Trop J Nat Prod Res, March 2023; 7(3):2487-2491 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Comparison of Nutritional Quality and Storage Stability of *β*-Carotene Fortified Soybean Oil and Olive Oil

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ARTICLE INFO

Article history:
Received 09 March 2022
Revised 20 February 2023
Accepted 17 March 2023
Published online 01 April 2023

ABSTRACT

Edible oils have made a significant contribution to people's diets by providing a valuable source of energy as well as a good source of protein, lipids, and fatty acids for nutrition. Additionally, these oils have helped to heal damaged tissues and produce new cells, in addition to being a good source of nutrition. Since vegetable oil is a frequent source of vital fat and is high in energy, polyunsaturated fatty acids, and vitamin E, it makes perfect sense to use vegetable oil as a vehicle for administering a vitamin A or beta-carotene supplement. The aim of the present study was to develop methods that to add *β*-carotene to soybean oil and olive oil. These methods are based on simple and inexpensive technologies. We have investigated the stability criteria of the prepared oils, including their moisture content, acid value, peroxide value, and beta-carotene content, during a period of one-year storage. According to the results of our study, olive oil is superior to soybean oil in terms of its stability and ability to retain beta-carotene. The content and stability studies of the oil indicates that daily ingestion of small quantities of the produced oils can assist in the prevention and/or mitigation of vitamin A deficiency, which is a problem that affects the public health of a number of countries as well as the health of particular people all over the world.

Keywords: *β*-carotene, olive oil, soybean oil, acid value, peroxide value

Introduction

Malnutrition in the Indian subcontinent, especially among women, children, and adolescents, is a serious problem that must be addressed immediately if the country is to achieve inclusive growth and development.[4–6] To address the global nutrition security issue, a variety of techniques are needed, including short-, intermediate-, and long-term sustainable approaches.[7–9] In addition to traditional vitamin supplementation, food-based nutrition assurance techniques are needed to enable appropriate micronutrient intakes by the majority of the population.

Food fortification with micronutrients is regarded as a viable technology and approach for ensuring adequate amounts of the required nutrients in the diet. Preventing vitamin deficiency through food fortification is a well-known strategy for addressing nutritional issues, particularly in developing countries. Edible oils are a vital source of nutrition for the world's population as they serve three functions in the human body: as an energy source, a structural element, and significant biochemical mediators. Oils and fats also play a significant role in the human body's metabolic processes. Vegetable oils are widely utilized in preparing meals, including frying, salad dressing, grilling, baking, and cooking. Vegetable oils and fats account for around 80 percent of the edible oils and fats consumed worldwide. On the other hand, vitamin A deficiency is a problem that impacts people's nutritional status and health. Provitamin A carotenoids are the predominant source of vitamin A.

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Citation: Ahmed S, Arefin P, Mondal K, Miah MAS. Comparison of Nutritional Quality and Storage Stability of β-Carotene Fortified Soybean Oil and Olive Oil Trop J Nat Prod Res. 2023; 7(3):2487-2491 http://www.doi.org/10.26538/tjnpr/v7i3.3

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Only about 10 percent of the 600 carotenoids identified from natural sources serve as vitamin A precursors.[3] Carotenoids, particularly β-carotene from vegetables, is a significant source of vitamin A.[10,11] β-carotene acts as a physiological antioxidant, safeguarding cells or tissues against free radicals and singlet oxygen damages.[2,12,13]

Vitamin A and β-carotene are fat soluble and vegetable oil production and supplementation with vitamin A or β-carotene technology are uncomplicated.[14–16] In our present study, we have used soybean oil and olive oil for vitamin A fortification. The current work focuses on β-carotene extraction, encapsulation, and fortification in edible oils to address micronutrient deficiencies.

Materials and Methods

Raw material collection

Fresh matured carrots (*Daucus carota* L) vitamin E-fortified soyabean oil, and extra virgin olive oil were collected from the local market on June 2021 by Salma Ahmed, Principal Scientific Officer and Kanika Mondal, Principal Scientific Officer of IFST, BCSIR. Carrots were washed thoroughly in tap water to remove surface dust and reduce the number of microorganisms.

Preparation of raw materials

Carrots were sorted and peeled. Then they were cut into small pieces (0.8 mm in diameter) by a mechanical slicer. Then they were blanched in a steam blancher at 100°C temperature for three to five minutes, which is required to deactivate the enzymes present and avoid undesirable changes in texture. The water was drained out. Then carrot cuts were cooled and dried in an air oven at 50^0-60^0C for 6-8 hours, until the moisture content was 4-5%. The cuts were then ground by an electrical grinder to form a fine, homogenous powder. The carrot powder was stored in an airtight container in a refrigerator at 8°C.

Preparation of the Beta Carotene Enriched Oil
Carrot powder was mixed with soybean oil and olive oil separately in a 1:3 ratio and left to soak in. Carrots powder and oil mixture was placed on rotatory shaker for overnight. In our experiment, we took 2 kg carrot powder in 6 Litre of each types of oil separately. The oils were centrifuged at 50,000 rpm for 5-10 minutes, and the carrots' debris settled down. The supernatant oil layer was separated and collected. β-Carotene Enriched Soybean Oil (β-CFSO) and Beta Carotene Enriched Olive Oil (β-CFOO) were kept in sealed amber color glass bottles at room temperature (25-27^{o0} C) and observed for 12 months for stability and β-Carotene retention.

Analysis
Moisture, acidity, peroxide value, and beta-carotene contents of the oil were estimated. The oil bottle was sealed. Moisture, acidity, peroxide value, and beta-carotene content were measured every three months over a 12-month storage period.[17–20]

Determination of Moisture
Moisture in oils was determined by the method described by Arefin et al. [21]In a weighted crucible, ten grams of oil sample were poured. The product was dried for 1 hour at a constant weight in an oven with the temperature set at 105°C. Then the oil was cooled in desiccators for 15 minutes. The moisture content was computed using the equation below.

$$\% \text{ Moisture} = \frac{\text{W1 x 100}}{\text{W2}}$$

Where,
W_1 is the weight loss (g) due to drying, and
W_2 is the weight (g) of the oil sample.

Determination of Acidity
The acidity of the oil was measured as its free fatty acid (FFA) content was determined byInternational Organization for Standardization (ISO) 660, 2009). [22] Approximately 5 grams of oil were weighted, and 50 mL of ethanol were added. 2 mL of 1 % phenolphthalein indicator was added to ethanol. Titration was performed using a 0.1 N potassium hydroxide solution with vigorous shaking until a faint pink color appears and persists for at least 30 sec. The percentage of free fatty acids expressed as oleic acid corresponds to acidity. The FFA content was calculated using the equation:

$$\text{FFA } (\%) = 0.5 \text{ x AV}$$

Where, AV= Acid Value in mg/gm of oil
and

$$\text{Acid Value} = \frac{\text{VxNx 56.1}}{\text{m}}$$

Where,
V = volume of potassium hydroxide used until a pink color appears in mL,
N = normality of the potassium hydroxide solution,
56.1 is the molecular mass of potassium hydroxide,
m = the mass of the oil sample in grams.

Determination of Peroxide Value
The peroxide value is measured as milliequivalents of oxygen per kilogram of oil (mEq O_2/kg of oil). The peroxide value (PV) of oils and fats is a measurement of the extent of primary oxidation occurring when hydroxyl groups combine with molecular oxygen to form hydroperoxides and peroxides. Heat and light can expedite the generation of organic peroxides, which then decompose into low-molecular-weight molecules, including alcohols, aldehydes, and ketones, resulting in auto-oxidative rancidity. The peroxide value of oil was determined by AOAC 965.33.[23] Chloroform and acetic acid are mixed together in a 2:3 ratio, and 50 mL of the solvent mixture were taken.[24] Then the pre-weighted oil was put in it. It was allowed to react with 1 mL of freshly saturated potassium iodide (KI) solution for 60 seconds. The amount of free iodine (I_2) was measured by titrating the desired mixture with standard sodium thiosulfate ($Na_2S_2O_3$; 0.01 M).[25] 1mL of a 1% starch solution was used as the indicator. [26] Blank titration was performed. The peroxide value was measured by the equation:

Peroxide value (milliequivalents peroxide/1000 g sample) =

$$\text{Peroxide value (milliequivalents peroxide/1000 g sample)} = \frac{(\text{S} - \text{B})\text{x N x } 1000}{\text{m}}$$

Where—
B = Sodium-thiosulphate volume used for blank in mL
S = sodium-thiosulphate volume consumed by the oil in mL
N = normality of sodium thiosulfate solution
m = mass of oil in gram

Determination of beta-carotene
β-carotene in the oil was determined by the method described in [27]. 5 ml of petroleum ether and 5 ml of acetone were added to 10 mL of oil to transfer the pigments to the petroleum ether. Then it was passed through anhydrous sodium sulfate, and the volume was adjusted to 25 ml of petroleum ether. To determine β-carotene, the column was first attached to a Buchner flask and vacuum was applied.[28] The glass column was next compactly packed with the Alumina, the adsorbent to a height of about 10 cm. Anhydrous Sodium Sulfate (Na_2SO_4) was added to the top of the column to a height of 1 cm. The column was then washed with 30 ml petroleum ether by suction. When the petroleum layer had almost reached the Na_2SO_4 surface, the suction pump was disconnected and the column tube was attached to another clean and dry Buchner flask. 10 ml of the petroleum ether extract was taken into the column, and suction was applied. The column was continuously fed with 3% acetone in petroleum ether. β-carotene comes out of the column before any other pigments. [28] Once β-carotene band has come out entirely, the suction was removed and the contents of the flask were taken into a volumetric flask and the volume was made up to volume with petroleum ether, the eluent. The absorbance of the solution was measured at 452 nm using 3% acetone in petroleum ether as a blank. β-carotene is measured. [28]

$$\% \beta - \text{carotene} = \frac{\text{C1 x V2 x Vx 100}}{\text{V1 x W}}$$

Weight of oil taken for extraction = W
Volume of the petroleum ether extract of oil= V
Volume of the petroleum ether extract taken for chromatography = V1
Volume of β- carotene band made up to = V2
Concentration of β- carotene in the solution = C1

Statistical evaluation
One Way ANOVA followed by Post Hoc Tukey test was done to observe the means of the observations with standard deviation and determine the significance of variance in the parameters results of the oils prepared at each interval over the observation period. A paired comparison (t-test) between the means was used in IBM Statistical Packages for Social Science (SPSS) version 22.0 for statistical analysis.[18] The level for statistical significance was established at 0.05. OriginPro 2018 was used to make graphic illustrations.[29]

Results and Discussion

Maximum permissible limit of moisture for edible oils is 0.2%.[1,30] The moisture content for two types of fortified oils, β-CFSO and β-CFOO were found to be in the range of 0.04 ± 0.003 and 0.07 ± 0.002 and 0.03 ± 0.01 and 0.06 ± 0.00, respectively. The difference in moisture content between β-CFSO and β-CFOO is significant over the whole observation period. According to the analysis of comparison paired *t*-test at 95% confidence interval ($P < 0.05$), moisture content for β-CFSO and β-CFOO were significantly different ($P< 0.001$) from each other over the whole observation period.
The acid value is commonly used as a fundamental estimate of the oil's quality and edibility. The maximum acid value for all edible oils should be less than 0.6 mg KOH/g (reported as potassium hydroxide per gram) [22,30] as recommended by FAO/WHO. The acid value increased gradually over the storage time (Figure 2). The acid value for β-CFSO was found to be in the range of 0.48± 0.01 to 0.68± 0.01 mg KOH/g, the value was found to be 0.57 ± 0.01 mg KOH/g at 6 months observation period, but increased to 0.63 ± 0.02 mg KOH/g at 9 months observation period (Table 1). The acid value for β-CFOO was found to

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

be in the range of 0.44 ± 0.11 to 0.57 ± 0.02 mg KOH/g, which was under the permissible limit over the whole observation period. But, β-CFSO are observed to have a higher acid value than β-CFOO. The acid value for β-CFSO at each interval of observation were significantly different (P< 0.001) from each other. For β-CFOO, the acid value observed after three months were not significantly different with the acid value observed after six months. According to the analysis of comparison paired t-test at 95% confidence interval (P < 0.05), results of peroxide value for β-CFSO and β-CFOO were significantly different (P = 0.001) from each other over the whole observation period. As the higher acid value indicates the more oxidation and deterioration of the oil, β-CFSO was found to be deteriorate earlier than β-CFOO.

FAO/WHO recommends peroxide values (PV) of less than 10 mEq/kg for fresh oils. The maximum PV for extra virgin oil is 20 mEq/kg. But PV, more than 10–20 mEq/kg can cause rancidity and bad smell. The peroxide value in two types of fortified oils (β-CFSO and β-CFOO) were consistent with FAO/WHO recommended values. In general, the peroxide value increased over the storage time (Figure 3). But, β-CFSO are observed to have a higher peroxide value (PV) than β-CFOO. The PV for β-CFSO and β-CFOO were found to be in the range of 2.51 ± 0.18 to 5.77 ± 0.29 mEq/kg and 2.25 ± 0.08 to 3.8 ± 0.07 mEq/kg respectively, which was under the permissible limit over the whole observation period. The PV for β-CFSO and β-CFOO at each interval of observation were significantly different (P< 0.001) from each other. According to the analysis of comparison paired t-test at 95% confidence interval (P < 0.05), results of peroxide value for β-CFSO and β-CFOO were significantly different (P = 0.001) from each other over the whole observation period. As the higher PV indicates the more oxidation and deterioration of the oil, β-CFSO was found to be deteriorate earlier than β-CFOO.

The stability of the carotenoids in the β-CFSO and β-CFOO was evaluated over 12 months of storage (Figure 4). The β-Carotene content for β-CFSO and β-CFOO at each interval of observation were significantly different (P< 0.001) from each other. The β-Carotene content for β-CFSO decreased from 4925 ± 58.49 to 2920.16 ± 55.77 µg/ 100g. The results of the study showed almost 60% β-carotene retention in soybean oil after 12 months of preparation, whereas 78% β-carotene retention after 6 months. The β-Carotene content for β-CFOO decreased from 4992.3 ± 6.30 to 4100.58 ± 50.48 µg/ 100g which indicates almost 82% β-carotene retention in olive oil after 12 months of preparation. The β-Carotene content for β-CFSO and β-CFOO at each interval of observation were significantly different (P< 0.001) from each other. According to the analysis of comparison paired t-test at 95% confidence interval (P < 0.05), β-Carotene content for β-CFSO and β-CFOO were significantly different (P< 0.001) over the whole observation period.

Therefore, it can be posited that β-Carotene is more stable in olive oil than soybean oil. We have used extra virgin olive oil to prepare β-CFOO. According to Velasco and Dobarganes (2002), extra virgin olive oil has been found to have more resistance to oxidative deterioration due to the lower polyunsaturated fatty acids (PUFA) content and higher phenolic compounds content.

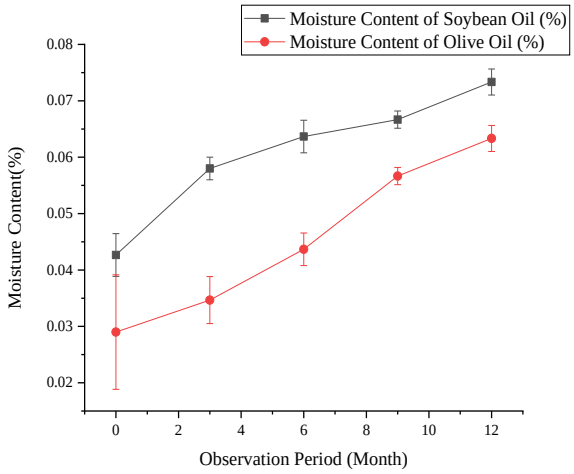

Figure 1: Moisture content of β-Carotene fortified soybean oil (β-CFSO) and β-Carotene fortified olive oil (β-CFOO) over 12 months of storage period

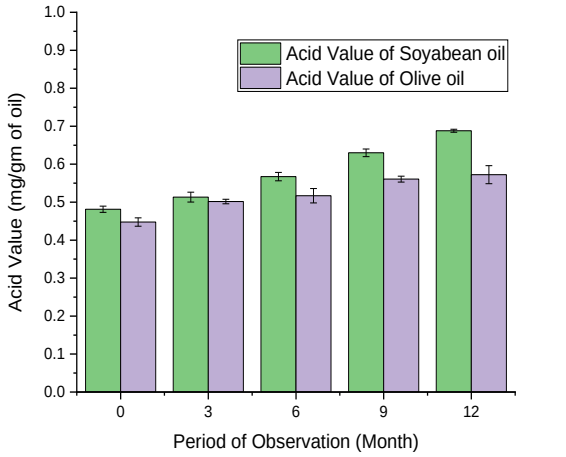

Figure 2: Acid Value of β-Carotene fortified soybean oil (β-CFSO) and β-Carotene fortified olive oil (β-CFOO) over 12 months of storage period

Table 1: Acid Value and Peroxide Value of β-CFSO and β-CFOO over 12 months of storage period

Observation Period (Month)	Acid value (mg in KOH/ g)		Peroxide values (mEq/Kg oil)	
	β-carotene fortified Soybean oil (β-CFSO)	β-carotene fortified Olive oil (β-CFOO)	β-carotene fortified Soybean oil (β-CFSO)	β-carotene fortified Olive oil (β-CFOO)
0	0.48 ± 0.01^{a}	0.44 ± 0.11^{a}	2.51 ± 0.18^{a}	2.25 ± 0.08^{a}
3	0.51 ± 0.01^{b}	0.50 ± 0.01^{b}	2.88 ± 0.05^{a}	2.84 ± 0.05^{b}
6	0.57 ± 0.01^{c}	0.52 ± 0.02^{c}	3.78 ± 0.14^{b}	3.18 ± 0.07^{c}
9	0.63 ± 0.02^{d}	0.56 ± 0.01^{d}	4.79 ± 0.07^{c}	3.22 ± 0.09^{d}
12	0.68 ± 0.01^{e}	0.57 ± 0.02^{e}	5.77 ± 0.29^{d}	3.80 ± 0.07^{e}
p-value	<0.001	<0.001	<0.001	<0.001
Level of Significance	***	***	***	***

Values were recorded in triplicates (n=3). Values are expressed as M ± SD of data. M= Mean, SD= Standard Deviation. Means in a column with same superscript are not significantly different at (p < 0.05) for the respective samples. *= Significant (P< 0.05); **=Significant (P< 0.01); ***=Significant (P< 0.001); NS= Non-significant.

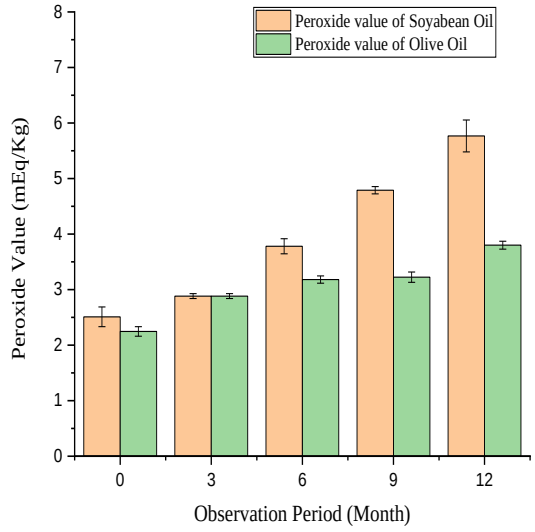

Figure 3: Peroxide Value of β-Carotene fortified soybean oil (β-CFSO) and β-Carotene fortified olive oil (β-CFOO) over 12 months of storage period

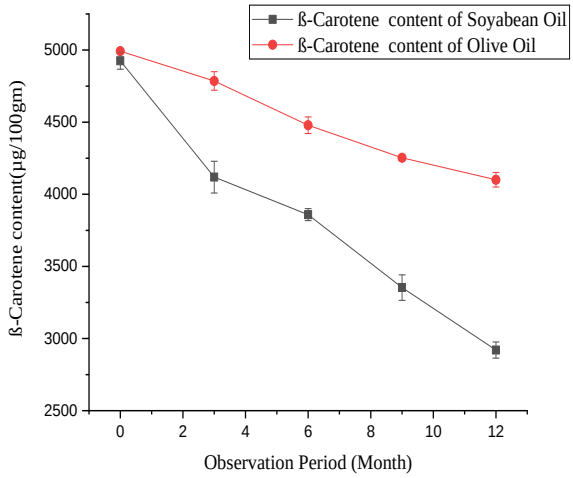

Figure 4: β-Carotene retention of β-Carotene fortified soybean oil (β-CFSO) and β-Carotene fortified olive oil (β-CFOO) over 12 months of storage period.

There is a greater amount of phenolic compounds in extra virgin olive oil compared to other refined oils, as they are eliminated or significantly reduced during the refining process in case of other oils. These phenolic compounds in extra virgin olive oil probably improve the stability of the carotenoids.

Conclusion

The carotenoids extraction and oil fortification method confirmed that edible vegetable oils may be fortified with carotenoids produced from carrots utilizing vegetable oils as the extraction medium.The peroxide and acid values of β-CFSO and β-CFOO increased significantly over a 12-month period, however β-CFOO was more stable than β-CFSO over longer time periods. However, the residual β-carotene level was sufficient to meet the recommended daily amount (RDA).In contrast, as carrots are susceptible to rotting in the presence of air, moisture, light, temperature, and microbial activity, the formulation of beta-carotene-enriched oil can also prevent massive post-harvest losses in carrots and produce goods with additional value. The beta-carotene levels in both oils indicate that 10 mL of each oil is sufficient to meet the daily recommendation (RDA). Therefore, this study suggests that vegetable oils such as soybean oil and olive oil might be employed as matrices for beta-carotene supplementation, with olive oil being the superior option.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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