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Effect of drying, storage temperature and air exposure on astaxanthin stability from *Haematococcus pluvialis*

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Abstract

Astaxanthin is a powerful antioxidant with various health benefits such as prevention of age-related macular degeneration and improvement of the immune system, liver and heart function. To improve the post harvesting stability of astaxanthin used in food, feed and nutraceuticals industries, the biomass of the high astaxanthin producing alga *Haematococcus pluvialis* was dried by spray- or freeze-drying and under vacuum or air at -20ºC to 37ºC for 20 weeks. Freeze-drying led to 41% higher astaxanthin recovery compared to commonly-used spray-drying. Low storage temperature (-20ºC, 4ºC) and vacuum-packing also showed higher astaxanthin stability with as little as 12.3±3.1% degradation during 20 weeks of storage. Cost-benefit analysis showed that freeze-drying followed by vacuum-packed storage at -20ºC can generate AUD$600 higher profit compared to spray-drying from 100 kg *H. pluvialis* powder. Therefore, freeze-drying can be suggested as a mild and more profitable method for ensuring longer shelf life of astaxanthin from *H. pluvialis*.

Keywords

Astaxanthin; cost-benefit analysis; freeze-drying; *Haematococcus pluvialis;* spray-drying.

Abbreviations

DNA = Deoxyribonucleic acid

UV = Ultra violet

PCPLC = Poly(ethyleneoxide)-4-methoxycinnamoylphthaloylchitosan

NaCl = Sodium chloride

HPLC-PDA = High Performance liquid chromatography photo diode array

DPAC = Degradation percentage of astaxanthin content

SD = Standard deviation

ANOVA = Analysis of variance

1. Introduction

Astaxanthin is a widely-distributed keto-carotenoid in nature and is predominantly produced in the red yeast *Xanthophyllomyces dendrorhous,* the plant *Adonis annua* and in the microalga *Haematococcus pluvialis* (Miao, Geng, Lu, Zuo and Li, 2013). Astaxanthin is the highest value carotenoid that is currently used in various industries, such as the food, feed, nutraceutical and pharmaceutical sectors and has been receiving great attention from the scientific community and consumers due to the various health benefits recently discovered. New studies have suggested that astaxanthin is a powerful antioxidant having higher capabilities than other carotenoids (10 times higher than zeaxanthin, lutein, β-carotene and cantaxanthin; Miao *et al.,* 2013) and vitamins and therefore can be highly effective in reducing DNA damage, improving the immune system, liver and heart function by causing higher production of antibodies and T-cells, protecting UV-light photooxidation, inflammation, age-related macular degeneration and cancer, eliminate lipid peroxidation in cell membrane and promoting cardiovascular health by modifying cholesterol levels in the plasma (Miao *et al.,* 2013; Bustos-Garza, Yáñez-Fernández, & Barragán-Huerta, 2013).

Astaxanthin is in high demand by several industries, such as the 130 tons of annual worldwide requirement in salmonid aquaculture (Bjerkeng, 2008). Although artificially synthesized astaxanthin, which has lower antioxidant capacity than the natural compound, constitutes most of the supply (around 95%), the natural astaxanthin produced by *H. pluvialis* is becoming increasingly popular (Miao *et al.,* 2013). Several companies, such as Cyanotech Corporation, Algatechnologies and Fuji

Health Science are currently commercially producing and marketing astaxanthin from *H. pluvialis* (Borowitzka, 2013; Bhattacharjee, 2014).

Astaxanthin is well-known to possess a high sensitivity to light, oxygen, and temperature, creating a need to develop methods to ensure long-term storage stability of this high-value carotenoid. Based on the limited water solubility of astaxanthin, different esters, such as disodium disuccinate, tetrasodium diphosphate, divitamin C disuccinate and fatty acid astaxanthin have been prepared in attempts to improve astaxanthin solubility and stability (Tachaprutinun, Udomsup, Luadthong and Wanichwecharungruang, 2009). Studies have suggested that incorporation of astaxanthin with compounds, such as β-cyclodextrin, hydroxypropyl-β-cyclodextrin, and chitosan, can provide protection against temperature and improve the stability of astaxanthin (Miao *et al.,* 2013). Encapsulation in compounds such as poly(ethyleneoxide)-4-methoxycinnamoylphthaloylchitosan (PCPLC), or chitosan was reported as another effective strategy to solve the solubility and stability issue with astaxanthin (Tachaprutinun *et al.*, 2009). Moreover, storage of dry algal meal under vacuum conditions was able to retain 90% of the initial astaxanthin content (Gouveia & Empis, 2003). A long-term storage study lasting for 96 weeks using spray-dried *H. pluvialis* powder suggested that the most economical and applicable storage condition would be storing the powder under vacuum at 4ºC in the dark (Miao *et al.,* 2013). It was reported that vacuum-drying could retain better astaxanthin content than hot air-drying in shrimp (Niamnuy, Devahastin, Soponronnarit and Vijaya Raghavan, 2008), but such comparison of various drying methods involving *H. pluvialis* powder has not been reported so far.

In this study, we report for the first time to our knowledge, about the impact of drying methods (freeze-drying versus spray-drying) on astaxanthin content in *H. pluvialis* powder. We also report here how the astaxanthin esters, and free (cis/trans) astaxanthin content in the dried powder change over time under vacuum and non-vacuum conditions and at different storage temperatures. This will help to understand how the different drying methods as well as temperature and vacuum conditions could affect astaxanthin stability in *H. pluvialis* powder.

2. Materials and Methods

2.1. Culture conditions

Haematococcus pluvialis biomass for the test was supplied by James Cook University-MBD Energy Pty Ltd Microalgae R&D Facility (Townsville, QLD, Australia). The biomass was harvested when the cells reached the red cyst aplanospore stage via centrifuge dewatering process. Then the biomass was preserved at -80ºC and delivered to the Algae Biotechnology Laboratory at the University of Queensland (St. Lucia, QLD, Australia) for the comparative drying tests.

2.2. Drying of biomass

The carotenized cultures of *H. pluvialis* were dried by spray- or freeze-drying. Around 100 g of *H. pluvialis* biomass was dried by each drying process. The inlet/outlet temperatures of the spray dryer (Anhydro 7067, Copenhagen, Denmark) were selected as 180ºC/110^{o}C based on the findings of (Raposo, Morais & Morais, 2012).

Freeze-drying was conducted in a Dynavac freeze dryer (Model FD1, Melbourne, VIC, Australia) at -40ºC for a minimum of 16 hours. Moisture contents in the spray- and freeze-dried biomass were measured by calculating the losses in the weights of powder after storing them in a drying chamber (Roband Food Services Equipment, Roband Australia Pty Ltd, Sydney, NSW, Australia) at 110ºC overnight.

2.3. Storage of dried powders

The dried powders collected from the two drying methods were stored in Ziploc bags in aliquots of 1 g. Half of the bags used for the experiments were vacuum-packed while the rest were closed and kept at -20ºC, 4ºC, 20ºC or 37ºC. The highest temperature used in our study (37ºC) is common in many parts of Australia and also in many tropical and subtropical countries during summer. The objective was to understand the level of astaxanthin degradation at such a temperature and whether vacuum-packing could reduce/prevent the degradation. For the low temperature storage (-20ºC and 4ºC), freezer and cold rooms, respectively, with these temperatures at the School of Agriculture and Food Science of the University of Queensland, St. Lucia Campus, were used. For storage at other temperatures, incubators (Model NB-205, N-Biotek Inc., Bucheon, Korea) were used. All bags were covered by aluminium foil to avoid the effect of light. Three replicates were stored for each temperature, packing (vacuum or non-vacuum) and drying method. Sampling (25-50 mg) was conducted at 3, 6, 9, 12 and 20 weeks after the start of the storage of the dried powders.

2.4. Astaxanthin extraction and analysis

The extraction of astaxanthin was conducted following the protocol described previously (Ahmed, Fanning, Netzel, Turner, Li and Schenk, 2014) with slight modifications. In brief, the biomass was crushed with mortar and pestle and treated with 10 mL acetone, 10 mL hexane and 5 mL 10% NaCl. The mixture was centrifuged (Eppendorf 5804R, Hamburg, Germany) at 3000 x g at 4ºC for 3 min. The process was repeated until the mixture became colourless. The hexane fractions were collected and dried in a centrifugal evaporator (miVac Duo Concentrator, Genevac Inc., Stone Ridge, New York, USA) prior to being reconstituted in 5 mL methanol/dichloromethane (50/50, v/v) for HPLC analysis. The HPLC-Photodiode Array (HPLC-PDA; Shimadzu, Kyoto, Japan) analysis was undertaken as previously described (Fanning, Martin, Wong, Keating, Pun and O'Hare, 2010) with minor modifications to the gradient: The 54 min gradient used for astaxanthin analysis was as follows: 0 min, 80% phase A; 48 min, 20% phase A; 49 min, 80 % phase A; 54 min, 80% (phase A - 92% methanol/8% 10 mM ammonium acetate, phase B - 100% methyl tert butyl ether). The identification and quantification of astaxanthin was conducted based on comparison of the retention times, mass spectra and calibration curves of authentic standards (Sigma, Sydney, NSW, Australia).

2.5. Cost-benefit analysis

A cost-benefit analysis was conducted to understand the costs incurred for the drying of 100 kg of *H. pluvialis* powder by the use of a spray dryer or a freeze dryer

and the profit margins after 20 weeks of storage of the powder. The energy consumption of spray-drying was calculated based on the technical specification of an APV PSD 55 Spray dryer (APV Anhydro, New York, New York, USA). It was calculated that drying of 100 kg *H. pluvialis* powder in this dryer would require 50 kW of power. For freeze-drying, the technical specification of an F100 freeze dryer (Frozen in Time Ltd., York, England) was used for calculations (www.freezedriers.com). It was calculated that freeze-drying in this dryer of the same biomass would consume 360 kW of power, considering 24 h running of the dryer (15 kW/h) to obtain the powder. The retail values of astaxanthin from 100 kg *H. pluvialis* powder on week 0 and week 20 were calculated considering astaxanthin content produced by the two drying methods and the rate of astaxanthin degradation at different storage conditions observed in the current study.

2.6. Statistical analyses

All statistical analyses were carried out in IBM SPSS 20. The differences in the degradation percentages of total, free, monoester and diester astaxanthin were analyzed by three way analysis of variance (ANOVA) with drying methods being the between subject factor and packaging and temperature being the within subject factors. Bonferroni test was used for multiple comparisons of significant treatment effects. Values are given as means ± SE. A significance level of 0.05 was used for all tests.

3. Results

3.1. Effects of drying methods, packaging and temperature on *Haematococcus pluvialis* astaxanthin degradation

The main purpose of this study was to establish the best post-harvest processing conditions for high stability of astaxanthin from freshly-produced *H. pluvialis* culture. The first step includes rapid drying. The moisture content in the spray-dried *H. pluvialis* powder (6.86 ± 0.39%) was slightly lower than in the freeze-dried powder (8.22 ± 0.28%) at the beginning of the storage treatments (Table 1). The total astaxanthin content in the spray-dried powder (4578.6 ± 14.5 µg/g dry weight (DW)) was also lower than that of the freeze-dried powder (6454.9 ± 23.8 µg/g DW) at the beginning of the storage treatments (referred to as week 0 in Table 1), demonstrating that already the drying method has a big influence on astaxanthin contents.

Next, astaxanthin stability was assessed following storage in sealed Ziploc bags (either vacuum-packed or air-packed) at different temperatures. During the 20 weeks of storage, degradation percentage of astaxanthin content (DPAC) in the spray-dried samples kept at different temperatures rose from 4.14 ± 0.99% (-20ºC storage vacuum-packed; Week 3) to 88.2 ± 0.08% (37ºC storage vacuum-packed; Week 20; Fig. 1). Three way ANOVA showed significant effects of drying methods, temperature and packaging on the DPAC of *H. pluvialis* powder during the storage period (p<0.001; Table 2). Although no interactive effect of all three factors was

found (p>0.05), significant effects of the interaction of drying method and temperature, and packaging and temperature were found (p<0.05) for total astaxanthin as well as free and monoester astaxanthin components (Table 2). As expected, the samples kept at 37ºC had the highest DPAC (35.7 ± 0.9% on week 3 to 88.2 ± 0.08% on week 20) throughout the whole storage period, irrespective of the packaging condition. In the normal non-vacuum packed samples stored at 20ºC, the DPAC was also very high (35 ± 2.4% on week 3 to 81 ± 4.3% on week 20) staying close to the degradation (rates) of the samples stored at 37ºC (Fig. 1). The DPAC of the samples kept at -20ºC and 4ºC rose to 24.7 ± 4% and 35.9 ± 3% respectively under vacuum and to 38.5 ± 2.4% and 50.2 ± 1%, respectively, under normal non-vacuum packaging conditions on week 20 (Fig. 1).

The freeze-dried powder showed a similar pattern as the spray-dried samples at different storage conditions. The DPAC was also the highest at 37ºC (28.2 ± 3.3% on week 3 to 91.2 ± 2.1% on week 20), irrespective of the packaging condition (Fig. 1). Similar to spray-dried algal biomass, the DPAC of the freeze-dried samples kept at 20ºC under normal packaging (23.3 ± 6.7% on week 3 to 70 ± 5.3% on week 20) were close to that of 37ºC during the 20 weeks of storage (Fig. 1). The DPAC of the samples kept at -20ºC and 4ºC rose to 12.3 ± 3.1% and 28 ± 4.2%, respectively, under vacuum and to 18.5 ± 0.9% and 35 ± 6.8%, respectively, under normal non-vacuum packaging conditions on week 20 (Fig. 1).

Raposo *et al*. (2012) described DPAC through a polynomial model of second order and termed it as “ratio of astaxanthin degradation”. The model was expressed as follows:

$$\text{Ratio of astaxanthin degradation} = at^2 + bt + c$$

where t = time of storage (weeks)

a, b, c = coefficients.

The ratio of astaxanthin degradation calculated from the model as well as rates of degradation (2at + b) and acceleration of degradation constants (2a) are shown in Table 3. The model fits very well with the degradation data in most storage conditions as expressed by the R^2 values (0.9 - 0.99), but in the case of freeze-dried vacuum powder stored at -20ºC and 4ºC the model did not fit well (R^2: -20ºC = 0.72, 4ºC = 0.59) and therefore was not considered for the calculations. The acceleration of degradation constant was the lowest at -20ºC under all packaging conditions. Moreover, the constants were higher at normal packaging at temperatures from 4ºC and above.

3.2. Changes in diester/monoester astaxanthin ratios

The ratio of diester to monoester astaxanthin was determined as it presents an indication of asthaxanthin degradation because monoesters are reported to degrade more rapidly than diesters (Miao *et al.,* 2013). It was slightly higher in the freeze-dried (0.163±0.002) than spray-dried (0.148±0.001) powder on week 0 (Table 4). Among the spray-dried samples, the ratio remained similar for the ones stored at -20ºC and 4ºC and vacuum-packed during the 20 weeks of storage. The ratio increased to a much higher level on week 20 in the vacuum-packed, spray-dried

powder at 20ºC whereas at 37ºC the ratio kept going up with the increase in storage time and reached the highest level among all storage conditions (0.418±0.03) on week 20 (Table 4). A different pattern was observed in the normal non-vacuum packed spray-dried samples. The ratios were similar to the initial level at all storage temperatures except 37ºC (Table 4). In the case of freeze-dried powder, the ratio remained similar to the initial one at week 0 at all storage temperatures except 37ºC, irrespective of normal or vacuum packing (Table 4).

3.3. Cost-benefit analysis of *H. pluvialis* drying methods

A cost-benefit analysis was carried out to establish the best drying conditions in a commercial scenario. As expected, the energy cost of producing freeze-dried powder (AU$101.4) was much higher than that of spray-dried powder (AU$14.9). However, due to lower astaxanthin retention capacity of spray-dried powder, the retail value of remaining astaxanthin content in powder that was vacuum-packed and stored at -20ºC is AU$685 lower than freeze-dried powder kept at similar storage conditions. Despite the higher energy cost, the freeze-dried powder can still generate more profit margins than spray-dried powder as long as it is kept at or below 4ºC in the dark with minimal access to air.

4. Discussion

To our knowledge, this is the first report on the effect of spray- and freeze-drying on the stability of astaxanthin from the highly productive microalga *H. pluvialis*. We also conducted a cost-benefit analysis of the two drying processes that could be highly

useful for the decision makers of the astaxanthin production industry. As expected, high temperatures played a significant role in the degradation of astaxanthin, irrespective of the drying and packaging methods. The results are in agreement with Raposo *et al.* (2012), Miao *et al.* (2013), and Niamnuy *et al.* (2008) who also reported high degradation rates of astaxanthin at temperatures from 15ºC to 25ºC.Our results also confirmed the findings of these authors that air exposure can significantly degrade astaxanthin, as vacuum-packing was found to retain more astaxanthin during the 20 weeks of storage, except for 37ºC storage where the degradation was the highest, irrespective of whether vacuum or non-vacuum packaging was used. Our results therefore indicate a stronger effect from high temperature (e.g. 37ºC) than air exposure on astaxanthin degradation.

Our results also confirmed that the interaction of temperature and air exposure (non-vacuum packaging) can significantly degrade astaxanthin, irrespective of the drying process used. Raposo *et al.* (2012) reported that *H. pluvialis* powder with higher moisture content showed better astaxanthin retention capacity and inferred that the moisture within the powder (termed as “wet character of the powder”) might have contributed to the prevention mechanism of astaxanthin degradation. Our results are in agreement with Raposo *et al.* (2012), as the freeze-dried biomass in this study had higher moisture content and exhibited slower astaxanthin degradation. This validates the postulation put forward by Raposo *et al.* (2012), although it remains unclear how moisture may help in the prevention mechanism of astaxanthin degradation. One possibility is that a slightly higher moisture content may improve the extractability of astaxanthin by enhancing the grinding capability of the powder. Follow-up studies using a broader range of moisture levels are required to confirm this hypothesis.

In this study, the dried powder was vacuum-packed and the lowest storage temperature used was -20ºC which resulted in the degradation of 25% and 12% of astaxanthin in spray- and freeze-dried powder, respectively, over a 20 week-period. Our results have similarities with the findings of Miao *et al.* (2013) and Raposo *et al.* (2012) who also reported low astaxanthin degradation when stored at -18ºC to -21ºC. Unlike Miao *et al.* (2013) who vacuum-packed the samples, Raposo *et al.* (2012) flushed the powder in nitrogen gas as a means of removing air. The same authors also reported varying levels of astaxanthin degradation in powder dried at different inlet/outlet temperature setups of the spray-dryer, even though the samples were stored at the same condition. However, when using a similar inlet/outlet temperature regime (180ºC /110ºC) as Raposo *et al.* (2012), our study found lower astaxanthin degradation (4-6%; Fig 1A & 1C) in the vacuum-packed samples when compared to nitrogen-flushed powder (10%) reported by Raposo *et al.* (2012) on week 9. Our results therefore suggest potentially higher effectiveness of vacuum-packing for retention of astaxanthin from *H. pluvialis* powder.

Miao *et al.* (2013) reported that monoester and diester astaxanthin comprised 75% and 15% of total astaxanthin, respectively in the *H. pluvialis* cysts. This ratio of diester to monoester (0.2) is reported to increase in correlation with higher degradation of astaxanthin, due to the higher stability of astaxanthin diesters (Miao *et al.* 2013). In the present study, the diester to monoester ratio was below 0.2 on week 0 and remained below 0.2 in the powder stored at -20ºC, 4ºC, and 20ºC from both packaging and drying methods, with one exception (spray-dried normally packed powder at 20ºC on week 20). However, the astaxanthin degradation was more than 20% on many occasions under those storage conditions. Our results are

therefore in disagreement with the inference of Miao *et al.* (2013) that a value of around 0.2 of the ratio is a good indication of a proper preservation status of astaxanthin. Moreover, the diester to monoester ratio and the astaxanthin degradation rate in the powder stored at 37ºC indicate that both esters degrade at similar rates at such high temperatures and that diesters are also susceptible to degradation when they are exposed to high temperatures even in the absence of air.

We conclude that freeze-drying of *H. pluvialis* can yield more astaxanthin than spray-drying and freeze-dried powder can retain more astaxanthin under vacuum packaging and storage at -20ºC. The long-term exposure of the *H. pluvialis* cells to low temperature (-40ºC) during the freeze-drying process may have caused lower degradation of the temperature-sensitive astaxanthin, resulting in higher yields of these compounds. Moreover, the cost-benefit analysis showed that freeze-drying followed by vacuum-packing and storing at -20ºC could generate around AU$600 higher profit per 100 kg biomass than spray-drying. The results clearly show that freeze-drying, vacuum-packing and storage at -20ºC of *H. pluvialis* is a preferred procedure in terms of maximizing profit per unit biomass and retention of bioactive astaxanthin.

In addition to producing a high amount of astaxanthin (1-4% dry weight under optimum conditions; Spiller & Dewel, 2003), *H. pluvialis* is also a good source of protein (23%), carbohydrates (38%), lipids (13%) and various vitamins and minerals (4%; Lorenz, 1999). Based on the presence of high contents of astaxanthin, this alga is also an excellent source of antioxidants (Bhattacharjee, 2014). In this study, we primarily focused on the stability of astaxanthin from dried powder. However, due to the nutraceutical value of this alga, further studies should focus on the effect of drying methods on the nutrients (e.g. protein, lipids, carbohydrates), antioxidant

capacity, as well as the microstructures and physical properties of the dried powder, in order to obtain conclusive evidence on the most suitable drying method for *H. pluvialis* cultures.

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Figure caption

Figure 1. Degradation of total astaxanthin content (%) in relation to initial levels of (A) spray-dried and vacuum-packed, (B) spray-dried and normal-packed, (C) freeze-dried and vacuum-packed, and (D) freeze-dried and normal-packed *Haematococcus pluvialis* powder stored at -20ºC, 4ºC, 20ºC and 37ºC for 20 weeks. Data are means +/- SD, *n* = 3 independent measurements.

Table 1. Moisture, free, monoester, diester and total astaxanthin contents of spray-dried or freeze-dried *Haematococcus pluvialis* powder on week 0 before the initiation of storage treatments. Data are means +/-SD, *n* = 3 independent measurements; DW = dry weight.

Drying method	Moisture content (%)	Free astaxanthin (µg/g DW)	Monoester astaxanthin (µg/g DW)	Diester astaxanthin (µg/g DW)	Total astaxanthin (µg/g DW)
Spray-drying	6.86 ± 0.39	173.5 ± 2.5	3838 ± 8.8	567.05 ± 5.4	4578.6 ± 14.5
Freeze-drying	8.22 ± 0.28	235.1 ± 5.9	5350.4 ± 9.3	869.5 ± 10.1	6454.9 ± 23.8

Table 2. Three way ANOVA of 20 weeks-stored *Haematococcus pluvialis* powders.

	df	**Total astaxanthin**		**Free astaxanthin**		**Monoester astaxanthin**		**Diester astaxanthin**	
		F	***p***	**F**	***p***	**F**	***p***	**F**	***p***
Test of between-subjects effects									
Drying	1	327.7	<0.001	111.4	<0.001	332.6	<0.001	104.25	<0.001
Packaging	1	16.9	<0.001	11.1	<0.01	14.99	<0.001	10.43	<0.01
Temperature	3	127.8	<0.001	119.5	<0.001	129.94	<0.001	32.02	<0.001
Drying x packaging	1	0.337	0.565	0.672	0.418	0.262	0.612	0.912	0.347
Drying x temperature	3	6.3	<0.05	6.7	<0.001	5.29	<0.05	4.74	<0.01
Packaging x temperature	3	4.03	<0.05	3.99	<0.05	4.23	<0.05	0.99	0.411
Drying x packaging x temperature	3	1.75	0.175	2.05	0.127	1.94	0.142	0.68	0.572
Error 1	32								
Test of within-subjects effects									
Day	5	309.248	<0.001	181.618	<0.001	276.408	<0.001	142.056	<0.001
Day x Drying	5	7.911	<0.001	1.940	0.091	7.197	<0.001	10.083	<0.001
Day x Packaging	5	2.457	0.036	1.663	0.147	1.902	0.097	3.286	0.007
Day x Temperature	15	28.251	<0.001	23.642	<0.001	24.163	<0.001	22.851	<0.001
Day x Drying x packaging	5	1.712	0.135	0.308	0.907	1.121	0.351	4.881	<0.001
Day x Drying x temperature	15	4.843	<0.001	1.932	0.024	4.104	<0.001	40244	<0.001
Day x Packaging x temperature	15	0.567	0.897	0.991	0.467	0.556	0.904	1.074	0.384
Day x Drying x packaging x temperature	15	1.201	0.276	0.723	0.758	1.266	0.230	1.835	0.134
Error 2	160								

Table 3. Ratio of astaxanthin degradation ($at^2 + bt + c$), rates of astaxanthin degradation ($2at + b$), and acceleration of degradation constants ($2a$) at different temperatures, vacuum- and normal packaging of spray-dried and freeze-dried *Haematococcus pluvialis* powder.

Drying method	Packaging	Temperature (°C)	Ratio of astaxanthin degradation	Rate of astaxanthin degradation	Acceleration of degradation (x 10% of the content/week)	R^2
Spray drying	Vacuum	-20	$0.0184t^2+0.8827t+0.1466$	$0.0368t+0.8827$	0.0368	0.94
		4	$-0.0118t^2+1.8628t+2.6713$	$-0.0236t+1.8628$	0.0236	0.96
		20	$-0.1251t^2+4.5062t+5.3649$	$-0.2502t+4.5062$	0.2502	0.91
		37	$-0.2t^2+8.1015t+5.0211$	$-0.4t+8.1015$	0.4	0.98
	Normal	-20	$-0.0085t^2+1.8829t+3.4469$	$-0.017t+1.8829$	0.017	0.94
		4	$-0.0536t^2+3.2785t+4.6379$	$-0.1072t+3.2785$	0.1072	0.93
		20	$-0.0652t^2+4.7568t+9.2041$	$-0.1304t+4.7568$	0.1304	0.9
		37	$-0.2489t^2+9.0833t+4.2109$	$-0.4978t+9.0833$	0.4978	0.99
Freeze drying	Vacuum	-20	-	-	-	-
		4	-	-	-	-
		20	$-0.0205t^2+3.765t-2.9367$	$-0.041t+3.765$	0.041	0.97
		37	$-0.2169t^2+9.2238t-5.0592$	$-0.4338t+9.2238$	0.4338	0.98
	Normal	-20	$0.0228t^2+0.45t+0.5319$	$0.0456t+0.45$	0.0456	0.96
		4	$-0.1221t^2+4.2364t-0.385$	$-0.2442t+4.2364$	0.2442	0.98
		20	$-0.2281t^2+8.0858t-0.0933$	$-0.4562t+8.0858$	0.4562	0.99
		37	$-0.1977t^2+8.3356t+0.644$	$-0.3954t+8.3356$	0.3954	0.99

Table 4. Changes in the ratios of diester to monoester astaxanthin at different temperatures, vacuum or normal packaging of spray-dried or freeze-dried *Haematococcus pluvialis* powder during 20 weeks of storage. Data are means +/-SD, *n* = 3 independent analyses.

Drying method	Packaging	Temperature (°C)	Diester/monoester ratio					
			Week 0	Week 3	Week 6	Week 9	Week 12	Week 20
Spray drying	Vacuum	-20	0.148±0.001	0.138±0.03	0.142±0.02	0.139±0.01	0.149±0.005	0.149±0.01
		4	0.148±0.001	0.154±0.02	0.149±0.009	0.147±0.007	0.137±0.006	0.164±0.01
		20	0.148±0.001	0.114±0.003	0.127±0.003	0.132±0.005	0.159±0.002	0.189±0.02
		37	0.148±0.001	0.18±0.04	0.207±0.03	0.233±0.02	0.299±0.07	0.418±0.03
	Normal	-20	0.148±0.001	0.121±0.02	0.134±0.01	0.145±0.01	0.16±0.02	0.176±0.01
		4	0.148±0.001	0.111±0.02	0.127±0.02	0.136±0.02	0.155±0.02	0.183±0.02
		20	0.148±0.001	0.116±0.01	0.122±0.01	0.127±0.01	0.145±0.002	0.251±0.08
		37	0.148±0.001	0.215±0.11	0.239±0.04	0.226±0.02	0.149±0.006	0.242±0.02
Freeze drying	Vacuum	-20	0.163±0.002	0.132±0.02	0.136±0.01	0.144±0.008	0.151±0.005	0.157±0.008
		4	0.163±0.002	0.144±0.04	0.144±0.03	0.147±0.02	0.15±0.008	0.154±0.01
		20	0.163±0.002	0.108±0.02	0.112±0.02	0.114±0.008	0.124±0.005	0.154±0.005
		37	0.163±0.002	0.208±0.07	0.2±0.08	0.182±0.05	0.182±0.01	0.243±0.01
	Normal	-20	0.163±0.002	0.108±0.02	0.131±0.01	0.148±0.01	0.168±0.01	0.177±0.02
		4	0.163±0.002	0.103±0.02	0.121±0.01	0.145±0.008	0.154±0.01	0.156±0.005
		20	0.163±0.002	0.101±0.01	0.108±0.01	0.112±0.01	0.121±0.01	0.13±0.007
		37	0.163±0.002	0.153±0.01	0.143±0.02	0.131±0.02	0.193±0.006	0.234±0.04

Table 5. Cost-benefit analysis of spray-drying and freeze-drying of 100 kilogram *H. pluvialis* powder for long term storage.

Drying method	Energy cost of drying powder (AUD)[a]	Packaging	Temperature (ºC)	Retail value of astaxanthin (Week 0)[b]	Retail value of astaxanthin (Week 20)[c]	Profit margin at week 20 (AUD: retail value – energy cost of drying powder)
Spray-drying (APV PSD 55 Spray dryer)	(50 kWh x 0.27916) + 0.91755 (daily service fee) = 14.9	Vacuum	-20	1380	1035	1020.1
			4	1380	880	865.1
			20	1380	730	715.1
			37	1380	170	155.1
		Normal	-20	1380	840	825.1
			4	1380	690	675.1
			20	1380	260	245.1
			37	1380	180	165.1
Freeze-drying (F100 Freeze Dryer)	(360 kWh x 0.27916) + 0.91755 = 101.4	Vacuum	-20	1950	1720	1618.6
			4	1950	1400	1298.6
			20	1950	740	638.6
			37	1950	180	78.6
		Normal	-20	1950	1580	1478.6
			4	1950	1270	1168.6
			20	1950	585	483.6
			37	1950	234	132.6

a = based on the current price of electricity in Queensland, Australia (http://www.dews.qld.gov.au/energy-water-home/electricity/prices/current-prices) accessed 28 October, 2014. AUD $1 equals approx. USD $0.82 or EUR €0.67.
b = based on the astaxanthin content obtained in the current study (4.6 mg/g from spray-drying and 6.5 mg/g from freeze-drying) assuming a retail value of astaxanthin of $3000/kg.
c = based on the astaxanthin degradation rate observed in the current study.

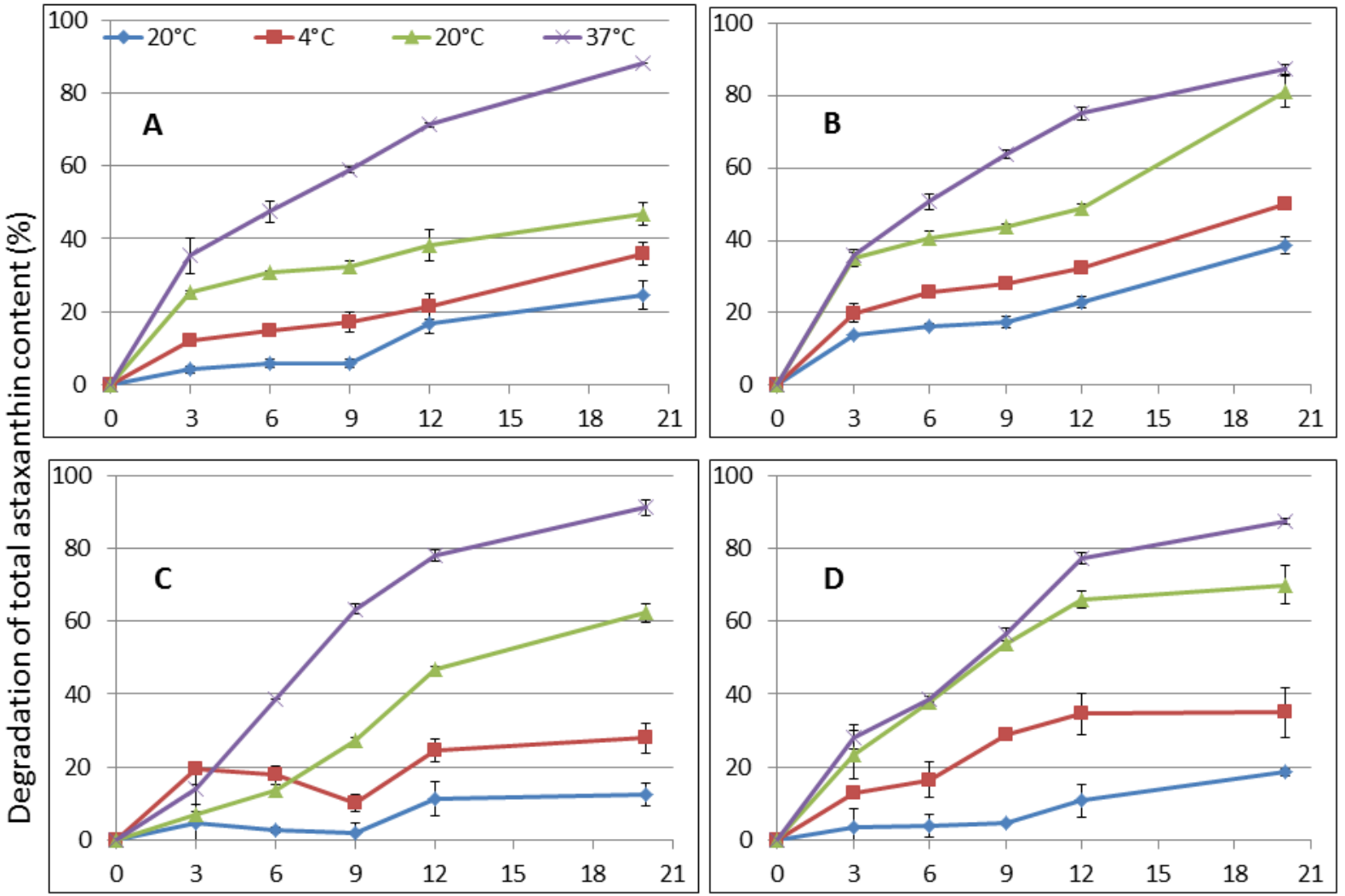

Figure 1

Highlights

- Freeze-drying led to higher astaxanthin recovery from microalgae than spray-drying
- Freeze-drying can increase the shelf life of astaxanthin
- Storage below 4ºC and vacuum packing showed higher astaxanthin stability
- Freeze-drying and vacuum-packed storage at -20 ºC can generate higher profits