Available online at www.sciencedirect.com
ScienceDirect
Separation and Purification Technology 61 (2008) 130–135
Separation and Purification Technology
www.elsevier.com/locate/seppur

Supercritical CO_2 extraction of biological active compounds from loquat seed

Yukari Kawahito [a,c], Mikako Kondo [b], Siti Machmudah [a,d], Katsunari Sibano [c], Mitsuru Sasaki [a], Motonobu Goto [a,*]

[a] *Graduate School of Science and Technology, Kumamoto University, Kurokami 2-39-1, Kumamoto 860-8555, Japan*
[b] *Department of Applied Chemistry and Biochemistry, Kumamoto University, Kurokami 2-39-1, Kumamoto 860-8555, Japan*
[c] *Hyper Plants Co. Ltd., 4F, 1-7-5 Shiba, Minato-ku, Tokyo 105-0014, Japan*
[d] *Department of Chemical Engineering, Sepuluh Nopember Institute of Technology, Kampus ITS Keputih, Sukolilo, Surabaya 60111, Indonesia*

Received 12 July 2007; received in revised form 13 September 2007; accepted 15 September 2007

Abstract

Amygdalin and β-sitosterol from roasted powder and ground unroasted loquat seed were extracted using supercritical CO_2. Loquat seed is the waste of loquat fruit (*Eribotrya japonica*), that called biwa in Japan. Loquat seed contains some active compounds, such as amygdalin, sterol and β-sitosterol. Amygdalin is used for medical purposes, mainly as drug in cancer. β-Sitosterol is found in health supplement for various physical ailments. In this work, extraction was conducted at pressure of 20, 30 and 45 MPa, temperature of 40, 60 and 80 °C, and CO_2 flow rate of 3 ml/min for 180 min. Roasted powder and ground unroasted loquat seeds were used as materials. Component in extract was analyzed by HPLC carried out on 5C18-MS column at 27 °C for amygdalin, and 35 °C for β-sitosterol using acetonitrile:water (85:15 v/v) as mobile phase. Based on results, a change in temperature and pressure affected the yield of total extract, amygdalin and β-sitosterol recovery. For comparison between extract from roasted powder and ground unroasted loquat seed, total extract and β-sitosterol recovery from roasted powder loquat seed were higher than that from unroasted one. On the other hand, amygdalin recovery from ground unroasted seed was much higher than that from roasted powder. The optimum condition of extract from roasted powder and ground unroasted loquat seed and amygdalin recovery were obtained at 80 °C and 20 MPa.

Keywords: Supercritical CO_2 extraction; Loquat seed; Amygdalin; β-Sitosterol

1. Introduction

Loquat (*Eriobotrya japonica*) has been used as a medicinal plant for a long time, and its leaves are known to have many physiological actions such as anti-inflammatory, anti-tussive and expectorant. On the other hand, loquat seeds, like those of apricots and peaches of the same genus (Rosaceae), contain amygdalin as the main constituent; therefore, they were used as a substitute medication for the latter seeds in prewar Japan. Recently, Hamada et al. [1] have investigated that loquat seed extract has an inhibitory effect on liver disorders. They also reported that loquat seed extract has direct anti-oxidative action. In pharmaceutical industry, amygdalin has been applied as a drug in cancer directly injected into humans blood [2]. Loquat seed also contains sterol, β-sitosterol, triglyceride, sterolester, diglyceride and fatty acids, mainly linoleic, palmitic, linolenic and oleic [3,4]. β-Sitosterol has secured an important place in the realm of health supplements with extensive scientific support for their prophylactic and therapeutic use for various physical ailments like atherosclerosis benign prostatic cancer and colon cancer [5,6].

The extraction of active ingredients of herbs has called for a gentle technique that does not pollute and damage these biologically active compounds. Supercritical fluid extraction (SFE) complies with these requirements. For this reason, the current applications of supercritical fluid (SCF) include cleanings, coatings, extractions, impregnations, particle formation, reactions and separations. Carbon dioxide is probably the most widely used as SCF solvent. Its critical temperature (31 °C) makes it an ideal solvent for extracting thermally labile materials and it eliminates from the extract after extraction. CO_2 is also non-toxic, non-flammable, environmentally acceptable and inexpensive.

* Corresponding author. Tel.: +81 96 342 3664; fax: +81 96 342 3679.
E-mail address: mgoto@kumamoto-u.ac.jp (M. Goto).

1383-5866/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.seppur.2007.09.022

These properties of SFE make the products more advantageous in the fields of foods, pharmaceuticals and cosmetics. SFE has been proposed for the extraction of active compounds from herb seeds [7,8].

The extraction of active compounds from loquat seed with some organic solvents have been reported [1,6], but literature lacks information on the extraction using SCF. Furthermore, the present work shows the extraction of active compounds, such as amygdalin and β-sitosterol, from loquat seed using SC-CO_2. The effect of extraction conditions, such as temperature and pressure, on the yield of total extract, amygdalin and β-sitosterol extracted were studied.

2. Materials and methods

2.1. Materials and chemicals

Sample of roasted powder and unroasted loquat seed were purchased from local market in Japan. Prior to processing, the unroasted seeds were dried in oven for 48 h at 50 °C and ground in a coffee grinder. Standard amygdalin and β-sitosterol, HPLC grade of acetonitrile and methanol used for analysis were purchased from Wako Pure Chemical Industries Ltd., Japan. CO_2 was obtained from Uchimura Co., Japan.

2.2. SC-CO_2 extraction

Extraction apparatus used in this work was similar as previous work [8]. Extraction was conducted under pressures of 20–45 MPa, temperatures of 40–80 °C and CO_2 flow rate of 3 ml/min. In each experiment, approximately 4 g of samples were loaded into a 10 ml extraction vessel and the remaining volume was filled with glass beads in the bottom and upper of the cell. The cell was placed in the heating chamber to maintain the operating temperature. The extract was collected in the vial at ambient temperature and 0.1 MPa in every 30 min for 180 min, and weighed immediately after the collection. To determine total amount of amygdalin and β-sitosterol present in the seed, 6 g of seed was extracted with 200 ml of methanol using a soxhlet apparatus for 8 h.

2.3. Chemical analysis

Amygdalin and β-sitosterol content in the extract were determined by high-performance liquid chromatography (HPLC) equipped with PDA detector (Shimadzu, Japan). Extract dissolved in methanol was injected through a 20 μl loop and separated with a reversed-phase 5C18-MS Waters column at 27 °C for amygdalin, and 35 °C for β-sitosterol using acetonitrile:water (85:15 v/v) as mobile phase at a flow rate of 1 ml/min. The separated compounds were monitored at 210 nm. The signals of the detector were recorded in LC solution software. Peak identification of compounds profile was based on comparison of retention times and scanned spectra of amygdalin and β-sitosterol standards. Weight of amygdalin and β-sitosterol in the extract was calculated by comparison of peak area and weight of amygdalin and β-sitosterol standard, respectively.

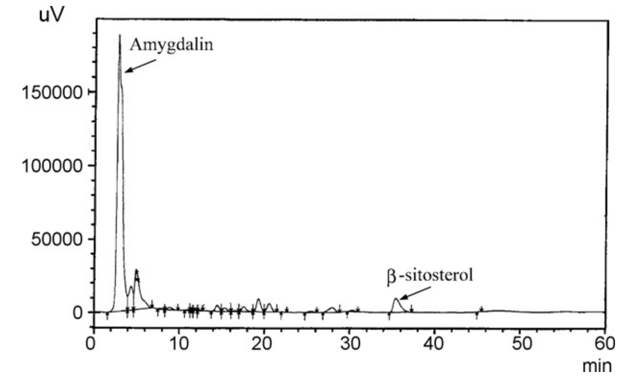

Fig. 1. HPLC chromatogram of loquat seed extract at 80 °C and 30 MPa.

3. Results and discussion

Generally, the extract obtained was white to light yellow of solid with special smell of loquat seed. As chromatogram peak of HPLC analysis in Fig. 1, amygdalin and β-sitosterol peaks appeared at 3.02 and 35.25 min, respectively. On the basis of the soxhlet extraction with methanol, the total amount of amygdalin and β-sitosterol in the unroasted ground loquat seed were 16.38 and 0.59 mg/g, respectively, and in the roasted powder seed were 11.27 and 0.45 mg/g, respectively.

3.1. Effect of temperature

The effect of temperature on the yield of total extract, recovery of amygdalin and β-sitosterol was studied at 30 MPa and 3 ml/min for ground unroasted seed as shown in Fig. 2(a–c), respectively. The yield of extract was obtained by dividing weight of extract with weight of sample loaded in the extractor (w/w %). Recovery of amygdalin and β-sitosterol was defined by dividing weight of amygdalin and β-sitosterol in the extract with weight of initial amygdalin and β-sitosterol contained in the seed (w/w %). In Fig. 2(a), at 40–60 °C yield of total extract significantly decreased with increasing temperature for the short extraction time, and then dramatically increased with increasing temperature at 80 °C. It indicated that at low to moderate temperature extraction was controlled by SC-CO_2 density which decreased with increasing temperature. At this condition, the density effect predominates and the solubility of solute decreases with increasing temperature, which is referred to as retrograde behavior [9]. Contrary at low to moderate temperature, at moderate to high temperature, there was cross-over effect where solute vapor pressure became dominant in the extraction process. Thus extraction yield increased with increasing temperature.

Recovery of amygdalin is shown in Fig. 2(b). As shown in the figure, at 40–60 °C amygdalin recovery was independent on temperature, but at higher temperature amygdalin recovery dramatically increased with increasing temperature. The result indicates that extraction is dependent on solute vapor pressure which increased with increasing temperature. Instead of that, the higher temperature contributed to the decomposition of cell walls, and as a result extractable compounds availability for extraction was increased [10]. In contrast, as

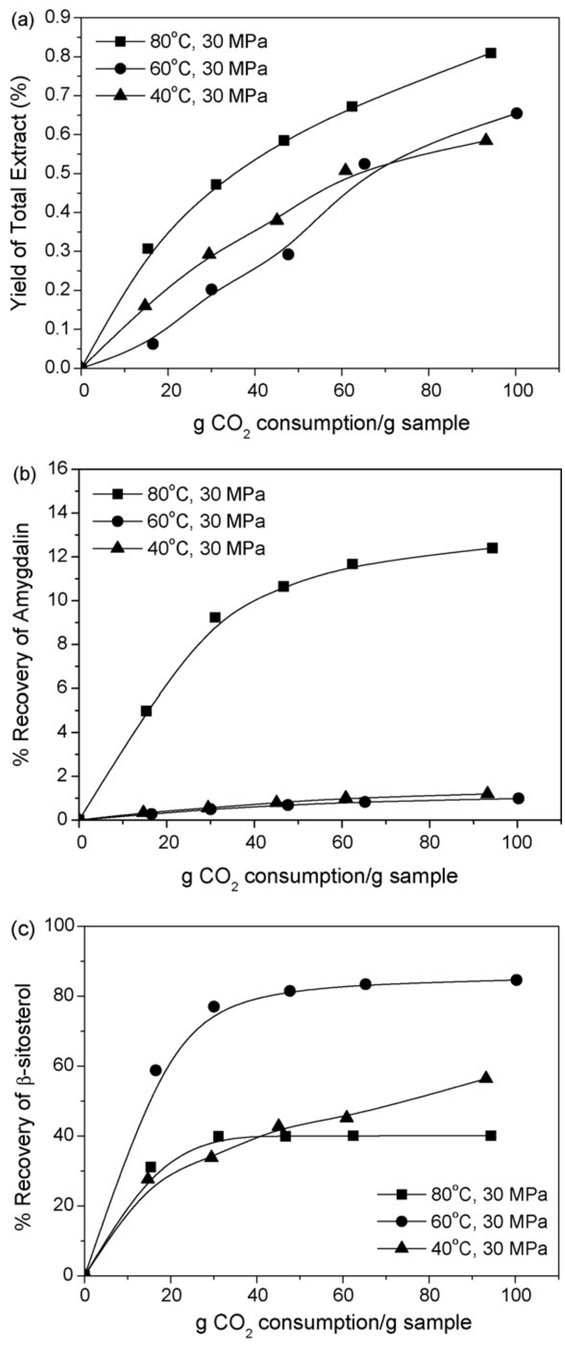

Fig. 2. Effect of temperature on the yield of total extract (a); % recovery of amygdalin (b); and % recovery of β-sitosterol (c) at 30 MPa for ground unroasted seed.

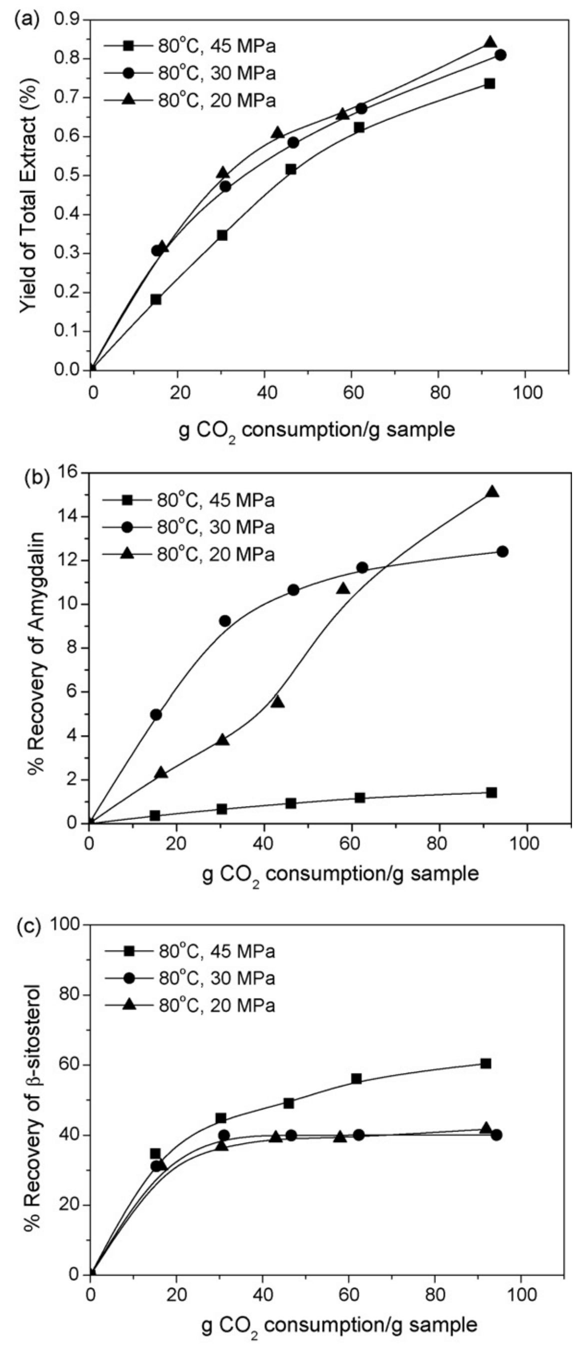

Fig. 3. Effect of pressure on the yield of total extract (a); % recovery of amygdalin (b); and % recovery of β-sitosterol (c) at 80 °C for ground unroasted seed.

shown in Fig. 2(c) for the effect of temperature on the recovery of β-sitosterol, at lower to moderate temperature β-sitosterol recovery dramatically increased with the increasing temperature, and then dramatically decreased at higher temperature. The result evidences that at lower to moderate temperature solute vapor pressure was dominant. At higher extraction temperature, selectivity of β-sitosterol seems to be lower than amygdalin that increased dramatically as temperature increased.

At all conditions, amygdalin recovery was much lower than that of β-sitosterol. It can be explained that amygdalin is polar compound and β-sitosterol is unpolar compound easily extracted by SC-CO_2.

3.2. *Effect of pressure*

The effect of pressure on the yield of total extract, recovery of amygdalin and β-sitosterol were studied at 80 °C for ground unroasted seed as shown in Fig. 3(a–c), respectively. In Fig. 3(a), the increasing pressure almost did not affect to the yield of total extract. The increasing pressure usually causes increasing extraction rate due to the increasing SC-CO_2 density. But in this case solubility of loquat seed extract in SC-CO_2 seems to be limited, thus it is independent on the pressure.

As shown in Fig. 3(b), at 20–30 MPa the increasing pressure caused the increasing amygdalin recovery, but it decreased at 45 MPa. At lower to moderate pressure, the dependency on the pressure was expected as the SC-CO_2 density increases as increasing pressure, and therefore the solvent power to dissolve the substances increases. But at higher pressure, the increasing SC-CO_2 density may not affect to the solubility of amygdalin in SC-CO_2. To understand the effect of high pressure on the amygdalin extraction, pure amygdalin was extracted using SC-CO_2 at 45 MPa. As a result in HPLC analysis of the extract, except amygdalin peak, other peak also appeared at 18.13 min. It indicated that amygdalin was decomposed into other compound that could not be identified. The result could explain the decreasing amygdalin recovery at high pressure, where high pressure caused decomposition of amygdalin. On the contrary, higher pressure increased the recovery of β-sitosterol as shown in Fig. 3(c). It indicated that the increasing SC-CO_2 density caused the increasing solubility of β-sitosterol in SC-CO_2, and then increased the extraction rate. At lower and moderate pressure, the increasing pressure did not affect to the β-sitosterol recovery.

3.3. Comparison between extract from roasted powder seed and ground unroasted seed

Fig. 4(a) shows comparison between yield of total extract of roasted powder and ground unroasted seed. As shown in the figure, total extract of roasted powder seed was higher than the ground unroasted one. It can be explained that particle size of roasted powder (about 0.3 mm) is much smaller than the ground unroasted one (about 1.7 mm), which was only ground by a coffee grinder. Smaller particle size caused larger surface area of the seed to contact with SC-CO_2 and more broken cell availability of loquat seed, thus extractible compounds increased.

The comparison between recovery of amygdalin from roasted powder and ground unroasted seed is shown in Fig. 4(b). Amygdalin recovery from ground unroasted seed was much higher than that from roasted powder one. It might be the decreasing amygdalin content in the roasted powder seed during roasting process as indicating from the initial amount of amygdalin in the seed. As known, the roasting process needs high temperature that can decompose heat sensitive labile compounds in the seed. Amygdalin is also relatively unstable compound that easy to decompose into other compounds [2].

Fig. 4(c) shows the comparison between recovery of β-sitosterol from roasted powder and ground unroasted seed. As shown in the figure, β-sitosterol recovery from roasted powder loquat seed was higher than that from unroasted one. As expected, for smaller particle size β-sitosterol recovery is high due to the reduction particle size can open the plant cells and increase the surface area of the seed to contact with SC-CO_2.

3.4. Optimum condition of loquat seed extraction

To determine the optimum condition in the range of experimental condition, yield of total extract from roasted powder and ground unroasted seed and recovery of amygdalin were plotted with temperature and pressure at constant CO_2 flow rate, as shown in Fig. 5(a), (b) and (c), respectively. As shown in Fig. 4(a) the highest yield of total extract from roasted powder loquat seed was obtained at high temperature and low pressure. The increasing temperature caused

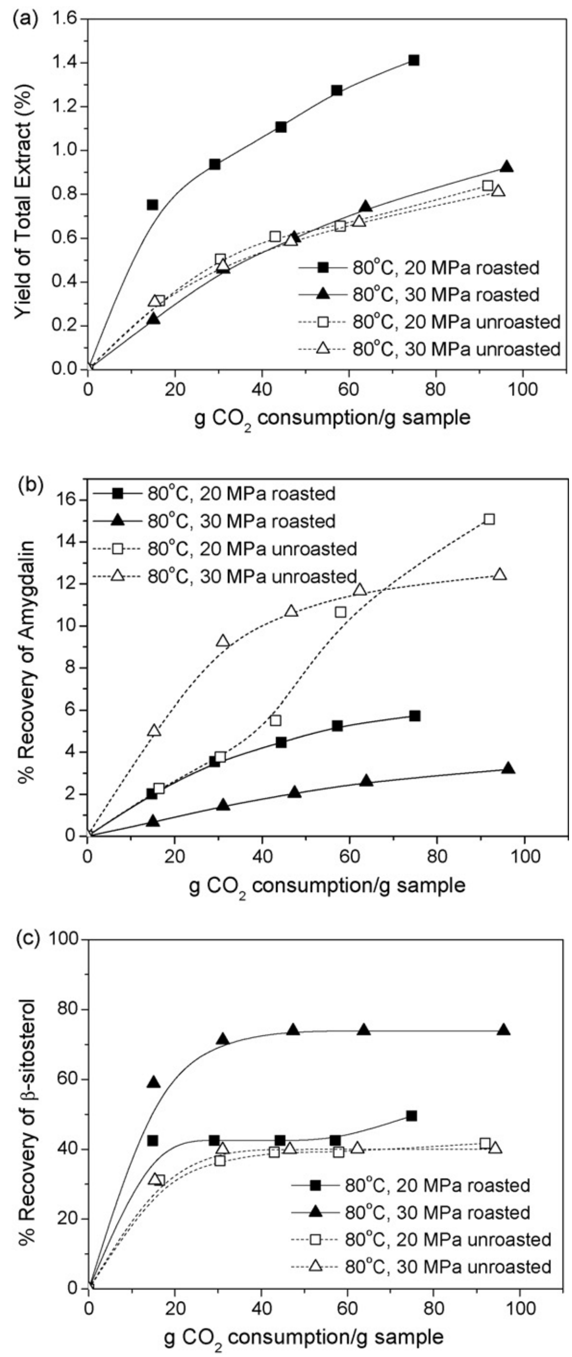

Fig. 4. Comparison of total extract yield (a); amygdalin recovery (b); and β-sitosterol recovery (c) between extract from roasted powder and ground unroasted loquat seed.

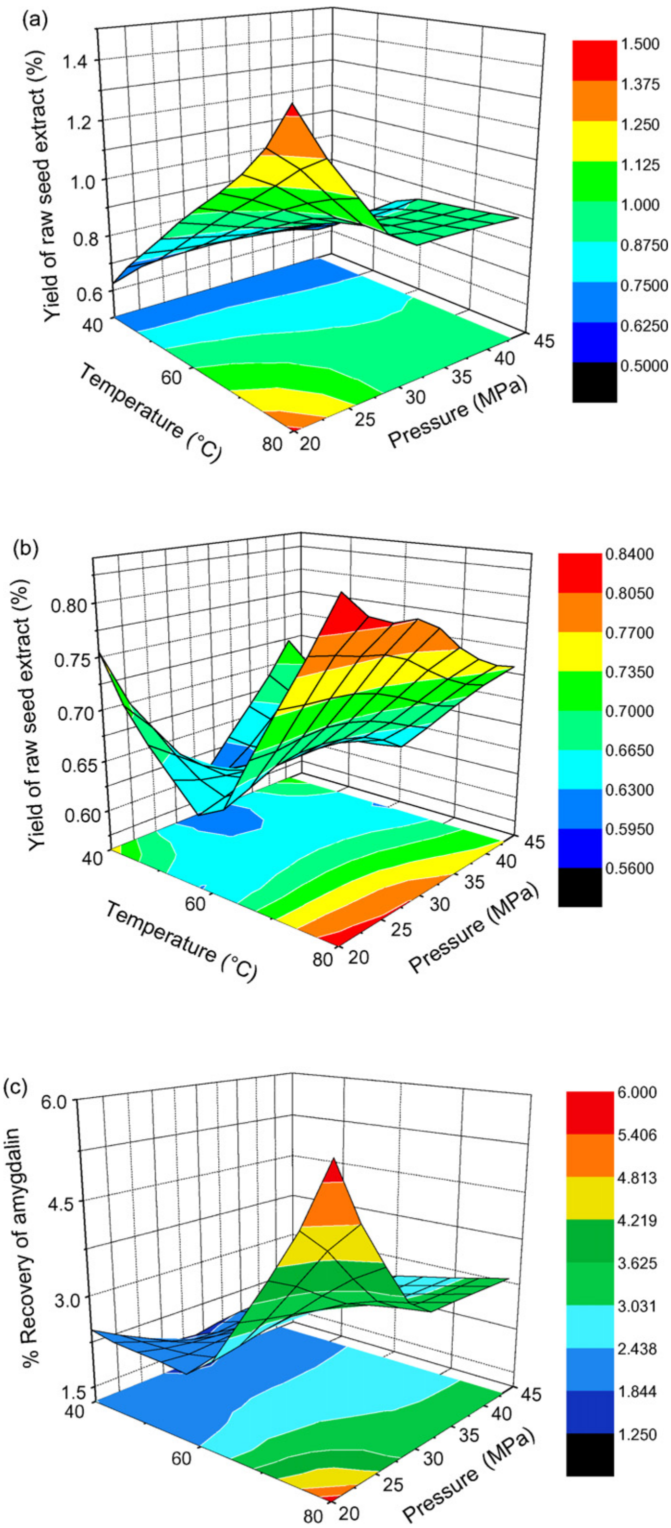

Fig. 5. Yield of total extract of roasted powder (a); yield of total extract of ground unroasted (b); and amygdalin recovery of ground unroasted loquat seed (c) as function of temperature and pressure.

the increasing yield, but the increasing pressure caused the decreasing yield. The optimum condition of the extraction process for roasted powder loquat seed was found at 80 °C and 20 MPa.

In Fig. 5(b) maximum yield of total extract from ground unroasted loquat seed was found at high temperature and low pressure. Moderate temperature at all pressures resulted minimum yield of total extract. To obtain the highest yield for extraction of ground unroasted loquat seed, 80 °C and 20 MPa of extraction condition appeared to be the optimum one.

As shown in Fig. 5(c), maximum recovery of amygdalin from ground unroasted loquat seed was obtained at high temperature and low pressure. The increasing temperature caused the increasing recovery of amygdalin; on the other hand the increasing pressure caused the decreasing amygdalin recovery due to decomposition of amygdalin. The optimum condition of amygdalin recovery was found at 80 °C and 20 MPa.

4. Conclusion

Extraction from roasted powder and ground unroasted loquat seed has been conducted at various temperatures and pressures to obtain the optimum condition of the process. The change of temperature and pressure affected to the yield of total extract, amygdalin and β-sitosterol recovery. For the comparison between extract from roasted powder and ground unroasted loquat seed, total extract and β-sitosterol recovery from roasted powder loquat seed were higher than that from unroasted one. On the other hand, amygdalin recovery from ground unroasted seed was much higher than that from roasted powder. The optimum condition of extract from roasted powder and ground unroasted loquat seed and amygdalin recovery were obtained at 80 °C and 20 MPa.

Acknowledgment

This work was partly supported by Kumamoto University 21st Century COE Program "Pulsed Power Science".

References

[1] A. Hamada, S. Yoshioka, D. Takuma, J. Yokota, T. Cui, M. Kusunose, M. Miyamura, S. Kyotani, Y. Nishioka, The effect of *Eriobotrya japonica* seed extract on oxidative stress in adriamycin-induced neophrapathy in rats, Biol. Pharm. Bull. 27 (2004) 1961–1964.

[2] A. Silem, H.-O. Gunter, J. Einfeldt, A. Boualia, The occurrence of mass transport processes during the leaching of amygdalin from bitter apricot kernels: detoxification and flavor improvement, Int. J. Food Sci. Technol. 41 (2006) 201–213.

[3] H. Tsuyuki, S. Itoh, Y. Nakatsukasa, Lipids in loquat seed, Nihon Daigaku Nojuigakubu Gijutsu Kenkyu Hokoku 34 (1977) 155–161.

[4] A. Femenia, M. Garcia-Conesa, S. Simal, C. Rossello, Characterization of the cell walls of loquat (*Eriobotrya japonica* L.) fruit tissues, Carbohydr. Polym. 35 (1998) 169–177.

[5] V.D.P. Nair, I. Kanfer, J. Hoogmartens, Determination of stigmasterol. β-Sitosterol and stigmastanol in oral dosage forms using high performance liquid chromatography with evaporative light scattering detection, J. Pharm. Biomed. Anal. 41 (2006) 731–737.

[6] J. Yokota, D. Takuma, A. Hamada, M. Onagawa, S. Yoshioka, M. Kusunose, M. Miyamura, S. Kyotani, Y. Nishioka, Scavenging of reactive oxygen species by *Eribotrya japonica* seed extract, Biol. Pharm. Bull. 29 (2006) 467–471.

[7] S. Machmudah, A. Sulaswatty, M. Sasaki, M. Goto, T. Hirose, Supercritical CO_2 extraction of nutmeg oil: experiments and modeling, J. Supercrit. Fluid 39 (2006) 30–39.
[8] S. Machmudah, Y. Kawahito, M. Sasaki, M. Goto, Supercritical CO_2 extraction of rosehip seed oil: fatty acids composition and process optimization, J. Supercrit. Fluid 41 (2007) 421–428.
[9] O. Guclu-Ustundag, F. Temelli, Correlating the solubility behavior of fatty acids, mono-, di-, and tri-glycerides, and fatty acid esters in supercritical carbon dioxide, Ind. Eng. Chem. Res. 39 (2000) 4756–4766.
[10] S. Machmudah, A. Shotipruk, M. Goto, M. Sasaki, T. Hirose, Extraction of Astaxanthin from *Haematococcus pluvialis* using supercritical CO_2 and ethanol as entrainer, Ind. Eng. Chem. Res. 45 (2006) 3637–3652.