Ecovalorization of *Haematococus pluvialis* using carbon dioxide expanded ethanol

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1. Introduction

Astaxanthin has been approved as a food supplement by FDA (Food and Drug Administration) since 1999. Among all of the microalgae that can produce astaxanthin, *Haematococcus pluvialis* produces the most. It could produce up to 4% per dry mass of microalgae. Quantum Synergy Solutions (QSS) is a young Canadian company that aims to produce astaxanthin. QSS developed modular microalgae production systems in photobioreactors as well as extraction and purification methods specifically adapted to the selected strains. The developed method **CO_2 expanded ethanol** was compared with a supercritical fluid extraction (SFC) method and a conventional solid-liquid extraction method (SLE).

2. Materials and Methods

2.1. Conventional extraction

Conventional extraction[1,2] was carried out as the reference, to compare with supercritical extraction (SFC) and CO_2 expanded ethanol.
200 mg of microalgae were mixed with 20 ml of acetone containing 0.1% (w/v) of butylated hydroxytoluene in a 50 ml Falcon tube protected from light by a sheet of aluminum and stirred at 500 rpm for 24 hours in a thermostatic bath at 20°C. The mixture was then centrifuged for 10 minutes at 4° C at 10,000 rpm followed by separation of the supernatant. The solvent was then evaporated by a nitrogen stream.

2.2. Supercritical extractions

A design of experiments was developed using the following factors (see Table 1). The measured response was the concentration of astaxanthin in the extract and in the raw material [1,3].

Table 1. Experiment plan

Experiment	Pressure (bar)	Temperature (°C)	Ethanol (%)
1	300	50	5
2	300	50	15
3	300	70	5
4	300	70	15
5	500	50	5
6	500	50	15
7	500	70	5
8	500	70	15
9	400	60	10
10	400	60	10
11	400	60	10

- ✓ Pressure: 300-500 bars, Temperature: 50-70°C, % ethanol: 5-15%.
- ✓ The extraction time was set at 4 hours
- ✓ The CO_2 flow rate was set at 5g/min considering the scale-up factors
- ✓ Mass: 25 grams of microalgae powder.

2.3 Carbon dioxide expanded ethanol

We also worked using the "CO_2 expanded ethanol" mode, i.e. injecting a mixture of ethanol and liquid CO_2 (at 70 bars) into the reactor[4,5]. By using this technique, we could obtain more interesting astaxanthin recoveries ranging from (90-100%) with an ethanol percentage of 30 to 50% in CO_2 and a pressure of 700 bars.
Operating conditions:

Raw material (g)	P (bar)	T °C	Time (h)	CO_2 Flowrate (g/min)	Ethanol Flowrate (g/min)		
25	70	45	4	5	2.14 (30 %)	3.33 (40%)	6.3 (50%)

3. Results and discussion

Several types of conventional extraction have been performed. They all give an astaxanthin value of around 4%, as indicated in the literature[1,3].

The supercritical extraction gives an astaxanthin yield of 3.45% g/g of raw material with a recovery rate of 78.5%, compared to a conventional extraction with acetone (SLE), which is of the order of 4.25%. Extraction with the "CO_2 expanded ethanol" mode gives better results since a yield of 4.2% is obtained with a recovery rate of 95.5%.

4. Conclusions

It has been shown that the carbon dioxide expanded ethanol could provide faster and more efficient extraction than SFC and SLE methods.

This approach made it possible to optimize the complete process of producing microalgae oil rich in 10% astaxanthin and polyunsaturated fatty acids. The oil was encapsulated in capsule form and marketed for the natural health product (NHP) market.

References

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