Influence of sugars on the reddening of triturated florets of dyer's saffron flowers

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Abstract

Pentoses, hexoses and disaccharides were administered to the triturated florets of dyer's saffron (*Carthamus tinctorius*) and their effects on enzyme-catalyzed floret reddening examined spectrophotometrically. Among the sugars tested, hexoses promoted reddening most prominently. On average, a 4.2-fold increase in the carthamin content was observed when hexoses were added to the crushed materials. Disaccharides followed these. Upon incubation of the triturated tissues with 10 mM sugars, the pigment producing activity was raised to 3.4-fold or more. Pentoses were also potent stimulators, although their efficiencies were somewhat lower (3.2-fold). On the whole, D-forms were more stimulative than L-forms (D-forms — 3.6-fold, L-form — 2.8-fold). Based on these comparisons, we concluded that sugars play a role in floret reddening through interaction with endogenous precarthamin-oxidizing enzyme(s) in the floral tissues of the garden plant.

Key words: *Carthamus tinctorius*, dyer's saffron, floret reddening, sugar

INTRODUCTION

The red florets of dyer's saffron (*C. tinctorius*) are the essential material for preparation of carthamin, which has been used as a red dye stuff for textiles and cosmetics or other purposes (Saito and Fukushima 1988, Saito et al. 1989, Kanehira et al. 1990). To obtain the reddish material, the florets are usually processed after being picket from the matured capitula gathered during July and August in some districts of our country. Currently, in order to be successful, very complicated and time-consuming methods of floret processing must be employed. However, as far as we are aware, no attempt has yet been

made to improve the old-fashioned and wasteful techniques. From this view point, it is necessary to carry out basic studies on some experimental models.

As a preliminary to this study, the effect of sugars on the reddening of triturated florets was tested by using pre-matured capitula from dyer's saffron, whose colouration remains almost bright-yellow or orange-yellow.

MATERIALS AND METHODS

MATERIALS

D- and L-galactose were obtained from Sigma Chemicals Co. (St. Louis, Mo., U.S.A.). D-xylose, D-ribose, 2-deoxy-D-ribose, D- and L-arabinose were supplied by Wako Pure Chemical Inc., Ltd. (Osaka, Japan). L-xylose and L-ribose were purchased from Aldrich Chemical Co., Inc. (Milwaukee, Wis., U.S.A.). D-glucose, D-fructose, maltose and lactose were obtained from Kanto Kagaku Co., Ltd. (Tokyo, Japan). Sucrose was provided by Nakarai Kagaku Yakuhin Co., Ltd. (Kyoto, Japan). Avicel cellulose was a product of Asahi Kasei Kogyo Co., Ltd. (Tokyo, Japan). Seeds of dyer's saffron were sown on our experimental field in April, 1990. The plants bloomed in August, bearing orange-yellow cylindrical flowers. Yellow florets were harvested from the pre-matured capitula and used at once for the experiments.

ADMINISTRATION OF SUGAR

Each 4.5 g florets was frozen in liquid nitrogen and crushed into small pieces with a pestle and mortar. To the powdered florets, 20 cm³ of 10 mM sugar solution (0.2 mmol) were added separately and incubated for 5 min at $30 \pm 1^\circ\text{C}$ by agitating vigorously at 180 strokes per min. At the end of incubation, the floret suspension was heated at $99 \pm 1^\circ\text{C}$ for 5-7 min and stocked frozen at -20°C before using for pigment extraction.

EXTRACTION AND DETERMINATION OF CARTHAMIN CONTENT

The floret suspension was stirred with K_2CO_3 ($0.1 \text{ g} \times 3$) for 3 min after being thawed in a water-bath at $33 \pm 2^\circ\text{C}$ for a few minutes. The extracts were combined and acidified with 1 g citric acid. To this acid solution, 1 g Avicel cellulose was suspended and the suspension stirred with a magnetic stirrer for over 10 min at room temperature. The resulting reddish Avicel was washed 5 times with 200 cm³ deionized-distilled water on a Büchner funnel and then extracted with a total of 50 cm³ of 60% (by vol.) acetone. The so obtained red eluate was used for spectrophotometric measurement of carthamin content. The data from the reading of the absorption maximum at λ 521 nm were compared with a standard curve and the amount of carthamin in the eluate was calculated.

RESULTS AND DISCUSSION

Although some data has been obtained concerning the enzymatic (Saito et al. 1983a, b) and non-enzymatic (Saito and Takahashi 1985) red colour manifestation in detached floral tissues of dyer's saffron, the effect of exogenous carbohydrates on the transcolouration has not yet been clarified experimentally. This study shows that accumulation of a red colouring matter occurs in the presence of sugars at milimolar levels. As is the case with enzyme-catalyzed carthamin synthesis in experimental models (Saito et al. 1985), the transcolour reaction in the intact tissues exhibits various sensitivity to external additives. In this observation, 7 pentoses, 4 hexoses and 3 disaccharides, including some of their D- and L-isomers, were tested at a concentration of 10 mM.

The addition of pentoses to the crushed florets distinctly stimulated the production rate of a red colouring matter, which has been shown to be carthamin (Saito et al. 1983a, b) (Table 1). 2-Deoxy-D-ribose is the most effective stimulator among 7 pentoses used (4.2-fold, compared with no sugar added control). Arabionose is also promising (3.7-fold). Ribose follows this (2.9-fold).

Table 1

Effect of pentoses on the reddening of dyer's saffron florets

Sugar	Carthamin formed* μM		Specific value $\mu\text{M carthamin} \cdot \text{mM pentose}^{-1} \cdot \text{min}^{-1}$	
	D	L	D	L
Xylose	4.95	4.09	99.0	81.8
Arabinose	6.15	5.44	123.0	108.8
Ribose	5.27	3.82	105.4	76.4
2-Deoxy-ribose	6.65	—	133.0	—

*The amount of carthamin formed during 6 minutes of incubation.

The amount of carthamin from the control test was $1.59 \mu\text{M}$ per 5 min. The test was done with 4.5 g fresh florets in 20 cm³ deionized-distilled water for 5 min at 30°C. Little or no carthamin could be detected in the starting materials.

Xylose enhances the reaction to a sizable extent (2.9-fold). Thus, the ratios among test pentoses calculated were: xylose/ribose/arabinose/2-deoxy-D-ribose = 1:1.1:1.3:1.5. The above data also show that D-isomers stimulate the enzyme(s) more than L-forms. This is confirmed by the ratio of the carthamin content in the materials treated with D- and L-forms (1.3:1, in average). The results suggest the possibility that the enzyme(s) uses these optical isomers selectively. Pentoses with the dextro-rotatory configuration have a greater affinity for the enzyme(s) than levo-forms, which may reflect on the higher productivity of carthamin. L-pentoses promote the pigment synthesis to a lesser extent, indicating that the floral tissues retain a racemization system, which catalyzes the conversion of inert L-series to active D-forms.

More pronounced pigment induction was seen in the case of triturated florets incubated with hexose solutions. At 30°C, increments of the pigment content reached clearly higher levels, while only 0.32 $\mu\text{M}/\text{min}$ carthamin was produced by no sugar-added control (Table 2). The stimulatory effect is exerted predominately by D-fructose (4.7-fold). With D- and L-galactose, marked stimulation can also be achieved at a 0.2 mmol level (4.3-fold). D-glucose enhances the floret reddening to a much lesser extent (3.6-fold). In general, hexoses are stronger stimulators than pentoses. This is indicated by the carthamin productivity ratio between two sugars: pentose/hexose = 1: 1.3.

Table 2

Effect of hexoses on the reddening of dyer's saffron florets

Sugar	Carthamin formed μM		Specific value $\mu\text{M carthamin} \cdot \text{mM hexose}^{-1} \cdot \text{min}^{-1}$	
	D	L	D	L
Glucose	5.71	—	114.2	—
Fructose	7.42	—	148.4	—
Galactose	5.22	8.26	104.4	165.2

For details of explanation, see Table 1.

The effects of disaccharides on the development of the red colour is presented in Table 4. At a given 10 mM concentration, lactose is the most potent stimulator (4.8-fold). Sucrose is inferior to lactose (3.9-fold). Maltose elevates the carthamin content to a far lesser extent (1.5-fold).

Table 3

Effect of disaccharides on the reddening of dyer's saffron florets

Sugar	Carthamin formed μM	Specific value $\mu\text{M carthamin} \cdot \text{mM disaccharide}^{-1} \cdot \text{min}^{-1}$
Maltose	2.31	46.2
Lactose	7.58	151.6
Sucrose	6.18	123.6

For details of explanation, see Table 1.

The mechanism by which sugars accelerate the carthamin formation in triturated floral tissues is not clear. Sugars are naturally occurring components and are known to be used as key substances for a vast variety of enzymes which control vital reactions in living systems (Briggs 1973, Conn and Stumpf

1976). Such a system involves coupled mechanisms through which resulting products are transformed to subsidiary diverged components affecting directly and/or indirectly the physiological status through alteration of metabolic processes in cellular compartmentations (Barz et al. 1985, Kashimura et al. 1988).

The reddening of dyer's saffron capitula has been shown by Saito et al. (1983a, b) to be induced through an oxidative process at the late flowering stage. Soon after blooming, the florets dwindle and then wither gradually. This indicates that a certain drastic physiological change could be introduced into the normal metabolic process at the transcolouration stage (Saito 1989). Consequently, various endogenous components, including sugars must be obligatorily caught up into the abnormal metabolic flow during the transition and affect directly and/or indirectly the floret colour manifestation.

Sugars added externally also enter various degradation processes, under which they must obligatorily be broken down and/or transformed to many catabolites of different types, some of which exert their stimulatory effects on the enzyme(s) to produce carthamin; these interesting mechanisms will be clarified in time.

The current results may become helpful in the study of the mechanism of flower colour transition in dyer's saffron capitula and also to produce reddened florets from the plant material for carthamin preparation. Additional studies on evaluating other substances which have potential efficiencies ought to be carried out more extensively, so as to give these data a former base.

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Wpływ cukrów na czerwienienie sproszkowanych kwiatów szafranu

Streszczenie

Badano wpływ pentoz, heksoz i dwucukrów na katalizowany enzymatycznie proces czerwienienia, które określano spektrofotometrycznie. Największy efekt zaczerwienienia wywoływały heksozy, powodując 4,2-krotny wzrost tego zjawiska. Następne w kolejności były dwucukry — inkubacja sproszkowanych płatków z 10 mM cukrów wzmacniała proces wytwarzania barwnika ponad 3,4-krotnie. Pentozy również okazały się niezłymi stymulatorami czerwienienia — 3,2-krotny wzrost zabarwienia.

Ogólnie można powiedzieć, że formy D cukrów były tutaj bardziej aktywne, niż formy L (D — 3,6-krotnie; L — 2,8-krotnie). Stąd wniosek, że cukry biorą udział w procesie czerwienienia płatków, współdziałając z endogennymi enzymami utleniającymi prekartaminę, obecnymi w tkankach kwiatów.