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Influence of Vegetable Oils on Micellization of Lutein in a Simulated Digestion Model

Bhatiwada Nidhi · V. Baskaran

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Abstract Lutein and zeaxanthin are selectively accumulated in the macula of the retina, yet their bioavailability is influenced by various dietary factors. Insights regarding the effects of dietary lipids on lutein micellization that is available for absorption are limited. This study investigated the influence of vegetable oils on the relative efficiency of lutein micellization using in vitro digestion procedure. Lutein dispersed in either olive oil (OO), corn oil (CO), soybean oil (SBO), sunflower oil (SFO), groundnut oil (GNO), rice bran oil (RBO) or palm oil (PO) was subjected to simulated gastric and small intestinal digestion. Results showed that the efficiency of micellization of lutein dispersed in olive oil exceeds the other vegetable oils. The percent lutein micellization was in the order of OO > GNO > RBO > SFO > CO > SBO > PO. In comparison, the values for OO were higher than GNO (11%), RBO (18.3%), SFO (19%), CO (21.7%), SBO (30.5%) and PO (35.2%), respectively. These results suggest that OO rich in oleic acid may favor the incorporation of lutein into micelles at the intestinal level. To conclude, the type of vegetable oil in which carotenoids are dispersed is important to achieve an enhanced bioavailable lutein. The correlation between the micellizable lutein and fatty acid composition of vegetable oils are discussed.

Keywords Fatty acids · In vitro digestion · Lutein · Micellization · Mixed micelles · Vegetable oils

B. Nidhi · V. Baskaran (✉)
Department of Biochemistry and Nutrition,
Central Food Technological Research Institute,
CSIR, Mysore 570 020, Karnataka, India
e-mail: basrev@yahoo.co.in

Introduction

There is increasing evidence that dietary carotenoids may prevent certain degenerative and nutritional deficiency disorders. In particular, lutein has been strongly implicated as being protective against the AMD (age-related macular degeneration), and cataracts [1], although the mechanism of its action at the molecular level still remains unclear [2]. In addition, there is evidence to support the protective effect of lutein against certain cancers and heart diseases [1]. The mechanism by which lutein is involved in protecting the eyes from light irradiation is not known, but it may function as an antioxidant and act as a blue light filter to protect the underlying tissues from phototoxic damage [3]. Lutein from the plant food materials is the primary source of the macular pigment, since it is not synthesized in vivo. The absorption of lutein decreases when ingested with other food components [4]. Herden et al. [5] reported that intestinal absorption of carotenoids, in general, depends on the concentration and origin of the dietary fat consumed. Dietary fat is reported to improve the absorption of lutein ester by influencing the release of bile and the formation of mixed micelles in the intestine [6]. Brown et al. [7] showed that use of fat-free or reduced-fat salad dressings limits the absorption of α-carotene, β-carotene and lycopene. Plasma lutein response was enhanced after a meal with sufficient fat, but reduced when fat is absent or too low [8]. From the literature it is evident that dietary lipids are an essential factor that improves carotenoid absorption.

The intestinal absorption of dietary carotenoids from food involves several steps: the breakdown of the food matrix to release the carotenoids, dispersion in lipid emulsion particles, solubilization in mixed micelles, movement across the unstirred water layer adjacent to the

microvilli, uptake by the cells of the intestinal mucosa and incorporation into chylomicrons [8, 9]. Hu et al. [10] studied the effect of dietary sunflower oil and beef tallow on the levels of β-carotene in plasma triacylglycerides-rich lipoprotein in women subjects. Clark et al. [11] reported that lycopene and astaxanthin absorption is greater in olive oil compared to corn oil when these emulsions were directly infused into the duodenum of rats. Dietary olive oil significantly enhanced the plasma lutein level in rats [12]. None of these reports say how certain vegetable oils favor the intestinal absorption of lutein. Hence, we hypothesize that the type of fatty acid micelles influences intestinal absorption of lutein. However, the fate of micellar lipids carrying hydrophobic carotenoids after their intestinal uptake is unknown. To improve our understanding of micellizable lutein with different vegetable oils, we determined the micellizable lutein in vitro. Vegetable oil utilization, particularly soybean oil, corn oil, sunflower oil and rice bran oil, has increased rapidly not only in India but also worldwide, especially among low socio-economic groups. Vegetable oil appears to be a suitable carrier for enhancing the bioavailability of lipophilic nutrients like carotenoids. Although lutein disperses well in vegetable oils, its intestinal uptake may depend on the fatty acid composition of vegetable oils. Previous studies from our laboratory [12] have shown olive oil to be a good vehicle to enhance dietary lutein absorption. Therefore, it is important to choose a suitable lipid medium for the delivery of lutein. This study reports the influence of vegetable oils on the micellization of lutein in vitro.

Materials and Methods

Chemicals and Materials

Lutein (96%) was extracted and purified from marigold flowers for experimental purpose. Standard lutein (99%), butylated hydroxyl toluene (BHT), pepsin (porcine), bile extract (porcine) and pancreatin (porcine) and fatty acid standards were purchased from Sigma-Aldrich (St. Louis, USA). High-performance liquid chromatography (HPLC) grade acetonitrile, methanol and dichloromethane and analytical grade solvents were purchased from Sisco Research Laboratories (Mumbai, India). Refined olive (OO), rice bran (RBO), sunflower (SFO), groundnut (GNO), soybean (SBO), corn (CO) and palm (PO) oils were obtained from a local super market (Mysore, India).

Extraction and Purification of Lutein

Lutein was extracted from marigold flowers (*Calendula officinalis*) according to the procedure described by Lakshminarayana et al. [13] with slight modification. In brief, fresh marigold petals (10 g) were ground using a pestle and mortar along with sodium sulfate (5 g) and 0.1% BHT. Carotenoids were extracted in ice-cold acetone until the sample turned colorless (crude extract). The pooled extract (volume 250 ml) was dried over anhydrous sodium sulfate (20 g) and filtered through Whatman No.1 filter paper. The crude acetone extract was saponified with methanolic KOH (30%) at room temperature (27 °C) for 3 h in the dark to extract the lutein. After incubation, carotenoids were extracted three times with 50 mL of hexane and washed with deionized water. The hexane extract was evaporated to dryness by using a rotary evaporator (Buchi, Switzerland). The residue was re-dissolved in a known volume of hexane (2 mL) and applied on to an activated silica column (60–120 mesh) for purification of the lutein. β-carotene was eluted with hexane; the lutein and zeaxanthin fraction was eluted with methanol/dichloromethane (1:1, v/v) and the fractions rich in violaxanthin and neoxanthin were eluted with ethyl acetate/hexane (1:1, v/v) and ethyl acetate/hexane (1:9, v/v), respectively. The purity of the lutein was clarified and quantified by HPLC, based on the peak area of standard lutein at 445 nm. Purified lutein was blanketed with nitrogen and stored at −70 °C for further use. The HPLC conditions adapted to separate lutein are given elsewhere in this text.

In Vitro Digestion

To determine the role of vegetable oils on lutein micellization in vitro, purified lutein in hexane was evaporated to dryness under a stream of nitrogen and then solubilized in different vegetable oils ($n = 7$). The schematic representation of the experimental protocol describing the in vitro digestion of lutein is shown in Fig. 1. In brief, in a 15-mL screw cap test tube, 50 mg of olive, corn, soybean, sunflower, groundnut, rice bran or palm oil containing 600 nmol lutein was added. The samples were subjected to in vitro digestion simulating the gastric and small intestinal phase of digestion according to the method proposed by Garret et al. [14] with slight modification. In brief, 3 mL of 0.5% pepsin (porcine gastric mucosa 88–2,500 units/mg protein) in phosphate buffer (3.6 mmol/L $CaCl_2$, 1.4 mmol/L $MgCl_2.6H_2O$, 49 mmol/L NaCl, 12 mmol/L KCl, 6.4 mmol/L KH_2PO_4) was added to the lutein-oil mixture. The pH was adjusted to 2.02 with 2 mol/L HCl. The tubes were screw capped under a stream of nitrogen and incubated for 1 h at 37 °C in a shaking water bath (Scigenics Orbitek, India) at 120 strokes/min (gastric phase). On cooling, the pH was raised to 5.0 with 1 mol/L $NaHCO_3$ followed by the addition of 6 mL 0.1 mol/L $NaHCO_3$ containing 16 g/L pancreatin (porcine pancreas 8× U.S.P. specifications) and 25.38 g/L bile extract

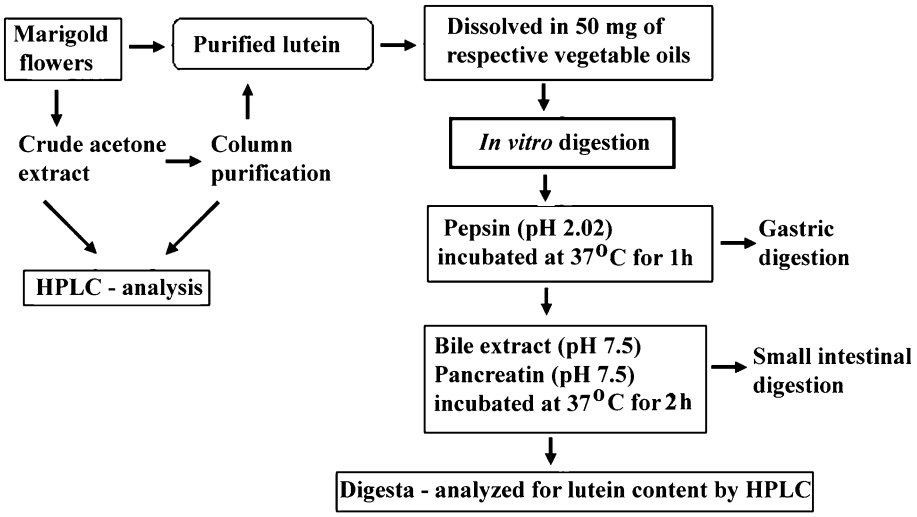

Fig. 1 Schematic representation of the methodology employed in the in vitro digestion process to estimate the micellization of lutein with different vegetable oils

(porcine). Then the pH of the digesta was further adjusted to 7.5 by 1 N NaOH. The test tubes were blanketed with a stream of nitrogen and subjected to incubation at 37 °C with shaking at 120 strokes/min for 2 h (intestinal phase). After incubation, an aliquot of digesta (1 mL) was withdrawn from each sample, centrifuged (Z 360 K, BHG Hermle, Gosheim) at 12,000×*g* at 4 °C for 120 min to separate the aqueous fraction that contain micelles and this fraction was used for quantification of micellized lutein by HPLC.

Extraction of Lutein from Digesta

Lutein from the digesta was extracted according to the procedure of Lakshminarayana et al. [12]. In brief, to the digesta (1 mL), 3 mL of dichloromethane/methanol (1:2, v/v) containing 0.1% BHT was added and vortexed. To the mixer, 1.5 mL hexane was added, mixed and centrifuged (Z 360 K, BHG Hermle, Gosheim) at 1,000×*g* for 5 min at 4 °C. The resulting upper hexane/dichloromethane layer was collected. The extraction procedure was repeated three times with 1 mL dichloromethane and 1.5 mL hexane. The extracts were pooled, evaporated to dryness using nitrogen gas, redissolved in a known volume of acetonitrile/methanol/dichloromethane (60:20:20, v/v/v) containing 0.1% ammonium acetate (mobile phase) and analyzed by HPLC.

HPLC Analysis of Lutein

Lutein extracted from the digesta (micellizable lutein) was quantified with an HPLC system (LC-10A; Shimadzu, Kyoto, Japan) equipped with a photodiode array detector (SPD-M20A, Shimadzu). Lutein was separated on a Princeton SPHER C-30 (ODS) column (250 mm × 4.6 mm; 5 μm) by isocratically eluting with the mobile phase of acetonitrile/methanol/dichloromethane (60:20:20, v/v/v) containing 0.1% ammonium acetate at 1 mL/min. Lutein was monitored at 445 nm (Shimadzu Class-VP version 6.14 SP1 software). The peak identity of lutein was confirmed by its characteristic spectrum and was quantified by comparing its peak area with an authentic lutein standard.

Analysis of Fatty Acids

The fatty acid profiles of vegetable oils used in this study were analyzed using boron trifluoride in methanol as described by Morrison and Smith [15] to obtain fatty acid methyl esters (FAME). A gas chromatograph (Shimadzu GC 2014; Shimadzu, Kyoto, Japan) fitted with a flame ionization detector (FID) was used to identify the individual fatty acids. The FAME dissolved in hexane were separated on a fused silica capillary column, 30 m × 0.32 mm × 0.25 μm (Omegawax™ 320; Supelco, Bellefonte, USA) with a split ratio of (1:30). The column, injector and detector temperatures were 200, 250 and 260 °C respectively; with nitrogen as the carrier gas at 1 mL/min. The fatty acids were identified by comparing them with authentic standards. The data presented are means of duplicate analyses.

Statistical Analysis

Results obtained on the percentage of micellization and the fatty acid profile of the vegetable oils are from duplicate analyses and expressed as means and standard deviations of the means. Further, the percentage difference in micellizable lutein was calculated in comparison with groundnut oil since it is commonly used for cooking.

Results and Discussion

In this study, simulated gastric and small intestinal digestion in vitro was used to determine the influence of vegetable oils on the micellization of lutein that is available for absorption by the intestinal mucosa. Micellization of nutrients with fat is an essential step in the process of food digestion before cellular uptake [9]. Lutein extracted from the digesta and spectrum of lutein and zeaxanthin with λ_{max} are shown in Fig. 2, while the percentage micellization of lutein, after the intestinal phase of digestion with different vegetable oils is shown in Fig. 3. The influence of vegetable oils on the percentage of the micellization of lutein was in the order of OO > GNO > RBO > SFO > CO > SBO > PO. In comparison, the value for micellizable lutein in OO was not significantly higher than GNO (11%), RBO (18.3%) and SFO (19%), but was significantly higher than CO (21.7%), SBO (30.5%) and PO (35.2%), respectively. Furthermore, the micellizable lutein was almost similar in RBO (74.8%), SFO (74.2%) and CO (71.7%) samples unlike PO (59.4%).

Among the processes involved before absorption of the carotenoids, the formation of mixed micelles is dependent on the nature and amount of fat present in the intestine. Dietary fat induces the secretion of bile and its hydrolysates such as monoacyl glycerol and free fatty acids are also included in the mixed micelles. Bile salts are formed in the liver and secreted by the gall bladder and allow micelles of fatty acids to assimilate carotenoids that may affect their bioavailability, which involves the incorporation of released carotenoids into mixed micelles [16]. The formation of micelles is an essential precondition implicating the importance of dietary fat intake for the absorption of carotenoids [17]. Therefore, ingestion of fat along with carotenoids is thought to be crucial [8].

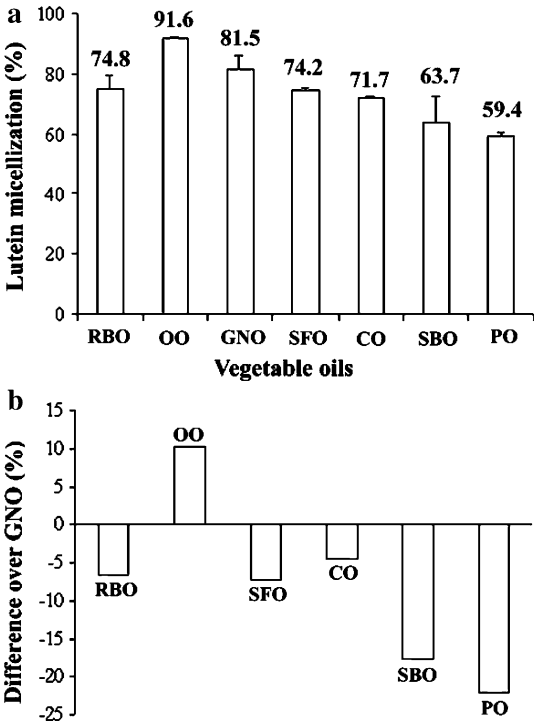

Fig. 3 Percentage lutein micellization after in vitro digestion process with different vegetable oils (**a**) and percent difference in micellizable lutein in comparison with groundnut oil (**b**). Values are means ± SD of duplicate analyses

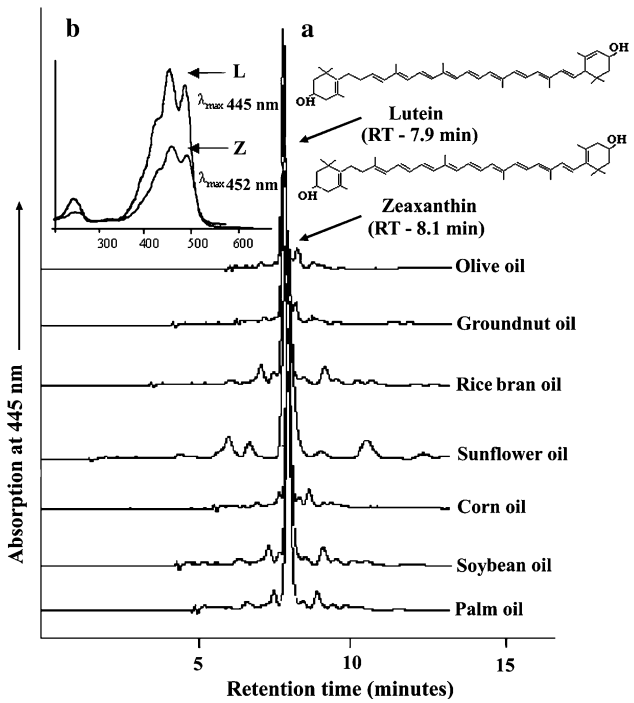

Fig. 2 HPLC profile of lutein and zeaxanthin along with their chemical structure, extracted from the digesta after in vitro digestion with different vegetable oils (**a**) and spectrum of lutein and zeaxanthin with their λ_{max} (**b**)

In this study, we used equal amounts of fat (vegetable oils) having different fatty acid profiles and exhibiting varying effects on the percentage micellization of lutein. The extent of carotenoid solubilization into the micelles may be affected by the micellar fatty acid composition and the extent of saturation [18]. Results indicate that the extent of lutein micellization in oleic acid rich olive oil was higher than the other vegetable oils. This is in agreement with our previous study [12], which reported that micellar oleic acid and dietary olive oil enhanced plasma and liver levels of lutein in rats, suggesting that oleic acid may influence the intestinal accessibility of lutein. Hollander and Ruble [19] also reported that oleic acid in micellar perfusates significantly enhanced the β-carotene absorption compared to linoleic acid, indicating that the rate of transport depends upon the hydrophobicity of the fatty acid.

Table 1 Fatty acid profile of vegetable oils used for in vitro digestion of lutein

Fatty acid (%)	Rice bran oil	Olive oil	Groundnut oil	Sunflower oil	Corn oil	Soybean oil	Palm oil
16:0	18.19 ± 0.269	12.88 ± 0.035	13.62 ± 0.297	6.75 ± 0.622	14.25 ± 0.115	11.49 ± 1.202	41.41 ± 1.280
16:1	0.347 ± 0.002	0.652 ± 0.033	0.108 ± 0.001	ND	ND	ND	0.191 ± 0.001
18:0	1.75 ± 0.156	3.51 ± 0.148	3.40 ± 0.021	3.42 ± 0.092	1.85 ± 0.071	3.00 ± 0.297	3.62 ± 0.007
18:1	42.14 ± 0.537	73.32 ± 0.354	41.51 ± 0.170	23.08 ± 0.728	29.45 ± 0.092	23.84 ± 0.404	42.06 ± 1.181
18:2(n-6)	34.08 ± 1.860	8.56 ± 0.092	36.76 ± 0.580	65.85 ± 0.141	53.47 ± 0.460	53.95 ± 0.849	11.03 ± 0.325
18:3(n-3)	0.30 ± 0.021	0.52 ± 0.064	ND	ND	0.69 ± 0.043	5.53 ± 0.028	0.17 ± 0.004
20:0	0.54 ± 0.092	0.34 ± 0.046	1.16 ± 0.014	ND	0.35 ± 0.004	0.23 ± 0.001	0.24 ± 0.014
22:0	ND	ND	1.72 ± 0.034	ND	ND	ND	ND

Values are means ± SD

ND Not detected

The fatty acid compositions of the vegetable oils used in this study are given in Table 1. The predominant fatty acid in OO is oleic acid (73.3%), whereas linoleic acid is most common in SFO (65.8%), SBO (53.9%) and CO (53.5%). Both GNO and RBO have both oleic (41.5 and 42.1%, respectively) and linoleic acid (36.8 and 34.1%, respectively) in almost the same proportion (Table 1). Vegetable oils used in this study were chosen mainly because of their common use in India and other parts of the world. Furthermore, the differential fatty acid composition of these oils allows for the determination of the effect of vegetable oil, with varying fatty acid profile, in lutein digestion and micellization before it is available for intestinal absorption. The difference in micellarated lutein found in this study may be attributed to the varying fatty acid profile of vegetable oils. Ahuja et al. [20] and Clark et al. [11] reported that the rate of lycopene and astaxanthin absorption in humans and rats was higher in an OO enriched diet as compared to CO and SFO diets. They also affirmed that the rate of carotenoid bioavailability depends upon the hydrophobicity of fatty acids in which they are mixed. There may be several events where dietary vegetable oil could influence carotenoid absorption. Lipids high in PUFA might increase carotenoid oxidation in the chyme resulting in less carotenoid available for absorption [11]. It has been speculated that micelles containing PUFA are larger in size, thus reduce the diffusion of micelles through the unstirred water layer adjacent to the enterocyte, and hence decrease the rate of β-carotene absorption when compared to micelles containing oleic acid [19]. Results suggest that OO influence the mixed micelle formation in the intestine and thereby may enhance the uptake of lutein. These vegetable oils differ in the degree of unsaturation (Table 1). Because of differences in their properties, vegetable oils may act differently at all stages of digestion, gastric and intestinal micellization, the permeation process through enterocytes, metabolism and absorption. These specific properties of fatty acids could make it possible to modify the bioavailability of hydrophobic compounds such as lutein.

Conclusion

Lutein dispersed in OO and GNO increased the micellization of lutein which in turn may enhance the rate of intestinal absorption. Hence, OO and GNO can be suggested as the best dietary lipids to increase macular pigment densities in patients having AMD. Further, to achieve a higher level of lutein absorption, selection of vegetable oil rich in oleic acid is vital.

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