EXTRACTION AND ENCAPSULATION IN β-CYCLODEXTRINE OF AROMA FROM PINEAPPLE HUSK USING SUPERCRITICAL CARBON DIOXIDE

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Abstract. Husks from a variety of pineapple (*Ananas comosus L.*) that is produced at industrial scale in Colombia can be a source of valuable aroma compounds useful for incorporating this exotic flavor into several kinds of food products. In this work, samples of pineapple husk were subjected to cryogenic milling and were freeze dried. The resulting material was extracted with supercritical carbon dioxide in a lab-scale apparatus to establish operating conditions at which a maximum extraction yield is obtained. Experiments were conducted following a factorial design, with temperature (33 and 55 °C) and CO_2 density (0.7 and 0.9 g/cm^3) as controlled variables, and extraction yield as response variable. Extraction yields from 2 to 3 wt% were obtained. Three sets of operating conditions were then selected for performing experiments in which the extract is obtained and then immediately encapsulated by passing the stream of CO_2 + extract through a capsule loaded with β-cyclodextrine.

The main aroma components present in the pineapple extracts (up to 80 components) were identified by gas chromatography-mass spectrometry (GC-MS), and the formation of inclusion complexes extract/β-cyclodextrine was verified by differential scanning calorimetry (DSC). Analyses of the thermograms allowed us to quantify the inclusion efficiency, which was 98%. Thus, supercritical fluid extraction-encapsulation is a technology that might be useful to produce solids loaded with tropical-fruit aroma suitable for the development of food products from inexpensive raw materials.

Keywords: pineapple, encapsulation, supercritical extraction, natural aroma, cyclodextrine

1. Introduction

Byproducts of the industrial processing of tropical fruits can be a source of valuable products such as aroma compounds, which might be useful for incorporating exotic flavors into several kinds of food products. Among many of such byproducts, husks from a variety of pineapple (*Ananas comosus L.*) that is produced at industrial scale in Colombia are readily available since they constitute about 45 wt% of the whole fruit and constitute a low added value by product of this industry.

Conventionally, aroma compounds might be obtained by liquid extraction, vacuum distillation and cryoconcentration. These processes are known for having low yields and high production costs [1]. In addition, due to the volatile nature and sensitivity to oxygen, temperature and light of most aroma compounds, it is necessary to stabilize them either by dissolving them in a solvent or by encapsulation in a solid matrix.

Supercritical fluid extraction might provide a technological platform to accomplish both the separation and the encapsulation in one single process. As it is well known, many of the common applications of this technology are in the field of extraction of natural products. Using supercritical carbon dioxide it is possible to carry out processes at near ambient temperatures, avoiding the degradation of thermo labile substances [3]. This solvent is appreciated because it is inexpensive, not toxic or flammable, it is available in high purity, and is approved without restrictions for use in the food industry [4]. Supercritical extraction of flavors and fragrances has been reviewed by Stahl et al. [5], by Moyler [6], and by Kerrola [7].

The first encapsulation processes were developed in 1930 for the textile industry, and applications were then developed for pharmaceutical, medical and food industries [8]. Recently, applications in the food industry have been growing, as encapsulation helps to prevent degradation or losses by evaporation during processing and packaging, improving the flavor, aroma and nutritional value of the final product [9].

Encapsulation can be prepared by several techniques [10]. One that is gaining popularity is the use of cyclodextrins (CD) [11], which are oligosaccharides with an annular structure made of 6, 7 or 8 sugar molecules (namely, α-, β- or γ-cyclodextrin, respectively). To illustrate, Figure 1 shows the molecular structure of β-cyclodextrin. As the external surface of this molecule is hydrophilic while the internal surface is hydrophobic, cyclodextrins are able to include in their cavity large organic molecules by non-covalent interaction forces such as hydrogen bonds and van der Waals forces. Physical and chemical properties of the included molecules may thus be favorably modified, and in particular the physical stability and the aqueous solubility can be improved [12].

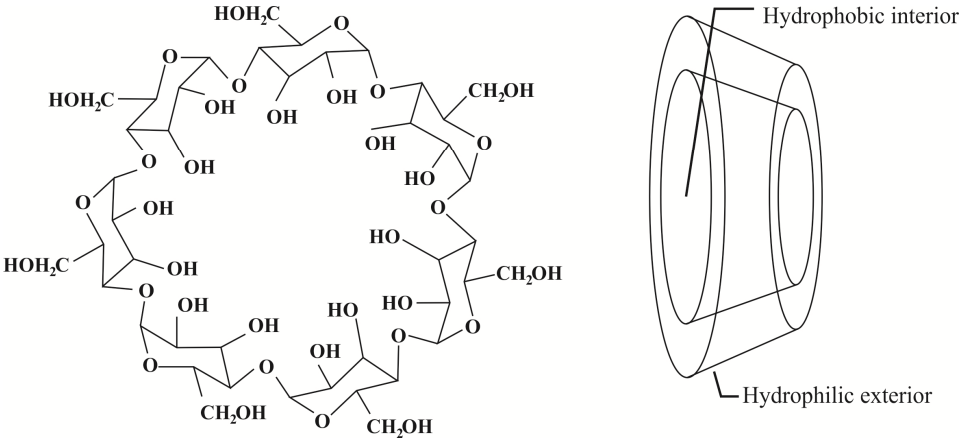

Figure 1. Molecular structure of β-cyclodextrin

Several researchers have been studying the formation of inclusion complexes in the presence of compressed fluids. For example, Kamihira et al. [13] used CD for the encapsulation of volatile aromatic compounds after extraction with pressurized CO_2 at 10 MPa and 20 °C [13]. Van Hees et al. [14] prepared an inclusion complex of piroxicam, a pharmaceutical product in β-CD by pressurization of a mixture of the two compounds in the presence of carbon dioxide at 45 MPa and 150 °C. Marangiou et al. [15]. prepared another inclusion complex of the fungicide imazalil in β-CD by using supercritical carbon dioxide as a solvent for imazalil. More recently, different drug/CD complexes have been successfully prepared in supercritical media by dynamic or static methods for several molecules [16].

We have been studying the encapsulation of natural aroma and flavor compounds in β-CD using supercritical fluids technology. In this paper we describe our experimental efforts for the encapsulation of aroma compunds present in the husks of a pineapple variety (*Ananas comosus L.*). This variety is produced at industrial scale in Colombia and due to its exotic flavour and aroma is accepted by international markets as one of the most important tropical fruits [17].

2. Experimental apparatus and procedure

2.1 Materials

Pineapple husks were obtained from a local company (Valfruth Ltda., Cali, Colombia). CO_2 (99.9% purity) was obtained from Cryogas (Cali, Colombia). Food grade β-Cyclodextrin (98% purity), was provided by Quick Suppliers (Cali, Colombia). They were used as received without further purification.

2.2 Experimental apparatus

Figure 2 shows the experimental apparatus that was used in this research for the extraction and encapsulation process. This is a laboratory scale apparatus that was designed and built in a previous work [18] but it was modified in this work by adding a cell loaded with the inclusion agent (β-CD).

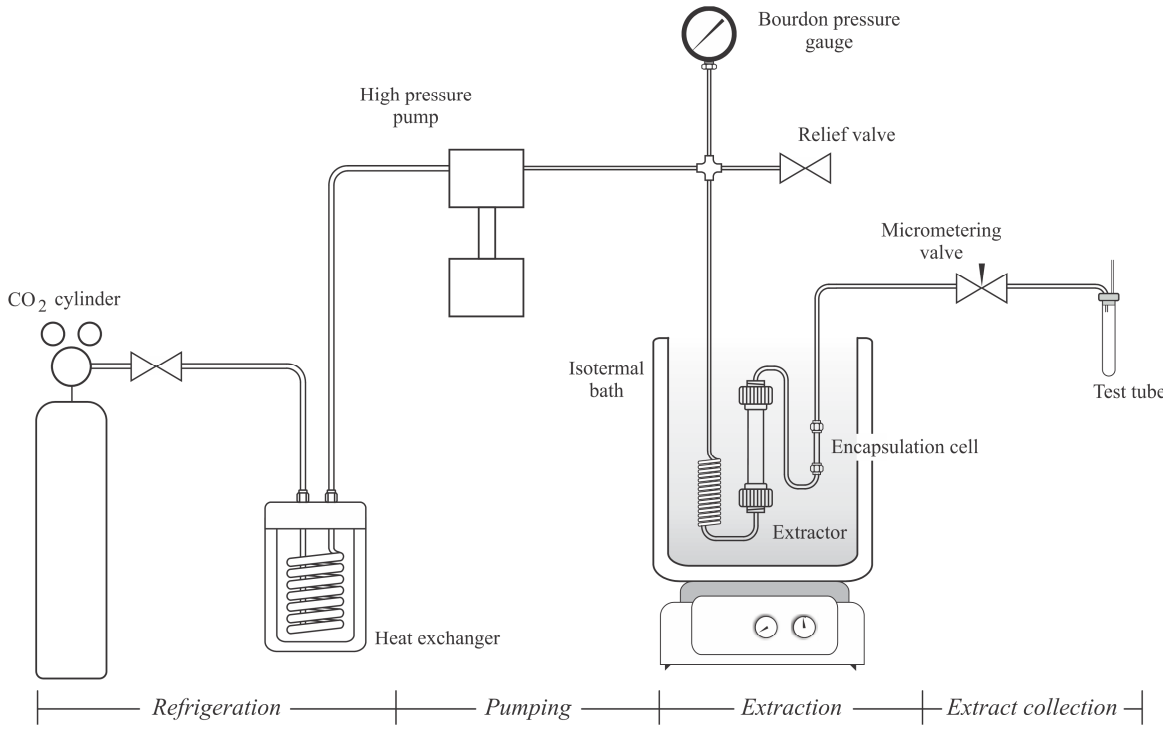

Figure 2. Experimental apparatus that was used for extraction and encapsulation.

Carbon dioxide from a cylinder is passed through a heat exchanger which operates with ethylene glycol at -10 °C to maintain the CO_2 in liquid phase before entering the pump. Pumping of CO_2 at high pressure is performed with a Williams- Milton Roy pneumatic pump (model CP250/V225, rated for pressures up to 7000 psi). The pressure at the exit of the pump is registered with a Bourdon pressure gauge (Ashcroft, model 3005HL, 0 to 5000 psi, with marks every 100 psi) located in the extractor feed line. The CO_2 passes through a cylindrical extractor, which is located inside an isothermal water bath. The extractor has a useful volume of 15 cm^3. It was fabricated in stainless steel 316, and is rated for pressures up to 10000 psi at 200 °C. For extraction experiments, after the extractor the CO_2 with the dissolved extract passes through a micrometering valve and the sample is collected in a 10 mL test tube. The micrometering valve and the exit line are wrapped with an electric resistance to prevent plugging due to freezing of the extract or CO_2 as a consequence of the cooling produced by expansion of the supercritical fluid in the valve. The extract is collected after the micrometering valve in a 10 mL test tube, fitted with a rubber stopper to protect the tube in case of a sudden pressure increase during the sample collection.

For encapsulation experiments, a 3 mL encapsulation cell was fabricated from a piece of ¼" O.D. SS316 tubing and was inserted in the line right at the exit of the extractor, inside the isothermal bath as shown in Figure 2. The isothermal water bath is equipped with a type-K thermocouple and a temperature controller (DISAN, Model BS 1400), which acts on a 1000 W resistance as final control element. The water bath and thus the extraction temperature are controlled to ± 0.3 ºC.

2.2 Experimental procedure

Pineapple husks were obtained from a local company and were frozen using liquid nitrogen. Then, they were milled, freeze dried, and sieved. Freeze drying was conducted at -77 °C and 26 Pa. The pineapple husks lost 88 wt% of moisture during this process, which guarantees that no volatile compounds are lost, and also that no degradation of thermally labile compounds occurs before the supercritical extraction. The material with an average particle size of 1.2 mm (i.e., retained in No. 20 mesh) was used as raw material for the extraction and encapsulation experiments. Figure 3 shows photographs of the raw material in different treatment steps before the supercritical extraction.

(a) (b) (c)

Figure 3. Photographs of the raw material in different treatment steps before the supercritical extraction. (a) Pinneaple husks received, (b) after freeze drying, (c) after sieving.

In a typical extraction experiment, pineapple husks were loaded into the extractor (with an empty encapsulation cell), which then was immersed into the isothermal bath. After few minutes in which the temperature reached a stable value, carbon dioxide was pumped with the micrometering valve fully closed until the desired pressure was reached. At this moment, the micrometering valve was slowly opened, until steady values of both pressure an exit flow rate were obtained. The extraction time in each run was one hour. Similar procedure was followed in the encapsulation experiments. The encapsulation cell was loaded with 0.4 g of β-CD and was placed immediately after the extractor, inside the isothermal bath.

2.3 Experimental design

An experimental plan was prepared to explore the effect of extraction temperature and CO_2 density on the extraction yield, which was defined as the percentage of the raw material that is extracted by the supercritical fluid. The experimental runs were organized according to an augmented 2^2 factorial experiment in which temperatures between 35 and 55 °C, and CO_2 densities between 0.7 and 0.9 g/cm^3, were used as the conditions for the factorial experiment. At each one of the four possible combinations of these conditions one experimental run was made. Three more runs were planned at 45 °C and 0.8 g/cm^3 (i.e., the so called "augmentation") to obtain an indication of the reproducibility of the results. For each combination of temperature and CO_2 density, we used the Bender equation of state [4] to calculate the pressure at which the corresponding run was to be made. Conditions of temperature and density were chosen to obtain pressures in the range of the operational capacity of our apparatus.

At the conditions at which the higher yields of extraction were obtained, encapsulation experiments were planned. The extracts obtained at these conditions were characterized by gas chromatography coupled to a mass spectrometer (GC-MS) and the formation of inclusion complexes was verified by differential scanning calorimetry (DSC).

3. Results and discussion

Table 1 shows the experimental conditions and extraction yields of supercritical carbon dioxide extraction of aroma from pineapple husks. Note that the extraction yields increase with increasing CO_2 density and also with increasing temperature. The results obtained at the central point of the experimental design (2.82, 2.37, 2.91 wt%) corresponding at 45 ºC and 2736 psi, indicate the reproducibility of the experiments.

3.1 Characterization of the pineapple husk extract

All the extracts obtained looked very similar: viscous yellow oil with a strong sweet caramel smell. Runs 3, 5 and 6 were chosen for analysis by GC-MS because at these conditions we obtained the higher extraction yields. Figure 4 shows the main functional groups identified in the extracts. Note that proportion of the extracts are composed by esters, followed by hydrocarbons (alkanes) and low relative amounts of

Table 1. Experimental conditions and extraction yields of aroma from pineapple husks.

Run	Temperature (°C)	Pressure (psi)	CO_2 density (g/cm^3)	Extraction yield (wt. %)
1	35	1412	0.7	2.01
2	45	2736	0.8	2.82
3	35	3583	0.9	2.52
4	45	2736	0.8	2.37
5	55	2446	0.7	2.79
6	45	2736	0.8	2.91
7	55	5583	0.9	3.01

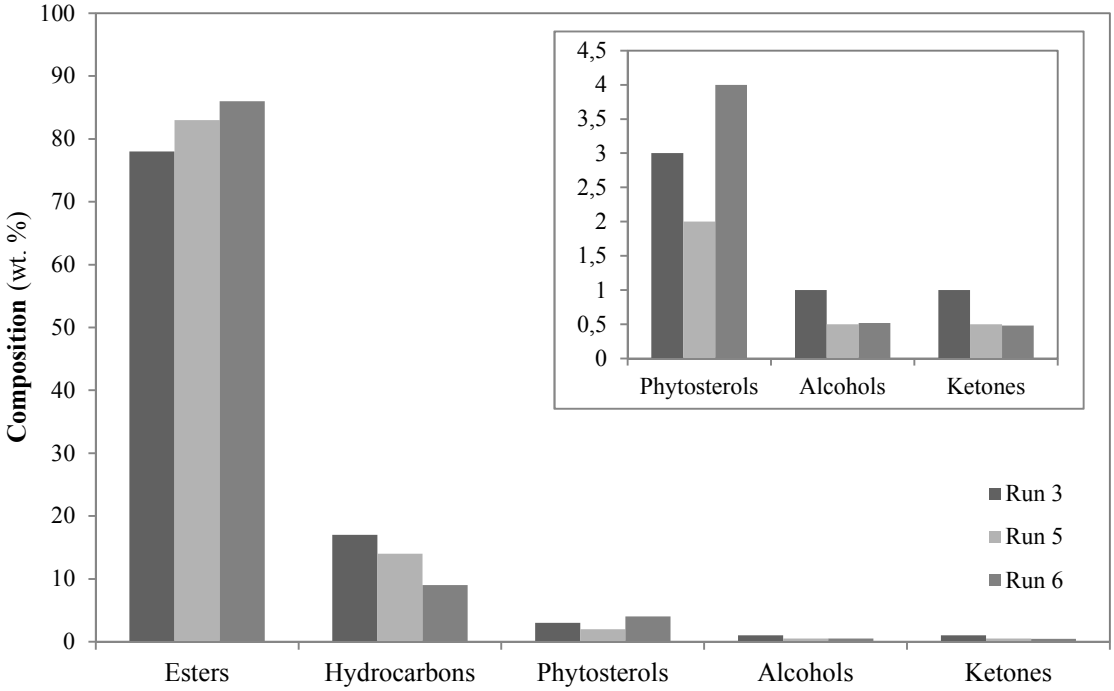

Figure 4. Main functional groups identified in the extracts.

phytosterols, alcohols and ketones. It is important to point out that although low concentrations of phytosterols were identified, such as β-sitosterol, campesterol, stigmasterol and β-sitostanol, these compounds are not found in other important Colombian pineapple varieties like India and Perolera [1]. A recent study has shown that such compounds might prevent the development of colon cancer and benign prostatic hyperplasia [19]. The major components of the three extracts were similar but they were present in different quantities in the three extracts due to the different operating conditions at which the experiments were carried out.

3.2 Verification of the inclusion complex

The formation of the inclusion complex extract/β-CD was verified by differential scanning calorimetry (DSC). DSC has been shown to be a very powerful analytical tool in the characterization of complexes formed with cyclodextrins. When an inclusion complex is formed, phase transition temperatures usually shift to different values or disappear as a result of the guest molecules being embedded into the inclusion agent cavities [20]. In our case, the melting point of the supercritical extract should not appear in the thermogram of the solid that is obtained after encapsulation of the extract, for formation of the inclusion complex.

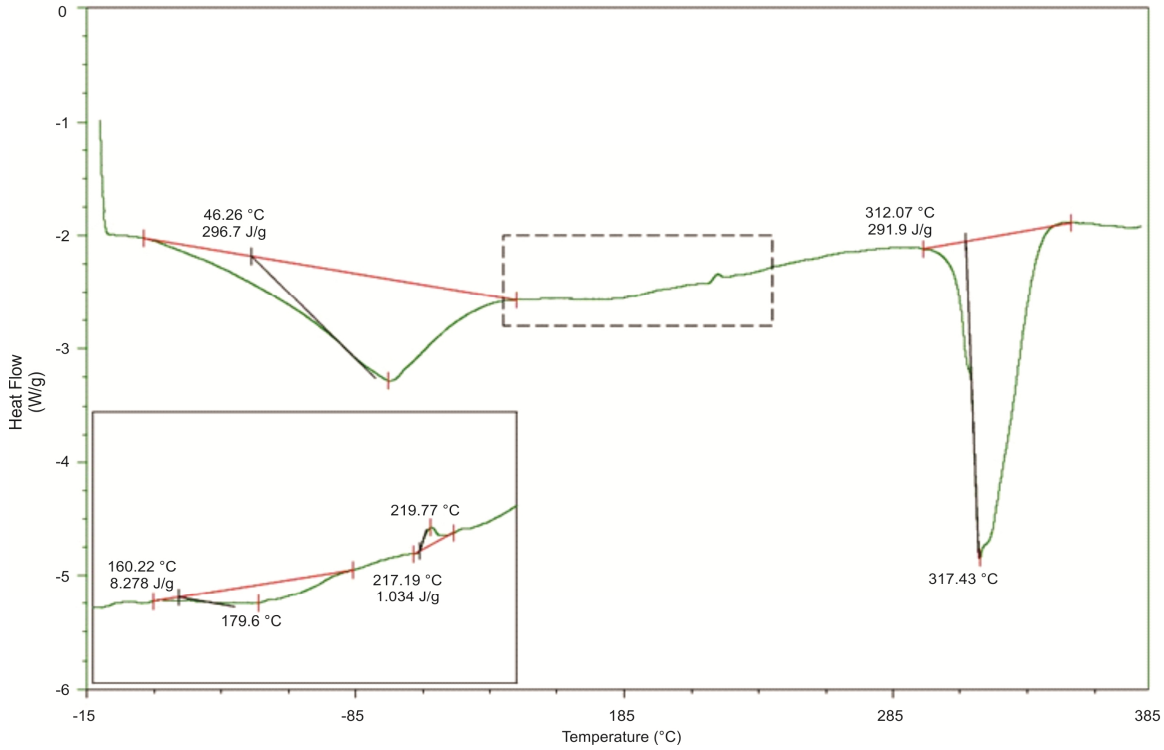

Figure 5. Thermogram of β-CD

Figure 5 shows the thermogram of pure β-CD that was used as encapsulating agent. Three endothermic peaks are shown: 102.98 °C, which is related to the evaporation of water; 226.21 °C, which is related to the β-CD melting point; and 312.17 °C, which is the decomposition temperature of β-CD.

Figure 6a shows the thermogram of the extract that was obtained at 45 °C and 2700 psi. The melting point of the extract is identified at 54.34 °C, while Figure 6b shows the thermogram of the β-CD/extract complex obtained at same conditions. In this thermogram we identify three important transitions: the water evaporation (120.36 °C), the complex melting point (225.11 °C) and the complex decomposition temperature (311.24 °C). Note the absence of the peak that corresponds to the melting point of the extract, which is clearly observed in Figure 6a, indicating that the molecules of the extract were embedded into the β-CD cavities.

Table 2 shows the thermal transitions of inclusion complex, extract and cyclodextrin obtained by the DSC analysis. Note that at the different operating conditions at which the extraction- encapsulation process was carried out, the analysis results of the thermal transitions allow us to verify the formation of the inclusion complex, the melting point of the extract disappear.

The peaks related to the water boiling point can be explained by the formation of the inclusion complex mechanism, when host and guest molecules approach one another, desolvation first occurs not only around the guest but also for the host cavity; then, host–guest interactions take place, replacing host-water and guest-water interactions. Essentially, the binding between β-CD and the organic compunds of the supercritical extract depends on both hydrophobicity of the guest molecules and their geometric accommodation into the CD cavity [21].

Table 2. Thermal transitions temperatures of inclusion complex, extract and cyclodextrin.

	SC extracts			**Inclusion complex**			**β- CD**
	Run 3	Run 5	Run 6	Run 3	Run 5	Run 6	
Boiling point (°C)	---	---	---	132.1	108.07	120.36	102.98
Melting point (°C)	62.09	73.23	54.34	224.54	221.02	225.11	226.21
Decomposition (°C)	102.64	177.33	153.28	297.54	309.81	311.24	312.17

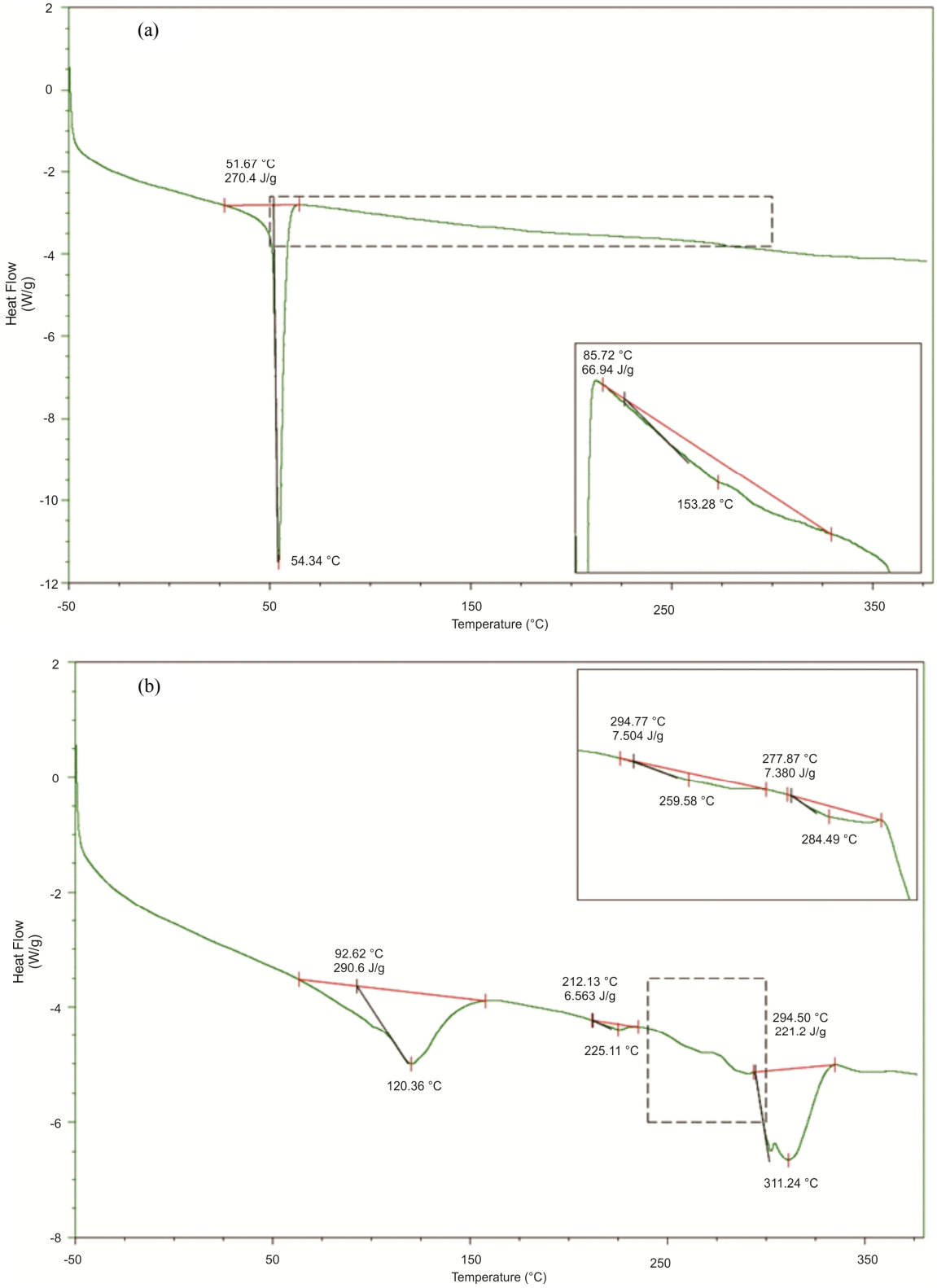

Figure 6. Thermograms of the extract (a) and the solid β-CD/extract (b) obtained at 45 °C and 2700 psi

3.3 Inclusion efficiency

The inclusion efficiency (IE) of the complex was estimated directly by the equation [22]:

$$IE = \frac{\Delta H_e - \Delta H_{e\beta}}{\Delta H_e} \times 100\% \quad (1)$$

Where ΔH_e is the enthalpy of melting of the supercritical extract and $\Delta H_{e\beta}$ is the enthalpy of melting of the inclusion complex. Table 3 shows the inclusion efficiency for the different extraction- encapsulation operating conditions. Note that in all cases a high efficiency (>97%) was reached.

Table 3. Inclusion efficiency of complexes formed in this study.

Run	**Temperature** (°C)	**Pressure** (psi)	**Melting ethalphy** (J/g)		**IE** (%)
			SC extract	Inclusion complex	
3	35	3583	265.800	5.341	98.0
5	55	2446	563.000	8.089	98.6
6	45	2736	270.400	6.563	97.6

4. Conclusion

Supercritical fluid extraction-encapsulation is a technology that might be useful to produce solids loaded with tropical-fruit aroma suitable for the development of food products, from inexpensive raw materials. The operating conditions are moderate, in the range of 35 to 55 °C, with a carbon dioxide density in the range of 0.9 g/mL that allows us to obtain extraction yields close to 2 % and inclusion efficiencies higher than 97%.

Acknowledgments

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