Impact of Composition and Quantity of Triglycerides on Micellarization of Dietary Carotenoids during Simulated Digestion

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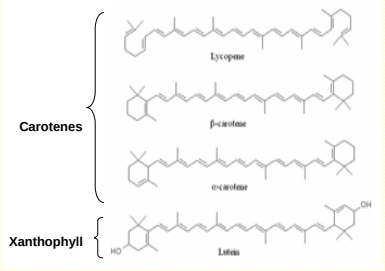
Abstract

A carotenoid-rich salad purée with varying amounts of either a structured lipid or dietary oil was digested using simulated gastric and small intestinal conditions. Lutein and carotenes (α-carotene, β-carotene and lycopene) in chyme and micelle fraction were quantified to determine digestive stability and efficiency of micellarization ("bioaccessibility"). Relative micellarization was as follows: lutein > α- and β-carotene > lycopene. Micellarization of carotenes, but not lutein, was enhanced (P<0.05) by addition of lipid (2.5% v/w) to purée and dependent on fatty acid chain length in structured TG (c18:1 > c8:0 > c4:0). Micellarization efficiency for each carotenoid was similar when equivalent amounts of tri-oleate (c18:1), tri-linoleate (c18:2), and tri-linolenate (c18:3) were added to purée. Relatively low amounts of tri-oleate and canola oil (0.5-1.0%) were required for maximum micellarization of carotenes, but more oil (~2.5%) was required when TG with medium chain saturated fatty acids (e.g., tri-octanoate and coconut oil) was added to salad. The results suggest transfer of carotenoids from chyme to mixed micelles during digestion is inversely correlated with hydrophobicity of the pigment, generally requires minimum (0.5-1%) lipid in the purée, and is influenced by chain length, but not degree of saturation, of dietary fatty acids in TG. (Supported in part by OARDC Graduate Student Scholarship to TH)

Introduction

Carotenoids are lipophilic plant pigments with various biological properties that include pro-vitamin A activity, antioxidant activity, photoprotection of eye and skin, and vitamin A independent regulation of cell signaling and gene transcription. In order to deliver carotenoids and their metabolites to target tissues to modulate such activities, these compounds must be a) released from the food matrix and incorporated into micelles, b) taken up by enterocytes and c) incorporated in chylomicrons and secreted into lymph for distribution to target tissues . [1]

The absorption of carotenoids is affected by numerous post-harvesting, physicochemical, dietary, physiological and pathological factors. [2] Dietary lipid is recognized as a potent promoter of carotenoid bioavailability. [3-5] This is likely associated with the ability of dietary fat to a) provide a "sink" for transfer of carotenoids from food matrix to oil droplets, b) stimulate secretion of bile and pancreatic enzymes, and c) promote the synthesis and secretion of chylomicrons. The effects of quantity and composition of dietary lipids on processes required for the absorption of carotenoids have not been systematically investigated. The goal of this research is to clarify the influences of amount and type of dietary triglycerides (TG) on the following processes: micellarization; uptake of micellarized carotenoids by enterocytes and carotenoid secretion across the basolateral membrane of enterocytes. This study investigated the influence of composition and quantity of dietary TG on micellarization of carotenoids using simulated gastric and small intestinal digestion.



Abbreviations used: TG, triglyceride; LUT, lutein; BC, β-carotene; AC, α-carotene; LYC, lycopene.

Hypothesis

Efficiency of micellarization of carotenoids during digestion will be dependent on chain length and degree of saturation of fatty acids in TG, as well as amount of the dietary TG.

Materials and Methods

1. Materials

1.1 Test Salad purée

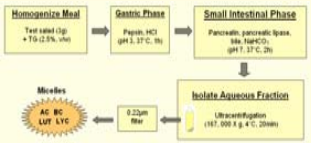
Salad purée was prepared by homogenizing carotenoid rich vegetables and fruits (spinach, tomato, carrot, romaine lettuce and orange pepper) and stored in -80°C. The frozen salad purée contains LUT (1.95mg/100g) AC (1.17mg/100g), BC (3.83mg/100g) and LYC (3.72mg/100g). Carotenoids were stable in the frozen puree for at least 6 months. Lipid extracts of salad puree were saponified to determine fatty acid composition by Gas Chromatography. Fatty acids accounted 0.1% of the wet weight and the majority of the esterified fatty acids in the purée were c18:2 (29%) and c18:3 (37%). Indicated quantities of test triglycerides were added to 3.0g salad purée immediately before initiating simulated digestion.

1.2 Test Triglycerides

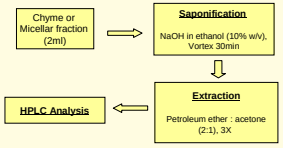
Structured lipids includes tributyrate (c4:0), trioctanoate (c8:0), trioleate (c18:1), trilinoleate (c18:2), trilinolenate (c18:3) and *cis* and *trans* isomers of conjugated linoleate. Safflower oil (rich in long chain polyunsaturated fatty acids), canola oil (long chain monounsaturated) and coconut oil (medium chain saturated) were also tested.

2. Methods

2.1 Simulated gastric and small intestinal digestion [6,7]



2.2 Carotenoid extraction and analysis [6,7]



$$\text{Digestive stability (\%)} = \frac{\text{carotenoids in the digesta}}{\text{carotenoids in salad purée}} \times 100\%$$

$$\text{Efficiency of micellarization (\%)} = \frac{\text{carotenoids in the micellar fraction}}{\text{carotenoids in salad purée}} \times 100\%$$

2.3 Statistical analysis

Statistical analysis was performed using SPSS/Win 14.0. The efficiency of micellarization was calculated for each carotenoid in each purée sample. Values are expressed as means ± SD. Significant differences for effects of amount and type of oil were tested by one-way ANOVA followed by Dunnett's post hoc test. Three or six observation was made to determine whether there are significant differences between groups (depending on the experiment). The differences are considered significant at $P < 0.05$.

Results

Experiment 1: Chain length, but not degree of saturation of fatty acids in TG influences micellarization of carotenoids from salad purée.

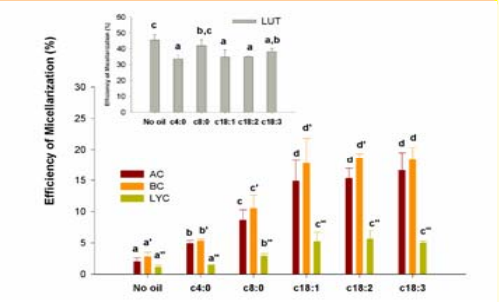


Figure 1 Effect of structured lipids on efficiency of micellarization of carotenoids from salad purée. The salad purée (3g) was digested *in vitro* with 2.5% (v/w) of structured lipids which contain fatty acids with different chain length and degree of saturation. (See Materials and Methods) Each structured lipid has identical fatty acids in all three sn-positions of the glycerol backbone. Data was expressed as the mean ± SD from two independent experiments with three replicates per experiment. (n = 6). It is considered significantly different between groups when $p<0.05$.

Experiment 2: Dietary oils enhanced micellarization of carotenes from salad purée.

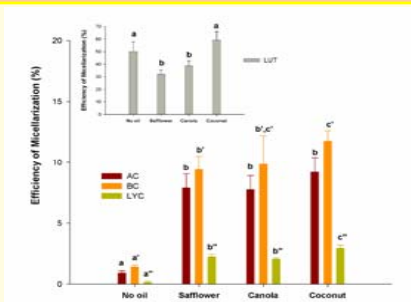


Figure 2 Effect of dietary oils on efficiency of micellarization of carotenoids from salad purée. The salad purée (3g) was digested with 2.5% (v/w) of either safflower, canola or coconut oil, which contain fatty acids with different chain length and saturation. The compositions of the dietary oils were shown in Table 1. Data was expressed as the mean ± SD from two independent experiments with three replicates per experiment. (n = 6). It is considered significantly different between groups when $p < 0.05$.

Experiment 3: Maximum micellarization of carotenoids requires relatively low amounts of TG and the amount of required TG is dependent on the type of TG.

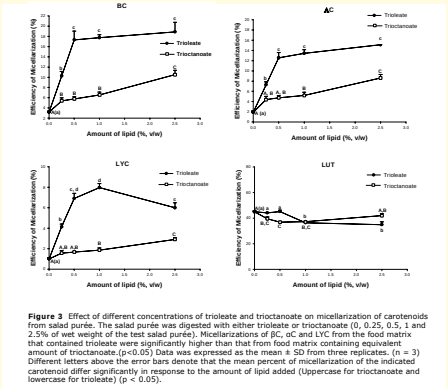


Figure 3 Effect of different concentrations of trioleate and trioctanoate on micellarization of carotenoids from salad purée. The salad purée was digested with either trioleate or trioctanoate (0, 0.25, 0.5, 1 and 2.5% of wet weight of the test salad purée). Micellarizations of βC, αC and LYC from the food matrix that contained trioleate were significantly higher than that from food matrix containing equivalent amount of trioctanoate.($p<0.05$) Data was expressed as the mean ± SD from three replicates. (n = 3) Different letters above the error bars denote that the mean percent of micellarization of the indicated carotenoid differ significantly in response to the amount of lipid added (Uppercase for trioctanoate and lowercase for trioleate) ($p < 0.05$).

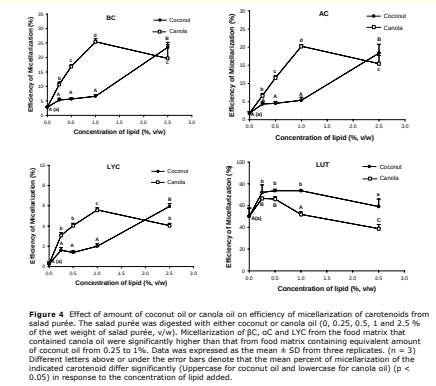


Figure 4 Effect of amount of coconut oil or canola oil on efficiency of micellarization of carotenoids from salad purée. The salad purée was digested with either coconut or canola oil (0, 0.25, 0.5, 1 and 2.5 % of the wet weight of salad purée, v/w). Micellarization of βC, αC and LYC from the food matrix that contained canola oil were significantly higher than that from food matrix containing equivalent amount of coconut oil from 0.25 to 1%. Data was expressed as the mean ± SD from three replicates. (n = 3) Different letters above or under the error bars denote that the mean percent of micellarization of the indicated carotenoid differ significantly (Uppercase for coconut oil and lowercase for canola oil) ($p < 0.05$) in response to the concentration of lipid added.

Summary

- **Partitioning of carotenoids in aqueous fraction (i.e., micellarization) during *in vitro* digestion of salad purée was enhanced by addition of TG in salad purée.**

- **Efficiency of micellarization of carotenoids from the salad is influenced by carotenoid structure : lutein > α-carotene, β-carotene > lycopene.**

- **Micellarization of carotenes is dependent on chain length, but not the number and position (data not shown) of double bonds, of TG fatty acids.**

- **Relatively low amounts (approx. 0.5-1.0%, v/w) of trioleate and canola oil are required for maximum micellarization of carotenes, but more oil is required (approx. 0.5%, v/w) if TG contains medium chain saturated fatty acids, e.g., trioctanoate and coconut oil.**

References

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Acknowledgement

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