LWT - Food Science and Technology 44 (2011) 1809–1813

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

A simple UV-Vis spectrophotometric method for determination of β-carotene content in raw carrot, sweet potato and supplemented chicken meat nuggets

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ARTICLE INFO

Article history:
Received 31 March 2010
Received in revised form
14 March 2011
Accepted 16 March 2011

Keywords:
β-carotene
UV-Vis spectrophotometry
Carrot
Sweet potato
Chicken meat

ABSTRACT

A simple, rapid and low cost analytical method for the determination of β-carotene content in four different food categories is developed and validated. This method is based on solvent extraction followed by UV-Vis spectrometric detection. Target compound was extracted with four different solvents, in which acetone appears to be an excellent extractant as recovery of the analyte at 1 μg/g concentration in spiked samples was in the range of 67.8–98.8%, with relative standard deviation (RSD) ranging between 4.8 and 6.6%. Intra- and inter-day assay precisions of the method at 0.1, 1.0 and 5.0 μg/g concentrations ranged in between 3.4 and 8.9%. The linearities for β- carotene in the pure acetone, raw carrot, sweet potato, and fortified raw and cooked chicken meat nuggets were 0.994, 0.995, 0.990, 0.984 and 0.984, respectively. For robustness, the analytical method was applied to 24 samples of four different food categories namely carrot, sweet potato and carrot and sweet potato supplemented chicken meat nuggets.

1. Introduction

β-carotene is one of the most important functional ingredients for development of value added meat products because of its efficacy in providing vitamin A activity from vegetable sources in the human food supply. In addition, it is accepted that carotenoids in general and carotenes in particular provide significant antioxidant activity to the human food and animal feed supply, and thus may be responsible for some of the significant correlations between increased intake of vegetables providing significant carotenoid content and improved health status (Akhtar & Bryan, 2008). Carotenoids represent a large group of phytochemicals that may contribute to health and disease prevention (DeNardo,Shiroma- Kian, Halim, Francis, & Rodriguez-Saona, 2009; El-Sohemy et al., 2002). Among all carotinoids, β-carotene is widely distributed in fruits and vegetables such as carrots, sweet potatoes, carrot and sweet potato based products, tomatoes and tomato-based products (Baranska, Schutze, & Schulz, 2006), apricot, cantaloupe and oranges. Recently it has been reported that the world carotenoid market is expected to reach U.S. $1.06 billion by the end of 2010 as consumers continue to look for natural ingredients (Nutraingredient, 2007). As carotenoids are rich in phytochemicals, and many food processors are incorporating them into development of functional based processed products, so ever increasing demands for carotenoids, especially β-carotene, is bound to increase misuse of the term carotenoids and adulteration with sources that may not contain bioavailable carotenes. Such abuse of carotenoids has necessitated the need for better analytical methods that are simple, inexpensive, rapid, robust and require only a little toxic solvent for their extraction and quantification. Spectrophotometry method is widely used technique for the analysis of β-carotene in commercial products (Schierle, Schellenberger, Fizet, & Betz, 2002). But in most cases it has limitation because of exhaustive extraction methods, requires large amount of toxic solvents which is very difficult to dispose off considering recent environmental issues, and finally the methods can be applied only for specific food components (Schierle, Pietsch, Ceresa, Fizet, & Waysek, 2004; Barba, Hurtado, Mata, Ruiz, & de Tejada, 2006). A number of liquid chromatography (LC) methods for the analysis of carotene and vitamin A have been published (Akhtar & Bryan, 2008; Ye, Landen, & Eitenmiller, 2000). Although high performance liquid chromatography (HPLC) is the method of choice for separation and quantification of β-carotene (Barba et al., 2006), but requires exhaustive sample purification steps for its detection, and developed methods are complicated than UV–Vis spectrometric methods. Recently, there is a very limited report on the determination of β-carotene in variety of vegetables and fortified meat samples. For this is reason a simple, sensitive, low cost and reliable UV–Vis spectrometric method for the determination of β-carotene content in different food categories (carrot, sweet potato, and carrot and sweet potato fortified chicken meat nuggets) was developed and validated.

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doi:10.1016/j.lwt.2011.03.017

2. Materials and methods

2.1. Chemicals and reagents

Standard of β-carotene Type I (95% purity, UV) was obtained from Sigma–Aldrich. All of the solvents and chemicals used in this study were of analytical grade and were procured from SRL, s d fine chemicals and Rankem, India.

2.2. Standard preparation

The standard stock solution at 1 mg/ml concentration was prepared by dissolving standard in acetone. The working standard solutions of 32, 16, 8, 4, 2, 1, 0.50, 0.25, 0.125, 0.062, and 0.03 μg/ml were prepared daily in the same solvent and were used to spike raw carrot, sweet potato and blank meat samples. All solutions were protected against light with aluminium foil and maintained at 4 °C for one month maximum, because greater periods of storage may produce irreproducible results due to degradation or reaction processes.

2.3. Sample collection

Raw carrots (red variety) and sweet potatoes (orange variety) were purchased from local grocery stores. Chicken meat samples were collected from experimental slaughterhouse of Department of Livestock Products Technology, College of Veterinary Science, GADVASU, Ludhiana, India. The collected vegetables were cleaned, washed and then packed in colourless low density polyethylene (LDPE) bags. Chicken meat samples were collected from the deboning table where the chilled carcasses were cut, deboned, trimmed, and packed. About 2 kg of the sample was cut aseptically during deboning operations and transferred to self-sealing LDPE. Meat emulsion of control sample was prepared by blending of meat mince, salt, sugar, phosphates, nitrite, spice mix, condiments, refined wheat flour, etc (Bhosale, Biswas, Sahoo, Chatli, Sharma & Sikka, in press). Emulsions for treated samples were prepared by adding carrot (10 g/100 g) and sweet potato (10 g/100 g) along with other ingredients as mentioned for control sample. The meat emulsions prepared as above formulations were filled up in rectangular shape aluminium molds and cooked in an autoclave at 15lb pressure, 121 °C temperature for 20 min. The cooked samples were cooled to room temperature, cut into nuggets, packed in colourless low density polyethylene bags (150–200 gauges), sealed, labeled and then deep-frozen. All types of samples were stored at −20 °C before analysis, separately.

2.4. Sample preparation and extraction

Frozen samples were thawed overnight in a refrigerator (4 ± 1 °C). For vegetables, outer most thin layer (skin or cuticle) and central white (only for carrot) portion were removed and remaining portions were sliced. The components so obtained were then ground separately into a spice grinder until become fine paste. The muscle samples (10–15 g) were diced into small pieces after being trimmed of external fat and fascia and then blended properly using pestle and mortar for 2 min.

For extraction, a representative portion of this sample (1 g) was accurately weighed in a glass test tube. Then 5 ml of chilled acetone was added to it, and the tube was held for 15 min with occasional shaking at 4 ± 1 °C, vortexed at high speed for 10 min, and finally centrifuged at 1370 × g for 10 min. Supernatant was collected into a separate test tube, and the compound was re-extracted with 5 ml of an acetone followed by centrifugation once again as above. Both of the supernatants were pooled together and then passed through the Whatman filter paper No. 42. The absorbance of the extract was determined at 449 nm wavelength in a UV–Vis spectrophotometer. Other extracts of diethyl ether, acetonitrile and methanol also prepared in similar manner as mentioned for acetone extraction.

Carrot and sweet potato fortified nuggets as well as control (without added carrot/sweet potato) sample were extracted with acetone alone after identification of best extractant from above extraction mehods.

2.5. Fortification of blanks and preparation of calibration curves

Blank samples for raw carrot (RC), sweet potato (SP), raw chicken meat (RCM) and cooked chicken meat (CCM) were prepared as described above. A working standard containing 32 μg/ml was prepared from the 1 mg/ml stock solutions kept at 4 °C. From this working standard different dilutions were made to spike the samples. Blank samples of 1.0 g were spiked with working standards to obtain final concentrations 16.0, 8.0, 4.0, 2.0, 1.0, 0.5, 0.25, 0.125, 0.062, 0.031 and 0.015 μg/g of β- carotene and extracted as described previously. Calibration curves were plotted by taking Optical Density (O. D.) value to the respective concentrations by back extrapolation methods. These curves were used to quantify the β-carotene content in the samples analyzed.

2.6. Analytical recovery and precision

Analytical recoveries were determined by spiking β-carotene to blank samples to yield concentrations of 1 μg/g by back extrapolation method and then analyzed. The amount of β-carotene found by the assay method for each concentration was estimated using a linear regression equation after calibration of standard curves considering O. D. values. Five determinants were made for each concentration, and the percent recovery was calculated. Both intra- and inter-day assay precisions [repeatability relative standard deviation (RSD_r) and reproducibility relative standard deviation (RSD_R)] were determined by analyzing three spiked concentrations of 0.1, 1.0, and 5.0 μg/g, five sets each with blank. However, intraday assay precision was determined at three occasions at least 6 h apart, whereas inter-day precision was determined at three successive days.

2.7. Statistics

The recovery and precision data were evaluated with an in-house statistical software program making use of robust statistic concepts of Snedecor and Cochran (1994).

3. Results and discussion

3.1. Linearity

Pure solvent (acetone) based calibration and four matrix-matched (carrot, sweet potato, raw and cooked chicken meat) calibration curves of β- carotene were generated by plotting O. D. value versus the concentration of analyte, and linear regression analysis was performed using Microsoft Excel. The calibration curves for all samples were linear from 0.015 to 8 μg/ml or μg/g, and the equations for the standard curves were as follows:

$y = 0.108x - 0.008$ ($R^2 = 0.994$) for β- carotene in the acetone matrix
$y = 0.103x - 0.005$ ($R^2 = 0.995$) for β- carotene in carrot matrix
$y = 0.096x - 0.003$ ($R^2 = 0.990$) for β- carotene in sweet potato matrix

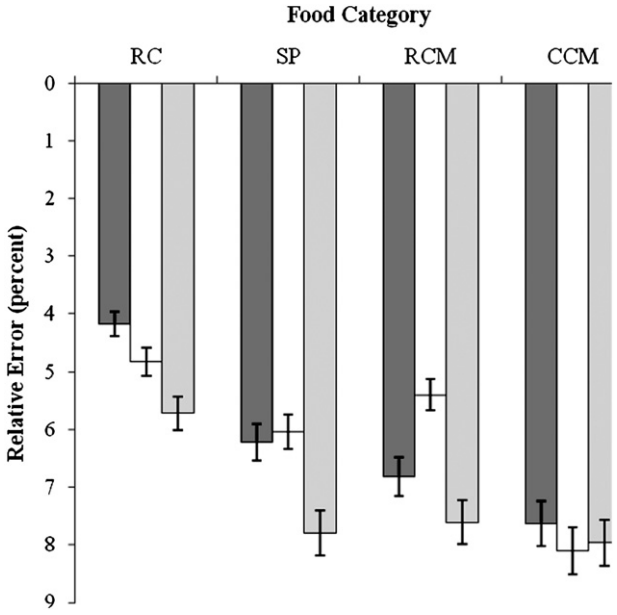

Fig. 1. Matrix-induced errors in O. D. values of four different matrixes extracts at different β-carotene concentration (0.1–5.0 μg/g).5.0μ/g ■;1.0μ/g □;0.1μ/g □. n=5; RC=raw carrot, SP=sweet potato,RCM=raw chicken meat and CCM=cooked chicken meat.

$y = 0.086x + 0.002$ ($R^2 = 0.984$) for β- carotene in raw chicken meat matrix
$y = 0.080x + 0.016$ ($R^2 = 0.985$) for β- carotene in cooked chicken meat nuggets matrix

Where y = O. D. value; and x = concentration (μg/ml).

3.2. Matrix effects

The use of matrix-matched calibration solutions is the most common approach that is used to circumvent errors associated with matrix-induced enhancement during quantification (Fig. 1). The occurrence of matrix-induced effects depends on whether or not extracts contain compound that will significantly influence the quantity of analyte molecules that present in the UV path. This may be due to competition between the analyte and coeluting components for the available charge, which mostly results in increase of O. D. value. Therefore we have studied the errors in UV–Vis spectrophotometer due to matrix effects by comparing the O. D obtained from matrix-matched calibration solutions with O. D. obtained from the pure solvent based solutions containing the same β-carotene concentration. Fig. 1 shows the relative errors of the method with different spiked concentrations of β- carotene (0.1, 1.0 and 5.0 μg/g). The significant error occurred in the chicken meat extracts comparing to that of carrot and sweet potato about 7.8–11.6%. Furthermore there are no major differences in error for O. D. values at different β- carotene concentrations in same matrix. Thereby, in this study, we employed matrix-matched calibrations so that this error could be circumvented.

Table 1
Recovery of β-carotene at 1 μg/g concentration.

Extractant	Mean recovery ± RSD, %			
	Carrot	Sweet potato	Raw meat (Spiked)	Cooked meat (Spiked + boiled)
Acetone	98.82 ± 4.8	95.44 ± 6.6	85.40 ± 5.9	67.86 ± 4.9
Diethyl Ether	78.64 ± 6.4	67.80 ± 5.6	77.48 ± 6.7	58.25 ± 3.5
Acetonitrile	41.39 ± 7.2	36.48 ± 7.4	36.76 ± 7.8	32.11 ± 8.5
Methanol	28.77 ± 6.5	29.55 ± 8.8	26.88 ± 6.3	18.66 ± 7.2

$n = 5$.

3.3. Extraction procedure

To simplify the extraction method best effort was made to minimize processing steps and also to avoid usages of large quantities of toxic solvents like chloroform, *n*-hexane, and even dichloromethane (Seo, Burri, Quan, & Neidlinger, 2005; Zanatta & Mercandante, 2007). The well-documented solvent acetone was found to be suitable for the extraction of β-carotene compound

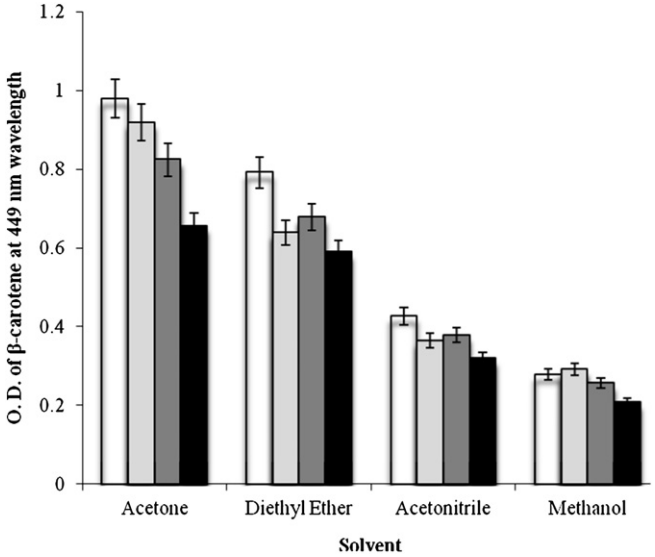

Fig. 2. Efficacy of different solvents for extraction of β-carotene. Raw carrot □;Sweet potato □;Raw chicken meat ■;Cooked chicken meat ■; No. of replication, n=5.

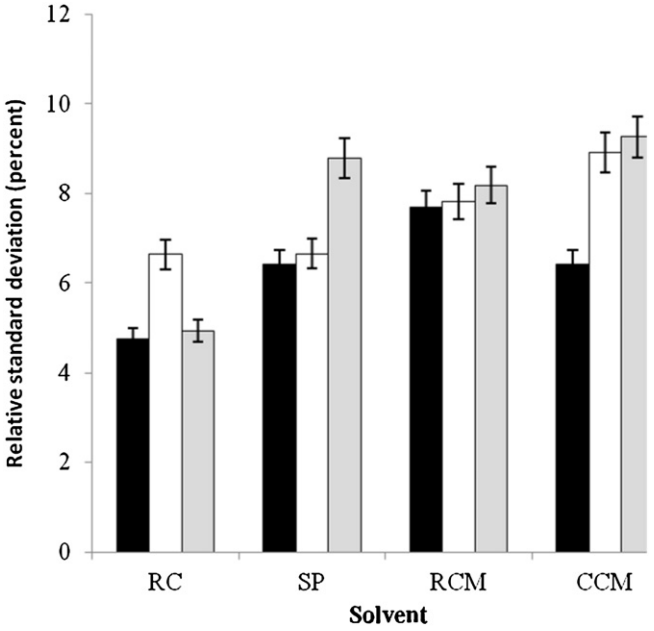

Fig. 3. Changes in relative standard deviation (percent) of O. D values at different concentrations of β-carotene. 5.0μ/g ■;1.0 μg/g □;0.10μg/g □. n=5; RC=raw carrot, SP=sweet potato, RCM=raw chicken meat and CCM=cooked chicken meat.

Table 2
Recovery, repeatability relative standard deviation (RSD_r) and reproducibility relative standard deviation (RSD_R) obtained for β-carotene at three spiked level.

Sample	Spiked Level,μg/g	Day1		Day2		Day3		RSD_R
		Average recoveries, %	RSD_r	Average recoveries, %	RSD_r	Average recoveries, %	RSD_r	
Carrot	0.1	91.7	8.1	89.8	7.6	89.2	7.1	6.8
	1.0	92.1	6.7	90.1	7.0	90.5	5.8	6.5
	5.0	98.9	3.2	97.7	3.5	97.2	3.7	3.4
Sweet Potato	0.1	75.2	9.3	75.1	8.9	75.7	7.9	7.5
	1.0	77.5	8.9	77.6	8.1	76.1	8.3	8.7
	5.0	78.8	8.1	79.6	7.3	78.1	6.5	6.5
Raw meat	0.1	82.4	7.2	82.6	6.8	86.5	8.3	6.7
	1.0	84.2	8.8	83.1	8.1	84.8	6.2	7.2
	5.0	89.2	8.0	87.2	7.1	90.4	4.9	6.3
Spiked + cooked meat	0.1	67.6	7.1	65.3	7.4	65.8	8.2	7.7
	1.0	68.4	6.5	66.2	6.9	64.1	7.9	6.8
	5.0	69.5	6.8	66.8	5.4	67.6	4.6	5.8

$n = 5$.

present in four very different types of samples (Fig. 2). As β-carotene is nearly non-polar compound, its extraction was improved with moderately polar solvent i.e. acetone. This solvent was found to have advantages over a number of organic solvents (methanol, acetonitrile, and diethyl ether) in terms of deproteinization (removal of >99% of protein), recovery (Table 1), co eluting interference (Fig. 1), and avoid emulsion formation. Diethyl ether shows extraction efficiency next to acetone, while methanol had least values. The relative standard deviation (RSD %) of O.D values at different concentrations of β-carotene for same food product did not vary significantly, though differences existed among the food products (Fig. 3). Indeed, this method could avoid many disadvantages of previous methods because they were principally based on cumbersome extraction steps (saponification) with large sample size, and required ample volumes of toxic solvents (Szpylka & Devries, 2005). Again it is worthwhile to mention here that some other researchers (Akhtar & Bryan, 2008; Barba et al., 2006) though avoid saponification step when analyzing carrot juice and oily samples but are time consuming and somewhat costlier. Hence, acetone was used as the extraction solvent, but in reduced quantities.

3.4. Recovery, repeatability and reproducibility

The analytical method was validated by evaluating percent recovery, precision, linear dynamic range, sensitivity, LODs, and LOQs of the analytes. In recovery study, carrot, sweet potato and raw chicken meat were all spiked at 1.0 μg/g concentration (Table 1), based on five replicates, before extraction by adding the appropriate volume of working standard solution, while that were spiked at 0.1, 1.0, and 5.0 μg/g concentrations for precision study. Another chicken meat sample was also spiked and then boiled simultaneously for determination of effect of heat on β-carotene content in fortified chicken meat sample (Table 2). The recoveries obtained were in the acceptable range of 67.8, 85.4, 95.44 and 98.82% in cooked meat, raw meat, sweet potato and carrot respectively with the relative standard deviation (RSD_r) of ranging from 4.8 to 6.6% as shown in the Table 1. Comparatively lower recovery was found in cooked samples could be due to degradation of β-carotene on heating. The recovery ranges found with other solvents were unacceptable though moderate values were recorded for diethyl ether.

The precision of the method was determined by repeatability and reproducible studies of method and expressed by relative standard deviation (RSD). The repeatability (RSD_r) was measured by comparing standard deviation of the recovery percentages spiked samples run in the same day. The reproducibility RSD_R was determined with analyzing spiked samples for 3 successive days for all four types of samples. In general, repeatability and reproducibility were in the ranges of 3.2–9.3% and 3.4–8.7%, respectively (Table 2).

Table 3
β-carotene (μg/g) in carrot, sweet potato and carrot (T_1) and sweet potato (T_2) fortified chicken meat nuggets.

Treatment	β-carotene content
Control (cooked nuggets)	–
Carrot	74.06 ± 3.8
Sweet potato	68.48 ± 4.1
T_1	5.65 ± 0.06
T_2	4.36 ± 0.08.

$n = 6$; "-" = Not determined.
Control = without carrot/sweet potato added chicken nuggets; T_1 = carrot added cooked chicken nuggets and T_2 = sweet potato added cooked chicken nuggets.

3.5. Application in real samples

A total of 24 samples comprising of ground raw carrot, sweet potato and carrot (10 g/100 g raw emulsion) and sweet potato (10 g/100 g raw emulsion) incorporated chicken meat nuggets were evaluated for the quantification of β-carotene content. The results of analytical data (Table 3) indicated that fortified chicken meat nuggets had lower levels of β-carotene than their corresponding values of nearly 7.4 and 6.8 μg/g of ground raw carrot and sweet potato samples, respectively. This could be attributed to lower recovery of β-carotene content from the fortified tissue samples (Table 2) coupled with degradation of that compound on heating during product processing. The degradation of β-carotene content during heat processing is well-documented (Edwards & Lee, 1986; Park, 1987).

4. Conclusions

UV–Vis spectrophotometry detection and acetone as the extraction medium was successfully employed for the simple and rapid determination of β-carotene content in four different types of tissue samples. In comparison to the saponification methods mentioned previously, the proposed UV–Vis spectrophotometry method is environmentally friendly and inexpensive and easily performed. In addition, simultaneous analysis was accomplished with high precision (repeatability and reproducibility). The method has great potential for adoption by many developing countries where limited funds are available for routine monitoring of such type of compound. Therefore the proposed method could be useful

and practical in the future for β- carotene detection from various tissue samples.

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