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Extraction of phytosterols from melon (*Cucumis melo*) seeds by supercritical CO_2 as a clean technology

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Abstract: Extraction with supercritical carbon dioxide (SC-CO_2) which is known as a clean technology was carried out to extract oil from melon (*Cucumis melo*) seeds. SC-CO_2 extraction technique does not contaminate extracts. SC-CO_2 is not a toxic and a flammable solvent. Phytosterols, natural and bioactive compounds, which is known to provide protection against various chronic diseases were examined in the seed oil by using gas chromatography – mass spectrometry (GC-MS). Stigmasterol and β-sitosterol were detected in the melon seed oil. SC-CO_2 extractions were performed in a range of 30-55°C, 150-240 bar, 7-15 g CO_2/min, 0.4-1.7 mm (mean particle size of the seeds) and 1-4 h. The optimal quantities of extracted oil, β-sitosterol and stigmasterol were 36.8 g/100 g seed, 304 mg/kg seed and 121 mg/kg seed, respectively, at 33°C, 200 bar, 11 g CO_2/min, 0.4 mm and 3 h.

Keywords: supercritical carbon dioxide extraction; melon (*Cucumis melo*) seeds; phytosterols; β-sitosterol; stigmasterol

1 Introduction

The interest in supercritical fluid extraction which has been applied to food and natural products to obtain nutritionally beneficial compounds such as phytosterols (plant sterols) has recently increased. Phytosterols, a triterpenes group, are natural and bioactive compounds found in all plant cell membranes [1]. They have similar structural and biological functions to cholesterol [2]. Different structures in their side chain, even though minor, make phytosterols and cholesterol differ from each other functionally and metabolically [3]. The structural similarity of phytosterols to cholesterol allows to reduce the absorption of cholesterol from the gut [4]. It is stated that they provide protection against various chronic diseases such as cardiovascular and liver diseases, diabetes, obesity and cancer [2,5]. There are more than 200 phytosterol species naturally found in the plant kingdom and many are found in edible foodstuffs [5]. Primary sources are vegetables, fruits, nuts and seeds [6]. β-sitosterol, campesterol, and stigmasterol are the most common phytosterols in the human diet [2]. In this study, oil was extracted from Kırkağaç melon seeds by using supercritical carbon dioxide (SC-CO_2). β-sitosterol, stigmasterol and campesterol, known as principal phytosterols, were investigated in the seed oil. According to Turkish Statistical Institute, melon production in Turkey in 2017 was over 1.8 million tonnes (http://www.turkstat.gov.tr/UstMenu.do?metod=temelist). Kırkağaç melon is the most important melon cultivated in Turkey.

Supercritical fluid extraction (SFE) offers several advantages especially for biotechnology. Mass transfer limitations are reduced due to high diffusivity of supercritical fluids, particularly for extraction from porous matrices such as plant material. Their low surface tension provides them to penetrate into smaller pores. Selectivity can be managed by changing solubility with pressure and temperature. Since it is possible to work at low temperatures, it is possible to extract the compounds, which cannot be distilled due to thermal instability [7]. Low viscosities allow for flow with less friction [8] CO_2 is the most widely used as a SFE solvent due to its low critical temperature and pressure, no contamination of extracts, its non-flammable and non-toxic properties. It is available in high purity at relatively low cost [9,10]. For these reasons, supercritical CO_2 extraction is an efficient and green technology for the extraction of bioactive compounds [11].

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2 Experimental

2.1 Raw materials

Melon seeds, naturally dried, were taken from a farmer. The seeds with their shells were milled by using a plant grinder and then grouped into various sizes (0.4 mm, 0.8 mm, 1.2 mm and 1.7 mm) by sieve analysis. CO_2 (99.99%) was used as a solvent in SFE.

2.2 SFE system and procedure

Spe-ed SFE series of "Applied Separations" was used as a SFE equipment to extract melon seed oil. The basic components of the SFE system consist of CO_2 tube, compressor, pump, extraction vessel, oven and extract accumulator. A more detailed description of SFE system is given elsewhere [12]. 5 g of melon seed was charged into a 100 mL extraction vessel. Glass wool plugs were placed in the entrance and exit of the extraction vessel in order to reduce dead volume then the extraction vessel was placed into the oven. The extraction was started after the desirable temperature, pressure and flow rate were achieved. The oil was collected in the collecting vessel at ambient temperature and pressure. The extractions were carried out between 30-55°C, 150-240 bar, 7-15 g CO_2/min, 0.4-1.7 mm mean particle size and 1-4 h.

2.3 Analysis of phytosterols

Saponification of oil was performed at 80°C similarly with the method of Nyam et al. [13]. Unsaponifiable part including phytosterols was extracted with petroleum ether and washed with neutral ethanol-water (1:1, v/v). A sample was taken and then dried under reduced pressure. Trimethylsilyl (TMS) ether derivatives of phytosterols were obtained in the way detailed by Cunha et al. [14]. After cooling of mixture formed from mentioned method, 1 µL of it was injected into the gas chromatography of GC-MS (Thermo Finnigan). The specifications of column, procedure and the temperature programme were given in the previous study [15]. The amounts of the phytosterols were determined as cited by Nyam et al. [13].

3 Results and discussion

As a result of the analysis with GC-MS, β-sitosterol and stigmasterol were found (Figure 1) in melon seed oil by comparison of their retention times according to the standards which have been purchased. 350-650 g/mol of measured molecular mass range in GC-MS and separating phytosterols from other compounds by saponification provided easy selectable peaks.

Other parameters were kept constant at a selected value according to literature research and preliminary experiments to examine the effect of a parameter. Both extraction and analysis trials were performed in triplicate at least.

3.1 Effect of temperature

To examine the effect of temperature on the amounts of oil and phytosterols, extractions were performed at a temperature varying between 30-55°C when the other parameters were fixed at 200 bar, 7 g/min, 0.8 mm and 2 h. The results are represented in Figure 2.

As seen in Figure 2, up to 33°C, a raise of amounts of both oil and total phytosterols was observed with temperature rising. On the contrary, when 33°C was

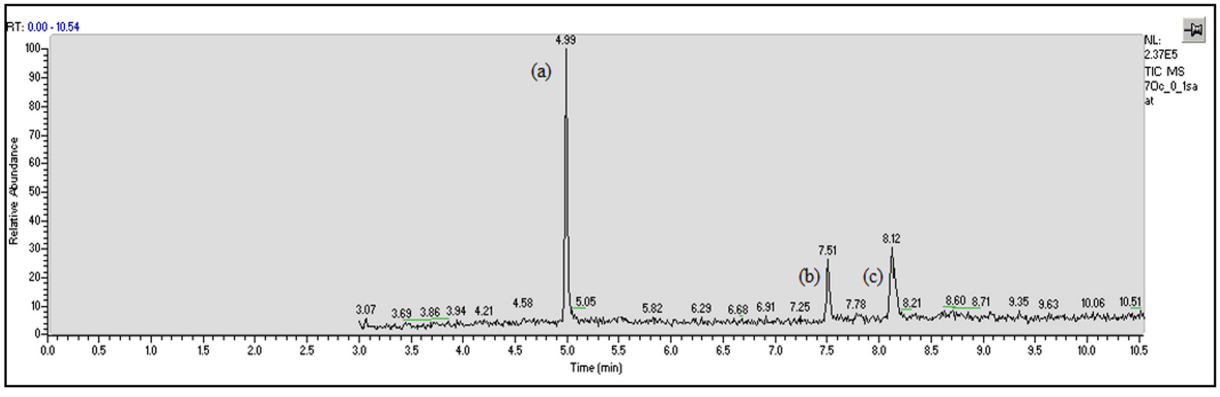

Figure 1: GC-MS chromatograms of derivatives (a) internal standard (5α-cholestane); (b) stigmasterol; (c) β-sitosterol.

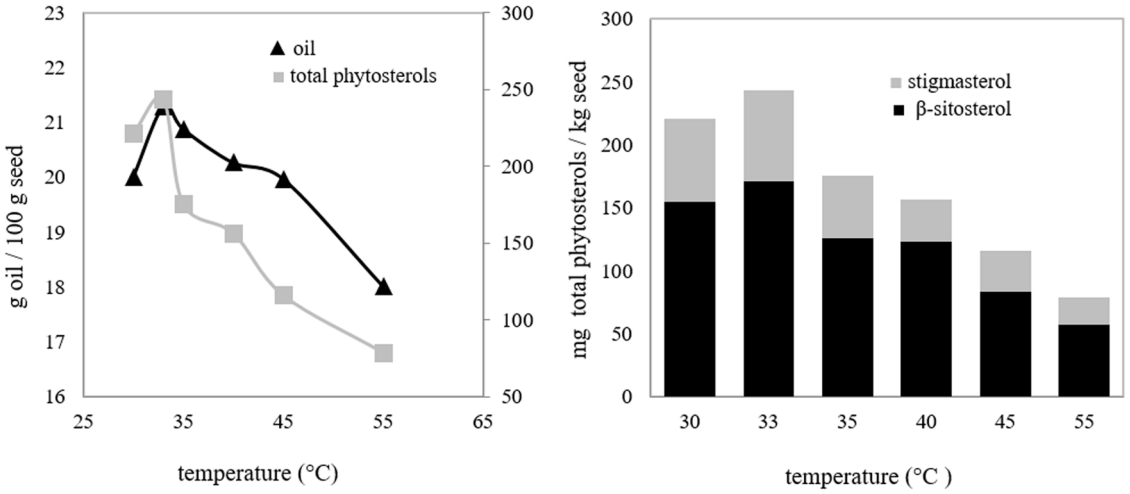

Figure 2: Effect of temperature on the amounts of seed oil and phytosterols: 200 bar, 7 g CO_2/min, 0.8 mm mean particle size, 2 h.

exceeded, amounts of oil and total phytosterols decreased while the temperature increased. Same tendency was also seen for β-sitosterol and stigmasterol. The temperature on the solubility has two opposing effects. With increasing temperature, the density of the solvent is reduced, resulting in lower solvent power. Contrarily, the vapor pressure of the solutes increases, thus increasing the solubility [16]. It indicated that from 30°C to 33°C, increasing vapor pressures of β-sitosterol, stigmasterol and the other active components in oil were dominant in comparison with the decreasing CO_2 density, thus decreasing solvent power. CO_2 is already subcritical fluid at 30°C but it has a lot of properties of supercritical fluid [17]. After 33°C, the reduction of SC-CO_2 density became more effective. Corroborative results were obtained in the literature. The quantities of oil and β-sitosterol extracted from peach seeds were increased with increasing temperature for the range of 35-40°C and then decreased [15]. In a study by Kawahito et al., β-sitosterol recovery strikingly increased first and then decreased with the rising temperature at 40-80°C and 30 MPa. This result proved that increasing vapor pressure of solute was preponderant at lower temperature [18]. According to another study, it was observed that the effect of temperature on the total wax yield and on the content of phytosterols in the wax extracted from flax straw was important. At a constant pressure in the range of 132-468 bar, yields of total wax and phytosterols first increased, and then decreased with increasing temperature [19]. Between 35-65°C, the amount of oil which was extracted from lotus bee pollen initially increased and then decreased at 200 bar. Higher temperature resulted in lower sterol yields [20]. Between 40-80°C, kenaf seed oil yield [21] and oil recovery from okara [22] declined with increasing temperature at 200 bar. Consequently, 33°C was determined to be preferred temperature value for subsequent experiments.

3.2 Effect of pressure

Effect of pressure was examined at 33°C, selected as optimum temperature value, 7 g/min, 0.8 mm and 2 h. The results are given in Figure 3.

As can be seen from the Figure 3 the higher the pressure was, the higher the amounts of oil and phytosterols extracted were. It was represented that depending on increasing of pressure, density of CO_2 increased so its solvent power increased. Between 150-180 bar, there were increments in the quantities of extracts but not significant when compared with the range of 180-200 bar. It is thought that great part of the solutes was partitioned into SC-CO_2 at 180-200 bar. There were insignificant increases in the amounts of extracts and the curve was approximately linear after 200 bar. As a result, 200 bar was determined as the appropriate pressure value. In previously published study [15], altering in the amounts of oil and β-sitosterol with pressure were similar to data which has been obtained in this study. The change in the amount of oil was consistent with the change in another study [22].

3.3 Effect of flow rate of SC-CO_2

The influence of flow rate of SC-CO_2 was investigated between 7 and 15 g CO_2/min (Figure 4). Optimum temperature and pressure values had already been determined as 33°C and 200 bar. The other parameter values were 0.8 mm and 2 h.

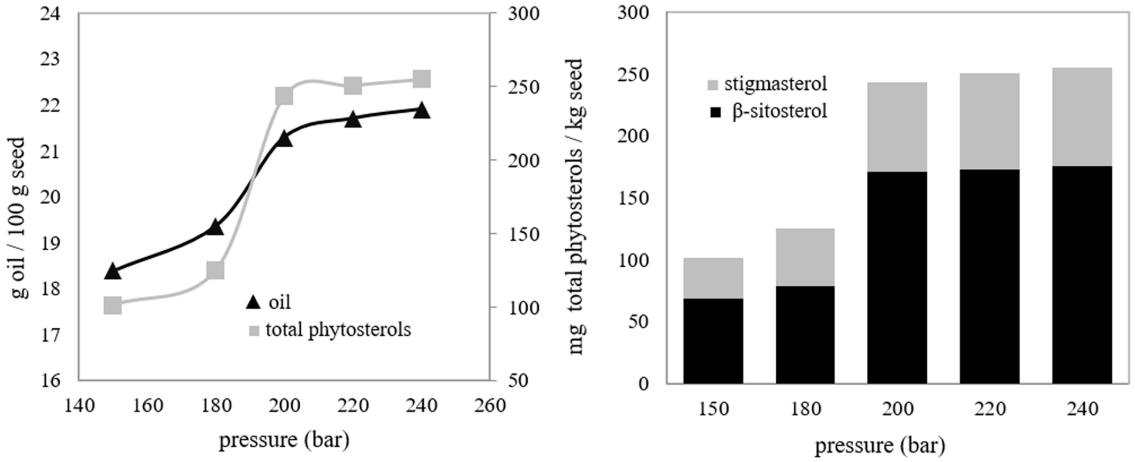

Figure 3: Effect of pressure on the amounts of seed oil and phytosterols: 33°C, 7 g CO_2/min, 0.8 mm mean particle size, 2 h.

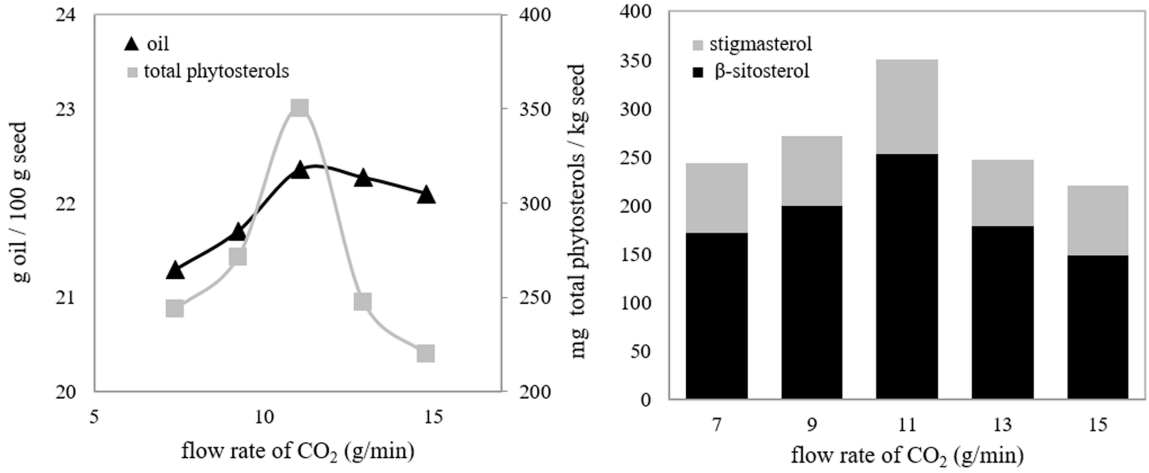

Figure 4: Effect of flow rate of SC-CO_2 on the amounts of seed oil and phytosterols: 33°C, 200 bar, 0.8 mm mean particle size, 2 h.

Figure 4 indicates that the quantities of oil and phytosterols increased from 7 to 11 g/min. It can be explained by the fact that in this range, the external mass transfer resistance or the equilibrium controlled the extraction process. Increased flow rate reduced the external mass transfer resistance. The extraction rate was determined by the flow rate and thus the amount of CO_2 fed the extraction vessel [23]. Conversely, further increases in flow rate caused decreases in the amounts of extracts. These decreases suggest that extraction was controlled primarily by diffusion of extracts and increasing the flow rate of SC-CO_2 could not control the extraction process hereafter. Increasing the flow rate and thus the linear velocity caused to decrease contact time between SC-CO_2 and the seeds resulted in decreases in the amounts of extracts.

It was reported that the flow rate of SC-CO_2 had a remarkable influence on the extraction of wax from flax straw [19]. In earlier studies about extraction of different seed oil, similar courses relevant to influence of the flow rate of SC-CO_2 had been obtained [15]. As a result, 11 g CO_2/min was determined as an optimal value.

3.4 Effect of particle size

Effect of mean particle size was investigated between 0.4 and 1.7 mm for 2 h of extraction time (Figure 5). Optimum temperature, pressure and flow rate had already been selected as 33°C, 200 bar and 11 g/min.

As it is understood from Figure 5 the quantities of oils and phytosterols were increased with decreasing mean particle size. High increases in the quantities of oil and total phytosterols (127% and 109%, respectively) were observed between 1.7 mm and 0.4 mm. Smaller

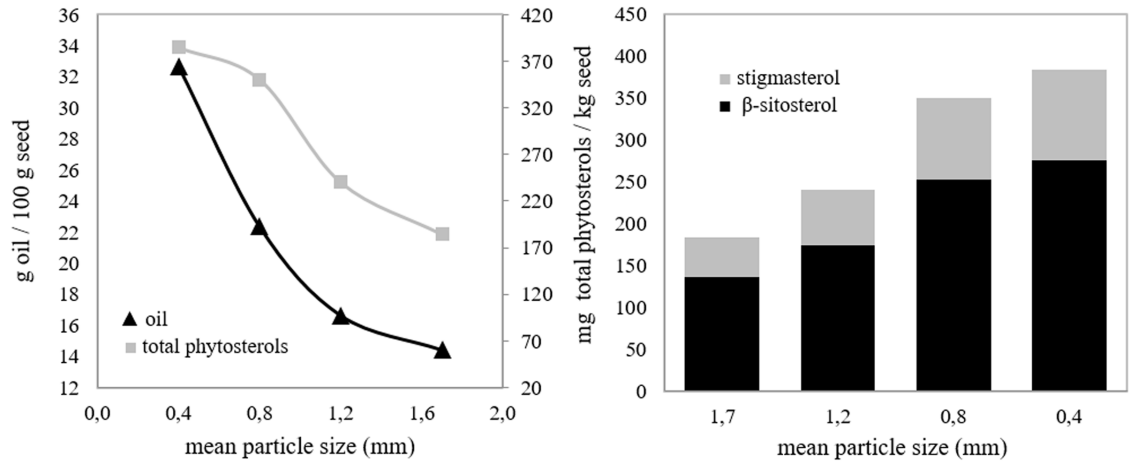

Figure 5: Effect of mean particle size of the seeds on the amounts of seed oil and phytosterols: 33°C, 200 bar, 11 g CO_2/min, 2 h.

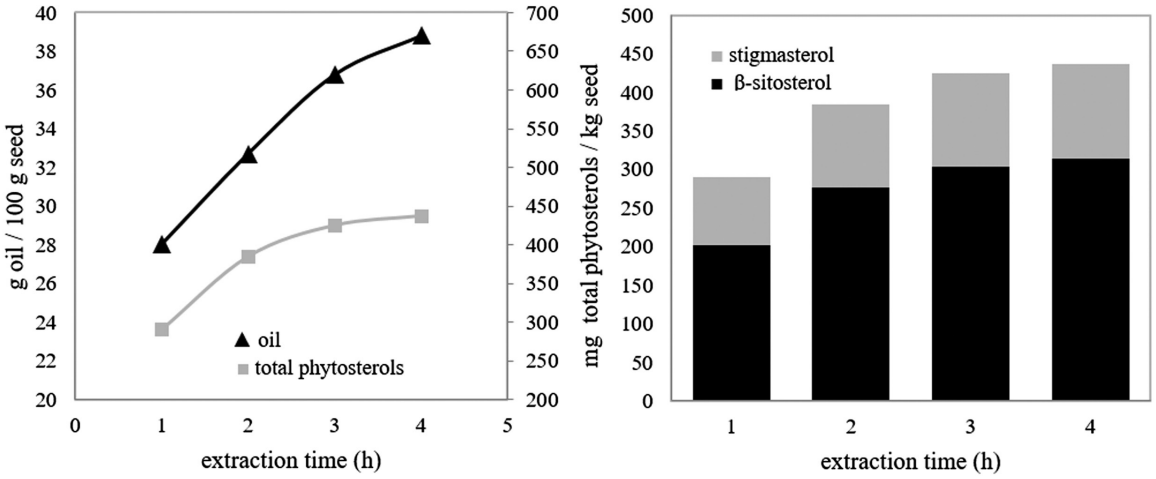

Figure 6: The changes in the amounts of seed oil and phytosterols with extraction time: 33°C, 200 bar, 11 g CO_2/min, 0.4 mm.

mean particle size provided higher contact area between SC-CO_2 and the seeds and also reduced the length of diffusion of the solvent [23]. Therefore, higher quantities of oil and phytosterols were extracted. On the other hand, the seeds were not further downsized to avoid channeling problems.

3.5 Effect of extraction time

After determining the optimal values of temperature, pressure, flow rate of SC-CO_2 and mean particle size (33°C, 200 bar, 11 g CO_2/min and 0.4 mm), the optimal extraction time was determined (Figure 6).

Figure 6 clarifies that the quantities of oil and phytosterols did not change meaningfully from 3 h to 4 h. There were more considerable increases in the quantities of oil and total phytosterols (13% and 10%, respectively) from 2 h to 3 h than from 3 h to 4 h (5% and 3%, respectively). 95% of the oil and 97% of the total phytosterols extracted from melon seeds were obtained at the end of the 3th hour in 4-hour period. Therefore, the optimal extraction time was determined as 3 h.

According to experiment results, optimal quantities of extracted oil, β-sitosterol and stigmasterol were 36.8 g/100 g seed, 304 mg/kg seed and 121 mg/kg seed, respectively, at 33°C, 200 bar, 11 g CO_2/min, a mean particle size of 0.4 mm and an extraction time of 3 h.

4 Conclusions

Phytosterols are very important components for human health because they are known to decrease LDL cholesterol and provide protection against various chronic diseases such as cardiovascular diseases, diabetes and cancer.

In this study, β-sitosterol and stigmasterol which are principal phytosterols were detected in melon seed oil. Melon seed oil was obtained by the supercritical CO_2 extraction which is a clean and an effective method. The optimal values of the extraction parameters were determined. As a result, obtaining of these healthful compounds with this outstanding method is considered as a promising study.

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