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ORIGINAL PAPER

# Extraction of Fatty Acids from *Boletus edulis* by Subcritical and Supercritical Carbon Dioxide

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**Abstract** Mushrooms contain many valuable compounds such as fatty acids, carbohydrates, lycopene, lovastatin, trace elements etc. As they are currently valued for biologically active substances, and as high pressure carbon dioxide fluid extraction has been documented as an effective method for preparing bioactive products from plant materials, subcritical and supercritical carbon dioxide extraction of *Boletus edulis* mushroom was performed. In the extracts obtained, the fatty acids were determined. Response surface methodology (RSM) was applied to investigate the effect of pressure and extraction time on the extraction yield. The analysis of variance showed that pressure and extraction time had a significant effect on the extraction yield in both investigated process. The interaction between pressure and extraction time had a significant effect only in supercritical extraction process of *B. edulis*. Higher extraction yields have been obtained by subcritical carbon dioxide, and higher linoleic acid content has been determined in extracts obtained by supercritical carbon dioxide.

**Keywords** *Boletus edulis* · Fatty acids · Subcritical extraction · Supercritical extraction

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## Introduction

Mushrooms are a valuable healthy food, rich in almost all amino acids, vegetable proteins, vitamins and minerals, but low in calories. Wild edible mushrooms have been traditionally used in many Asian countries. They were found to be a therapeutic food useful in preventing many diseases. Studies have shown cholesterol-lowering, antitumor, antiviral, antithrombotic and immunomodulating effects of different mushroom species [1]. Mushroom lipid content is low, but it has been found that many wild edible mushrooms contain high levels of several unsaturated fatty acids (UFA). Results from several papers dealing with fatty acids composition of edible mushroom, showed that polyunsaturated fatty acids (PUFA) are present in significant amounts [2]. The high content of these PUFA, particularly linoleic acid, contributes positively to the diets of people with high blood cholesterol [3]. Linoleic acid is a potential suppressor of tumor growth and metastasis [4], as well as having secondary prevention of coronary heart diseases, hypertension, rheumatoid arthritis, ulcerative colitis, Crohn disease, and chronic obstructive pulmonary diseases [5].

Usually plant materials, mushrooms as well, are extracted by organic solvents. Traditional solid-organic solvent extraction methods are very time-consuming, they required relatively large quantities of solvents, they leave toxic solvent residue, and cause degradation of unsaturated compounds due to the heat. Because of this fact there is an increasing demand for different extraction techniques with shorter extraction times, reduced organic solvent consumption, and decreased pollution [6]. Subcritical and supercritical carbon dioxide extraction, represent an alternative to classical organic solvent extraction.

Carbon dioxide is the most widely used compressed fluid, especially for the extraction of natural products,

because it is non-toxic, non-explosive, readily available, easy removable from the product and possesses convenient critical properties ($T_c = 31.1$ °C, $P_c = 73.8$ bar) [7]. It is a convenient solvent for obtaining valuable substances that could find use not only in food but also in the pharmaceutical industry. $CO_2$ is a generally recognized as safe (GRAS) solvent so that products extracted with "food grade" carbon dioxide are safe with respect to human health [8].

The solvent properties of carbon dioxide can be described as a function of fluid density [9]. The subcritical state can be reached under the critical temperature and pressure. Under these conditions, solute solubility, particularly non-volatile or more polar solutes can be much less than in supercritical fluids. The efficiency of both, supercritical and subcritical extraction, can be affected by several variables, alone or in combination, which includes the operation temperature and pressure, extraction time and carbon dioxide flow rate [10]. The most dominant effect on the extraction process is the effect of the operating pressure. Selection of the operating conditions in the process of carbon dioxide extraction for specific application is still an area of active research [11].

Although the prices of wild edible mushrooms are higher than cultivated ones, people prefer to consume them due to their flavor and texture [12]. *B. edulis* is a widely used wild edible mushroom, has a worldwide distribution, and its consumption is increasing from year to year primarily due to the nutritional value [13]. It is the most consumed wild mushroom in the Balkan region. There are no reports on supercritical extraction of *B. edulis* or extraction by subcritical carbon dioxide, even though much attention has been paid to its chemical composition, nutritional evaluation and lipid profiles [2, 3]. The aim of this research was to explore the feasibility of using carbon dioxide, in subcritical and supercritical states, to extract the valuable compounds from the investigated plant material. The extraction yield, at different pressures during the extraction time, was monitored. The response surface methodology was applied to investigate the effects of the pressure and the extraction time on the yield of *B. edulis* extracts obtained by supercritical and subcritical carbon dioxide extraction. The fatty acid composition of the extracts obtained was determined by GC/MS and quantified by GC/FID analysis.

## Experimental Procedure

### Chemicals

Commercial carbon dioxide (Messer, Novi Sad, Serbia), $CH_2Cl_2$ (Supelco), methanol and hexane (J. T. Baker), $Na_2SO_4$ and $NaHCO_3$ (Sigma) were used. All other chemicals were of analytical reagent grade.

### Plant Material and Sample Preparation

*B. edulis* mushroom samples were collected from the Istra region, Croatia, in late summer 2008. The mushroom fruiting bodies were cleaned to remove any residual compost. Fresh mushrooms were dried and then stored in airtight plastic bags at room temperature. The humidity of dry mushroom material was determined as 12.61 ± 0.02%. All the dried mushrooms samples were ground in a blender before the extraction. Particle size was determined using sieve sets (Erweka, Germany).

### Soxhlet Extraction

To determine the total oil content, ground mushroom, *B. edulis*, was extracted by a Soxhlet device for 10 h using hexane as the solvent, then evaporated to obtain mushroom oil. Experiments were conducted in triplicate.

### Liquid and Supercritical Carbon Dioxide Extraction

The extraction process was carried out on a laboratory-scale high pressure extraction plant (HPEP, NOVA Swiss 565.0156, Effertikon, Switzerland). The main plants parts (Fig. 1) and properties, by manufacturer specification, were: gas cylinder with $CO_2$, the diaphragm type compressor (with pressure range up to 1,000 bar, made of stainless steal), extractor with heating jacket for heating medium (with internal volume 200 ml, internal diameter of 40 mm, maximum operating pressure of 700 bar and temperature of 100 °C, made of stainless steel), separator with heating jacket for heating medium (with internal volume 200 ml, internal diameter 40 mm, maximum operating pressure of 250 bar, made of stainless steel), pressure control valve (self-contained, direct-acting, spring-loaded reducing valve with self-relieving and venting, Series 26-1000, Tescom corporation), temperature regulation system made using thermostats and liquid water, regulation valves and control and operating panel (with two pressure digital indicator and two digital temperature indicator). Maximum carbon dioxide mass flow rate is 5.7 kg/h.

The ground mushroom samples (60.0 g), mean particle size of 0.283 mm, were placed in an extractor vessel. The extraction process was carried out and the extraction yield was measured. A flow rate of carbon dioxide, expressed under normal conditions, was 97.725 $dm^3$/h. Investigated values of pressure were 100, 200 and 300 bar at a temperature of 27 °C for subcritical extraction, and 40 °C for supercritical carbon dioxide. Separator conditions were

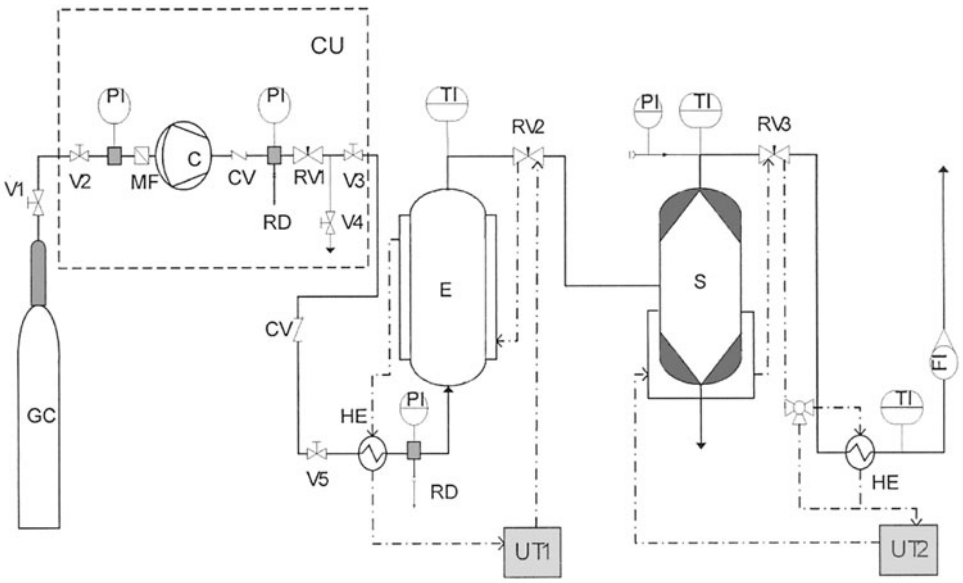


**Fig. 1** Laboratory-scale high pressure extraction plant. *GC* gas cylinder, *CU* compressor unit, *C* compressor with diaphragm, *E* extractor, *S* separator, *HE* heat exchanger, *UT* ultra thermostat, *RV* regulation valve, *V* on–off valve, *MF* micro filter, *CV* cut-off valve, *RD* rapture disc, *PI* pressure indicator, *TI* temperature indicator, *FI* flow indicator

15 bar and 23 °C. Extractions were conducted in duplicate. After extraction, the extracts obtained were placed in glass bottles, sealed, and stored at −4 °C to prevent any possible degradation.

## Chromatographic Procedure

By extracting 200 mg of each extract sample with 1 ml of acetonitrile for 15 min, using an ultrasonic bath, filtration, and addition of 10% methylene chloride, samples were prepared for GC/MS analysis.

Transesterification was carried out by dissolving 200 mg of each sample in 2 ml 3% $H_2SO_4$ methanol solution. The vials with reaction mixtures were sealed and heated in a water bath for 2 h. After cooling, the mixture was neutralized with 2 ml 10% $NaHCO_3$ solution and 2 ml $CH_2Cl_2$ was added. The organic layer was dried over anhydrous $Na_2SO_4$, filtered and evaporated. The residue was dissolved in $CH_2Cl_2$ after what volume was evaporated to the final volume of 1 ml and than injected in GC system. After transesterification procedure, methyl esters of carboxylic acids were detected by GC/MS and quantified by GC/FID.

GC/MS analysis was run on Agilent 7890 GC system coupled to a quadruple mass spectrometer model Agilent 5975C. For mushroom extract analysis, the GC was fitted with a capillary HP-5MS column (0.25 μm film thickness, 30 m length and inner diameter 0.25 mm). The operating conditions were as follows: injector temperature 250 °C; split ratio, 20:1; detector temperature, 300 °C; carrier gas He with constant pressure of 21.956 psi (1.5 bar). The temperature program was: from 60 to 300 °C (3 °C/min). The mass spectrometry (MS) conditions were as follows: ionization voltage, 70 eV; ion source temperature, 230 °C; scan range, *m*/*z* 35–550. Then, 2 μl of acetonitrile extracts were injected into the system. Total analysis time was 80 min. The identification of the components was carried out based on computer matching with Adams and NIST/EPA/NIH version 2.0d mass spectral libraries. The components of the extracts were also identified by comparing their retention times to those in the Adams library.

For methyl esters analysis the GC was fitted with a capillary DB-23 column (0.25 μm film thickness, 30 m length and inner diameter 0.25 mm). The operating conditions were as follows: injector temperature, 250 °C; split ratio, 20:1; detector temperature, 300 °C; carrier gas, He with constant pressure of 37.7 psi (2.6 bar). The temperature program was: from 200 to 240 °C (5.6 °C/min), and held at 240 °C for 15 min. The mass spectrometry (MS) conditions were same as those for intact extract analysis. Total analysis time was 22.14 min. Injected sample volume was 0.2 μl.

For GC/FID analysis of methyl esters Hewlett-Packard chromatograph model HP 5890 Series II equipped with FID and fitted with the same column as those for GC/MS analysis (DB-23) was used. The operating conditions were as follows: injector temperature, 250 °C; split ratio, 30:1; detector temperature, 300 °C; carrier gas, $H_2$ with 1.0 ml/min flow rate. The temperature program was: from 150 to 240 °C (4 °C/min), and held at 240 °C for 10 min. 1 μl of the sample dissolved in $CH_2Cl_2$ (1:100; v/v) was injected. Total analysis time was 32.50 min. The percentage composition was computed by the normalization method from the GC (FID) peak areas.

One-way analysis of variance (ANOVA) and multiple comparisons (Duncan's post-hoc test) were used to evaluate the significant difference of the data at $p < 0.05$. Data were expressed as means ± standard deviation.

Experimental Design

In this study, response surface methodology (RSM) and central composite design were used to determine the optimal extraction time and pressure for *B. edulis* subcritical and supercritical extraction. Response surface methodology is a statistical method, which used quantitative data from an appropriate experimental design to determine or simultaneously solve multivariate equations [14]. This experimental methodology can generate a mathematical model. The main interaction effects of two investigated factors, pressure ($X_1$) and extraction time ($X_2$), on extraction yield, were also evaluated. Extraction temperature and carbon dioxide flow rate were fixed values. Twelve experiments for subcritical and the same number of experiments for supercritical carbon dioxide extraction were performed. Investigated factors and levels tested are reported in Table 1.

The experimental data were fitted to a second order response surface model (Eq. 1) with the following form:

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum \sum_{i<j=1}^{3} b_{ij} X_i X_j \qquad (1)$$

where $Y$ is response (extraction yield, %), $b_0$, $b_i$, $b_{ii}$, $b_{ij}$ are constant coefficients of intercept, linear, quadratic, and interaction terms, respectively. $X_i$ and $X_j$ are coded independent variables (pressure and time). Analysis was performed using STATISTICA 8.0, StatSoft (Europe) GmbH, Hamburg, Germany. The analysis of variance (ANOVA) was also used to evaluate the quality of the fitted model. The test of statistical difference was based on the total error criteria with a confidence level of 95.0%.

**Table 1** The tested factors and levels for the design experiment for subcritical and supercritical carbon dioxide extraction of *B. edulis*

| Factor | Low level (−1) | Medium level (0) | High level (+1) |
|---|---|---|---|
| Pressure ($X_1$, bar) | 100 | 200 | 300 |
| Time ($X_2$, h) | 1 | 2 | 3 |

## Results and Discussion

The samples of *B. edulis* mushroom were extracted by subcritical and supercritical carbon dioxide under different pressures during the different extraction time. The extraction process of *B. edulis* by subcritical carbon dioxide was investigated at a temperature of 27 °C and at the three different pressures (100, 200 and 300 bar). The extraction process by supercritical carbon dioxide was investigated at a temperature of 40 °C and at the same three different pressure values as in subcritical case. The central composite design was used to optimize two important operating variables (pressure and time), for both processes of the extraction, in order to achieve the optimal yield of *B. edulis* extract. The applied design required 12 experiments in subcritical and 12 experiments in supercritical case (Table 2), with three replicates for the central point.

From the experimental results obtained for an applied pressure interval from 100 to 300 bar and a time interval from 1 to 3 h, it was obvious that the highest experimental extraction yield had been reached at a pressure of 300 bar after 3 h of extraction for subcritical carbon dioxide extraction (2.367%). At the same pressure and for the same time using supercritical carbon dioxide extraction, the extraction yield achieved was 2.013%. A significant difference in extraction yield, for these two extraction processes, was noted at a pressure of 100 bar for an extraction

**Table 2** Experimental matrix and values of observed response

| Run number | Pressure (bar) | Time (h) | Extraction yield (%) by subcritical $CO_2$ | Extraction yield (%) by supercritical $CO_2$ | Coded pressure variable $x_1$ | Coded time variable $x_2$ |
|---|---|---|---|---|---|---|
| 1 | 100 | 1 | 1.484 | 0.550 | −1 | −1 |
| 2 | 100 | 2 | 1.848 | 0.829 | −1 | 0 |
| 3 | 100 | 3 | 2.030 | 0.972 | −1 | 1 |
| 4 | 200 | 1 | 1.840 | 1.172 | 0 | −1 |
| 5 | 200 | 2 | 2.143 | 1.684 | 0 | 0 |
| 6 | 200 | 3 | 2.236 | 1.905 | 0 | 1 |
| 7 | 300 | 1 | 1.929 | 1.228 | 1 | −1 |
| 8 | 300 | 2 | 2.249 | 1.760 | 1 | 0 |
| 9 | 300 | 3 | 2.367 | 2.013 | 1 | 1 |
| 10 | 200 | 2 | 2.133 | 1.692 | 0 | 0 |
| 11 | 200 | 2 | 2.101 | 1.673 | 0 | 0 |
| 12 | 200 | 2 | 2.162 | 1.685 | 0 | 0 |

time of 3 h. Under these conditions, a much higher extraction yield was obtained using subcritical carbon dioxide extraction, 2.030%, than by using supercritical carbon dioxide, 0.972%. The extraction yield obtained by subcritical carbon dioxide, at 100 bar after 3 h of extraction, was almost identical to the extraction yield obtained by supercritical carbon dioxide, after the same extraction time, but at a much higher pressure of 300 bar. There was no significant difference in extraction yields obtained after 3 h at higher pressure (200 bar and 300 bar) between these two extraction processes. Furthermore, it was observed that the extraction yield for the supercritical process rapidly increased by increasing the pressure from 100 to 200 bar.

The extraction yields obtained were compared to the yield obtained using Soxhlet extraction using hexane. This extraction yield was higher, 3.025 ± 0.004%, thus, subcritical and supercritical extraction by carbon dioxide can offer a comparable yield to that extracted by hexane, and the extracts obtained, which is important, are without any trace of toxic residues.

According to the fact that various parameters potentially affected the extraction process, an investigation of their effects and interactions is needed. The fluid pressure and dynamic extraction time has been generally considered as the most important factors. This factors and their influence on extraction process were analyzed by RSM.

The effect of the linear, quadratic or interaction coefficients on the response was tested for significance by analysis of variance (ANOVA). Regression coefficients of intercept, linear, quadratic, and interaction terms of the model were calculated using the least square method. The degree of significance of each factor is represented in the Table 3 by its $p$ value. When the $p$ value of a factor is smaller then 0.05, the factor has a significant influence on the process (for a confidence level of 0.95). In Table 3 $X_1$ is the linear term of pressure, $X_2$ is the linear term of time, $X_1^2$ is the quadratic term of pressure, $X_2^2$ is the quadratic term of time and $X_1X_2$ is the interaction between pressure and time. Table 3 shows that, in the case of subcritical extraction, the linear term of pressure and the extraction time had the most significant effect on the extraction yield, followed by the quadratic term of pressure and the extraction time. From the results obtained it can be seen that the extraction yield increased when the operating pressure and extraction time increased. The interaction between extraction pressure and time, $X_1X_2$, was not significant ($p > 0.05$). The best way to visualize this influence of independent variables on the dependent one was to draw surface response plots of the model [15]. Figure 2 shows a three-dimensional plot of the response surface for the extraction yield obtained by subcritical carbon dioxide.

In Fig. 2 it can be observed that the yield of extract significantly increased with increased pressure and increased time, at the given temperature and flow rate. The increase of pressure at the constant temperature increased the density of supercritical carbon dioxide, and thus its solvating power [16]. It seems that the same effect was happening in the case of subcritical carbon dioxide.

**Table 3** Regression coefficient of polynomial function of response surface of *B. edulis* extraction yield obtained by subcritical and supercritical carbon dioxide

| Term | Coefficients | SD | $t$ | $p$ |
|---|---|---|---|---|
| Subcritical extraction | | | | |
| Constant | 2.134625 | 0.013253 | 161.0678 | 0.000000 |
| $X_1$ | 0.197167 | 0.011854 | 16.6332 | 0.000003 |
| $X_2$ | 0.230000 | 0.011854 | 19.4031 | 0.000001 |
| $X_1^2$ | −0.085875 | 0.017781 | −4.8297 | 0.002911 |
| $X_2^2$ | −0.096375 | 0.017781 | −5.4202 | 0.001632 |
| $X_1X_2$ | −0.027000 | 0.014518 | −1.8598 | 0.112259 |
| $R^2 = 0.992$ | | | | |
| Supercritical extraction | | | | |
| Constant | 1.676625 | 0.017294 | 96.9485 | 0.000000 |
| $X_1$ | 0.441667 | 0.015468 | 28.5532 | 0.000000 |
| $X_2$ | 0.323333 | 0.015468 | 20.9031 | 0.000001 |
| $X_1^2$ | −0.368375 | 0.023202 | −15.8767 | 0.000004 |
| $X_2^2$ | −0.124375 | 0.023202 | −5.3605 | 0.001727 |
| $X_1X_2$ | 0.090750 | 0.018945 | 4.7903 | 0.003031 |
| $R^2 = 0.996$ | | | | |

*SD* standard deviation

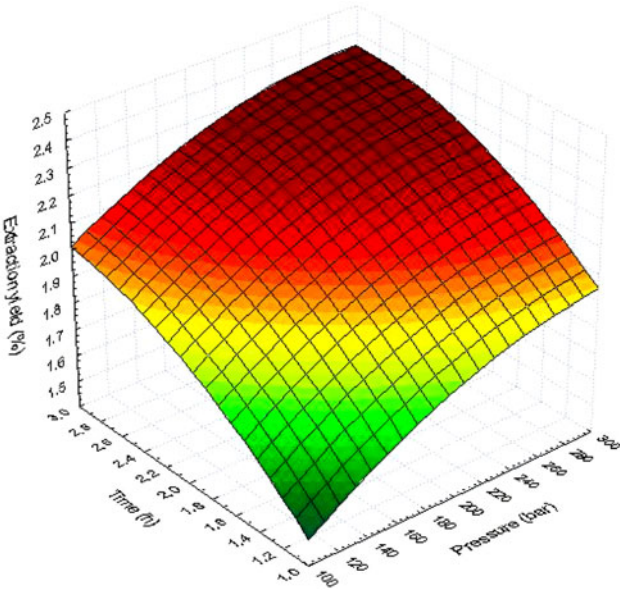


**Fig. 2** Three-dimensional plot of the response surface for *B. edulis* extraction yield ($Y$), obtained by subcritical carbon dioxide, as related to pressure and time at a fixed temperature and carbon dioxide flow rate

Furthermore, the increase in the extraction yield with increasing dynamic time was obvious.

The second order polynomial model used to express the extract yield ($Y$) obtained by subcritical carbon dioxide as a function of two independent variables is shown below (Eq. 2):

$$Y = 2.134625 + 0.197167X_1 + 0.23X_2 - 0.085875X_1^2 - 0.096375X_2^2 \quad (2)$$

Based on this model, the optimal condition for *B. edulis* subcritical carbon dioxide extraction were determined to be at a pressure of 344.396 bar and a time of 3.580 h at a fixed temperature of 27 °C. These conditions are outside the experimental research range. Under these conditions the total extract yield was 2.385%. For confirmation of the calculated values for the extraction yield, the experiment was set up at the determined optimal extraction pressure and time and the yield was measured. The experimental extraction yield obtained of 2.389% corresponded well to the predicted yield.

The results in Table 3 also show that the linear term of pressure and time, the quadratic term of pressure and time, and the interaction between pressure and temperature, had a significant influence on the extraction yield obtained by supercritical extraction ($p < 0.005$). As in the subcritical process, the extraction yield increased with an increase in the operating pressure and with an increase in the extraction time. Figure 3 shows a three-dimensional plot of the response surface for the extraction yield obtained by supercritical carbon dioxide.

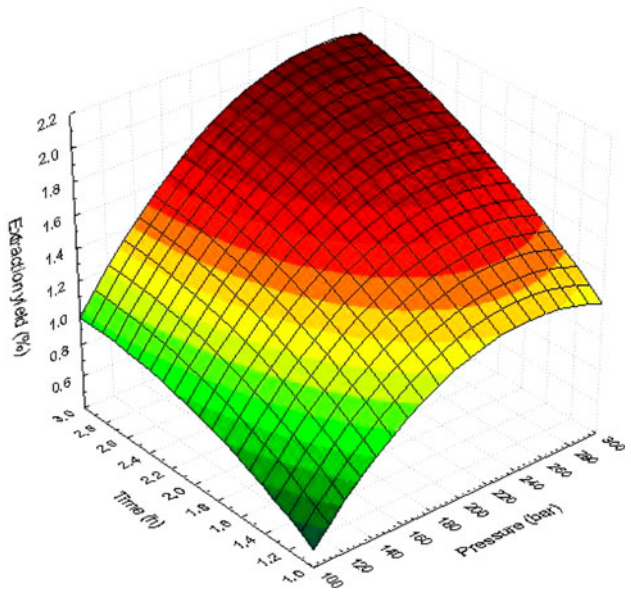


**Fig. 3** Three-dimensional plot of the response surface of *B. edulis* extraction yield ($Y$), obtained by supercritical carbon dioxide, as related to pressure and time at a fixed temperature and carbon dioxide flow rate

Again, a second order polynomial model was used to express the extract yield ($Y$) as a function of the independent variables (Eq. 3):

$$Y = 1.676625 + 0.441667X_1 + 0.323333X_2 - 0.368375X_1^2 - 0.124375X_2^2 + 0.090750X_1X_2 \quad (3)$$

Using the above equation, optimal conditions for *B. edulis* supercritical carbon dioxide extraction were determined as a pressure of 238.598 bar and a time of 4.770 h at the fixed temperature of 40 °C. Under these conditions, the total extract yield was 2.110%. For confirmation of previous experiment at the determined optimal extraction pressure and extraction time was set. The extraction yield of 2.116% confirms the polynomial model and determined parameters used.

The experimental data were analyzed by analysis of variance (ANOVA) to assess the "goodness of fit". The analysis showed that both models used for analysis were extremely statistically significant ($p < 0.0001$) at the 95% confidence level.

The mushroom extracts obtained were analyzed using GC/MS and quantified by GC/FID. After a transesterification procedure, methyl esters of fatty acids, as the dominant compounds in all extracts, were detected and quantified (Table 4). The extracts obtained in both process were very similar in quality and quantity. Three fatty acids were dominant in all the investigated extracts: saturated palmitic, unsaturated oleic, and linoleic acids. In all the investigated extracts, the content of saturated palmitic acid was similar, the highest content (11.78%) was determined in the extract obtained at a pressure of 200 bar after 3 h of extraction by supercritical carbon dioxide. A higher content of oleic acid was detected in all extracts obtained by subcritical carbon dioxide, compared to extracts obtained by supercritical carbon dioxide. The fatty acid that was present most in the investigated mushroom extracts was linoleic acid. Its content varied from around 41 to around 46%. In the supercritical extraction process, an increase in the yield of linoleic acid was achieved by an increase in the applied pressure, from 42.61%, at a pressure of 100 bar, to 46.59%, at a pressure of 300 bar. The content of linoleic acid was very similar for all extracts obtained by subcritical carbon, and the influence of pressure was not significant here.

The total yield of monounsaturated fatty acids (MFA) was higher in extracts obtained by subcritical carbon dioxide, while the case was different with the total yield of polyunsaturated fatty acids (PFA) as it was significantly higher in the extract obtained using the supercritical process.

Beside saturated palmitic acid, saturated stearic acid, 18:0, was detected in the extracts obtained in amounts

**Table 4** *B. edulis* total extract fatty acid composition

| Subcritical extraction | Temperature 27 °C | | |
|---|---|---|---|
| | 100 bar | 200 bar | 300 bar |
| Palmitic acid, 16:0 | 10.04 ± 0.03[a] | 9.87 ± 0.05[b] | 10.08 ± 0.01[a] |
| Oleic acid, 18:1 | 40.43 ± 0.02[a] | 40.47 ± 0.04[a] | 39.63 ± 0.03[b] |
| Linoleic acid, 18:2 | 41.62 ± 0.01[a] | 41.40 ± 0.03[b] | 41.27 ± 0.06[c] |
| Others | 0.97 ± 0.04[a] | 1.06 ± 0.05[a] | 2.46 ± 0.08[b] |
| Linoleic:oleic ratio | 1.03 ± 0.01[a] | 1.02 ± 0.01[a] | 1.04 ± 0.01[a] |
| Saturated fatty acid (SFA) | 13.65 ± 0.02[a] | 13.55 ± 0.04[a] | 14.28 ± 0.07[b] |
| Monosaturated fatty acid (MFA) | 43.49 ± 0.06[a] | 43.71 ± 0.02[b] | 43.08 ± 0.04[c] |
| Polysaturated fatty acid (PFA) | 41.89 ± 0.10[a] | 41.68 ± 0.11[a] | 40.18 ± 0.02[b] |
| UFA:SFA ratio | 6.25 ± 0.03[a] | 6.30 ± 0.06[a] | 5.83 ± 0.03[b] |
| Supercritical extraction | Temperature 40 °C | | |
| | 100 bar | 200 bar | 300 bar |
| Palmitic acid, 16:0 | 10.90 ± 0.09[a] | 11.78 ± 0.10[b] | 11.34 ± 0.06[c] |
| Oleic acid, 18:1 | 32.91 ± 0.08[a] | 33.34 ± 0.20[b] | 36.58 ± 0.03[c] |
| Linoleic acid, 18:2 | 42.61 ± 0.31[a] | 44.49 ± 0.06[b] | 46.59 ± 0.02[c] |
| Others | 6.48 ± 0.04[a] | 7.51 ± 0.08[b] | 1.97 ± 0.07[c] |
| Linoleic:oleic ratio | 1.29 ± 0.03[a] | 1.33 ± 0.01[a] | 1.27 ± 0.04[a] |
| Saturated fatty acid (SFA) | 13.73 ± 0.05[a] | 14.66 ± 0.08[b] | 14.13 ± 0.11[c] |
| Monosaturated fatty acid (MFA) | 37.18 ± 0.09[a] | 36.39 ± 0.11[b] | 38.76 ± 0.12[c] |
| Polysaturated fatty acid (PFA) | 42.61 ± 0.23[a] | 41.44 ± 0.04[b] | 45.14 ± 0.08[c] |
| UFA:SFA ratio | 5.81 ± 0.05[a] | 5.31 ± 0.04[b] | 5.93 ± 0.03[c] |

Data are expressed as mean values of 3 replications ± SD (standard deviation)

The same letter in the same row indicates no significant differences (Duncan's test, $p < 0.05$)

varying from 2.49 to 3.10%. Other saturated fatty acids, like pentadecanoic, 15:0, margaric, 17:0, arachidonic, 20:0, behenic acid, 22:0, and lignoceric acid, 24:0, were present in amounts lower than 0.5%. In the investigated extracts, the content of unsaturated palmitoleic acid, 16:1 9c, was from 0.6 to 0.82%. Other unsaturated fatty acids, such as omega 6 $\gamma$-linolenic, 18:3 c6 c9 c12, and gadoleic, 20:1 c11, were present in much lower amounts than oleic and linoleic acid, and they were lower than 0.5%.

Fatty acid profiles of the extract obtained using the usual Soxhlet procedure and classical solvents, from *B. edulis* from the Black Sea region of Turkey and *B. edulis* from Asia are available from recent publications. These reports show a that much lower levels of linoleic (33.6 and 33.8) and oleic acid (30.2 and 31.1) were obtained, in comparison to the investigated samples obtained using subcritical and supercritical carbon dioxide [2, 3]. The ratio of linoleic:oleic acid was similar in extracts obtained with subcritical carbon dioxide as in the report published by Kavishree et al. (2008), while in the extract obtained by supercritical carbon dioxide this ratio was slightly higher.

## Conclusion

Using carbon dioxide in subcritical and supercritical state, extracts of *B. edulis* mushroom were obtained. The applied response surface methodology explained the influence of the extraction time and the pressure on *B. edulis* subcritical and supercritical carbon dioxide extraction. This study showed that the second order polynomial model was sufficient to describe and predict the influence of the investigated parameters on the extraction processes. The results were very useful for the selection of pressure and time in order to obtain the highest extraction yield by pressurized carbon dioxide.

In both subcritical and supercritical carbon dioxide extraction, increasing the time and pressure leads to increases in extraction yield. The optimal extraction time and process pressure for *B. edulis* extraction, in both subcritical and supercritical state, were determined. For subcritical extraction the optimal extraction pressure and time, at 27 °C, were 344.396 bar and 3.580 h. Under these conditions, the predicted value of the extraction yield was 2.385%. For supercritical extraction, the optimal extraction

pressure and time at 40 °C were 238.598 bar and 4.770 h. Under these conditions the predicted value of the extraction yield was 2.110%. The process parameters have important effects on the extraction efficiency. Results obtained by ANOVA showed that the applied models were extremely statistically significant. The extracts obtained were rich in unsaturated fatty acids especially essential linoleic acid, which was the predominant fatty acid in all mushroom extracts. The subcritical extraction method was more efficient in terms of extraction yield and recovery of oleic acid, while extracts obtained by supercritical carbon dioxide were mostly richer in the content of linoleic acid. Significant differences in extraction yield, of these two extraction processes was noted at a pressure of 100 bar for an extraction time of 3 h, where much a higher extraction yield was obtained by subcritical extraction, 2.030%, in comparison to that obtained by supercritical carbon dioxide extraction, 0.972%. Under the same conditions, 100 bar and a 3-h extraction time, differences in oleic and linoleic fatty acid contents were not so significant. The fatty acid profile shows one of the possible uses of the mushroom extracts obtained. The high content of polyunsaturated fatty acids in the analyzed *B. edulis* extracts, particularly the essential ones, contributes to the recommendation of this mushroom and their extracts to be beneficial in the diets of people with high blood cholesterol.

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