Micellarization of *β*-carotene during *in vitro* digestion of maize and uptake by Caco-2 intestinal cells is minimally affected by xanthophylls

Sagar K. Thakkar[1] and Mark L. Failla[1, 2]

[1]Interdisciplinary PhD Program in Nutrition, The Ohio State University and [2]Department of Human Nutrition, The Ohio State University

INTRODUCTION

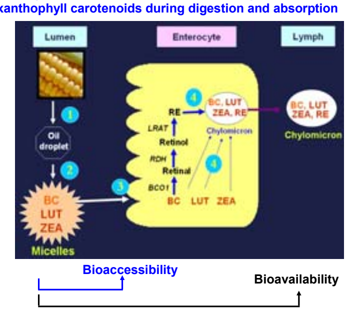
Vitamin A Deficiency

- **Most commonly occurs in developing countries, especially in Sub-Saharan Africa and South East Asia.**
- **Primarily affects children and women of child bearing age.**
- **Global impact of Vitamin A Deficiency.**
  - **250 million pre-school-age children are sub-clinically vitamin A deficient.**
  - **An estimated 250,000 - 500,000 become blind every year.**
  - **50% die within 12 month of loosing their sight.**
  - **It also is estimated that nearly 20 million pregnant women are diagnosed with sub-clinical deficiency of vitamin A each year.**

Strategies to Combat Vitamin A Deficiency

- **Supplementation, food fortification and dietary diversification have been used for last three decades to combat vitamin A deficiency, but lack sustainability particularly for the impoverished population in rural areas.**
- **Recently, biofortification, has been suggested as a complimentary approach to traditional interventions as a cost effective and sustainable source of micronutrients.**
- **Biofortification of a crop involves development of "nutrient dense", high yielding cultivars.**
- **This is achieved either by development of transgenic species expressing key genes required for the biosynthesis or transport of the compounds/ions of interest or by selectively breeding cultivars with high levels of micronutrients with varieties with agroeconomically important characteristics.**
- **Increasing the pro-vitamin A content in maize has the potential to also increase the concentration of xanthophylls which are oxidative products of carotenes.**
- **Increased concentrations of xanthophylls in maize cultivars may adversely affect the bioavailability of pro-vitamin A carotenoids.**

Potential processes for interactions between pro-vitamin A and xanthophyll carotenoids during digestion and absorption



SPECIFIC AIM

To evaluate potential pre-absorptive interactions between pro-vitamin A carotenoids (i.e., *β*-carotene and *β*-cryptoxanthin) and xanthophylls (i.e., lutein and zeaxanthin) in maize using simulated digestion and uptake by Caco-2 intestinal cell models

ABSTRACT

Currently consumed varieties of maize contain limited pro-vitamin A (VA) carotenoids despite relatively high levels of lutein (LUT) and zeaxanthin (ZEA). Here we determined the relative bioaccessibility of pro-VA carotenoids and their interactions with xanthophylls. First, we examined four accessions of maize containing 0.4 – 11.3 µg/g *β*-carotene (BC) + *β*-cryptoxanthin (BCX) with ratios of xanthophylls to pro-VA carotenoids ranging from 1.9 – 7.0. Recovery of carotenoids in cooked maize exceeded 80% after simulated digestion. Mean efficiencies of micellarization of BC, BCX, LUT and ZEA were 16.7, 27.7, 30.3 and 27.9%, respectively, and were independent of the ratio of xanthophylls to pro-VA carotenoids. We also digested white maize to which 3.3 µg/g BC in extra light olive oil (2% v/w) and increasing amounts of LUT (0 – 33.3 µg/g) were added. Efficiency of micellarization of BC was 21.7% in the absence of LUT and increased to 30.2% ($P < 0.05$) when LUT content was 7x that of BC. Incorporation of BC into synthetic micelles also increased ($P < 0.05$) when LUT to BC ratio was ≥10. Caco-2 cells accumulated 270, 240 and 180 pmol BC/mg cell protein ($P < 0.05$) in absence of LUT and at LUT to BC ratios of 7 and 13, respectively. These results suggest that the potential bioavailability of pro-VA carotenoids in maize does not appear to be markedly affected by the relative levels of xanthophylls found in cultivars of maize. Supported in part by HarvestPlus & OARDC.

MATERIALS & METHODS

Chemicals and supplies – Unless otherwise stated, all chemicals and supplies were purchased from Sigma-Aldrich and Fisher Scientific.

Maize varieties – Maize samples were provided by Dr. Torbert Rocheford (U. Illinois, Urbana-Champaign) and flour from four maize cultivars were prepared and forwarded by Dr. Sherry Tanumihardjo (U. Wisconsin, Madison). White maize meal was purchased from local supermarket.

Preparation of carotenoid rich oil – Known concentrations of BC and/or LUT (gift from Dr. Zoraida DeFreitas, Kemin Foods) and 2.3 mg phosphatidylcholine were added to 11 mL glass vial followed by 1 mL of extra light olive oil. Organic solvent was evaporated under a stream of nitrogen gas at 25 ºC.

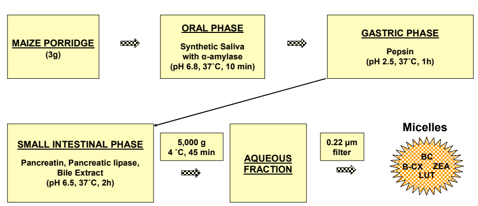
Preparation of maize porridge – Maize porridge was prepared in manner similar to that for consumption by subjects in human trial directed by Dr. Wendy S. White, Iowa State University (personal communication). Briefly, maize flour (20 g) was mixed with DI water (65 mL) and heated in a Teflon-coated pan at 95 °C for 12 min. The porridge was allowed to cool for 10 min at room temperature before storing in 50 mL polypropylene screw-cap tubes under nitrogen gas at -80 °C until analysis.

Extraction and analysis of carotenoids from maize flour and porridge – The extraction procedure was adapted from Howe and Tanumihardjo (1). Briefly, carotenoids were released from dried maize (0.6 g) by adding 6 mL of ethanol, mixing by vortex (30 sec), and placing in an 85 °C water bath for 5 min. Potassium hydroxide (500 µL, 80% w/v in water) was added to the heated mixture to saponify oil. Samples were then vortexed for 30 sec and returned to the 85 °C water bath for 10 min with additional vortexing after 5 min. After saponification, samples were next placed in ice bath and 3 mL ice cold DI water was added to the samples for rapid cooling. Internal standard (2 µg *β*-apo-8'carotenal in hexane) was added and carotenoids were extracted three times with 3 mL hexane. The combined hexane fraction was then washed three times with 3 mL of ice cold DI water. The washed organic layer was transferred to a new vial and the residual water was extracted twice with 3 mL of hexane. Hexane from the combined extraction was then evaporated under nitrogen gas and the film was reconstituted in 1 mL of mobile phase for HPLC analysis.

Extraction of carotenoids from digesta, micelle fraction, synthetic micelles and Caco-2 cells – Carotenoids from digesta, micelle fraction, synthetic micelles and Caco-2 cells were extracted as described by Thakkar et al. (5).

HPLC analysis – HPLC analysis of carotenoids in extracts from maize, porridge, digesta, micelle fraction, synthetic micelles and Caco-2 cells was performed as reported in Thakkar et al. (5).

Simulated digestion of porridge – Protocol for simulated digestion was slightly modified from Garrett et al. (2) and also included the oral phase of digestion described by Oomen et al. (3).



Preparation of synthetic micelles – Protocol of Chitchumroonchokchai et al. (4) was used to prepare synthetic mixed micelles.

Uptake of micellar carotenoids by Caco-2 cells – Cultures of Caco-2 small intestinal cells (passages 23-25, 12-14 days post confluency) were incubated with synthetic micelles containing 2.0 µM BC and 0 – 27 µM LUT. Cells were harvested and carotenoids were extracted and analyzed by HPLC according to Thakkar et al. (5).

Statistical Analysis – A minimum of six independent observations were made for *in vitro* digestion of maize porridge and preparation of synthetic micelles (n≥6). At least five (n≥6) independent observations were made for uptake of carotenoids from synthetic micelles by Caco-2 cells. All data are expressed as means ± SEM. Statistical analysis was performed using SPSS Release 14.0 for Windows (SPSS Inc., Chicago, IL). Means were compared using one way analysis of variance (ANOVA) followed by Fisher's protected LSD for pair wise comparison. The differences are considered significant at $P < 0.05$.

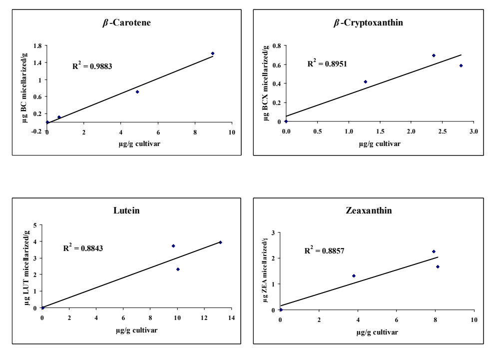
RESULTS

Concentration of carotenoids in maize flours*

Cultivar	LUT	ZEA	BCX	BC	Ratio of Xanthophylls to pro-VA carotenoids
	µg/g maize flour				
White	0.06 ± 0.005	0.05 ± 0.001	ND**	0.04 ± 0.001	**2.8**
Yellow	9.72 ± 0.33	3.81 ± 0.12	1.27 ± 0.09	0.67 ± 0.08	**7.0**
Light Orange	10.06 ± 0.29	8.12 ± 0.21	2.80 ± 0.07	4.90 ± 0.09	**2.4**
Dark Orange	13.18 ± 0.23	7.91 ± 0.51	2.36 ± 0.05	8.97 ± 0.12	**1.9**

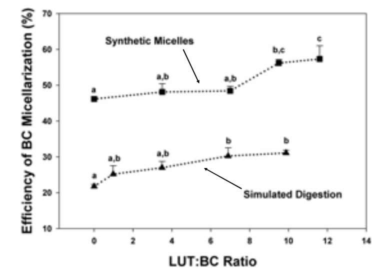
* Data are means ± SEM (n = 6). ** ND = Not detected

Micellarization of pro-vitamin A carotenoids during simulated digestion is highly correlated with their amounts in the cultivars and independent of amount of xanthophylls present in maize flour*



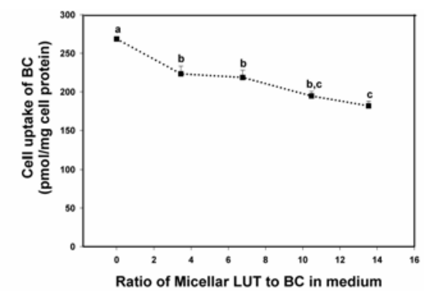
* Data are means for n = 6 replicate samples.

Efficiency of micellarization of BC increases during simulated digestion and during preparation of synthetic micelles when relative concentrations of LUT are high*



* Data are means ± SEM (n = 6).

Accumulation of BC by Caco-2 cells decreases with increasing micellar LUT*



* Data are means ± SEM (n = 6).

SUMMARY

- **Micellarization of pro-vitamin A carotenoids during digestion of cultivars of maize was proportional to their content in maize flour and independent of xanthophyll content.**
- **Incorporation of BC into micelles slightly, but significantly ($P < 0.05$), increases at LUT:BC ratios ≥ 7 during simulated digestion of maize meal containing carotenoid enriched oil and at ratios ≥ 10 during preparation of synthetic micelles.**
- **Increased efficiency of micellarization of BC in presence of high LUT is offset by decreased uptake of micellar BC by Caco-2 cells exposed to elevated concentrations of micellar LUT.**

CONCLUSION

Xanthophylls have minimal impact on bioaccessibility of pro-vitamin A carotenoids at ratios likely to be present in biofortified maize.

ACKNOWLEDGEMENTS

This study was supported by funding from HarvestPlus and Ohio Agricultural Research and Development Center (OARDC).

REFERENCES

1. **Howe JA, Tanumihardjo SA. (2006) Evaluation of analytical methods for carotenoid extraction from biofortified maize (Zea mays sp.). J Agric Food Chem. 54:7992-7.**
2. **Garrett, DA, Failla, ML, Sarama, RJ. (1999) Development of an in vitro digestion method to assess carotenoid bioavailability from meals. J. Agric. Food Chem. 47:4301-9.**
3. **Oomen AG, Rompelberg CJ, Bruil MA, Dobbe CJ, Pereboom DP, Sips AJ. (2003) Development of an in vitro digestion model for estimating the bioaccessibility of soil contaminants. Arch. Environ. Contam. Toxicol. 44:281-7.**
4. **Chitchumroonchokchai C, Schwartz SJ, Failla ML. (2004) Assessment of lutein bioavailability from meals and a supplement using simulated digestion and caco-2 human intestinal cells. J Nutr. 134:2280-6.**
5. **Thakkar SK, Maziya-Dixon B, Dixon AG, Failla ML. (2007) Beta-carotene micellarization during in vitro digestion and uptake by Caco-2 cells is directly proportional to beta-carotene content in different genotypes of cassava. J Nutr. 137:2229-33.**