion[a]	Safflor yellow B remaining (pmol safflor yellow B/ml/min)	Diminution rate (% of control)
Mn^{2+}	29·0	94·0
Mn^{3+}	60·2	87·6
Fe^{2+}	86·1	82·2
Fe^{3+}	96·7	80·0
Cu^{2+}	84·5	82·6
Zn^{2+}	310·2	35·9
Mg^{2+}	349·6	27·8

[a] Metal ions used were 0·3mM each.
A Safflor yellow B sample of 14·5 nmol/ml was tested.

Table 2. Effect of sugars on the heat stability of safflor yellow B

Sugar[a]	Safflor yellow B remaining (pmol safflor yellow B/ml/min)	Diminution rate (% of control)
Arabinose	384·2	20·7
Xylose	402·2	16·9
Glucose	397·5	17·9
Galactose	403·7	16·5
Fructose	398·5	17·7
Sucrose	400·9	17·2
Maltose	397·0	18·0
Lactose	366·1	24·4

[a] Sugars used were 5mM each.
A safflor yellow B sample of 4·5 nmol/ml was tested.

Table 3. Effect of oxidants and antioxidants on the heat stability of safflor yellow B

Compound[a]	Safflor yellow B remaining (pmol safflor yellow B/ml/min)	Diminution rate (% of control)
Potassium ferricyanide	6·6	98·6
Potassium permanganate	1·5	99·7
Hydrogen peroxide	300·0	38·0
D-Isoascorbic acid	390·3	19·4
L-Ascorbic acid	394·9	18·4
α-Tocopherol	480·1	0·85
Hydroquinone	383·3	20·8

[a] Compounds used were 1mM each.
A safflor yellow B sample of 14·5 nmol/ml was tested.

CONCLUSIONS

1. Heating of coloured products with safflor yellow B is best conducted at low temperatures for a short time.
2. Sugars are usefully added as sweetening agents for safflor yellow B-coloured foods.
3. Addition of metal ions, oxidants, antioxidants and other noxious contaminants should be avoided in any food containing safflor yellow B.

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