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RESEARCH NOTE

Stability of carthamin in calcium alginate beads

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Sodium alginate solution, containing carthamin, was added dropwise to calcium acetate solution. Calcium alginate beads thus prepared were freeze-dried and carthamin stability in the gel beads was examined. About 47% of carthamin was kept intact after 60-h pretreatment, when the moisture of the red beads was removed *in vacuo* at −40°C. The utility of the new technique for enclosing carthamin in alginate capsules is discussed on the basis of the experimental data.

INTRODUCTION

Carthamin exhibits a characteristic noble red colour, 'Carthamin-Red', in aqueous solutions. Due to its acceptable and fine colouration, a vast number of attempts have been made to prove that the colouring matter can be applied, not only to dye textile fabrics, but also to colour medicinal tablets and processed foods including beverages, soft drinks, pectic cakes, fruit jams and cooked confectioneries (Saito, 1990*a*; Saito *et al.*, 1993).

To use carthamin as a food colorant, the bio-dye must be stabilized by mixing with appropriate stabilizers (Saito & Fukushima, 1986, 1987, 1988). Our previous studies have provided evidence that polyethylene glycol, glycerine and gelatine allow good preservation of carthamin colour in solutions (Saito *et al.*, 1993). In continuation of a screening investigation into stabilizers and/or effective methods for carthamin preservation, this work aims to encapsulate the dye with calcium alginate. The experimental results described, indicate that the red colour can be stabilized within the gel beads.

MATERIALS AND METHODS

Chemicals

Carthamin was prepared from processed dyer's saffron florets through the methods of Saito (1991) and Saito *et al.* (1992). One type of sodium alginate (cp number for viscosity undetermined) was a gift from Kibun Food Chemiha (Sayama, Japan). Two types of sodium alginate (cp numbers: 100–150, 300–400) were purchased from Wako Pure Chemical (Osaka, Japan). Avicel cellulose was obtained from Asahi Kasei Kogyo (Tokyo, Japan). Other chemicals and reagents used were of analytical grade, and provided by several commercial suppliers.

Preparation of carthamin-containing calcium alginate beads

One milligram of carthamin was suspended in 10 ml of 50 mM citrate buffer, pH 5·0. To this carthamin solution (20 ml), 20 mg of sodium alginate was added (1% carthamin solution) to make a core solution, which was dropped carefully into 5% (w/v) calcium acetate solution from a 10 ml syringe with a needle of 0·6 mm i.d. After standing for several minutes with gentle stirring, reddish beads were transferred to 50 mM citrate buffer, pH 5·0, for washing and then spread over filter papers to absorb the residual buffer. The gel beads prepared were freeze-dried for 22–24 h at −40°C under reduced pressure. The dried beads were ground into small pieces in a porcelain mortar with a pestle and the powders in small vials kept for several successive hours in a freezer at −20°C in the dark before experimental use. This was done to keep carthamin from serious deterioration from the effects of light and/or temperature (Kanehira *et al.*, 1990).

Estimation of carthamin stability in gel beads

An aliquot of the fine powders was suspended in 2 ml of 1% (w/v) potassium carbonate solution and left for over 7 min under gentle stirring with a magnetic bar. The alkaline solution was poured into a beaker, in which 2·5 ml of 1% (w/v) citric acid solution had been added. The acid solution was made up to 5 ml and used for the determination of carthamin contents. The response (spectrophotometric reading at 521 nm) was monitored with a Hitachi double-beam UV/VIS spectrophotometer, model U-1100. The stability of

carthamin in calcium alginate beads was expressed as mg carthamin/mg gel bead, which was computed from the spectrophotometric data, and a calibration curve.

RESULTS AND DISCUSSION

Carthamin in food systems exhibits a fine red colour. To maintain this colouration, useful stabilizers are indispensable, because free dye is very unstable, especially when it is used in electrolytic solvents, in which the fine tincture changes irreversibly to reddish-orange, orange, orange–yellow and then light yellow.

In a series of observations on carthamin stability, we have shown that many substances affect the stabilization of carthamin red colour (Saito *et al.*, 1991). Certain polysaccharides and their derivatives are promising: they stabilize carthamin in a specified manner. With a view to discovering other stabilizers and/or methods for retaining carthamin tincture, we tested a new technique: calcium alginate encapsulation of the herbal dye. The findings in the present work reaffirm and expand our previous observations (Saito & Fukushima, 1986, 1987, 1988) that in the presence of a poly-mannurono-gulurono-glycan derivative, the red colour can be maintained at a high level. The results from the analyses, monitored with a spectrophotometric method, gave additional and conclusive evidence for the formation of a stable carthamin–alginate complex in the presence of divalent calcium ions. Dropping calcium acetate solution into the aqueous carthamin–sodium alginate mixtures resulted in uniform small reddish globular beads, which were freeze-dried, and carthamin preservation was measured after extracting with a buffered solution. The data summarized in Table 1 show that, when carthamin is fixed with sodium alginate, the red colour is retained effectively in the gel beads. The preservation rate averaged 47%. The cp number slightly affects the carthamin stability: cp 100–150 is most effective to maintain carthamin red colouration (51%). The results are indicative of the fact that groups such as —OH, >O and —COOH on the molecules of both carthamin and alginate interact with each other. Previously, we have reported that carthamin is stabilized on cellulose powders, where the primary alcoholic hydroxyl(s) of cellulose is suggested to play a leading role in the pigment stabilization process (Saito, 1990*b*). Of course, sodium and/or calcium ions used for the salt formation could influence the colour preservation rate, because these cations act as blanching agents (Kanehira *et al.*, 1990; Saito & Fukushima, 1991).

Table 1. Carthamin red colour preservation in calcium alginate beads

CP number	Carthamin content (μg)	Preservation rate (% of control[a])
Undetermined	17·2	46·6 ± 1·6
100–150	18·8	50·8 ± 2·5
300–400	15·9	43·0 ± 5·7

[a] Control valve 36·97 μg. Average values from three separate repetitions.

In one of our preceding reports, we have demonstrated that sugar alcohols are able to preserve carthamin colour in solutions (Saito *et al.*, 1993). These substances are expected to be smoothers, lubricants and softeners for processed foods as well as colour stabilizers. The present data furnish additional new evidence that sodium alginate encapsulates carthamin intact and stabilizes the colouration in the gel beads. The reddish beads are safely applicable as a colour additive for a vast variety of food products in both wet and dry states. Their colouration and sensory properties are satisfactory. When required, the dried beads can be used ornamentally without further processing. This is an excellent new technique for expanding the commercial value of the herbal dye-stuff.

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