 doi:10.20944/preprints201711.0005.v1

Obtaining of β-Sitosterol from *Cardamom* by Supercritical CO_2 Extraction

Hacer İçen[1], Hatice Tuğba Çelik[2], Mustafa Serhat Ekinci[2], Metin Gürü[2]

[1]Department of Chemical Engineering, Faculty of Engineering, Osmaniye Korkut Ata University, 80000, Osmaniye, Turkey; hacericen@osmaniye.edu.tr
[2]Department of Chemical Engineering, Faculty of Engineering, Gazi University, Maltepe, 06570, Ankara, Turkey; htgumusdere@gazi.edu.tr (H.-T.Ç.) mustafaserhatekinci@gmail.com (M.-S.E);.); mguru@gazi.edu.tr (M.G.)

Correspondence: mguru@gazi.edu.tr; Tel: +90-312-5823555

Abstract: Cardamom is plant of the Zingiberaceae family. It has been used for the treatment of many diseases such as migraine, bronchitis, stomach and intestinal disorders. Cardamom contains triterpenes, resins, starch and fatty compounds. Phytosterols (stigmasterol, campesterol and β-sitosterol) are a group steroid alcohol in plants. They are used food, medicine and cosmetic industry. They are protective effects against some types of cancer too. Phytosterols are found in the vegetable oil such as the spindle, corn and soybean oil. This paper deals with the maximum oil and β-sitosterol yield were investigated by means of the supercritical CO_2 extraction of cardamom. The effect of operating parameters as temperature, pressure and CO_2 flow rate were investigated on oil yield. The amount of β-sitosterol was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) at optimized conditions. The optimized conditions were recorded as temperature of 40^{o}C, pressure of 200 bar and CO_2 flow rate of 4 L/min. The maximum oil yield and amount of β-sitosterol were found as 74.83 mg oil/g seed and 4.73 mg β-sitosterol/g seed cardamom under these conditions.

Keywords: cardamom; supercritical CO_2 extraction; β-sitosterol

 doi:10.20944/preprints201711.0005.v1

1. Introduction

Cardamom is a general name of genera Elettaria and Amomum in the family Zingiberaceae. Cardamom seeds are dark brown, lentil-sized and pugnent smelling. Its triterpenes contain resin, starch and essential oils [1-3]. Cardamom oil has antimicrobial effect for this reason it is used for treatment in throat ache, earache and headache; asthma, bronchitis; snakebite, scorpion sting and disases of bladder, intestine, kidney. In addition, cardamom increases the number of healthy cells by fighting cancer cells, so that it shows anti-cancer properties [4-6].

Many important compounds which are used in various fields such as medicine and food and which are difficult to produce in laboratory conditions can easily be obtained by classical extraction method from plants [7]. However, organic solvents used during classical extraction are not completely removed from these compounds and the purification of the product becomes difficult [8]. The supercritical fluid extraction method, which shortens the extraction period, performs the separation at low temperature and has high separation efficiency compared to the classical extraction, has attracted interest in recent years. [9].

In this method known as environmentally friendly, carbon dioxide, which is generally an inert gas, is used as a chemical solvent. This gas is not flammable, explosive and doesn't lead to chemical reaction. Since high yields can be obtained at low temperatures, many compounds such as essential oils, vitamins are extracted without being decomposition [10].

Many studies have been reported in the literature such as obtaining essential oil from yarrow [11] and boldo leaves [12], obtaining β-sitosterol from melon and peach seed [13], obtaining silybin from thistle [14] and obtaining caffeine from tea [15, 16] by supercritical CO_2 extraction.

There is much research in the literature about oil and essential oils extraction from cardamom by different techniques. Compounds such as essential oils and / or oleresine have been obtained from the cardamom by methods such as solvent extraction and water vapor distillation [17]. In recent years, supercritical fluid extraction has been used to extract oleresine and essential oils [18-20]. However, in the literature, it has not been found any study about obtaining β-sitosterol from cardamom by supercritical CO_2 extraction.

The purpose of this study was to obtain maximum oil by supercritical CO_2 extraction from the cardamom and to determine the amount of β-sitosterol in this oil. For obtaining maximum yield of oil, extraction parameters effect such as temperature, pressure and flow rate was investigated and it was determined optimum conditions on the supercritical CO_2 extraction process. The amount of β-sitosterol in the optimum condition was analyzed by Gas Chromatography-Mass Spectroscopy (GC-MS).

2. Materıal and Method

2.1. Method

Cardamom; were separated from their shells and dried till fixed weighing at 25°C in a laboratory ambient. Separated and dried cardamom was ground with BOSCH MKM6000 just before the experiments

it is stated that the particle size should be within a minimum range of 0.25-2 mm at much research which is the supercritical fluid extraction of various natural sources in the literature. When working with a smaller particle size; it has been seen that some of the solvents did not come into contact with the extraction material as it penetrated

 doi:10.20944/preprints201711.0005.v1

through the channels in the extraction vessel, causing loss of efficiency in process and causing some obstructions in the channels in the extraction bed [9, 10]. Previous work by our group has reported that increasing the particle size (d_p) of seeded natural materials has a negative effect oil yield by supercritical CO_2 extraction [13, 14]. Taking into account the findings of these studies, grinded seeds were sieved from sieves complying with ASTM standards and particle size in the range of 0,185 <d_p <0,425 mm was obtained.

The experimental supercritical CO_2 extraction system consists of a supercritical fluid extraction unit, a compressor, a CO_2 tube with siphon, a modified pump, and a cooling water bath (Figure 1). The supercritical extraction device is "Applied Separations' Spe-ed SFE" oven model. The device consists of three parts; pump, extraction unit in the oven, collection and control units. The extraction vessel is a stainless steel cylindrical tube with a volume of 24 mL. Four special gaskets which are resistant to temperature and pressure were used between the connections of the extraction vessel for preventing leakage in the vessel. This vessel is placed vertically inside the oven. There are 3 valves, V_1 (inlet valve), V_2 (outlet valve) and V_3 (solvent discharging valve in the system) on the oven. There is a flow meter designed to measure the velocity of the fluid exiting the outlet valve V_2. A sample collection vessel is present at the exit of the oven to collect the product obtained.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 November 2017 doi:10.20944/preprints201711.0005.v1

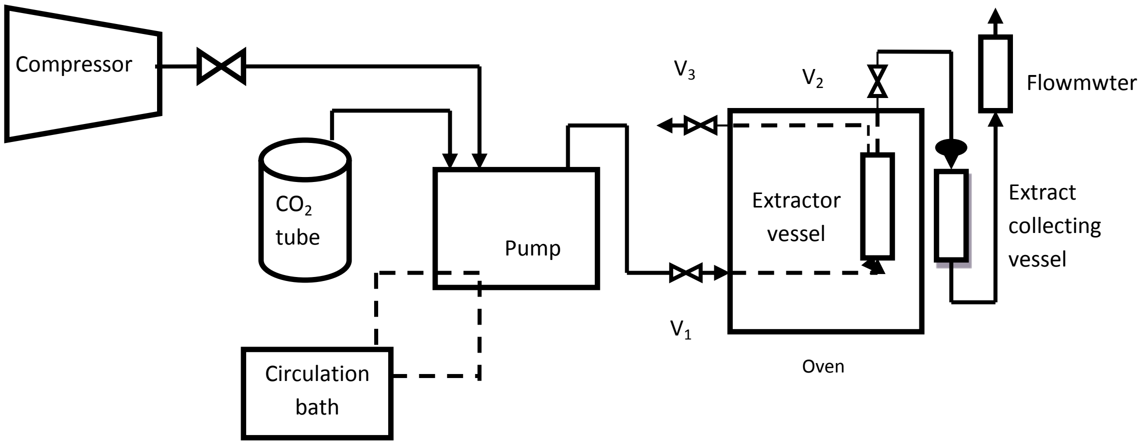

Figure 1. Flow diagram of the supercritical extraction set-up

By adjusting the temperature of the oven, it is possible to reach the desired temperature to work. 4 g cardamom pre-treated was placed in the extraction vessel in a volume of 24 mL. CO_2 pump is set to operating pressure. The inlet valve (V_1) and outlet valve (V_2) are opened, the V_3 valve is closed. During the extraction process, the extract is collected in the collecting vessel. After extraction process is finished, inlet and outlet valves are closed, V_3 valve is opened and CO_2 in the system is removed.

The effect of temperature, pressure and CO_2 flow rate parameters on the yield of maximum oil yield by supercritical CO_2 extraction of cardamom plants was investigated. Experiments were performed at 40-80°C temperature, 180-220 bar pressure, and 3-5 mL/min CO_2 flow rate ranges. Operating conditions were determined by using the previous work carried out by our group [13, 14]. On the whole of the experiments; oil and β-sitosterol analyzes were repeated 3 times.

2.2. Analysis of β-sitosterol

Saponification and derivatization pretreatments were performed to analyze the β-sitosterol contained in the extract. The amount of β-sitosterol in the extract obtained was analyzed by Gas Chromatography-Mass Spectroscopy (GC-MS). Details of the analysis were given in another study conducted by our group [13].

3. Results and Discussion

In this work, obtaining the maximum yield of oil was studied by supercritical CO_2 extraction of cardamom. The effects of temperature, pressure and CO_2 flow rate parameters on oil yield were investigated. Optimum conditions were determined and the amount of β-sitosterol in these conditions was analyzed.

3.1. Effect of Temperature

While the pressure and carbon dioxide flow rate parameters were kept constant, the effect of the temperature on the extraction process with supercritical CO_2 was investigated. Experiments were carried out at three different temperatures. The oil yield values obtained during the extraction process are shown in Figure 2.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 November 2017 doi:10.20944/preprints201711.0005.v1

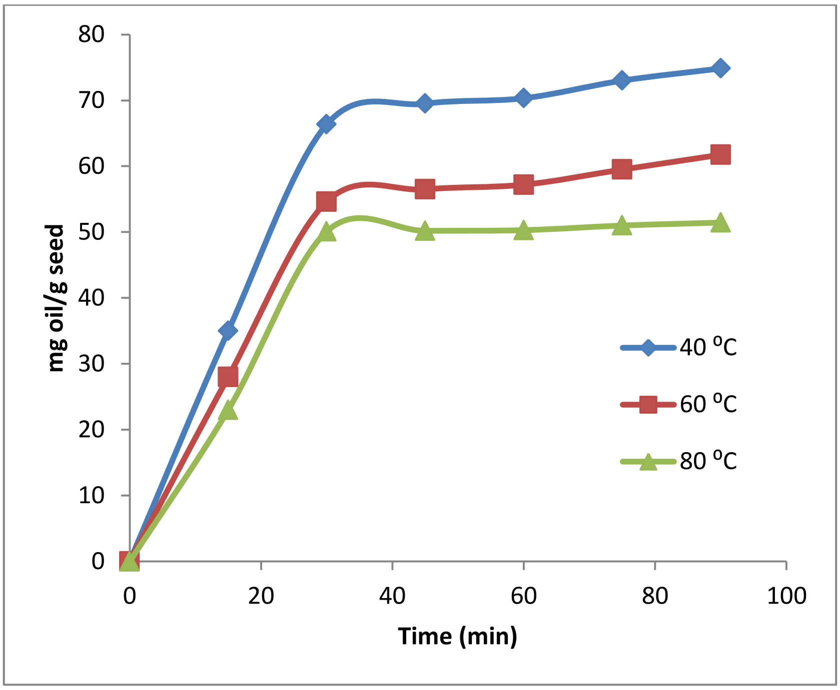

Figure 2. Effect of temperature on supercritical extraction processes (Pressure:200 bar; Carbon dioxide flow rate:4 L/min)

As can be seen in Figure 2, the rate and yield of extraction is increasing with decreasing temperature. As the temperature is reduced from 80 ^{0}C to 40 ^{0}C, the density of carbon dioxide decreases from 839.9 kg/m^3 to 594.1 kg/m^3 at 200 bar pressure. Supercritical carbon dioxide reduces the density of cardamom, so the solubility of the oil decreases in supercritical CO_2 [21]. Similarly, in the study by Goash et al., the crossover pressure was determined to be 200 bar at the eugenol extraction with supercritical CO_2 from the *Ocimum sanctum Linn* and it was stated that the extraction yield decreased with increasing temperature at lower pressures than 200 bar. At low pressures, the increase of vapor pressure with temperature is more effective than decrease of supercritical CO_2 density and solving power [22].

Figure 3 shows the amount of oil (mg oil / g seed), obtained at the end of the extraction period at temperatures of 40, 60, 80°C. At the end of the extraction period, the oil yields were determined 74.85, 61.75, 51.45 mg oil/g seed respectively in the temperatures 40, 60 and 80°C. It can be seen that the maximum oil is obtained at 40°C temperature. It was determined that in the extraction of peach seed with supercritical CO_2, both the amount of oil obtained and the amount of β-sitosterol increased up to 40°C at constant pressure and decreased after this temperature [13].

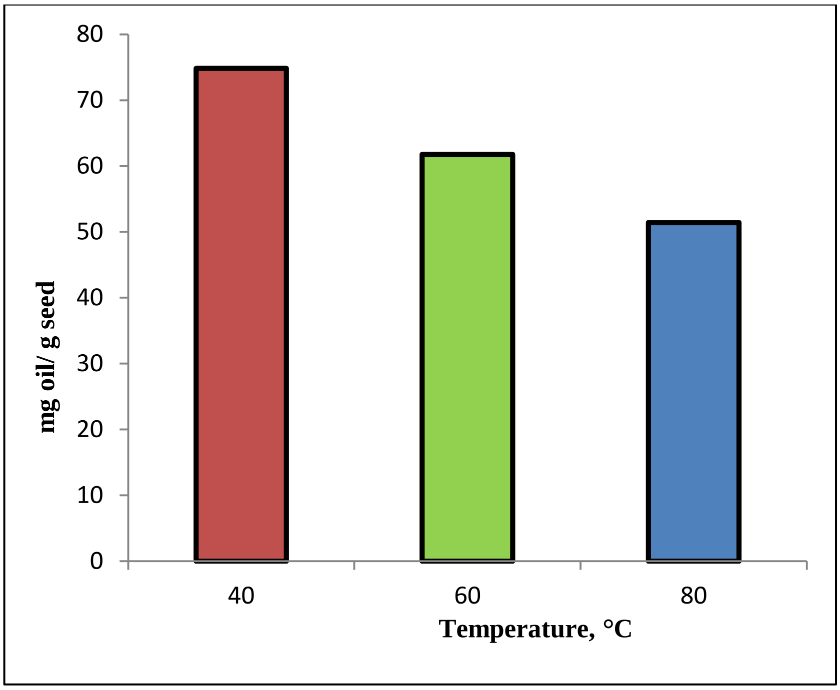

Figure 3. The temperature effect on oil yield at the end of the extraction process (Pressure:200 bar, Carbon dioxide flow rate: 4 L/min)

3.2. Effect of Pressure

The effect of pressure on the supercritical CO_2 extraction of cardamom plant was examined for values of 180, 200 and 220 bar, and the findings obtained were given in Figure 4.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 November 2017 doi:10.20944/preprints201711.0005.v1

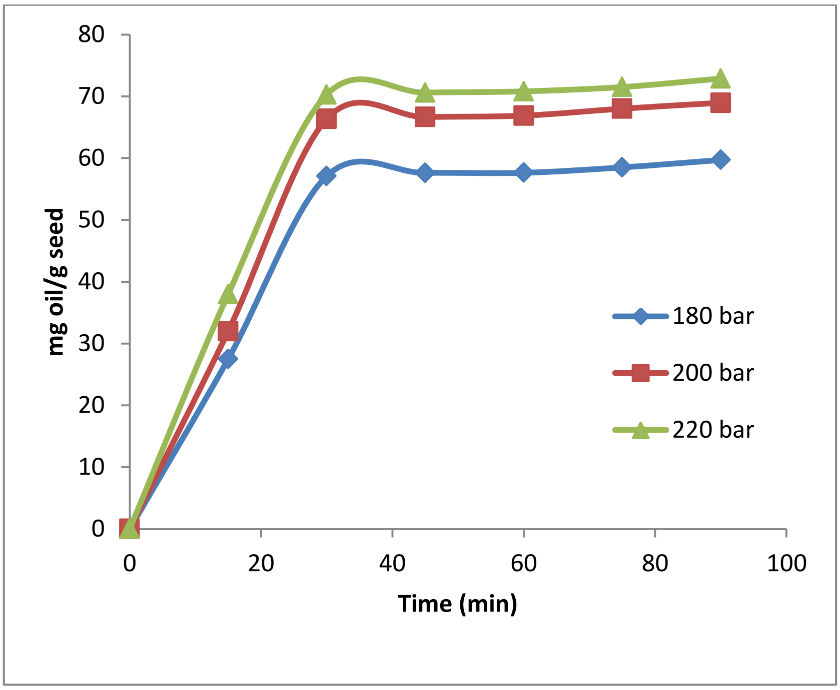

Figure 4. Effect of pressure on supercritical extraction processes (Temperature:40 °C; Carbon dioxide flow rate:4 L/min)

When the pressure is raised from 180 bar to 220 bar, the oil yield and the initial rate of extraction are observed to increase (Figure 4). At constant temperature, while the effective diffusivity decreases with increasing pressure, the solubility of the analyte and axial dispersion coefficient increases. At the same time, as the effective diffusivity and mass transfer coefficient decrease with increasing pressure, the internal and external mass transfer resistance increases. This behavior can be explained as, at the increasing pressure, the positive effects on the extraction process overcome the negative effects. [23].

The oil yield obtained at pressures of 180, 200 and 220 bar at the end of the extraction process is given in Figure 5. At 200 bar high pressures, the increase in efficiency loses its importance. From an economic point of view, as the pressure increases, the energy requirement also increases. Thus, the pressure was kept constant at

200 bar while other parameters was examined. Similarly, in the study conducted by Hamdan, it was determined that the amount of oil extracted by supercritical CO_2 extraction with pressure increased and the highest yield was obtained at 30 MPa [20]. The findings obtained are consistent with results of many studies such as obtaining β-sitosterol from melon and peach seed [13], obtaining silybin from thistle [14] and obtained caffeine from tea [15, 16] with supercritical CO_2 extraction.

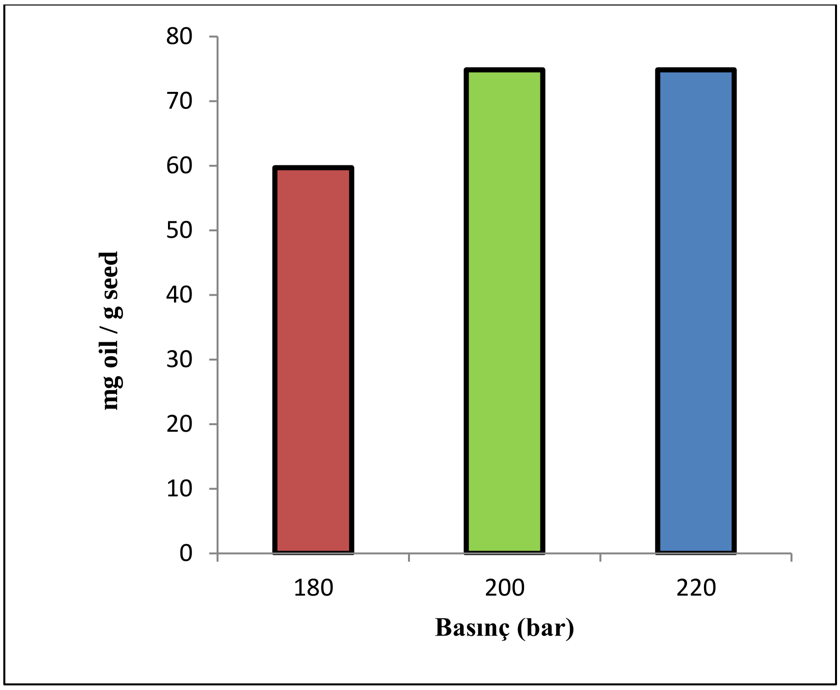

Figure 5. The pressure effect on oil yield at the end of the extraction process (Temperature: 40 °C, Carbon dioxide flow rate: 4 L/min)

3.3. Effect of Flow Rate

The effect of the flow rate of cardamom plant on the supercritical CO_2 extraction process was investigated. Experiments were carried out at 40°C constant temperature, 200 bar constant pressure 3, 4 and 5 L/min CO_2 flow rates (Figure. 6).

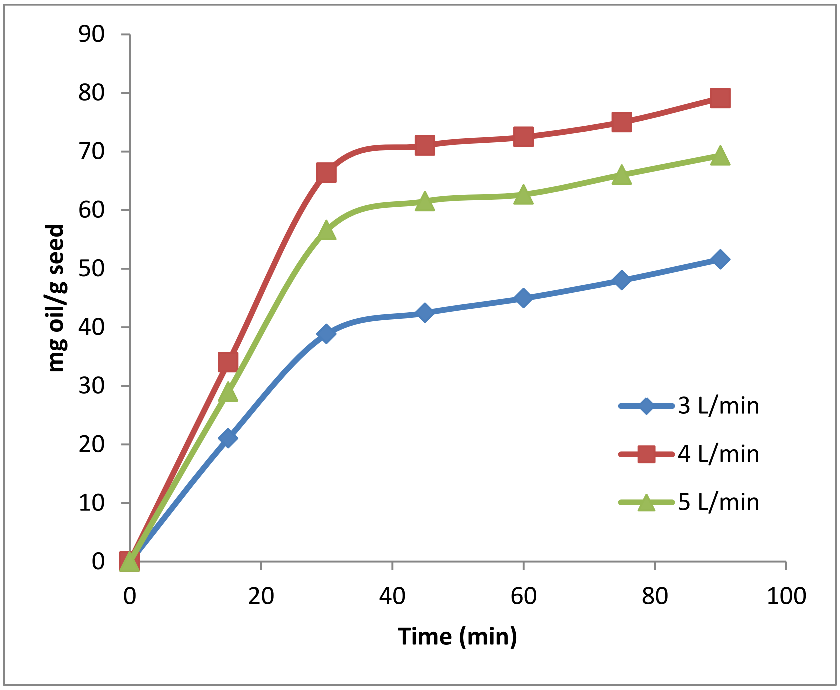

Figure 6. Effect of carbon dioxide flow rate on supercritical extraction processes (Temperature:40 °C; Pressure:200 bar)

It was determined that initial velocity increased by 71% as the flow rate increased from 3 L/min to 4 L/min (Figure 6). It is observed decrease of initial extraction rate when carbon dioxide flow rate is increased from 4 L/min to 5 L/min.

51.58, 74.85, 70.95 mg of oil were obtained at 3, 4 and 5 L/min CO_2 flow rates respectively and it was reached maximum oil yield at a flow rate of 4 L/min. CO_2 flow rate significantly affects the extraction process. On the one hand, the increase in flow rate leads to an increase in the vortices in the extraction chamber, a decrease in the film thickness between the solid and fluid, on the other hand, it shortens the interaction time between solid and fluid. The effect of solvent flow rate is closely related to solid matter structure. The increase of yield at 4 L/min flow rate can be explained by the reduction of film thickness between cardamom and carbon dioxide. As the CO_2 flow rate increases, the number of CO_2 molecules in the extraction vessel at unit volume

 doi:10.20944/preprints201711.0005.v1

increases, so the solubility of the CO_2 molecules increases by more contacting cardamom with CO_2 molecules. As the CO_2 flow rate increases, the intra-particle diffusion resistance dominates. In the increasing flow rate from 4 L/min to 5 L/min, while the external film transfer coefficient is decreased, the interaction time between cardamom and CO_2 is shortened. As a result of this situation, the second effect is predominant, so the efficiency is affected in the negative direction as shown in Figure7.

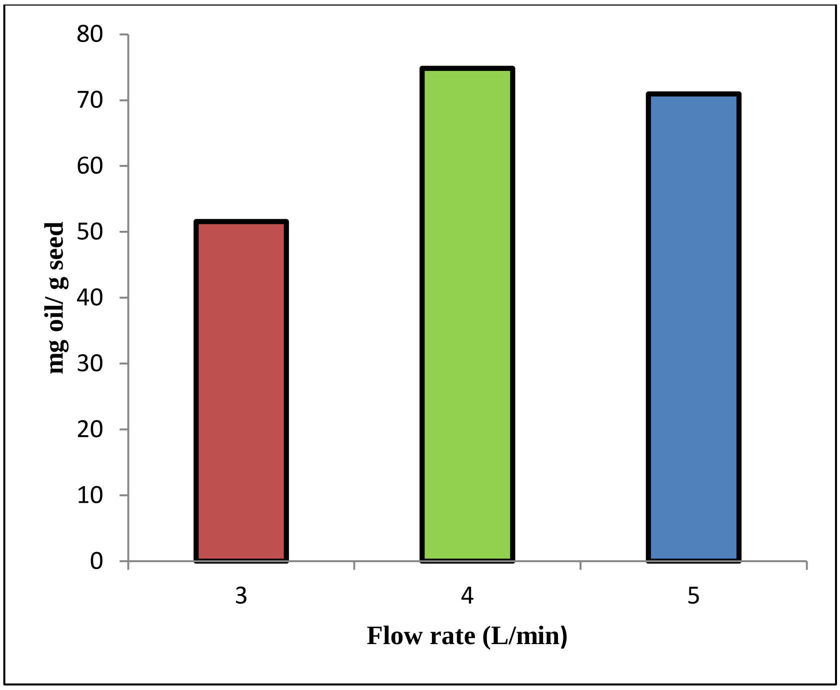

Figure 7. The carbon dioxide flow rate effect on oil yield at the end of the extraction process (Temperature: 40 °C, Pressure:200 bar)

Reverchon et al. investigated the extraction of rosehip seed oil with supercritical carbon dioxide and reported that the rate of extraction increased with increasing solvent flow rate in studies [24]. In the study on the extraction of coconut oil with supercritical carbon dioxide, it was emphasized that there is a slight increase in yield with increasing CO_2 flow rate. In these conditions, as CO_2 flow rate increases, mass transfer is affected and intra-particulate diffusion resistance dominates [25]. In the supercritical carbon dioxide extraction study of jojoba seed oil, it has been noted that the increase of the CO_2 flow rate increases the initial extraction rate and there is no significant change in the extraction efficiency [26]. Findings of the effect of flow rate on the yield are supported by similar results in the literature.

3.4. Analysis of β-sitosterol

For the determination of the amount of β-sitosterol contained in the oil sample obtained after cardamom with supercritical CO_2 extraction, the oil sample was purified by pre-treatment of saponification and derivatization [13] and analyzed by GC-MS. The amount of β-sitosterol in the extract obtained under optimal conditions was found to be 4.73 mg β-sitosterol/g seed.

4. Conclusion

In this study, it was investigated β-sitosterol extraction with supercritical CO_2 from cardamom which is used in the treatment of many diseases such as bronchitis, stomach and intestinal diseases, migraine etc. For this purpose, the effects of temperature, pressure and CO_2 flow rate on oil yield were investigated. Optimum conditions for the extraction of cardamom plant with supercritical CO_2 were found as 40°C temperature, 200 bar pressure and 4 mL/min carbon dioxide flow rate. The oil yield and β-sitosterol amount in this condition are 74.85 mg oil/g seed and 4.73 mg β-sitosterol/g seed.

Acknowledgements: This work was supported by Gazi University Scientific Research Projects 06/2011-01 and 06/2013-04.

Author Contributions: Hacer İçen, Hatice-Tuğba Çelik, Mustafa-Serhat Ekinci and Metin Gürü conceived and designed the experiments; Hatice-Tuğba Çelik and Mustafa Serhat Ekinci performed the experiments. All authors analyzed the data; Metin Gürü contributed reagents/materials/analysis tools. All authors wrote the paper. All authors have read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sardar, B.R.; Tarade, K.M.; Singhal. R.S. Stability of active components of cardamom oleoresin in co-crystallized sugar cube during storage. *J Food Eng*. **2013**, 117, 530–537.
   DOİ: 10.1016/j.jfoodeng.2013.03.035

2. Ravindran, M.K. (Ed.). *Cardamom: The Genus Elettaria*. New York, NY: Taylor&Francis, 2002; pp:1-10.
   Print ISBN:978-0-415-28493-6; eBook ISBN: 978-0-203-21663-7

3. Aaishwarya, B.D.; Natvarlal, J.P.; Rashwin, J.P. An ayurvedic strategy for treatment of dementia of Alzheimer's disease. *Pharmacogn Mag*. **2005**, 1, 144-151.
   DOİ: 10.1186/alzrt125

4. Majdalawieh, A.F.; Carr, R.I. In vitro investigation of the potential immunomodulatory and anti-cancer activities of black pepper (*piper nigrum*) and cardamom (E*lettaria cardamomum*). *J Med Food* **2010**, 13 (2), 371-381. DOİ: 10.1089/jmf.2009.1131

5. Benzie, I.F.F.; Wachtel-Galor, S. *Herbal Medicine: Biomolecular and Clinical Aspects*, 2nd edition, CRC Press/Taylor&Francis, Boca Raton (FL), 2011, pp:368, ISBN:13:978-1-4398-0713-2

6. Mutlu İngök, A.; Karbancıoğlu Güler, F. Cardamom, cumin, and dill weed essential oils: chemical compositions, antimicrobial activities, and mechanisms of action against campylobacter spp. *Molecules* **2017**, 22, 1191 DOI: 10.3390/molecules22071191.

7. Khaw, K.Y.; Parat, M.O.; Shaw, P.N.; Falconer, J.R. Solvent supercritical fluid technologies to extract bioactive compounds from natural sources: A review. *Molecules* **2017**, 22, 1186 DOI: 10.3390/molecules22071186.

8. Sánchez-Camargo, A.P.; Mendiola, J.A.; Ibáñez, E.; Herrero, M. *Supercritical Fluid Extraction, Reference Module in Chemistry, Molecular Sciences and Chemical Engineering*, Reedijk, J. (Ed.) Elseiver, Waltham, MA, 2014. DOI:1016/B978-0-12-409547-2.10753-X.

9. Reverchon, E., De Marco, I. Supercritical fluid extraction and fractional of natural matter. *J. Supercrit. Fluids* **2006**, 38, 146-166.
DOI: 10.1016/j.supflu.2006.03.020

10. Herrero, M.; Cifuentes, A.; Ibaniez E. Sub and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae and microalgae. *Food Chem*. **2006,** 98, 136–148.
DOI: 10.1016/j.foodchem.2005.05.058

11. Bocevska, M.; Sovov´a, H. Supercritical CO_2 extraction of essential oil from yarrow. *J. Supercrit. Fluids* **2007**, 360–367.
DOI: 10.1016/j.supflu.2006.07.014

12. Uquiche, E.; Huerta, E.; Sandoval, A.; Valle, J. M. Effect of boldo (peumus boldus m.) pretreatment on kinetics of supercritical CO_2 extraction of essential oil. *J Food Eng*. **2012**, 10, 9230–9237.
DOI: 10.1016/j.jfoodeng.2011.10.013

13. Ekinci, M.S.; and Gürü, M. Extraction of oil and β-sitosterol from peach (prunus persica) seeds using supercritical carbon dioxide. *J. Supercrit. Fluids* **2014,** 92, 319-323.
DOI: 10.1016/j.supflu.2014.06.004

14. Çelik, H.T.; Gürü, M. Extraction of oil and silybin compounds from milk thistle seeds using supercritical carbon dioxide. *J. Supercrit. Fluids* **2015,** 100, 105–109. DOI: 10.1016/j.supflu.2015.02.025

15. İçen, H.; Gürü, M. Extraction of caffeine from tea stalk and fiber wastes using supercritical carbon dioxide. *J. Supercrit. Fluids* **2009,** 50, 225–228. DOI: 10.1016/j.supflu.2009.06.014

16. İçen, H.; Gürü, M. Effect of ethanol content on supercritical carbon dioxide extraction of caffeine from tea stalk and fiber wastes. *J. Supercrit. Fluids* **2010**, 55, 156–160. DOI: 10.1016/j.supflu.2010.07.009

17. Goash, S; Paramitei B.; Das, S. 1,8-cineol-rich cardamom seed (Elettaria cardamomum) extracts using green technologies and conventional extractions: Process analysis, phytochemical characterization, and food application. *Sep. Sci. Technol.* ***2015***, 50, 1974-1985. DOI: 10.1080/01496395.2015.1016038

18. Gopalakrishnan, N.; Narayanan, C.S. Supercritical carbon dioxide extraction of cardamom. *J. Agric. Food Chem.* **1991**, 39, 1976–1978. DOI**:** 10.1021/jf00011a018

19. Marongio, B.; Piras, A.; Porcedda, S. Comparative analysis of the oil and supercritical CO_2 extract of *Elettaria cardamomum* (L.) maton. J. *Agric. Food Chem.* **2004**, 52, 6278–6282.
DOI: 10.1021/jf034819i

20. Hamdan, S.; Daood, H.G.; Toth-Markus, M.; Illés, V. Extraction of cardamom oil by supercritical carbon dioxide and sub-critical propane. *J. Supercrit. Fluids* **2008**, 44 (1), 25–30.
DOI: 10.1016/j.supflu.2007.08.009

21. Critical processes, calculation of density, enthalpy and entropy for supercritical carbon dioxide. http://www.criticalprocesses.com/Calculation%20of%20density,%20enthalpy%20and%20entropy%20of%20carbon%20dioxide.htm, 2017.

22. Goash, S.; Chatterjee, D.; Das, S.; Bhattacherjee, P. Supercritical carbon dioxide extraction of eugenol-rich fraction from *Ocimum sanctum Linn* and a comparative evaluation with other extraction techniques: Process optimization and phytochemical characterization. *Ind Crops Prod*. **2013**, 47, 78-85.
DOI: 10.1016/j.indcrop.2013.02.030

23. Döker O.; Salgın U.; Sanal I.; Mehmetoğlu Ü.; Çalımlı A. Modeling of extraction of β-carotene from apricot bagasse using supercritical CO_2 in packed bed extractor. *J. Supercrit. Fluids* **2004**, 28, 11–19.

DOI: 10.1016/S0896-8446(03)00006-8

24. Reverchon E.; Kaziunas, A.; Marrone C. Supercritical CO_2 extraction of hiprose seed oil: Experiments and mathematical modelling. *Chem. Eng. Sci*. **2000**, 55 (12), 2195–2201.
DOI: 10.1016/S0009-2509(99)00519-9

25. Machmudah, S.; Sulaswatty, A.; Sasaki, M.; Goto, M.; Hirose, T. Supercritical CO2 extraction of nutmeg oil: Experiment and modeling. *J. Supercrit. Fluids* **2006**, 39, 30-39.
DOI: 10.1016/j.supflu.2006.01.007

26. Salgın, U. Extraciton of jojoba seed oil using supercritical CO_2+ethanol mixture in green and high-tech separation process. J. *Supercrit. Fluids* **2007**, 39, 330-337.
DOI: 10.1016/j.supflu.2006.03.013