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Separation of Lycopene/Solvent Mixture by Chitosan Membranes

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Abstract

Acetone in view of lower toxicity could be used as extracting solvent of lycopene in tomato paste instead of hexane. The studied chitosan membranes were uncrosslinked and crosslinked types. It was found that the membrane morphology could be changed from porous to dense structure by increasing evaporation temperature or time or by crosslinking. They showed hydrophilicity but not preferred to both acetone and lycopene. The lycopene/acetone mixture could be separated by using water swollen chitosan membrane in pervaporation process. It was found that all studied membranes; i.e., both uncrosslinked and ionically crosslinked membranes prepared at 40 to 60°C for 2 to 6 hours, could separate acetone from lycopene solution as permeate with rejection of 100% or separation factor of infinity. The uncrosslinked membrane prepared at 40°C for 2 hours provided the highest acetone flux of 0.131±0.004 L m^{-2} h^{-1} by operating at –1 bar and 15 ppm feed concentration.

Key words: Chitosan membrane, Lycopene/solvent mixture, Pervaporation

Introduction

Lycopene is a 40-carbon acyclic open-chain carotenoid found in fruits and vegetables with red color, such as tomatoes and watermelon. Its molecular weight is about 536.88 Daltons. It has attracted attention due to its biological and physicochemical properties, especially related to its effects as a natural antioxidant. Lycopene in fresh tomato fruits occurs essentially in the all-*trans* configuration. The main causes of tomato lycopene degradation during processing are isomerization and oxidation. Isomerization converts all-*trans* isomers to *cis*-isomers due to additional energy input and results in an unstable, energy-rich station. For processing, tomatoes are washed, sorted, and sliced. Sliced tomatoes undergo a hot- or cold-break method for juice preparation. Juice from tomatoes is usually obtained using screw or paddle extractors. In the manufacturing of other tomato products such as pulp, puree, paste, and ketchup, tomato juice is concentrated with steam coils or vacuum evaporators. For canned tomatoes, sliced or whole tomatoes are retorted. For dried tomato slices and powder, tomatoes undergo a dehydration process. The thermal processing generally causes some loss of lycopene in tomato-based foods. Heat induces isomerization of the all-*trans* to *cis* forms. The *cis*-isomers increase with temperature and processing time [(5)]. Solvent extraction is one of the oldest methods of separation known. Because lycopene is liposoluble, it is usually extracted with organic solvents such as chloroform, hexane, acetone, benzene, petroleum ether, or carbon disulfide. Recently, supercritical fluid CO_2 has been applied. However, the capital costs necessary for the extraction setup are high and this limits the application of this technique both for research and industrial scale-up [(3)].

An environmentally friendly extraction and purification procedure on an industrial scale with minimal loss of bioactivity is highly desirable for the food, feed, cosmetic, and pharmaceutical industries. Taungbodhitham et al. [(6)] showed that two solvents of low biological hazard, ethanol and hexane, were the most suitable for extracting carotenoids from the matrix. The use of double extraction, each with 35 ml of ethanol:hexane mixture (4:3 (v/v)), resulted in good recoveries of carotenoids (lycopene 96%, α-carotene 102% and β-carotene 93±100%). Barba et al. [(1)] showed that a

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hexane:acetone:ethanol ratio of 2:1:1 (v/v/v) was the best solvent, with a virtually complete extraction of carotenoids. However, after extraction, the solvents were evaporated to dryness and this is problematic for the thermolabile lycopene. Further to this, the Threshold Limit Value – Time Weighted Average (TLV-TWA) of hexane, most effective lycopene solvent, is as low as 50 ppm. The objective of this study is to find an alternative friendly solvent for substitution of hexane. Membrane separation technologies are then applied to recover solvent. Lycopene could then be crystallized out without a heat evaporation step. Amongst the different membrane separation processes, either nanofiltration or pervaporation would be likely suitable methods in this case. Nanofiltration is a pressure driven process governed by the size exclusion mechanism (membrane) to retain molecules of 100 – 1000 Daltons in size, whereas pervaporation is based on the difference in sorption and diffusion properties of the feed components as well as the permselectivity of the membrane which maintains the vacuum in the downstream side of the apparatus. Chitosan is an insoluble derivative of chitin which can be distracted from shrimp and prawn shells. The chemical nomenclature of chitosan is Poly–β–(1,4)–2–amino–2–deoxy–D–glucose and the formula is $C_8H_{11}O_4N$. Chitosan is obtained through deacetylation of chitin in which single molecule of chitin (N–acetyl glucosamine) is converted into glucosamine. Deacetylation enables chitosan to be very active and can react with secondary amine and secondary alcohol [(4)]. Chitin is not soluble in water, dilute acid, dilute base, concentrated alcohol or solvent but will be soluble in concentrated hydrochloric, sulfuric, phosphoric or formic acids. Solubility depends on molecular densities and different functional groups. Chitosan is not soluble in water, base or organic solvent but can be dissolved in all organic acid. Acetic and formic acids are generally used. Chitosan is of interest in this research in view of its non-toxic biopolymer property.

Materials and Experimental Procedures

Materials

Chitosan with a deacetylation degree of 90±5 % was supplied domestically. Sulfuric acid (98%), sodium hydroxide (50%) and acetic acid (99.5% (w/w)) were commercial grade. Tomato pastes were purchased from a local supermarket. Commercial grade hexane, acetone and ethanol were purchased from local suppliers.

Lycopene Extract

Three solvents were used for comparison of extraction efficiency: (1) acetone, (2) ethanol, and (3) hexane. Tomato pastes were submitted to extraction with each solvent (1:10 v/v) by means of shaker for 30 minutes. The extraction was performed at room temperature with light protection by covering the vessels with aluminum foil. The lycopene contents were assayed for purity and concentration by UV–Vis spectroscopy at 472 nm (maximum of absorption for lycopene).

Membrane Preparation

Flat sheet chitosan membranes were prepared from 3wt% chitosan solution in 3% acetic acid (w/w). The 17–18 g of chitosan solution was cast onto the 15×15 cm^2 glass plates. The cast films were dried in oven with varying temperature and time. The evaporation temperature and time were varied from 40 to 60°C and 2 to 6 hours, respectively. The formed membranes were immersed in 4% (w/w) sodium hydroxide for 24 hours and then washed with water and dried at room temperature before using. The ionically crosslinked chitosan membranes were prepared by immersing uncrosslinked chitosan membranes in 4% (w/w) sulfuric acid at room temperature for 24 hours and then washed with water and dried at room temperature before using.

Membrane Characterization

The chemical structure of membranes was investigated by Fourier Transform Infrared Spectroscopy (Thermo, DF3C206A). The cross-section morphology of membrane was observed by scanning electron microscopy (Jeol JSM 6400). Pure water flux was measured at three different transmembrane pressures ranging from 100 – 300 psi. To indicate the degree of hydrophilicity of a membrane, equilibrium water content absorbed in membrane at room temperature was calculated by using Eq. (1). The membrane performances were performed by nanofiltration and pervaporation processes. A high pressure pump was used in the upstream side for the nanofiltration process, whilst a vacuum pump was used on the downstream side for pervaporation, as schematically shown in Figure 1. From the nanofiltration data, the flux (J) and rejection (R) were calculated by using Eqs. (2) and (3), respectively. The separation selectivity ($\alpha_{i/j}$), following Eq. (4), was used instead of Eq. (2) for evaluating the pervaporation performance.

$$\text{water content (\%)} = \frac{\text{weight of wet membrane} - \text{weight of dry membrane}}{\text{weight of dry membrane}} \times 100 \quad (1)$$

$$\text{Flux (J)} = \frac{Q}{A} \quad (2)$$

$$\text{\%Rejection (R)} = 1 - \left(\frac{C_{ip}}{C_{if}}\right) \times 100 \quad (3)$$

$$\alpha_{i/j} = \frac{C_{ip}/C_{jp}}{C_{if}/C_{jf}} \quad (4)$$

Where Q is the volume flow rate of permeate (l hour^{-1}), A the effective membrane area (m^2), C (ppm) the concentrations, and the subscripts i, j, p and f represented component i (more permeable component) and j (less permeable component) in the permeate (p) and feed (f), respectively.

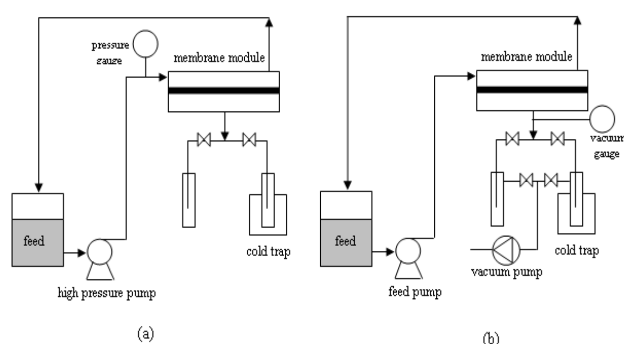

Figure 1. Schematic views of the apparatus for the (a) Nanofiltration and (b) Pervaporation processes.

Results and Discussion

Lycopene Extraction

The absorbance spectra of the extracted lycopene were shown in Figure 2. The extracted lycopene in acetone provided three characteristic peaks at 445, 471 and 500 nm. The use of hexane showed similar characteristic peaks at 445, 471 and 502 nm. They were absolutely consistent with those recorded for the standard lycopene as presented in Table 1. On the contrary, the UV–Vis spectra of the extracted lycopene from ethanol were different from the others. It could be stated that acetone with lower toxicity (TLV-TWA 500 ppm) was successfully used as extracting solvent of lycopene in tomato paste. The lycopene concentration of 37.5±3.2 ppm obtained from the extraction step was diluted to 15 ppm for studying the membrane performance throughout the experiments reported here.

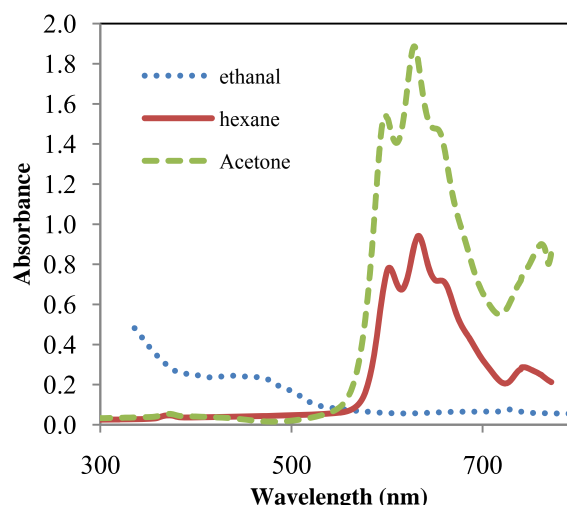

Figure 2. UV–Vis spectra of lycopene extracts from three different solvents.

Table 1. Absorbance spectra of lycopene extracts and standard lycopene samples.

Solvent	λ_1 (nm)		λ_2 (nm)		λ_3 (nm)	
	Standard[a]	Sample	Standard[a]	Sample	Standard[a]	Sample
Acetone	445	445	473	471	505	500
Hexane	445	445	471	471	502	502

[a]Data from Naviglio et al. (2)

Membrane Structure and Characteristics

Figure 3 showed the IR spectra of uncrosslinked and ionically crosslinked chitosan membrane. The NH_2 bending vibration at 1592 cm^{-1} of uncrosslinked chitosan membrane was changed to double peaks of NH_3^+ at 1533 and 1635 cm^{-1} in ionically crosslinked chitosan membrane. The broadened band between 3635 – 3150 cm^{-1} due to the interaction of NH_3^+ and SO_4^{2-} and the S–O band at about 618 cm^{-1} appeared in ionically crosslinked chitosan membrane.

The membrane morphology of uncrosslinked membrane evaporated at 40°C for 2 hours in Figure 4(a) showed the loose structure throughout the cross section whilst that evaporated for 4 hours in Figure 4(b) showed asymmetrically with the existence of a dense top layer or at least a finely porous thin skin layer. It appeared dense throughout the cross section when increasing the evaporation time to 6 hours in Figure 4(c). However, all the ionically crosslinked membranes provided dense structure. The pure water flux at 100 psi in Figure 5 showed correspondingly with highest flux in the uncrosslinked

membranes evaporated either at 40°C or 50°C for 2 hours and the least pure water flux in the ionically crosslinked membranes. The water permeabilities (obtained from the slope of the plot of pure water flux vs. driven pressure) of each membrane shown in Table 2 indicated more clearly that the dense structure in uncrosslinked membrane would be obtained by evaporating either at 50°C for at least 6 hours or at 60°C for at least 2 hours, otherwise by crosslinking the membranes with sulfuric acid solution. The different membrane structures had a significant influence on the membrane suitability for nanofiltration or pervaporation. The membranes with porous structure would be tested to separate lycopene molecules from hexane by nanofiltration process but dense structure would be used in pervaporation process.

The molecular weight cutoff (MWCO) curves in Figure 6 showed that the tentative chitosan membrane for separation of lycopene by nanofiltration process should be crosslinked type prepared by evaporating at 60°C for at least 8 hours with the MWCO of about 400 Da. Its water permeability was 0.002 L m^{-2} h^{-1} psi^{-1}. Too high MWCO obtained from other membranes although their dense structure and low water permeability was due to the high hydrophilicity of chitosan membrane as characterized in term of equilibrium water content as shown representatively in Figure 7.

Membrane Performances

By using conventional dry membranes, the experimental results showed that the flux was zero for all membranes either by the nanofiltration or pervaporation process. After equilibrating the membranes in water, it was found that only the uncrosslinked membranes evaporated either at 40°C or 50°C for 2 hours gave the permeate flux of 3.3±1.6 and 0.4±0.1 L m^{-2} h^{-1}, respectively, at the driven force of 200 psi and 15 ppm feed concentration. Their rejections according to Eq. (2) were as low as 2.6±3.7% and 39.5±13.1%, respectively. However, using pervaporation, in which the solvent could be vaporized by the action of a vacuum pump at room temperature and permeated through the membrane, complete separation was obtained with the results shown in Table 3. On FTIR examination of the pervaporation permeate as shown in Figure 8, the C=O of acetone molecule at 1701 cm^{-1} and broad peak at 3500 cm^{-1} of O–H in water molecule were found. It could be stated that only the acetone was evaporated out of the lycopene extracts and permeated through the membrane with water molecules absorbed in swollen membranes.

Table 2. Pure water permeability of chitosan membranes in L m^{-2} h^{-1} psi^{-1}.

Evaporation Condition	Uncrosslinked membrane			Ionically crosslinked membrane		
	40°C	50°C	60°C	40°C	50°C	60°C
2 h	0.039	0.012	0.008	0.006	0.007	0.005
4 h	0.027	0.014	0.006	0.004	0.006	0.002
6 h	0.018	0.007	0.007	0.005	0.005	0.004

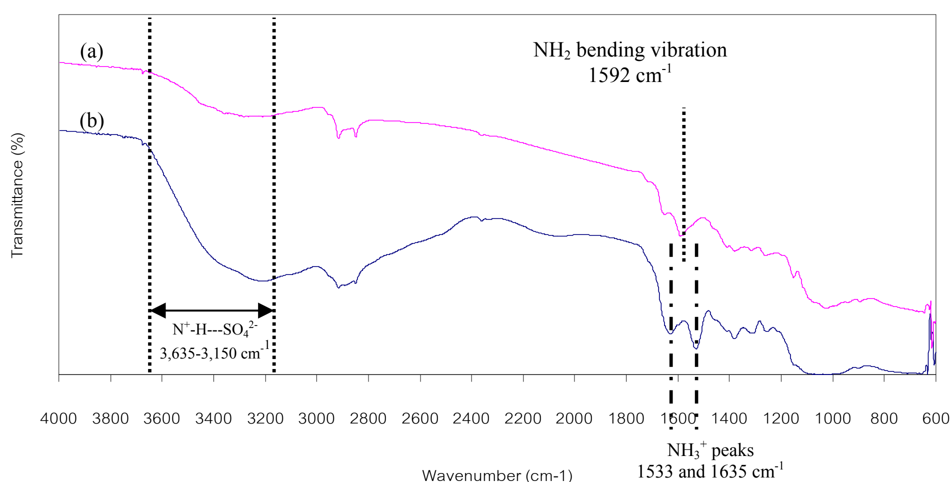

Figure 3. Infared spectrum of chitosan based membrane (a) uncrosslinked chitosan membrane (b) ionically crosslinked chitosan membrane.

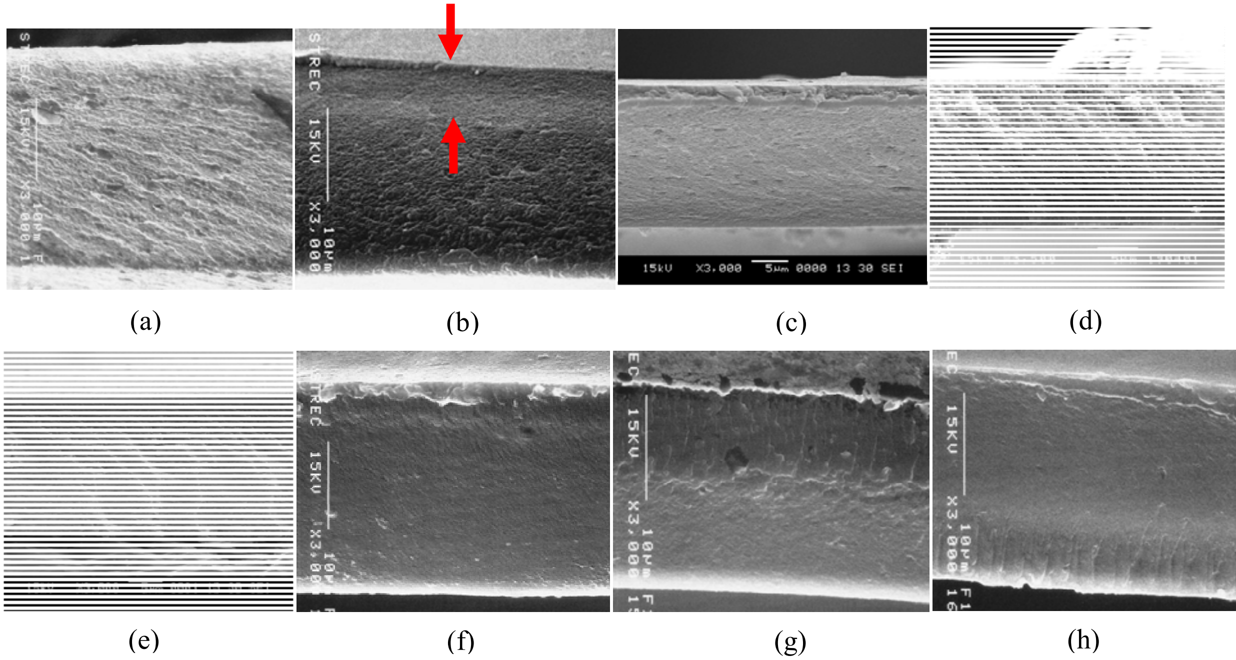

Figure 4. Cross-section morphologies of membranes from various conditions
(a) 40˚C, 2 hours uncrosslinked (b) 40˚C, 4 hours uncrosslinke (c) 40˚C,6 hours uncrosslinked
(d) 40˚C, 2 hours ionically crosslinked (e) 40˚C, 4 hours ionically crosslinked (f) 60˚C, 2 hours uncrosslinke
(g) 60˚C, 2 hours ionically crosslinked (h) 60˚C, 8 hours ionically crosslinked

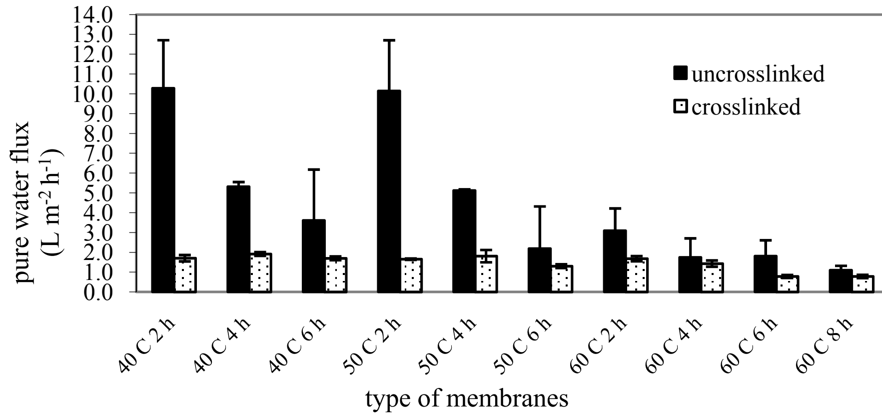

Figure 5. Pure water flux at 100 psi of membranes from various conditions.

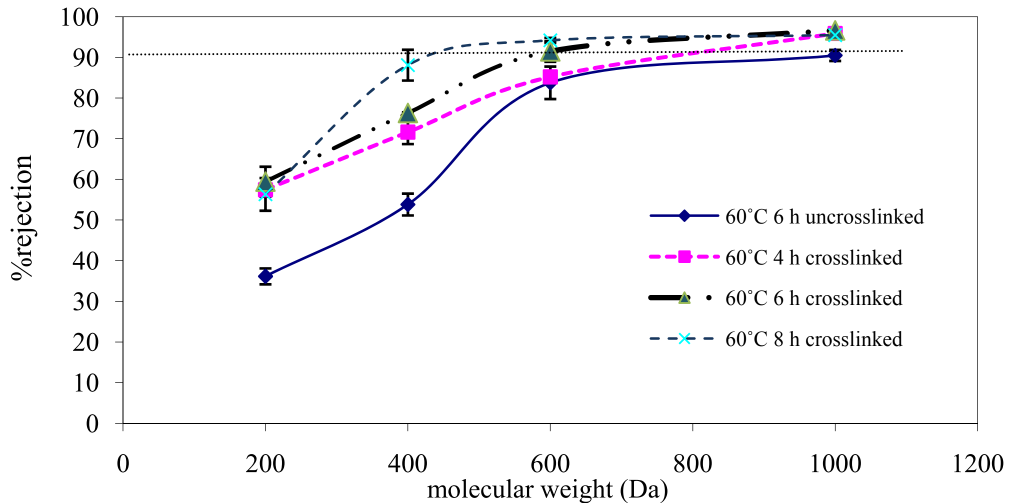

Figure 6. Molecular weight cutoff curve of membranes tested with PEG 1000 ppm.

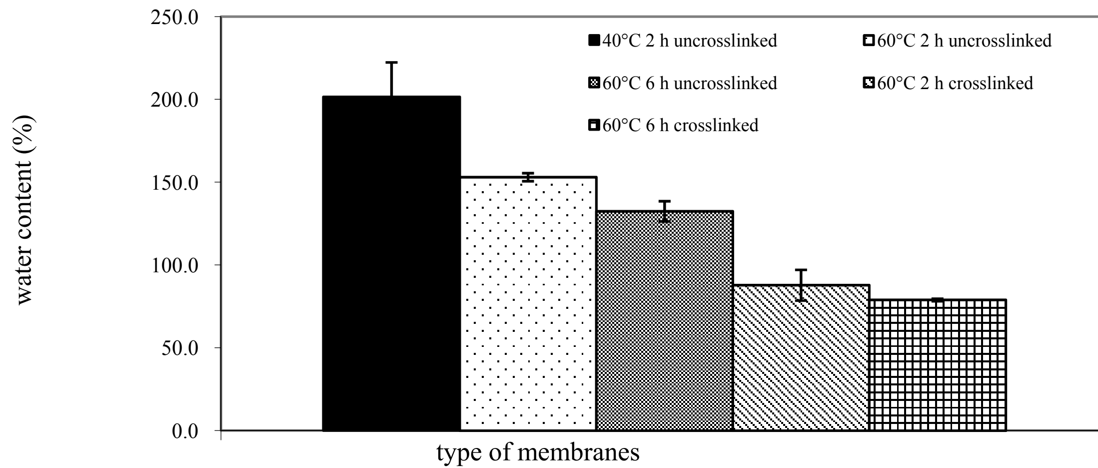

Figure 7. Equilibrium water contents in membranes.

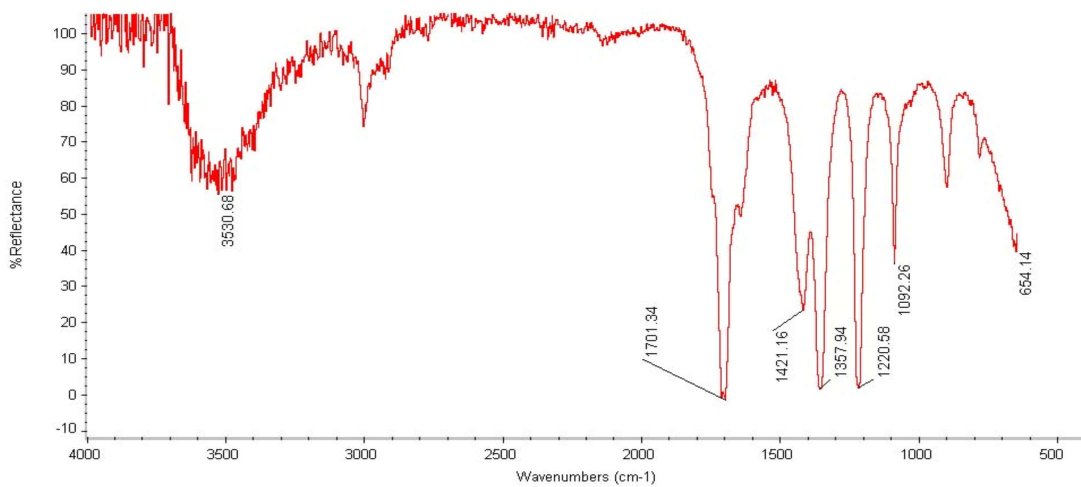

Figure 8. Representative FTIR spectrum of pervaporation permeate.

Table 3. Representative pervaporation results at −1 bar and 15 ppm feed concentration

Membrane	Flux (L m^{-2} h^{-1})	%Rejection	Separation selectivity ($\alpha_{i/j}$)
40°C, 2 h uncrosslinked	0.131±0.004	100.0±0.0	∞
50°C, 2 h uncrosslinked	0.093±0.025	100.0±0.0	∞
60°C, 2 h uncrosslinked	0.127±0.009	100.0±0.0	∞
60°C, 4 h uncrosslinked	0.038±0.012	100.0±0.0	∞
60°C, 6 h uncrosslinked	0.022±0.006	100.0±0.0	∞
60°C, 2 h ionically crosslinked	0.062±0.002	100.0±0.0	∞
60°C, 4 h ionically crosslinked	0.033±0.000	100.0±0.0	∞
60°C, 6 h ionically crosslinked	0.025±0.000	100.0±0.0	∞

Conclusions

Using canned tomato paste as a representative sample, it was shown that acetone of lower toxicity was an alternative solvent for extracting lycopene from tomato paste. In using hydrophilic chitosan membranes for the separation of lycopene from the acetone, the membranes must be pretreated in water becoming swollen membranes. A pervaporation membrane based process was successfully used to obtain the beneficial effect solving the instability problem of active lycopene contents during separation processes by conventional methods. The suitable chitosan membranes required for this pervaporation application were obtained by evaporating the membranes in the range of 40 – 60°C for 2 hours. The membranes had a high acetone flux of 0.093 – 0.131 L m^{-2} h^{-1} as well as a high rejection or separation selectivity. The pure lycopene crystals were precipitated from the retentate solution and could be filtered out for market availability.

Acknowledgements

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