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Supercritical Fluid Extraction of β-carotene from D. Salina Algae using C_2H_6 and C_2H_2

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Abstract. An experimental design procedure was used to investigate the response of the extractability of β-carotene from D. Salina algae using supercritical ethane and ethylene and making used of supercritical carbon dioxide as basis. A factorial experimental design was employed to establish the effects of the operating parameters such as temperature and pressureusing a dynamic extraction sequence. A three level approached was employed for each temperature and pressure and the experiments were repeated twice to ensure reliability of results. The extract was analyzed using a reversed-phase high-performance liquid chromatography where β-carotene is expected to elute in approximately 16 minutes with peaks at 450 nm. The mobile phase was a mixture of MtBE (methyl ter-butyl ether) and MetOH (methanol) on a 80:20 ratio. The solubilities of β-carotene in SCF carbon dioxide are far lower than those measured at SCFs ethane and ethylene as expected and predicted at differentTrand Prvalues. Ethane and ethylene make a better solvent than CO2 by virtue of their greater polarizabilities for β-carotene. Yield of β-carotene during the first 20 mins of extraction reached as high as 59% w-β-carotene/w-β-carotene in algae.

Keywords: Supercritical fluid, β-carotene, extraction, solubility

1. Introduction

Carotenoid pigments of the class polyenes represent the most unsaturated mass products of biosynthesis and ranged in color from yellow to red-orange. The carbon skeleton of carotenoids is highly branched and composed of isoprenic building blocks.

β-carotene belongs to a family of carotenoids along with α-carotene, lutein and other carotenes and is believed to be the principal precursor to vitamin A production. Ideally, one β-carotene molecule can produce two vitamin A molecules, however because of isomerization and oxidation, this is seldom observed. The pigment is being used as an essential component of many food supplements and dairy industries. Its potential for expanded applications to food industries is seen to grow and it brought concerns in the past because the pigment is traditionally extracted using solvent extraction. The danger pose by the presence of trace solvents, even after rigorous back extraction, evaporation and drying, led to the use of supercritical fluids as a more viable alternative. Aside from the presence of trace solvents to extract, the process requires heating, which facilitates the oxidation and isomerization of β-carotene, a known is a thermo-labile pigment.

Supercritical state is achieved by a pure component when its temperature and pressure reached values beyond the critical values of the substance. The gaseous phase and the liquid phase become identical ((Brunner, 1984; Taylor, 1990). In this region, the solvating power of the supercritical fluid, as determined by its density, is strongly dependent on the pressure and temperature. Such dependencies are very attractive as a tunable process solvents or reaction media where the basic philosophy of utilization is very much

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centered on the notion that supercritical fluid properties can be controlled using pressure and temperature as adjustable parameters (Kiran and Brennecke, 1993).

Solubility defines the ability of a solvent to dissolve or solvate a solute upon contact and generally a function of temperature and pressure for fluid-solid system. In supercritical fluid system, the solubility is expressed in terms of the supercritical fluid density, which at values of temperature and pressure above gas critical values is comparable to liquid.

Supercritical fluids are very good extraction medium due to their high molecular diffusivity, low viscosity, and liquid like densities, which provide wetting and solvating power comparable to the liquid. The most common and favored supercritical fluid used is carbon dioxide primarily because it is environmentally benign, readily available, non-toxic and cheap. However, its capacity to dissolve high molecular weight, polar pigment is limited and even after successive extraction; extract yield is not commensurate to the loading. This limiting behavior of carbon dioxide made the supercritical fluid extraction of β-carotene a challenge even a modifier like ethanol is added.

The researcher found it interesting to investigate the extraction of β-carotene using supercritical ethane and ethylene and compared the results to carbon dioxide. Ethane and ethylene have low or comparable critical parameters (critical temperature and critical pressure) to that of carbon dioxide, which allow processing at room temperatures.

2. Methodologies

2.1. Materials

- all-trans β-carotene; crystalline; synthetic was purchased from Sigma Chemicals Co., St. Louis, MO.
- Dunaliella Salina Algae was provided by Cyanotech Corporation, Kailua-kona, Hawaii.
- Carbon Dioxide, bone-dry, and research grade was supplied by Proxair Inc., Danburry, CI.
- Ethane, analytical grade was supplied by Union Carbide Corporation, Linde Division, Danburry, CI.
- Ethylene, analytical grade was supplied by Union Carbide Corporation, Linde Division, Danburry, CI.
- Hexane, 99%+ was bought from Aldrich, 1001 W. St. Paul Avenue, Milwaukee, WI.
- Methyl-ter-butyl ether, 99.8%+ HPLC grade was bought from Aldrich, 1001 W. St. Paul Avenue, Milwaukee, WI.
- Methanol, 99.9%+ HPLC grade was brought from Aldrich, 1001 W. St. Paul Avenue, Milwaukee, WI.

2.2. Extraction

- ISCO SFX 220 Supercritical Fluid Extractor
- ISCO 500D Syringe Pumps
- Brooks Mass Flow Meter (2slpm capacity)
- Deactivated Fused Silica Tubing from J and W Scientific, 91 Blue Ravine Rd. Folsom, CA

2.3. Analysis

- Waters 996 Photodiode Array Detector
  - C30 HPLC Reverse Phase
  - Millennium Software for Carotenoids
  - Waters 2690 Separation Modules
- UV-vis Spectrophotometer, Varian Cary 5

3. Results and Discussion

3.1. Solubility

The extraction system is composed of ISCO SFX 220 Supercritical Fluid Extractor, which allows both static and dynamic extraction programming. The supercritical fluid feed to the system issupplied by two ISCO 500D syringe pumps connected in series to each other, which ensure sufficient and ample supply of solvent during extraction. The temperature in the extraction vessel is kept constant during the entire extraction process and this was maintained by keeping part of the supply line immersed in a water bath prior to contacting the solute.

Syringe pumps are excellent feed-system to the extractor because of its ability to regulates changes in pressure due to sudden dropped as a result of changes in temperature. The pressure in the apparatus must be maintained at constant value during each experimental determination. This is most easily achieved using a pump that is equipped with a feedback pressure control facility (e.g. ISCO 260D and ISCO 500D syringe pumps).

It has been studied that dynamic method of solubility determination is sufficient as long as SCF flow rates is between 60 cm3/min up to 500 cm3/min (Temelli, 1998).

The solubility of pure β-carotene in supercritical carbon dioxide at carefully selected temperatures and pressures are determined and compared to published literature findings (Johannsen and Brunner, 1997; Jay and Steytler, 1992)in order to establish the reliability and validity of the system. Once the desired reliability is achieved, the solubilities of β-carotene in supercritical ethane and ethylene aredetermined. The process made used of identical reduced temperatures *(Tr)* and reduced pressure *(Pr)* instead of identical or similar temperature and pressure. The rationale behind this is the theory of corresponding state, which was also verified by the author in the earlier stage of the study.

The process made use of three (3) levels for each set of temperature and pressure and was repeated twice to ensure repeatability.

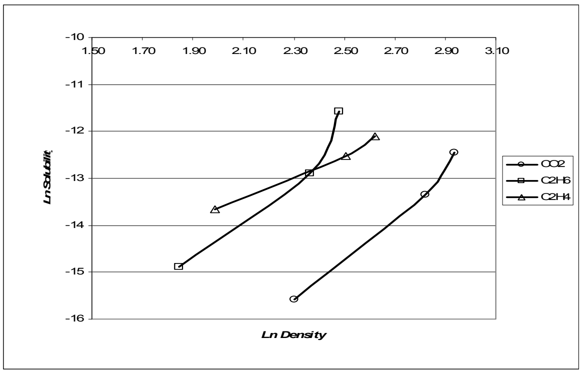

Fig. 1.0 Comparative solubility of β-carotene at different solvent at *Tr* = 1.06

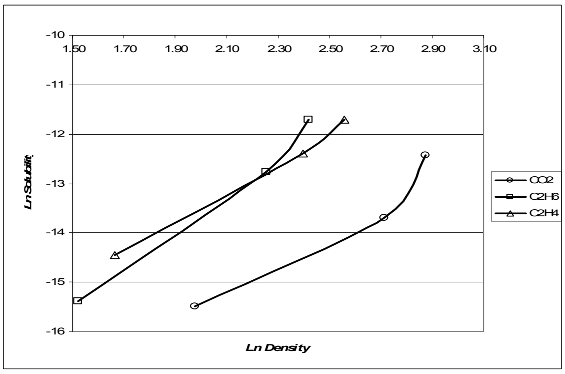

Fig. 2.0 Comparative solubility of β-carotene at different solvent at *Tr* = 1.09

As depicted in the figures, the solubilities of β-carotene in SCF carbon dioxide are far lower than those measured at SCFs ethane and ethylene at two *Tr* values, as expected and predicted. Ethane and ethylene make a better solvent that CO2 by virtue of their greater polarizabilities for β-carotene. A non-polar molecules like ethane and ethylene has no permanent dipole moment but when subjected to an electrical field, the electrons are displaced from their ordinary positions and a dipole is induced which give rise to polarizability.

Small amount of extracts was obtained in the low pressure due to the limiting solvating power of the supercritical fluids. This can be attributed to the inability of the supercritical fluids to attain liquid-like density and dissolve much of the solute.

Based on the results gathered, the researcher can generalize that both ethane and ethylene are better solvents than carbon dioxide for high molecular weight, non-polar solute like β-carotene.

There are two competing factors that to a greater extent affect the solubility of solids in supercritical fluids. These are supercritical fluid density and the solid sublimation pressure (Bruno, 1991). An increase in either parameter while holding the other parameter constant will result in enhanced solubility. Density affects the solute-fluid interaction while increase in the solute sublimation pressure decreases the solute-solute interaction.

3.2. Extraction

The calibration curve for all-trans β-carotene is generated and used in quantitative analysis of the extract. A stack solution was prepared and successively diluted to a fairly low concentration. The wavelength is monitored at 450 nm using a UV-vis spectrophotometer. Afterwards, the same set of solutions was dried up using liquid nitrogen and was dissolved in 50:50 ratios of MtBE and MetOH. The resulting solutions were then injected in the HPLC column equipped with C30 reversed-phase where β-carotene was expected to come out at around 16+ minutes. The mobile phase used was MtBE (methyl ter-butyl ether) and MetOH (methanol) on a 80:20 ratio.

Sample of *D. Salina* algae is kept refrigerated to retain the integrity of the sample. The sample is very fine, almost powdery and has uniform consistency, which allows easy loading to extraction cell. Once the sample is loaded to extraction chamber, the system is pressurized to the desired pressure and desired temperature. The extraction is conducted using dynamic sequence with the flow rate, being regulated by a silica flow restrictor, low enough to ensure saturation of the solvent prior to depressurization.

During extraction, samples of extract were collected at specific time interval to determine the amount of solute extracted over period of time.

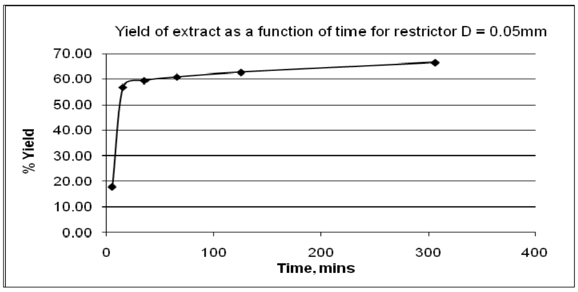

Fig 3.0 Extraction of β-carotene from algae

β-carotene seems to be loosely bound to the matrix of algae which make it is easily available for extraction. This is evident in the steep increase in the extraction profile during the first 5 to 20 mins of extraction time.

Extractions of β-carotene from algae using supercritical ethane were conducted at different temperatures and high-density values where solubilities are expected to be greatest. In terms of extractability from algae at different conditions, the density of the supercritical solvent affects the extraction of algae in increasing trends as can be seen in Fig 4.0. As such, we can expect more extract at higher density of supercritical ethane. In essence, the solvating power of the supercritical solvent as expressed by its density affects the extractability more than the vapor pressure of the solute.

In the extraction of β-carotene from the algal matrix making use of supercritical ethylene, β-carotene's yield is continuously increasing as the density of the supercritical solvent increases as shown in Fig. 4.0. However, the increase is not as sharp as the increase in ethane.

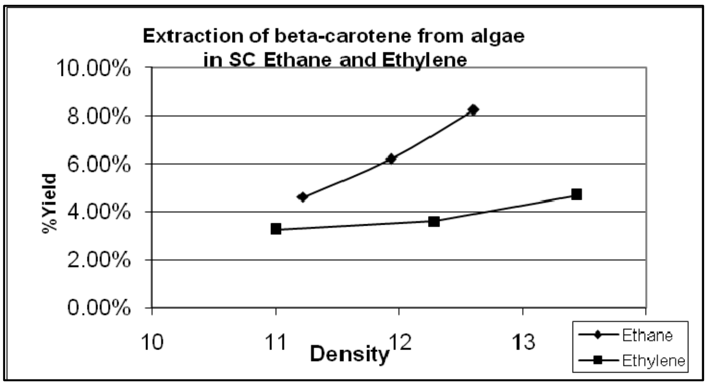

Fig 4.0 Extraction of β-carotene from algae by SCF ethane and ethylene

4. Conclusion

The solubility of the non-polar β-carotene in supercritical ethane and ethylene are higher than those in carbon dioxide at the same reduced temperature and reduced density. The values of solubilities at high temperature and high pressure for all the solvents investigated are the greatest because of the coupled effects of high solvating power of the supercritical fluids at high pressure and high solute vapor pressure at high temperatures.

Ethane and ethylene make better supercritical solvents than carbon dioxide by virtue of their greater polarizabilities which resulted in an induced dipole. The induced dipole served as an attractive force between the solute and solvent molecules and made the solvent to solubilize the solute molecules.

The yields of β-carotene from algae are higher at higher supercritical fluid densities which is a manifestation of the response on the changes in temperature and pressure of the system.

5. Acknowledgement

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