FINAL REPORT
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BIOTRANSFORMATION OF MEAT AND BONE MEAL (MBM) INTO HIGH-VALUE ASTAXANTHIN FOR ANIMAL FEED

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Lay Summary:

We propose a biological approach to transform meat and bone meal (MBM) into a high-value natural antioxidant, astaxanthin (price=$2500-7000/kg) which is commercially produced by a microalga, *Haematococcus pluvialis* and a yeast, *Phaffia rhodozyma*. Astaxanthin is an important pigment for the animal feed industry especially aquaculture and poultry industries as well as nutraceuticals and pharmaceuticals. For example, astaxanthin helps give salmon meat its rich pink color. The expanding market demand requires more affordable natural astaxanthin. MBM is rich in protein, vitamin and trace elements but has lower value than other animal protein ingredients. Our goal is to find out if MBM can replace the expensive conventional nitrogen source, yeast extract/peptone and if it can perform better than its competitor, soybean meal for natural astaxanthin production. In this project, we used *P. rhodozyma* as a natural astaxanthin producer to evaluate the technical feasibility and efficiency of MBM as a nitrogen source for astaxanthin production. Meanwhile, soybean meal will also be tested for comparison to demonstrate the potential compelling benefits of MBM. We conducted alkali and enzymatic hydrolysis of MBM and soybean meal to convert them into small peptides which were then used by the yeast to produce astaxanthin. The success of this project can establish an example high-value outlet for MBM, which would result in more profit for renderers in the future.

Objective (s):

The objective of this project is to investigate the potential of MBM as a less expensive alternative nitrogen source to produce high-value natural astaxanthin for animal feed.

Project Overview

Introduction

MBM is higher in protein than soybean meal and other plant proteins. In addition, MBM is higher in phosphorus, energy, iron, and zinc than soybean meal (Meeker, 2006). It has been used for animal feed for years. But, its value is lower than other animal protein ingredients such as blood and feather meals and also lower than soybean meal (Feed Ingredient Daily, 2016). Besides its vitamin and mineral contents, the high protein content of MBM renders it a potential nitrogen source to support microbial growth in the biomanufacturing industry to produce high-value products. However, little research has been done in this area. This team proposes an innovative biological technique to transform MBM (as a low-cost nitrogen substitute for yeast extract and peptone) to a high-value product, astaxanthin which can be applied to the animal feed industry (e.g., aquaculture and poultry). This project focuses on exploratory research to demonstrate the feasibility of a high-value outlet for MBM.

Astaxanthin is a secondary carotenoid from the same family as β-carotene. It is a highly potent antioxidant and has a wide range of applications in the food, feed, cosmetic, nutraceutical, pharmaceutical, and aquaculture industries. In terms of antioxidant activity astaxanthin is 65 times more powerful than vitamin C and 54 times stronger than β-carotene (Koller et al., 2014; Pérez-López et al., 2014; Cyanotech, 2017). Astaxanthin can be produced through chemical synthesis and natural biological metabolism (e.g., microalga and yeast). Synthetic astaxanthin

and natural astaxanthin from yeast *P. rhodozyma* are predominantly used in the aquaculture sector, while the other natural astaxanthin derived from *H. pluvialis*, a green microalga is the main source for human applications such as pharmaceuticals, nutraceuticals, cosmetics, and food. The *H. pluvialis* astaxanthin also has great potential in the aquaculture industry, due to increasing consumer demands for natural products and ability of *H. pluvialis* astaxanthin to provide necessary supplementation for adequate growth and reproduction of commercially valuable fishes (Salmon and Red sea bream), rainbow trout and shrimp (Shah et al., 2016). The *H. pluvialis* astaxanthin has been recognized as a safe and effective compound for flesh pigmentation of these fish (Torrissen and Naevdal, 1984; Tolasa et al., 2005). Utilization of *H. pluvialis* meal for pigmentation has resulted in significant astaxanthin deposition in flesh and skin, flesh coloration enhancement, enhanced antioxidant system, fish egg quality, better growth and survival of fry of Salmonid, sea bream, and rainbow trout (Arai et al., 1987; Sommer et al., 1991; Choubert and Heinrich, 1993; Sheikhzadeh et al., 2012a, b), ornamental fishes (Ako and Tamaru, 1999), and shrimp (Arai et al., 1987; Parisenti et al., 2011). A recent study indicated that *H. pluvialis* meal can improve the growth of large yellow croaker fish more than synthetic astaxanthin (Li et al., 2014). When added to the chick feed, the astaxanthin from *H. pluvialis* was found to be efficient in pigmentation of egg yolks, egg production (Elwinger et al., 1997) in hen and breast muscle tissue improvement and higher feed efficiency in broiler chicken (Inborr and Lignell, 1997; Inbbor, 1998). It has also been discovered to improve health and fertility of chicken and to decrease their mortality (Lignell and Inborr, 1999, 2000).

Several studies showed that MBM could be an inexpensive nitrogen source substitute for yeast extract and peptone to produce value-added products by microorganisms. For example, enzyme-hydrolyzed MBM was used to support *Escherichia coli* fermentation for biopolymer production (Solaiman et al. 2011). Both enzyme- and alkali-hydrolysates of MBM were found to be comparable to commercial yeast extract for Omega-3 production by a fungus, *Pythium irregular* (Liang et al., 2011). It was also reported that rendered proteins hydrolysate can be used as a low-cost substitute for commercial peptone (Garcia, et al., 2010). These results demonstrate an opportunity for exploring animal proteins as a low-cost nutrition source for microbial fermentation.

Materials and Methods

In this project, MBM and defatted soybean meal (DSM) were studied and compared as alternative nitrogen source for *P. rhodozyma* fermentation to produce astaxanthin. MBM and DSM were first defatted with hexane and hydrolyzed with alkaline or enzymes into amino acids and polypeptides which were used to replace standard nitrogen sources such as yeast extract and peptone in for fermentation (Figure 1). Since *H. pluvialis* and *Thraustochytrium striatum* did not show significant growth benefits on MBM compared to their standard medium with nitrogen sources of yeast extract and peptone, we did not do further research on them. So, only *P. rhodozyma* was researched in this project.

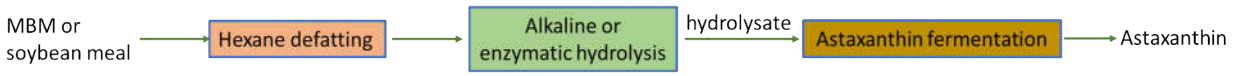

Figure 1. MBM and soybean meal processing for astaxanthin production

Culture Preparation

P. rhodozyma ATCC 74219 is a high astaxanthin-producing industrial strain and was selected for this research. It was purchased from the American Type Culture Collection (Manassas, VA). The received freeze-dried culture was reconstituted in 50 mL YM media (Sigma-Aldrich, St. Louis, MO) in 250-mL shake flasks. Following incubation at 22 °C and 200 rpm for 2 days, two volumes of the broth were mixed with one volume of sterile glycerol, and the stock cultures were stored at −80 °C. The YM contains (per liter): 10g/L of glucose, 3 g/L of malt extract, 5 g/L of peptone, and 3 g/L of yeast extract.

The inoculum for *P. rhodozyma* fermentation was prepared in YM medium. Each 250-mL inoculum flask containing 50 mL YM medium was inoculated with 1% (v/v) thawed glycerol culture. The inoculum flasks were incubated at 22 °C and 200 rpm for 2 days before 1 mL was used to inoculate the fermentation flasks. All flasks were sterilized by autoclaving at 121 °C and 20 min and allowed to cool prior to inoculation. Samples were taken daily, and cell growth was determined by measuring optical density (OD) at 600 nm. The experiments were typically run for 7-14 days when no further increase in OD was observed. The final samples were taken for analysis of dry cell mass (DCM), astaxanthin, and residual sugars. The DCM was measured at the same time by using the following procedures. An aliquot of 1 mL fermentation broth was withdrawn and centrifuged under 5,000 rpm for 5 mins, and the cell pellet was washed with deionized distilled water (DDI) for three times followed by drying at 105 °C overnight before weighing.

MBM and DSM Hydrolysate Preparation

Since the MBM contain a certain lipid content (approx. 12%), defatting step is needed to remove lipid to reduce the effect of lipid on protein hydrolysate processing and fermentation process (Figure 1). We used hexane for lipid extraction (15 mL hexane/1 g solid, dry basis). DSM was received as defatted. No lipid extraction was needed. Defatted MBM and DSM were milled into fine particles (60 mesh) followed by alkaline and enzymatic hydrolysis. All hydrolysis reactions consisted of 9% (w/w) MBM or DSM. The hydrolysates were freeze-dried or kept in liquid and used as nitrogen source for astaxanthin fermentation.

Alkaline hydrolysis

Defatted MBM and DSM samples were hydrolyzed in individual shaking flasks with calcium hydroxide (0.1 g calcium hydroxide/1 g MBM or DSM) under 85 °C for 4, 8 and 16 hours respectively. To terminate the alkaline hydrolysis reaction, carbon dioxide was sparged into the hydrolysate until the pH dropped to 9 and sulfuric acid was used to further reduce the pH to 7.0. Residual solid materials were removed by centrifuge at 4,000 rpm for 30 min under 4 °C. The hydrolysate were filtered through Whatman #1 filter paper three times to remove residual suspended particles completely.

Enzymatic hydrolysis

MBM and DSM samples were obtained from a rendering plant and Cargill Inc., respectively and were studied as alternative nitrogen source for *P. rhodozyma* fermentation. Four different types of proteinase needed for protein hydrolysis were purchased from Sigma-Aldrich (St. Louis, MO), including Alcalase 2.4L (AM) and proteinase bacterial Type VIII (Pb) from *Bacillus licheniformis*, Flavourzyme (FM) from *Aspergillus oryzaez*, and Proteinase K (Pk) from *Tritrachium album*. All chemicals used were of reagent grades. As generally known, temperature affects the density and density determines the mass of enzyme. Therefore, all enzymes were weighed and added to the hydrolysis reactors after the MBM suspension was preheated to the optimum temperature for enzyme. The enzymatic hydrolysis conditions are in Table 1. In order to adjust pH to the expected value, 8M sodium hydroxide solution and 37% hydrochloride acid were used. Reaction temperature was 90 °C for 10 minutes to terminate the enzymatic hydrolysis. Residual solid materials were removed by centrifuge at 4,000 rpm for 30 min under 4 °C. The hydrolysate were filtered through Whatman #1 filter paper three times to remove residual suspended particles completely.

Table1. Enzyme hydrolysis conditions

		Temp. (°C)	Time (h)	pH	Enzyme/substrate (dry basis)
Liquid enzyme	AM4	50	4	8.5	0.4 AU/g substrate
	AM8	50	8	8.5	0.4 AU/g substrate
	FM4	50	4	7	100 LAPU/g substrate
	FM8	50	8	7	100 LAPU/g substrate
	AFM	50	A for 4h + F for 4 h	8.5+7	0.4 AU/g substrate,100 LAPU/g substrate
Powder enzyme	Pb4	37	4	7.5	4 mg enzyme/g substrate
	Pb8	37	8	7.5	
	Pk4	37	4	8	
	Pk8	37	8	8	

Experimental Design

We tried to use both freeze-dried alkaline and enzymatic hydrolysates of MBM for fermentation, but precipitates always happened in the first 2-3 days of fermentation. The precipitate dissolved again after 2-3 days. However, it took more than 15 days for yeast to grow, which is much longer than the cultivation with regular media. The reason is not clear. Therefore, we decided to use the hydrolysate broth directly without drying. To keep the same loading level of MBM as a nitrogen source, we measured TKN of hydrolysate broth obtained under different hydrolysis conditions.

Experiment 1: Carbon/nitrogen ratio study

The objective of this was to study how carbon and nitrogen concentrations would affect the growth and productivity of *P. rhodozyma*. Two media were used for this study such as YM and YNB media. Since YM medium does not contain many mineral, vitamins and trace elements, enhanced media called YNB which aforementioned nutrients was also used. The C/N ratio was adjusted through two different methods. The first method is to keep constant glucose of 20 g/L and adjust nitrogen concentration; and the second method is to keep constant nitrogen concentration with varied glucose concentration (Table 2). Glucose was the only carbon source

and the nitrogen source included the mixture of yeast extraction, malt extraction and peptone (3:3:5, w/w/w). In addition, minerals, vitamin and trace elements were added to YDuring cultivation, the OD value, cell biomass and astaxanthin concentration were measured periodically.

Table 2. Compositions of media (YM and YNB) with different C/N ratio

	Method 1				Method 2			
	Varied nitrogen concentration				Varied glucose concentration			
C/N ratio (w/w)	5	10	20	30	5	10	20	30
Malt extraction (g/L)	1.1	0.55	0.27	0.18	1.1	1.1	1.1	1.1
Yeast extraction (g/L)	1.1	0.55	0.27	0.18	1.1	1.1	1.1	1.1
Peptone (g/L)	1.8	0.9	0.45	0.3	1.8	1.8	1.8	1.8
Glucose (g/L)	20	20	20	20	20	40	80	120

Experiment 2: Hydrolysate broth fermentation

The objective of this experiment was to study the suitability of MBM and DSM hydrolysates as nitrogen source to growth *P. rhodozyma* for astaxanthin production. The enzyme hydrolysis scenarios were shown in Table 1. The best C/N ratio determined in Experiment 1 was 5 and used here. The fermentation process was done in 125 mL shaking flasks with 25 mL fermentation broth. During cultivation, the OD value, cell biomass and astaxanthin concentration were measured periodically. The nitrogen in the hydrolysis broth was determined upon TKN which was used to adjust the C/N ratio by varying glucose concentration.

Analytical Methods

The intracellular carotenoids were measured by using HPLC method. The cells were harvested by centrifugation at 5,000 rpm for 5 mins, and the pellet was washed with DDI water for three times followed by freeze-drying. To extract carotenoids from cells, 5 mL of dimethyl sulfoxide (DMSO) was mixed with 20 mg freeze-dried cells and vortexed for 30 seconds in the presence of 200 mg glass beads for cell breakdown. The mixture was heated at 50 °C for 20 min in water bath, and then centrifuged at 4,000 rpm for 5 mins. The supernatants were collected into a series of new tubes. These steps were repeated until the color of cell pellets became pale (i.e., negligible pigment left). All collected supernatants were combined and subject to carotenoid analysis with a HPLC (Agilent 1100 series) equipped with a diode array detector (G1315B, Agilent). The used analytical column was ZORBAX Eclipse plus C18 (4.6×100 mm and 3.5 μm) which was put in a thermostatted column chamber at 25 °C. The mixture of dichloromethane, acetonitrile, methanol and 1% formic acid was used as a mobile phase with flow rate of 1.5 mL/min. Programed procedures included: (1) 0-2 min: dichloromethane/acetonitrile /methanol/formic acid=0/50/15/35 (v/v, %); (2) 2-5 min: dichloromethane/acetonitrile /methanol/formic acid=5/50/10/35; (3) 5-17 min: dichloromethane/acetonitrile /methanol/formic acid=40/30/10/20; and (4) finally the mobile phase composition resume to the initial values within 1 min. The peak signals were recorded at wavelength of 480 nm. Standard carotenoids including astaxanthin (Sigma-Aldrich), zeaxanthin (Sigma-Aldrich), canthaxanthin (ChromaDex, Inc.), β-cryptoxanthin (Alkemist Labs), echinenone (Sigma-Aldrich), and β-carotene (Alfa Aesar) were used to develop standard curves for carotenoid quantification.

Sugars (e.g., glucose, xylose, arabinose, and/or cellobiose) were analyzed with a HPLC (Dionex UltiMate 3000, Thermo Scientific, CA, USA) equipped with a refractive index (RI) detector (Shodex RI-101, Showa Denko America, NY, USA) and a Shodex carbohydrate analytical column (Sugar SP0810, Showa Denko America, NY, USA). Sugars were separated at 85 °C, and HPLC grade water was used as a mobile phase at a flow rate of 0.6 mL/min. A deashing guard column (Shodex Sugar SP-G 6B, Showa Denko America, NY, USA) was installed prior to the analytical column for preventing column from potential contamination. Total Kjeldahl nitrogen (TKN) is the total concentration of organic nitrogen and ammonia. A TKN kit (TNT 880) was used to measure TKNs and OD was analyzed by using HACH DR3900 spectrophotometer. The kit could measure the concentration of nitrate, nitrite, and total nitrogen. The results of TKN came from total nitrogen subtracted by nitrate and nitrite. All experiments on astaxanthin production were performed in duplicate, and the averages of the results are reported.

Results and Discussion

Experiment 1: Carbon/Nitrogen Ratio Study

YM media

When YM media with constant glucose of 20 g/L was used, cell growth has one day lag phase and reached the stationary phase after 3-4 days (Figure 2A). The maximum cell biomass increased with the decrease of C/N ratio, i.e., the yeast achieved the highest biomass at C/N ratio of 5. The C/N=5 also resulted in maximum growth rate. However, the C/N ratio did not have significant effect on the astaxanthin concentration, i.e., all C/N ration achieved similar astaxanthin concentration of approx. 15 mg/L (Figure 2B). These findings show the astaxanthin content of cells with high C/N ratio is higher than that with low C/N ratio. It can be also found that low C/N (i.e., high nitrogen source concentration) ratio favors cell growth while high C/N ratio is conducive for astaxanthin accumulation in cells. Therefore, we could use fed-batch cultivation mode (normal C/N ratio in the first stage for cell growth plus high/CN ratio in the second stage for astaxanthin accumulation) to replace batch mode to achieve both high cell mass and astaxanthin accumulation.

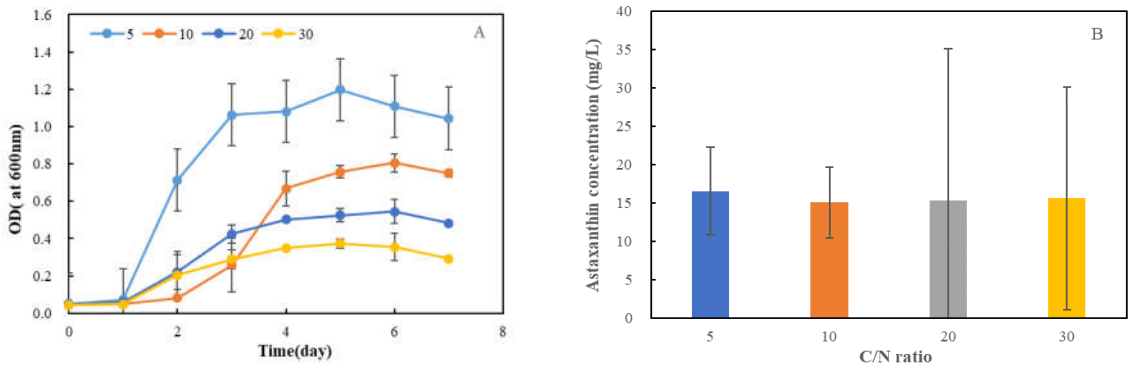

Figure 2. Effect of C/N ratio on yeast growth (A) and astaxanthin concentration (B) with YM media at constant glucose concentration

When nitrogen source concentration was consistent 4 g/L, the increase of glucose concentration resulted in increased C/N ratio. The cell growth trend on C/N ratio is simiar to the first C/N adjustment method, i.e., high C/N ratio had low cell growth (Figure 3A), while the astaxanthin concentration decreased signficantly with the increase of glucose concentration (Figure 3B). By comparing with the first method, we found the increasing glucose concentration resulted in higher cell growth with the same C/N ratio. Although the C/N ratio is the same, the astaxnathin concentration in lower in the 2nd method than the 1st method. Therefore, both C/N ratio and the absoluble glucose and nitrogen source concentrations are important to achieve high cell biomass and astaxanthin production.

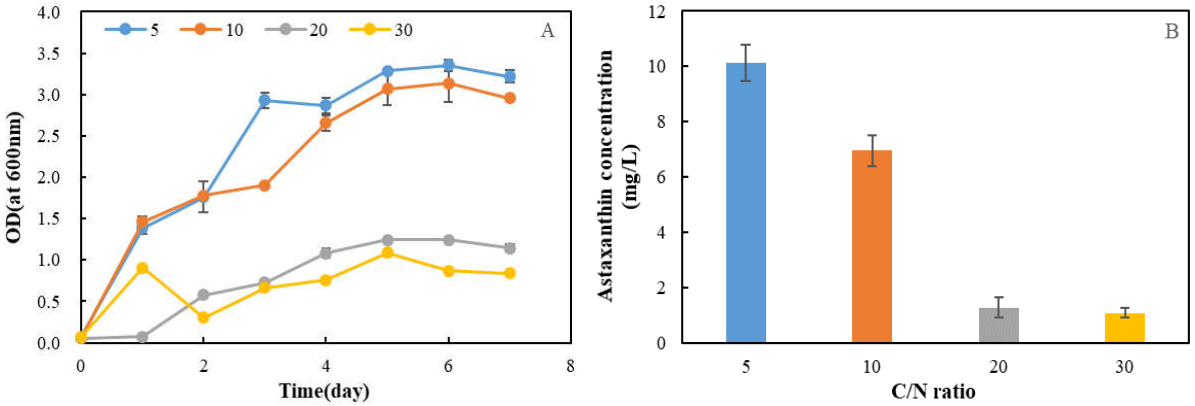

Figure 3. Effect of C/N ratio on yeast growth (A) and astaxanthin concentration (B) with YM media at constant nitrogen source concentration

YNB media

With the supplement of nutrients in the first method with constant glucose concentration, yeast cell biomass was increased significantly, and there was not lag phase (Figure 4A vs. Figure 2A). The higher C/N ratio caused higher cell biomass, which is different from YM media. These results revealed that the interaction between nutrients and carbon/nitrogen sources played a critical role in affecting cell growth. In addition, the presence of nutrients enhanced astaxanthin accumulation in cells, and the astaxanthin concentration increased from 50 mg/L to 1.8 g/L with the increase of C/N ratio from 5 to 30 (Figure 4B). Therefore, utilization of nutrients can be an effective approach to increase astaxanthin production during batch fermentation.

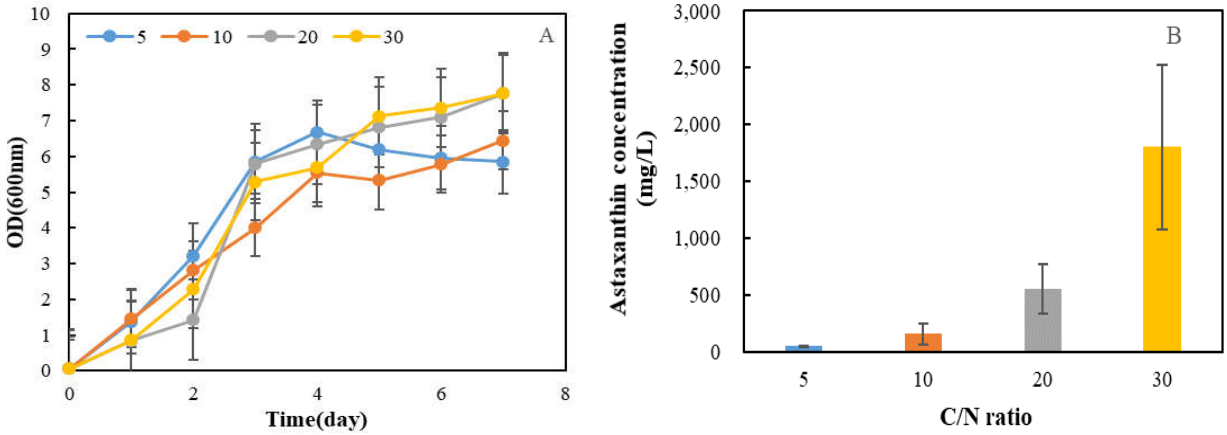

Figure 4. Effect of C/N ratio on yeast growth (A) and astaxanthin concentration (B) with YNB media at constant glucose concentration

When the 2nd method with constant nitrogen was used to adjust C/N ratio, the increase of glucose concentration reduced cell growth significantly (Figure 5A). Compared to the 1st method (Figure 4A), the 2nd method had lower cell growth. Compared to YM media (Figure 3A), YNB achieved higher cell growth at the C/N ratio. The astaxanthin production was in the reserve order to the C/N ratio (Figure 5B). Using the increasing glucose to vary the C/N ratio in the 2nd method reduced the astaxanthin significantly compared to the 1st method (Figure 4B). These finding could indicate the inhibition of high glucose on cell growth and astaxanthin accumulation.

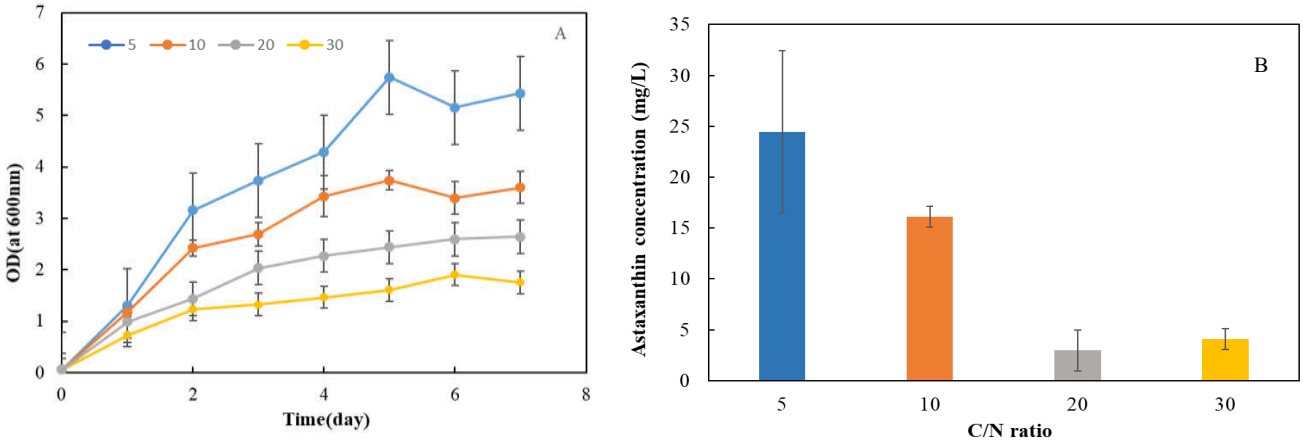

Figure 5. Effect of C/N ratio on yeast growth (A) and astaxanthin concentration (B) with YNB media at constant nitrogen source concentration

Overall, the nutrients including mineral, vitamins and trace element favor astaxanthin production, and C/N ratio of 5 achieved the maximum astaxanthin accumulation in most cases and can avoid potential glucose inhibition. Therefore, C/N=5 was selected for testing MBM and DSM hydrolysates. Given that MBM contains various vitamins and minerals, MBM was expected to provide a good nitrogen source for yeast fermentation for astaxanthin production.

Experiment 2: Fermentation of MBM Hydrolysates

Comparison between MBM and DSM

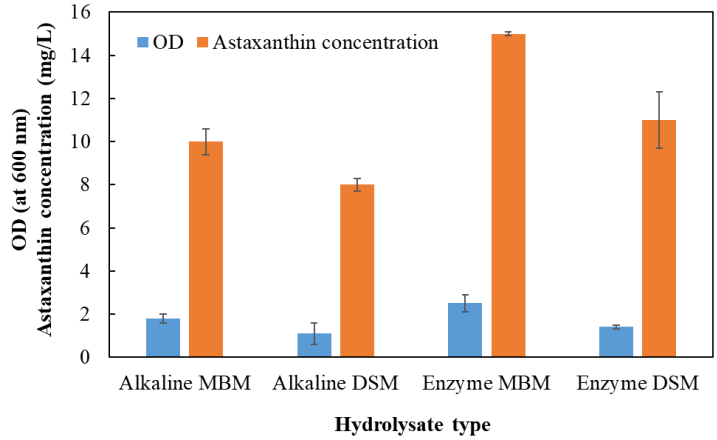

Figure 6. Comparison between MBM and DSM and alkaline (Pb 4 was used for enzyme hydrolysis, Table 1). C/N=5.0 and glucose=20 g/L for 4 days

Alkaline and enzymatic hydrolysis were both used to hydrolyze MBM and DSM, and the hydrolysates were used to replace yeast extract and peptone for *P. rhodozyma* fermentation. As shown in Figure 6, MBM was better than DSM to grow the yeast and produce more astaxanthin no matter which hydrolysis method was used. In addition, enzyme (e.g., PB 4) was better than alkaline hydrolysis for both MBM and DSM. When enzyme was used to hydrolyze MBM, *P. rhodozyma* growth reached OD=2.5 after 4 days of fermentation and astaxanthin concentration reached 15 mg/L. As a result, MBM but not DSM was selected for further test and enzyme but not alkaline was used for MBM hydrolysis.

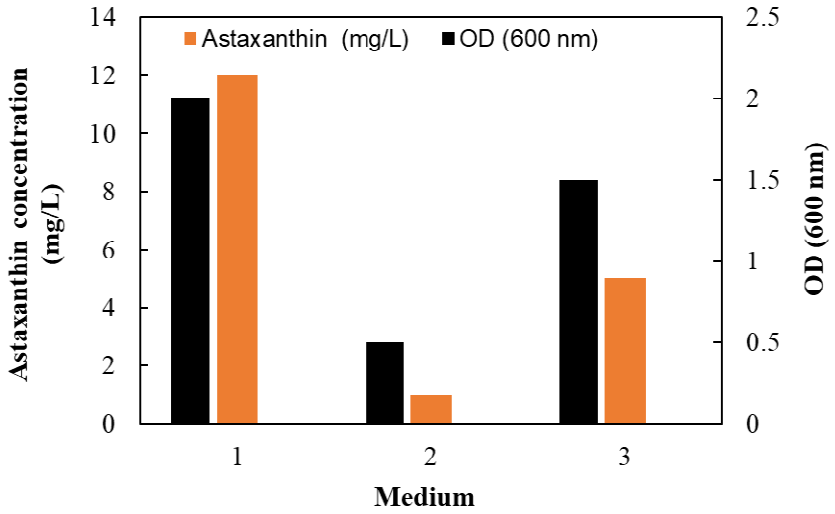

Figure 7. Yeast growth and astaxanthin production by *P. rhodozyma* on different media: 1=YM media, 2=YM without glucose and 3= MBM-based media without glucose

We made MBM-based media by replacing yeast extract/peptone and glucose in the YM media. The result obtained is very promising that MBM as alternative nitrogen achieved comparable yeast cell growth even though no glucose in MBM-based media (Figure 7), which means MBM serves as not only nitrogen source, but also carbon source. In addition, the vitamins and minerals in MBM may be beneficial to grow *P. rhodozyma* and accumulate astaxanthin. Therefore, we expect that MBM can be used to replace expensive yeast extract/peptone and partial glucose for astaxanthin production.

Enzymatic hydrolysates for astaxanthin production by P. rhodozyma

The effectiveness of enzyme hydrolysate varied with different enzymes and hydrolysis time. In general, longer time was better to achieve higher cell growth and more astaxanthin production (Table 3). The best situation was Pb enzyme for 8 h hydrolysis, which resulted in OD 3.5 and 24 mg/L of astaxanthin. Among all enzymes, the rank of different enzyme performance was Pb>Pk>AM>FM. The combination of AM and FM (AFM for 8 h) achieved better results than FM only but less than AM for 8 h. Enzymatic hydrolysis appeared to be a promising method to generate MBM-based nitrogen source for astaxanthin production by *P. rhodozyma*, but needs further optimization.

Table 3. Enzymatic hydrolysate for yeast fermentation (C/N=5.0 and glucose=20 for 4 days)

Enzyme	OD (at 600 nm)	Astaxanthin concentration (mg/L)
AM4	1.8	12
AM8	2.9	22
FM4	1.1	10
FM8	1.3	14
AFM	2.5	20
Pb4	2.6	16
Pb8	3.5	24
Pk4	2.4	13
Pk8	3.1	21

Conclusions

MBM can be used as an alternative nitrogen source to yeast extract and peptone for growing *P. rhodozyma* to produce natural astaxanthin, but not effective to support the growth of *H. pluvialis* and *Thraustochytrium striatum*. MBM is better than DSM for astaxanthin fermentation by *P. rhodozyma*. C/N ratio is a key parameter affecting astaxanthin production. The astaxanthin yield by using MBM reached more than 20 mg/L, comparable to the regular nitrogen source, i.e., yeast extract and peptone. The nutrients including vitamins and minerals play a critical role in promote yeast growth and astaxanthin accumulation. MBM is expect to be a promising alternative nitrogen source for yeast astaxanthin fermentation because it contains more vitamins and minerals than DSM. The MBM hydrolysis by using enzyme and alkaline did not achieve high hydrolysis yield, which needs to be improved further to optimize astaxanthin production.

Impacts and Significance:

MBM is expected to replace or partially replace expensive yeast extract/peptone with potential replacement of vitamin, trace elements and/or phosphorus in the "standard" media for natural astaxanthin production. Given that the price of bulk yeast extract ($3,000-8,000/ton) is much higher than that of MBM ($207/ton), it can be envisioned that using MBM as a nitrogen source can significantly reduce the cost of astaxanthin and increase the profit, which would be verified by the data generated through this project about the efficiency of MBM for astaxanthin production. Compared with soybean meal, MBM has a higher protein content and other nutrients which could make MBM a better alternative nitrogen source. In addition, the price of MBM is lower than that of soybean meal that is about $326/ton. Therefore, their efficiencies for astaxanthin production is a key factor determining which one is more cost-effective. This question will be answered in the course of this project. Depending on the efficiency of MBM, there may be a niche market for MBM to be used for natural astaxanthin production. The annual worldwide aquaculture market of astaxanthin is estimated at $200 million out of $257 million total global market with the price of $2500-7000/kg. In 2014, the global market for astaxanthin was estimated at 280 metric tons valued at $447 million most of which was used in fish coloration. The demand for natural astaxanthin is now emerging in the multi-billion dollar nutraceutical market owing to increasing consumer awareness of its health benefits. The potential market for animal feed and human consumption is estimated to be $800 Million and

$830 Million in 2020, respectively. Current market for natural astaxanthin is far from saturated. Therefore, more natural astaxanthin is needed to meet the fast-increasing market demand which will require a large quantity of low-cost media, which could be a niche market for MBM. Technoeconomic analysis was not proposed in this project, but may be needed to access the economic performance of MBM used for astaxanthin production, indicate the limiting factor, and/or maximize the profit of MBM for astaxanthin production.

Publications:

Li, S., Ladner, D., Zheng, Y., 2018. Investigation on meat bond meal as a nitrogen source to produce astaxanthin by *Phaffia rhodozyma*. Journal of Agricultural and Food Chemistry (In revision).

Outside funding:

Preproposal entitled "Biotransformation of food processing byproducts into high-value products" was submitted to the USDA NIFA AFRI Exploratory Research Program. (Under review)

Future Work:

This project has demonstrated that MBM hydrolysates are suitable nitrogen source for *P. rhodozyma* fermentation to produce natural astaxanthin. The further work would be focused on:

- Look for highly efficient MBM hydrolysis techniques and optimize the MBM hydrolysis (e.g., enzymatic hydrolysis) to maximize the yield of amino acids and peptides which can be digestible to *P. rhodozyma*
- Optimize the fermentation process to achieve high astaxanthin yield, e.g., medium, operational parameters and fermentation modes (e.g., batch, fed-batch and continuous)
- Study other astaxanthin producing microorganisms which can secrete proteinase. So, a simultaneous hydrolysis and fermentation process can be developed to improve the process efficiency
- Comprehensive economic analysis can be done to evaluate the financial feasibility of using MBM as a nitrogen source to produce astaxanthin
- Investigate other rendering proteins for astaxanthin production
- Test the MBM-astaxanthin for animal feed, e.g., chicken and aquaculture

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