AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2018 – 2020

TITLE: Utilization of crustacean shells as a source of natural astaxanthin with anti-obesity effect

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COOPERATORS:

EXECUTIVE SUMMARY:
Astaxanthin is a type of natural carotenoids and a powerful antioxidant found in marine organisms including microalgae, salmon, trout, and crustaceans such as shrimp, krill, lobsters, crabs, providing their recognizable red color. In crustaceans, astaxanthin is predominantly retained in their shells which are currently underutilized by-product and hence can be a valuable source for extracting natural astaxanthin. While astaxanthin has been extensively studied for its strong antioxidant activity and is currently being sold as a dietary supplement with over $200 million annual turnover, over 95% of astaxanthin products on the market contains synthetically produced astaxanthin. Synthetic astaxanthin retains chemical stereoisomer that is different from its natural counterpart. While natural astaxanthin mainly occurs as (3S, 3'S) stereoisomer based on the spatial bonding pattern of the atoms, synthesis of astaxanthin results into mixture of stereoisomers with 25% (3R 3'R), 50% (3R 3'S), and 25% (3S 3'S). Recently, different levels of bioactivities between natural and synthetic form of astaxanthin has been questioned and under a debate. With its well-established antioxidant effect, recent studies suggested that natural astaxanthin has significantly higher antioxidant capacity compared to the synthetic compound, one report indicating natural astaxanthin to be 20-30 times more effective than the synthetic.
The effect of astaxanthin on obesity-associated metabolic complications is a newly emerging research area. As obesity and related metabolic diseases have escalated to a global health crisis, research and development of strategies to combat obesity are in urgent demand. While its powerful antioxidant activity has brought great interest toward this marine compound, potential function of astaxanthin in obesity regulation is starting to uncover. Given the current wide availability of synthetic astaxanthin in the market, it is necessary to carefully examine the potential difference in efficacy or functional pattern of natural vs. synthetic astaxanthin in obesity regulation and chronic inflammation which is a perpetuating problem in obesity. Therefore, it is of interest to determine and compare the anti-obesity activity of natural and synthetic astaxanthin to assess the value of natural astaxanthin and subsequently, its source of extraction.

OBJECTIVES:
The main objective of this project is to determine the efficacy of natural astaxanthin in comparison to synthetic astaxanthin as an anti-inflammatory and/or anti-obesity compound in an effort to demonstrate the value of marine resources such as crustacean shell as a source of

natural astaxanthin and promote their utilization. The specific objectives of the project are as follows:

1. To confirm the differential anti-oxidant effects of natural vs. synthetic astaxanthin that has been previously reported.
2. To compare and determine anti-inflammatory effect of natural vs. synthetic astaxanthin.
3. To investigate the effect of natural vs. synthetic astaxanthin on lipid accumulation in adipocytes to determine associated molecular mechanisms in obesity control by astaxanthin.

PROCEDURES:

1. Antioxidant effect of natural vs. synthetic astaxanthin was determined by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity.
2. Inflammation in obesity is closely linked to the development of associated metabolic diseases. The anti-inflammatory effect of natural vs. synthetic astaxanthin was examined and compared by analyzing nitrite production level in RAW 264.7 macrophages stimulated by lipopolysaccharide (LPS) to induce inflammatory response.
3. The effect of natural astaxanthin on lipid accumulation in adipocytes was investigated to determine associated molecular mechanisms in obesity control by astaxanthin.

SIGNIFICANT ACCOMPLISHMENTS:

To verify the efficacy of natural vs. synthetic astaxanthin, the previously reported antioxidant effects has been analyzed by employing ABTS radical scavenging assay. Our result confirmed that natural astaxanthin exhibits significantly stronger antioxidant effect than its synthetic counterpart at concentrations 0.5 mg/ml and higher (Figure 1).

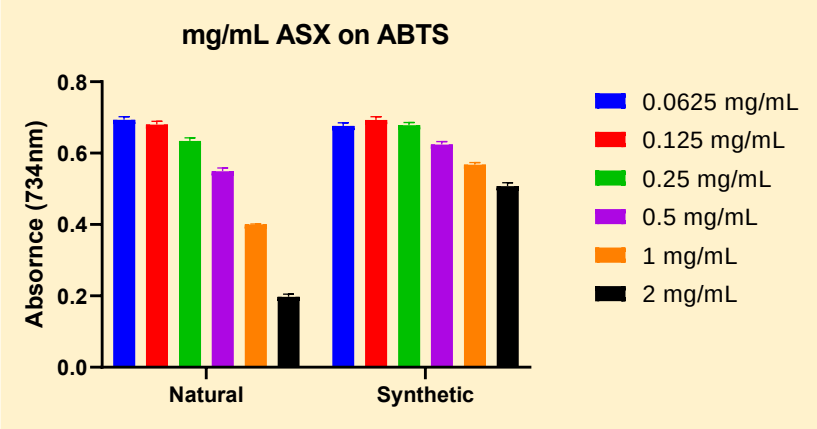

Figure 1. The ABTS radical scavenging effect of natural vs. synthetic astaxanthin (ASX)

To optimize the experimental model set up and procedures utilizing RAW 264.7 macrophages, various conditions of natural and synthetic astaxanthin treatment including pre-treatment, co-treatment, post-stimulation, time-dependent, and dose-dependent comparison have been tested. RAW 264.7 macrophage was stimulated with LPS to induce inflammatory response. Nitrite production in LPS-induced RAW 264.7 which are marker of inflammation has been investigated. While both natural and synthetic astaxanthin exhibited anti-inflammatory

response under pre-treatment and post-stimulation condition evidenced by decreased nitrite level, no significant difference between the effect of natural and synthetic astaxanthin has been observed (Figure 2). The cell viability determined by MTT assay confirmed that the observed anti-inflammatory effect of astaxanthin is not derived from cytotoxic effect in the cells (Figure 3).

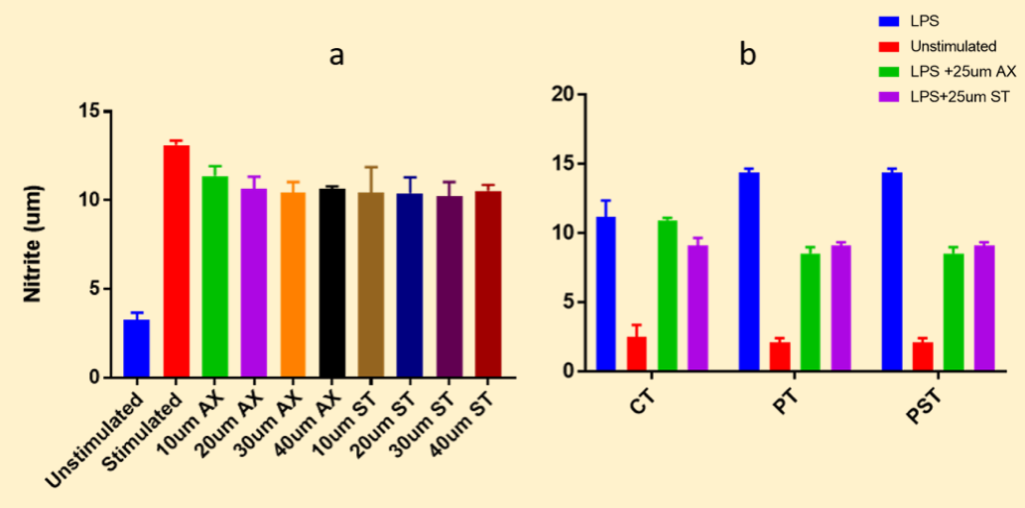

Figure 2. The effect of the modes of natural (AX) and synthetic (ST) astaxanthin treatment co-treatment (CT), pre-treatment (PT) and post-stimulation (PST) on the nitrite production of lipopolysaccharide-stimulated macrophages

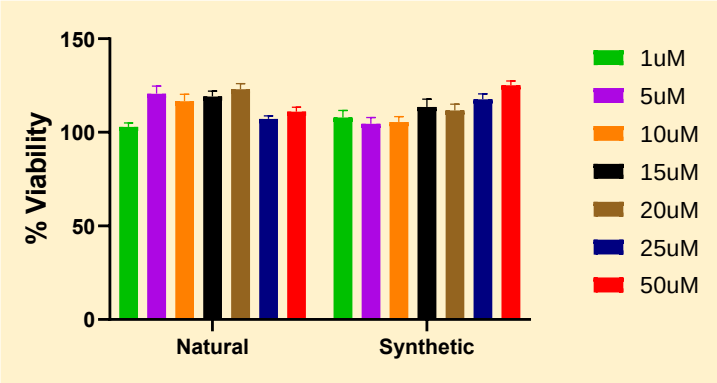

Figure 3. Effect of natural or synthetic astaxanthin treatment on the viability of RAW 264.7 macrophages.

To evaluate the effect of natural and synthetic astaxanthin on adipocyte differentiation and lipid accumulation, 3T3-L1 preadipocytes were differentiated into adipocytes in hypertrophic condition. Once fully differentiated, the mature adipocytes accumulate lipid droplet in the cells which can be observed morphologically (Figure 4) as well as quantitatively by employing lipid-binding dye Oil Red O. The level of triglyceride level in 3T3-L1 adipocytes has been investigated as a marker of differentiation. In this trial, both natural and synthetic astaxanthin did not suppress the level of lipid accumulation in the adipocytes, indicating the differentiation was not

effectively prevented (Figure 5). The cell viability determined by MTT assay confirmed that astaxanthin did not exhibit cytotoxic effect in the adipocyte cells (Figure 6).

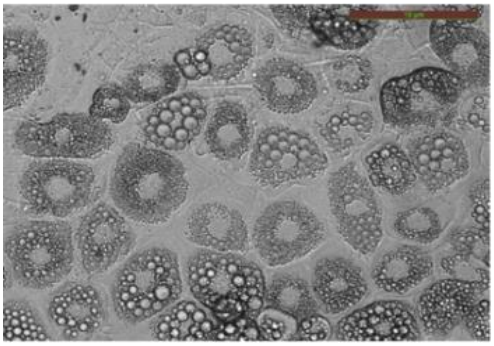

Figure 4. The morphology of differentiated 3T3-L1 adipocytes.

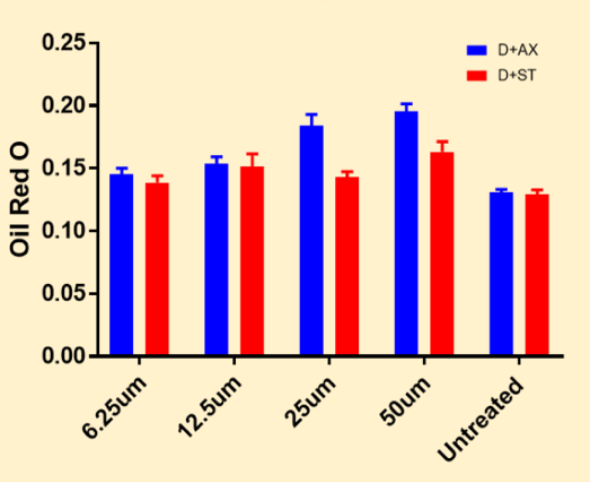

Figure 5. Effect of natural (AX) or synthetic (ST) astaxanthin treatment on lipid accumulation of differentiated 3T3L1 adipocytes.

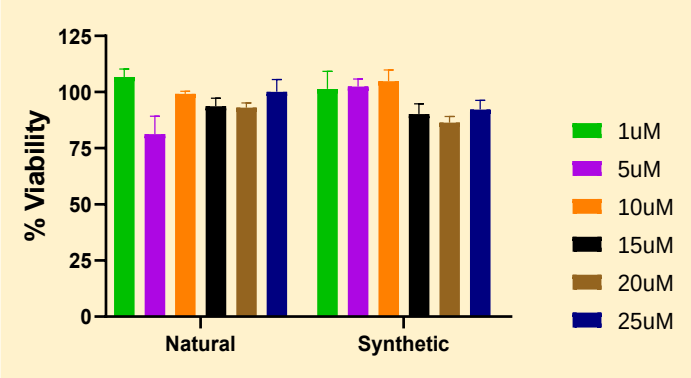

Figure 6. Effect of natural or synthetic astaxanthin treatment on the viability of 3T3-L1 adipocytes.

The results suggest that natural astaxanthin may possess stronger antioxidant effect compared to natural astaxanthin that may be due to the stereoisomeric difference. Anti-inflammatory

effect of natural and synthetic astaxanthin was comparable in the current investigation indicating the mechanism of action of astaxanthin in the inflammation inhibitory effect is not influenced by the spatial bonding patterns of different stereoisomers. Overall, our data support that natural astaxanthin is an effective anti-oxidant and anti-inflammatory agent with health beneficial implications. A future study is warranted to further determine the anti-oxidant effect of natural astaxanthin in biological model systems and to identify the regulatory mode of action of natural astaxanthin in inflammatory pathway to develop relevant biomedical applications.

BENEFITS & IMPACT:
Our study supports the evidences on the bioactivity of the natural marine carotenoid astaxanthin. Currently underused marine resources such as crustacean shells are an ideal candidate to extract astaxanthin, which infers additional value in the underutilized and wasted resources. Continued research will validate use of astaxanthin for its anti-oxidant and anti-inflammatory activities, which will contribute to human health improvement as well as resources valorization and utilization.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: None

FUTURE FUNDING POSSIBILITIES: None