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Stability studies on the enzyme extracted sweet potato carotenoproteins

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Abstract

Pectinase and cellulase were used for the extraction of carotenoid pigments. Sweet potato samples, either directly extracted or pretreated by blanching at 0.2% sodium-bisulfite solution, or combinations of these two were used. Carotenoproteins were stored at 25 °C light, 25 °C dark and at 4 and 40 °C. Stabilities of pigments under different processing and storage conditions were studied. Blanching of sweet potato pigments resulted in the highest retention after 120 days at 4 °C.

Keywords: Carotenoids; Extraction; Cellulase; Pectinase; Storage stability

1. Introduction

High commercial interest in carotenoid production methods is due to their natural origin, null toxicity, high versatility, providing both lipo- and hydro-soluble colorants with colours ranging from yellow to red, provitamin A activity, role in lipid oxidation and anticarcinogenic properties.

Carotenoids, being lipid in nature, require a mixture of polar (e.g., ethanol and acetone) and non-polar (e.g hexane and toluene) solvents for extraction (Bassi, Pineau, Dainese, & Marquardt, 1993; Chen & Yang, 1992; De Sio, Servillo, Loiuduce, Laratta, & Castaldo, 2001; Kopas & Warthesen, 1995; Tsimidou et al., 1993; Sadler, Davis, & Dezman, 1990). Also, some researchers have extracted carotenoids from samples by using supercritical CO_2; liquid CO_2 was pumped through the extraction vessel and extracted carotenoids were collected in the depressurized stream of supercritical CO_2 (Ambrogi, Cardarelli, & Eggers, 2003; Felix-Valenzuela, Higuera-Ciapara, Goycoolea-Valencia, & Arguelles-Monal, 2001; Gnayfeed, Daood, Illes, & Biacs, 2001; Spanos, Chen, & Schwartz, 1993).

The enzyme extraction of carotenoid pigments, being a fairly new procedure, has been studied by very few researchers (Aravantinos-Zafiris, Oreopoulou, Tzia, & Thomopoulos, 1992; Britton, Liaaen-Jensen, & Pfander, 1995; Bryant, McCord, Unlu, & Erdman, 1992; Chakrabarti, 2002; DelgadoVargas & Paredes Lopez, 1997a, 1997b).

Several studies have been performed on the carotenoid pigments; however, few have been focused on the stability of the pigments. There is some interest on the stability of pigments during frozen or cold storage of the fruit and vegetables. Sheean, O'connor, Sheeby, Buckley, and FitzGerald (1998) reported no visual colour changes in astaxanthin- and canthaxanthin-fed atlantic salmon during frozen storage for up to 12 weeks. Su, Rowley, and O'Dea (1999) studied the stability of individual carotenoids in human plasma during light exposure. Perkins-Veazie and Collins (2003) and Sanches-Mata, Camara, and Diez-Marques (2002) worked on the stability of greenbean and watermelon carotenoids, respectively, during storage but pigments were not isolated prior to the storage. Storebakken et al. (2004) observed a decrease in astaxanthin stability caused by increased enzymatic disruption of cell walls of red yeast used in fish feed.

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Tang and Chen (2000) and Çinar (2004) studied the stability of freeze-dried carotenoid pigments under different storage conditions. To the author's knowledge, there are no other reports on the stability of the extracted carotenoid pigments during storage. Enzyme-extracted pigments remain in their natural state, bound with proteins through covalent bonding or weak interactions, depending on their structure. This bonded structure prevents pigment oxidation, whereas solvent extraction dissociates the pigments from the proteins and causes water insolubility and ease of oxidation (Bassi et al., 1993). Although enzyme extraction provides more stable carotenoid pigments, the stability of carotenoids during storage is a very important objective, to make the final product attractive and acceptable. Degradation of carotenoids not only affects the attractive colour of foods but also their nutritive value and flavour.

Therefore, the aim of this work was to test the stability of extracted pigments under different storage conditions.

2. Materials and methods

2.1. Sample preparation

Sweet potato is an excellent source of carotenoids since all-*trans*-β-carotene is the major carotenoid and exhibits the maximum provitamin A activity. Sweet potatoes were purchased from a local grocery store. Commercial pectinase and cellulase from *Aspergillus niger* were purchased from Sigma Chemical Co., St. Louis, MO. Hexane, obtained from Baxter Health Care Corporation, Musketon, MI, was of analytical reagent grade and was used without further purification. Celite® Filter Cel was purchased from Fluka Chemical Corp., Ronkonkoma, NY.

Sweet potatoes were washed under tap water and peeled, diced, mixed, weighed and used immediately for further processing. The samples were homogenized in a laboratory Waring blender for 2 min with distilled water to increase the surface area for efficient enzyme treatment.

2.2. Treatments prior to extraction

For the stability tests, sweet potatoes were either directly homogenized or pretreated. Blanching, 0.2% sodium-bisulfite treatment and both blanching and 0.2% sodium-bisulfite conditions were used as pretreatments. For blanching and sodium-bisulfite treatment, diced sweet potatoes were wrapped in cheese cloth and immersed in either a water bath containing distilled water at 100 °C for 2 min to inactivate possible peroxidase activity or 0.2% sodium-bisulfite solution at room temperature for 5 min. Residual solution was removed by washing with distilled water.

2.3. Pigment extraction

Pretreated and homogenized materials were placed in a large beaker. Commercial pectinase and cellulase enzymes were added. Samples were stirred on the Corning PC 351 brand magnetic stirrer at medium speed for 24 h at room temperature. 2.5 ml pectinase and 2 g cellulase were used for the 100 g food sample in 200 ml distilled water. After 24 h of enzyme treatment, a Celite bed was prepared on a porcelain Buchner funnel on #1 Whatman filter paper. Approximately 2 g Celite were added to the enzyme-treated pigment mixture and left to stand for 10 min. The mixture was vacuum-filtered through the Celite bed. The first filtrate was the water-soluble pigment extract. The vacuum flask was changed and the Celite bed, containing carotenoid pigments, was washed with 95% ethanol. The washing procedure was repeated until the Celite bed had returned to its original grey colour. Distilled water was added to the ethanol extract until pigment precipitation occurred. Ethanol was then evaporated by using a Buchi EL 130 brand rotary evaporator at 50 °C. The remaining residue was carotenoid pigments (lipid-soluble extract) and water.

2.4. Stability tests

Pigment extracts were analyzed for stability by using a Perkin–Elmer brand UV–Vis spectrophotometer. For the stability tests, the effects of light, dark, temperature, blanching, 0.2% sodium-bisulfite treatment and both blanching and 0.2% sodium-bisulfite treatment were studied. For each condition, pigment extracts were stored in small bottles. Dark conditions were provided by wrapping a glass bottle with several layers of aluminium foil while samples were exposed to fluorescent room light during the day for the light effect. Pretreated and directly extracted samples were stored at 25 °C light, 25 °C dark, 4 and 40 °C for the temperature effect. Absorbance was measured with UV–Vis spectrophotometer at 450 nm and converted to percentage retention for accurate comparison among the treatments.

2.5. Statistical analysis

All data from sample stability studies were further subjected to analysis of variance (ANOVA) using a randomized block design procedure. Experimental variables were treatments (25 °C light and dark, 4 and 40 °C) and percentage retentions of pigments. Mean differences among treatments and percentage retentions of pigments were tested for significance using LSD (least significant difference).

3. Results and discussion

The data from overall stability tests were converted to half-life values for the sweet potato pigments (Table 1).

Untreated sweet potato pigment retention was 42.8% for the samples stored at 4 °C after 120 days. Zero retention was observed after 85 days for 25 °C light and dark samples and after 42 days for 40 °C samples, as shown in Fig. 1.

Craft (1992) spectrophotometrically determined 32–97% retention of β-carotene, depending on the extraction solvent type, over a 10-day period. These findings were consistent with these reported by Kopas and Warthesen (1995) and Pesek and Warthesen (1987) as a general trend. Tsimidou and Tsatsaroni (1993) stated that the half-life of the saffron pigments in aqueous solutions was 59 h in the dark at 40 °C and 32 days at 4 °C. Pesek and Warthesen (1987) reported 25% of the original colour after 4 days of light exposure, at 4 °C, for carrots. By comparison, enzyme-extracted pigments had higher stability, especially compared to pigments normally extracted.

Blanching was used both to inactivate possible lipoxygenase and peroxidase activities, due to their role in indirect oxidation of carotenoids by producing peroxides and to provide greater penetration of pectinase and cellulase into the cells, to enhance the release of pigments. Blanched sweet potato pigments showed 59.0% retention at 4 °C after 120 days. There was no colour retention after 66 days for 25 °C light or dark samples or 34 days for 40 °C samples (Fig. 2). Statistical data showed that light and dark conditions had no significantly different effects on pigment retention for any samples, whereas 4 and 40 °C had.

Table 1
Half-life of sweet potato under different treatment and storage conditions

Samples	Half life (days)			
	25 °C Light	25 °C Dark	4 °C Refrig	40 °C Oven
Untreated	22	23	97	8
Blanched	19	19	36	7
Na-bisulfite-treated	18	19	>120	6
Blanched + Na-bisulfite-treated	28	24	93	16

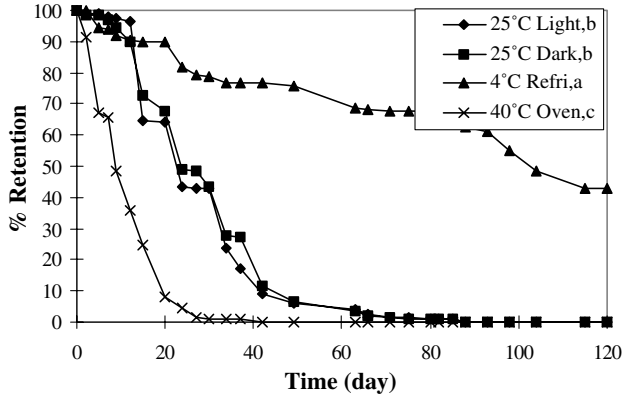

Fig. 1. % Retention of untreated sweet potato pigments at different storage temperatures. (The same letters in the legend are not significantly different.)

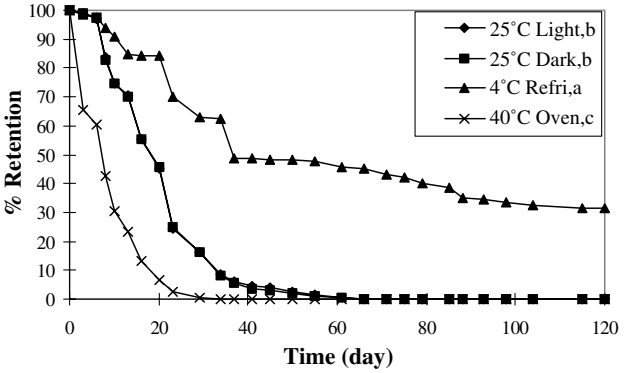

Fig. 2. % Retention of blanched sweet potato pigments at different storage temperatures. (The same letters in the legend are not significantly different.)

Chandler and Schwartz (1988) reported a 4–11.9% increase in carotene retention of sweet potato by blanching at 100 °C for 2–10 min. In contrast, Rahman and Buckle (1981) found that the percent retention of β-carotene was higher in unblanched frozen capsicums than in blanched samples after 12 months of storage.

Zhao and Chang (1995) reported that the use of 0.6% sodium-metabisulfite for 6 min retarded carotenoid breakdown, inhibited lipid oxidation and decreased discoloration of dehydrated carrots. Although this reaction is complex, the possible reaction of sulfite with carbonyl groups, reducing sugars and disulfide bonds in proteins may result in a protective effect. Sodium-bisulfite treatment enhanced the stability when compared to untreated samples. There was 54.2% pigment retention after 120 days at 4 °C; 25 °C light, 25 °C dark and 40 °C samples bleached completely in 60, 61 and 40 days, respectively (Fig. 3).

To determine if there was any synergistic effect of blanching and sulfite treatment on pigment retention, samples were first blanched and then treated with 0.2% sodium-bisulfite solution. Although blanching alone provided an improvement, combined treatment had no synergistic effect. Samples retained 47.6% after 120 days at 4 °C; 25 °C light samples lost all pigments after 80 days; 25 °C dark samples did so after 75 days and 40 °C samples after 38 days (Fig. 4).

For all samples, the difference between 25 °C light and dark samples was not significant, meaning that there was no light effect on pigment retention; 4 and 40

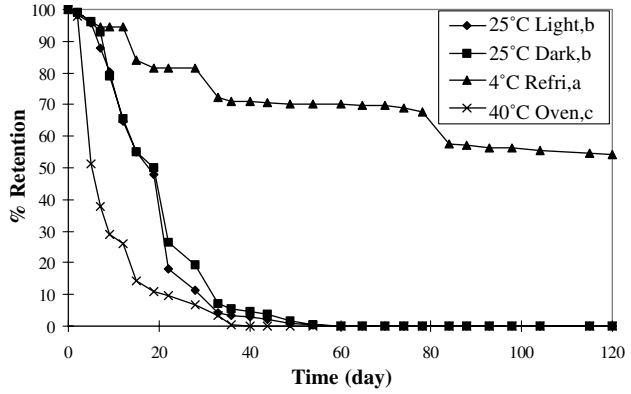

Fig. 3. % Retention of Na-bisulfite-treated sweet potato pigments at different storage temperatures. (The same letters in the legend are not significantly different.)

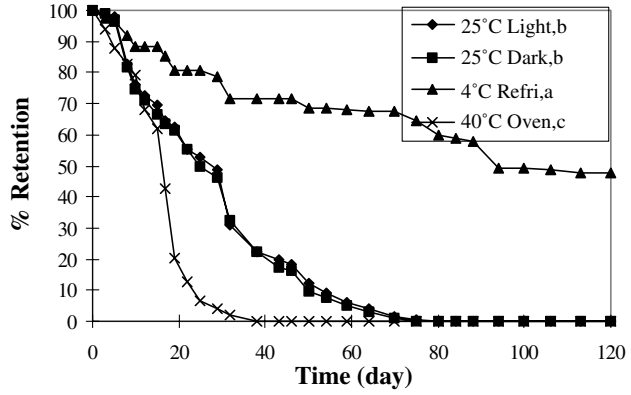

Fig. 4. % Retention of blanched + Na-bisulfite-treated sweet potato pigments at different storage temperatures. (The same letters in the legend are not significantly different.)

°C storage made a significant difference to retention ($p < 0.05$).

Contrary to our observation, Baloch, Buckle, and Edwards (1977) reported that blanching of carrot markedly protected the carotenoids and sulfite treatment gave further protection to carotenoids of blanched carrot. According to their study, higher pigment retention can be achieved by reducing the blanching time to 1 min. Optimization of blanching was highly recommended to gain the maximum benefit from sulfite treatment. This conclusion was in agreement with our results, since blanching alone was quite effective for stability of sweet potato pigments while the combination was not. Thus, optimization of combined treatments could be considered to be very important for stability.

4. Conclusion

This research introduces work on the stability of enzyme-extracted carotenoid pigments. Blanched sweet potato samples gave the highest colour retention after 120 days of storage at 4 °C. Storage of 25 °C light and dark samples gave reasonable retention rates compared to 4 °C and can be used as an alternative storage temperature. Contrary to our work, most of the previous studies on stability focus on storage of plant sample and carotenoid extraction prior to the analysis. Thus, this research may be helpful in demonstrating the higher stability of the enzyme-extracted pigments having possible use as colouring agents in food systems for commercial production.

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