Characteristics and antiproliferative properties of myrica rubra seed oil

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Abstract

Myrica rubra seed oil (MRSO) is a by-product of comprehensive processing and utilization of myrica rubra and has high nutritional value to human body. However, there are few researches on odor, physicochemical properties, stability, and functional nutrition of MRSO, especially compared with other vegetable oils. The characteristics of MRSO in terms of odor, physical and chemical indicators, fatty acid composition, etc., compared with other vegetable oils were studied in our paper. In addition, Oxidative stability and antiproliferative activity of MRSO were studied. The research showed that MRSO maintained lower free fatty acids content in comparison with the other vegetable oils, and was rich in unsaturated fatty acids and contained 2.48% of C22:2, which was not found in other vegetable oils. MRSO had the greatest effects on inhibiting the growth of HepG-2 and HT-29 by as much as 46% and 57% at 96 h. The findings demonstrated that MRSO is an edible oil with high nutritional value, and can be further processed to obtain oil products with high added value.

Characteristics and antiproliferative properties of *myrica rubra* seed oil

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Abstract *Myrica rubra* seed oil (MRSO) is a by-product of comprehensive processing and utilization of *myrica rubra* and has high nutritional value to human body. However, there are few researches on odor, physicochemical properties, stability, and functional nutrition of MRSO, especially compared with other vegetable oils. The characteristics of MRSO in terms of odor, physical and chemical indicators, fatty acid composition, etc., compared with other vegetable oils were studied in our paper. In addition, Oxidative stability and antiproliferative activity of MRSO were studied. The research showed that MRSO maintained lower free fatty acids content in comparison with the other vegetable oils, and was rich in unsaturated fatty acids and contained 2.48% of $C_{22:2}$, which was not found in other vegetable oils. MRSO had the greatest effects on inhibiting the growth of HepG-2 and HT-29 by as much as 46% and 57% at 96 h. The findings demonstrated that MRSO is an edible oil with high nutritional value, and can be further processed to obtain oil products with high added value.

Keywords *Myrica rubra* seed oil; Physicochemical properties; Oxidative stability; Antiproliferative properties

Introduction

Myrica rubra are mainly utilized for their juice, which is used in beverage-making. *Myrica rubra* seed oil (MRSO) is properly processed from fresh and mature high quality *myrica rubra* seeds and conveys pleasant aroma. MRSO is rich in oleic acid and linoleic acid, and the diet composed by which has plasma cholesterol-lowering effects compared with habitual diets. In China, consumers tend to desire oils produced or available in the region. MRSO is favored for providing nutrition in Zhejiang province and conveys pleasant aroma, *torreya grandis* seed oil (TGSO) and *camellia* seed oil (CSO) in Fujian province, *hylocereus undatus* seed oil (HUSO) in Hainan province, and *carya nutt* seed oil (CNSO) in Zhejiang and Anhui provinces. Many researches have distinctly revealed the odor profiles of vegetable oils [1-3], however, that of MRSO is still unknown. Electronic nose appeared around the late 1980s, which consisted of chemical sensor arrays and an appropriate pattern recognition system, capable of recognizing simple or complex odor. Many publications reported the application of electronic nose for the characterization of vegetable oils [3, 4]. Besides, the array of sensors, combined with principal component analysis (PCA), allow the distinction of olive oils of different qualities (extra virgin, virgin, ordinary and lampante), different varieties, and different geographic origins [5, 6]. Evaluation of sensory odor profiles of vegetable oils by using electronic nose is a preparatory research to detect the quality of vegetable oils. In this study, profiles of MRSO, TGSO, HUSO, CSO, CNSO and EVOO were evaluated. The objective was to acquire sensory odor fingerprints and odor characterization of MRSO, which also provided a choice to grasp the quality of the edible oils in the regions.

Lipid peroxidation is the metabolic process in which reactive oxygen species (ROS) result in the oxidative deterioration of lipids. This may significantly influence the nutritional and economic value of food products. Many factors may influence their oxidative stability, including temperature, light [7, 8], metal ion [9], et al. Only after these factors are studied are lipid peroxidation stopped, which is good to the storage of lipids. Antioxidants are used in the food industry especially like BHA, BHT and PG [10, 11]. Transition metal ions, particularly Fe^{2+} and Fe^{3+}, commonly found in food emulsions, are major pro-oxidants, accelerating the decomposition of primary lipid oxidation products [12]. Additionally, the storage conditions are also important to resolve lipid peroxidation during storage [13].

Currently, few researches on MRSO have been reported. As part of our ongoing efforts to develop value-added exploitations of *myrica rubra* seed, the objective of this study is to determine: (1) physical and chemical properties, oxidative stability; (2) the antiproliferative activities of the seed oil against HepG-2 and HT-29 human colon cancer cells.

Materials and methods

Materials and reagents

Supercritical CO_2-extracted MRSO was obtained from our laboratory. Hexane and methanol were chromatographic grade obtained from Tedia (USA). HepG-2 and HT-29 colon cancer cell lines were purchased from Boster (Wuhan, China). All components of the cell culture media were purchased from Thermo Scientific (Massachusetts, USA), except biological grade dimethyl sulfoxide from Sigma (St Louis, USA).

Oil samples

Biqi *myrica rubra* and red pulp *hylocereus undatus* were purchased from Xianju and Fujian, China, respectively. After removing the defectives, seeds were separated from the flesh and pressed to remove the juice. Then seeds were washed with water and dried at 40 to reach constant moisture content. *Torreya grandis V. merrilli* , *camellia* and *Carya nutt* were purchased from Fujian and Anhui, China, respectively. Seeds were dried at 40 after peeling off the coats to reach constant moisture content. *Myrica rubra* seed oils (MRSO), *torreya grandis* seed oil (TGSO),*hylocereus undatus* seed oil (HUSO), *camellia* seed oil (CSO), *carya nutt* seed oil (CNSO) were extracted by supercritical carbon dioxide extraction in our laboratory. The supercritical CO_2 extraction in bench-scale was used to extract 10 g of each of the ground seeds at a pressure of 360 bar, temperature of 40 , static and dynamic extraction time of 30 min and 2 hours [14, 15]. Extra virgin Olive oil (EVOO) was purchased from a local grocery, originating from Hojiblanca olive in Andalucía, Spain. And the trees were with 20 years old of age. All samples were stored at -20 in screw-cap amber bottles and thawed prior to further analysis.

Electronic nose experiment

The portable electronic nose PEN3 (Win Muster, Airsense, Germany) with a detector array of 10 metal oxide semiconductor (MOS) type chemical sensors was used. The electronic nose consists of three parts, sampling vessel, sensors array and data acquisition system (Win Muster v.3.0). The MOS is composed of MOS1 (aromatic), MOS2 (broadrange), MOS3 (aromatic), MOS4 (hydrogen), MOS5 (arom-aliph), MOS6 (broad-methane), MOS7 (sulphur-organic), MOS8 (broad-alcohol), MOS9 (sulph-chlor) and MOS10 (methane-aliph). The results were reported by the data of the responses of ten metal oxide semiconductors, and then analyzed through principal component analysis (PCA), a procedure permitting to project the data in a reduced hyperspace determined by primary components.

Electronic nose analysis was carried out according to the method of Buratti et al. with slight modification [2, 16]. Briefly, samples, each of 0.5 mL, were placed in 15 mL glass jars and then incubated at 40 for 10 min prior to injection. After the headspace equilibration, the running time was 500 s with the program set to include 20 s of referencing, 300 s of sampling, 60 s of washing, followed by 120 s of referencing. Referencing is a procedure zeroing the background noise of the sensors to correct the baseline. Each sample was analyzed in triplicate.

Chemical analysis

The chemical analyses, namely indices like acid value (AV), peroxide value (PV), iodine value (IV) and saponification value (SV), were carried out according to the international standard ISO 660:2009, ISO 27107:2008, AOCS official methods (Cd 1b-87) and ISO 3657:2002, respectively. All chemicals and solvents used were analytical grade. And AVs, PVs, IVs and SVs of every kind of oils were analyzed in triplicate.

Fatty acid composition of MRSO

Fatty acid profiles were determined by preparation of fatty acid methyl esters (FAMEs). Briefly, 50 mg of the seed oil was reacted with 10% H_2SO_4-MeOH at 70 , before n-hexane being added, and then FAMEs were extracted with n-hexane by adding water to stop the transesterification. The FAMEs were analyzed with an Agilent 7890A gas chromatograph-mass spectrometer-computer (GC-MS) equipped with a flame ionization detector (FID), controlled by TotalChrom Workstation version. A silica capillary column DB-WAX (30m, i.d. 0.25mm, d_f, 0.25 μm) was used with a temperature program started at 150, increased to 200 at a rate of 20/min and held at 200 for 5 min, and then increased to 240 at a rate of 5/min and held at 240 for 10 min. The injector temperature was 250, that of the detector 260. Electron ionization (EI) spectra were obtained at 70 eV at 210. The injection volume was 1 μL, with a split ratio of 1:10 [17]. FAMEs were identified by comparing their retention times with those of mass spectral library from Wiley. Area under each fatty acid peak relative to the total area of all fatty acid peaks was used to quantify the fatty acids identified. Results are reported as g fatty acid/100g of total fatty acids. All samples were analyzed in triplicate.

Oxidative stability of MRSO

The purposes of the presented investigation being identifying the oxidative stability of MRSO were determined as the functions of the (1) storage temperature, (2) different illumination, (3) antioxidants, and (4) metallic ion. Briefly, using a high throughput laboratory assay [18] added a certain quality of MRSO into sealed reagent bottles. Respectively, every other 24 hours, the PV of MRSO treated in the temperature of thermostat box 25, 40, 50, 60 was detected in accordance to the IUPAC method 2.102. Added a certain quantity of MRSO into sealed reagent bottles and divided into 3 groups to investigate the effects of ultraviolet sunlight, sunshine and dark at room temperature on the oxidative stability of MRSO, according to the PV of samples determined every other 24 hours. A certain quantity of MRSO was added into glass tubes and separately treated under exposure and sealed conditions in 60 . Every other 24 hours, the PV of MRSO was detected to study the effect of air conditions on its oxidative stability. Certain qualities of MRSO were put into the number of copies. 0.02% of TBHQ, 0.02% of BHA, 0.02% of BHT and 0.02% of PG were respectively added into the copies, which then were placed in 60. Every other 24 hours the PV was detected to study the effect of antioxidants on the oxidative stability of MRSO. All samples were analyzed

in triplicate.

Antiproliferation properties on human colon cancer cells

Human colorectal adenocarcinoma cells proliferation inhibitions were investigated on the basis of the method descripted by Wang et al. and Xie et al. [19, 20]. The cells were maintained in RPMI-1640 supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (100 unit/mL of penicillin G and 100 µg/mL of streptomycin sulfate) and cultured at 37 in 5% CO_2.

Cells (10,000 cells/ well) were plated in 96-well culture plates, in total volume of 100µL culture media. After incubation of 24 h, the medium was replaced with 100 µL of the appropriate treatment medium (1, 3, 5, 7 mg of seed oil equivalents/ml). Treatment media were prepared by dissolving MRSO in DMSO to make a concentration of 2 g oil equivalent/ml of DMSO. The seed oils in DMSO were then mixed with culture media to achieve the concentrations of 1, 3, 5, 7 mg of MRSO equivalents/ml of treatment media. Media, for both treatment levels and the control, had a final concentration of 0.2% DMSO, and were filtered through a 0.22 µm pore retrograde cellulose filter prior to treatment of cells [21]. Cells proliferation was studied via 3- [4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) [22, 23]. The absorption values of treatment and control media at 490 nm were taken at 24, 48, 72 and 96 h after initial treatment.

Statistical analysis

The tests for statistical significance of difference between treatments for volatile compounds data was performed by analysis of variance (ANOVA) at significance level of $P = 0.05$. The effect of treatments on means of samples between treatments were compared using Turkey's test ($P = 0.05$). Results were reported

as means and standard deviation. SPSS (version 20.0) was used for statistical analysis. Each sample was analyzed in triplicate.

Results and discussion

Electronic nose analysis

The odor of oils is one of the crucial factors influencing quality. Aroma profiles of these vegetable oils were obtained using electronic nose. The responses of ten electronic nose MOS sensors to the oils were shown in **Table 1** , and the radar charts of the vegetable oils in the odor amplitude were shown in **Fig. 1** . As was shown by**Fig. 1** and **Table 1** , all of the oils had more intensive responses to the electronic nose MOS2, MOS7 and MOS9, which meant that the oils contained the same major fragrance compounds. However, different oils held variations in the amounts of the compounds. Furthermore, MRSO and EVOO with lower odor amplitudes on ten electronic nose MOS sensors than others illustrated that MRSO and EVOO were different from the other oils in the odor profiles. To distinct the differences, principal component analysis was carried out.

Principal component analysis of odor compounds

The data of electronic nose matrix with 10 rows (responses from ten sensors) and 60 columns (ten samples of one kind of oils) was analyzed by means of principal component analysis (PCA) to reduce the number of variables down to important factors only [24]. Principal component analysis (PCA) and the correlation analysis of MRSO and other vegetable oils in odor character were shown in **Fig. 2** and **Table 2** , respectively. The first principal component (PC 1) and the second principal component (PC 2) described 96.99% of the total variance, which were sufficient to build a good model. Among the percentage, PC 1 and PC 2 explained 54.90% and 42.09%, respectively. Examining the score plot in the area defined by PC 1 and PC 2, the oils were separated into four groups. Specifically, MRSO, EVOO and CNSO formed three groups separately, and the others formed another group. TGSO, CNSO and HUSO had high positive scores along PC 1, while MRSO and EVOO had high positive scores along PC 2. As was shown in **Table 2** , MRSO possessed highest correlation with EVOO in odor characters than the others. Some literatures reported that the sensory properties of EVOO mostly depended on the odor descriptors of C_6 and C_5compounds grouped in 9 different odorant series: grass, leaf, wood, bitter-like, sweet-like, pungent-like, olive fruit, apple and banana [25-27]. Therefore, the odor descriptors of these compounds may contribute to the odor properties of MRSO.

Chemical analysis of basic properties

The AVs, PVs, IVs and SVs of MRSO and other vegetables oils determined in our study were given in **Table 3** . And they were in good quality before off-flavors were encountered. The AV of MRSO (0.42) was significantly lower (*p¡0.05* = than others except EVOO (0.34) and CNSO (0.71), followed by CNSO (0.71), TGSO (0.95), HUSO (2.57) and CSO (4.19), which indicted the content of free fatty acid in MRSO is significantly lower (*p¡0.05* = compared with others. PV of MRSO (1.43) was close to that of EVOO (1.21) and significantly lower (*p¡0.05* = than those of TGSO (7.46), HUSO (3.78), CSO (3.21) and CNSO (2.36). This may be due to the presence of natural antioxidants such as β-carotene, and α-tocopherol which had been found in olive oil [28] and grape seed oil [21]. In addition, fatty acid profiles of MRSO may influence its PV, which may have antioxidant activity just like grape seed oil [21, 29]. MRSO had the highest IV of 107.43, followed by CNSO (105.68). MRSO and CNSO were better sources of polyunsaturated fatty acids which possess health benefits, such as regulating blood cholesterol levels and lowing elevated blood pressure, since unsaturated fatty acids were richer in MRSO and CNSO than others. Among these vegetable oils, SVs were in order of EVOO¿ MRSO ¿ CNSO ¿ TGSO ¿ HUSO ¿ CSO. Therefore, the molecular of fatty acids in MRSO was smaller than those of others except EVOO, and was more easily absorbed by human body.

Fatty acid composition

Fatty acid composition of the vegetable oils studied in this research was shown in **Table 4** . The content of unsaturated fatty acids in these vegetable oils was over to 80%, and that of MRSO was 84.92% only behind EVOO (84.96%). Even α-Linolenic acid ($C_{18:3}$) (0.62%) was not found in MRSO but in EVOO,

HUSO, CSO and CNSO, the content of docosadienoic acid ($C_{22:2}$) was up to 2.48% in MRSO rather than the other. TGSO contained a larger amount of long-chain unsaturated fatty acids (12.53%) than the other. Squalene ($C_{30:6}$) was found and up to 2.39% of fatty acids in HUSO, which was unusual in vegetable oils. Besides, MRSO, TGSO and HUSO were rich in long chain polyunsaturated fatty acids in comparison with other vegetable oils studied in the paper. Monounsaturated fatty acids like oleic acid had been revealed in decreasing low-density lipoprotein (LDL) levels in blood [30-32], while some polyunsaturated fatty acids played important roles in reducing the risks of cancer, heart disease, cardiovascular disease, autoimmune and inflammatory disorders and disrupted neurological functions [33, 34]. Therefore, these vegetable oils especially MRSO may be beneficial to human health.

Oxidative stability of MRSO

Oxidative stability of MRSO was presented in **Fig. 3** . The effect of storage temperature on oxidative stability of MRSO at 25, 40, 50, 60 was determined, PV was measured as a function of time (1-10 days) and the results for PV were shown in **Fig. 3 (A)** . Briefly, when the temperature was less than 60 , the PV of MRSO changed slowly. However, it increased sharply when the temperature exceeded 60 . Therefore, Temperature is one of important factors of oil oxidation, because the high temperature not only promotes the OTM middle resulting from the base, but also the decomposition and polymerization of hydrogen peroxide, speeding up the oxidation process of the unsaturated fatty acid. As was shown in **Fig. 3 (B)** , in the dark condition, the PV of MRSO increased more slowly six days later than other illumination conditions, especially sunshine. Although PVs of MRSO were lower in lightshine and ultraviolet sunlight than those in dark and sunshine before five days, then they rose quickly and were higher than those in dark and sunshine. Based on the research by Zhao et al., 27 PAH-diones generate a dose-related lipid peroxidation, which is mediated by ROS [10]. **Fig. 3 (C)** provided information about the effect of added antioxidants on the PV of MRSO. The PVs of antioxidants-added groups were higher than that of control group, suggesting that antioxidants had certain delayed effect on the oxidation of MRSO and improved the stability of the oil. The effects of antioxidants on the seed oil were in order of PG > TBHQ > BHT > BHA. As was shown in **Fig. 3 (D)** , Fe^{2+} and Cu^{2+} can accelerate the oxidation of MRSO. Therefore, MRSO should be stored avoiding those metal ions.

Antiproliferation properties on human colon cancer cells

DMSO solution of the seed oil at final dose levels of 1, 3, 5 and 7 mg of oil equivalents/ml in the cell culture media, were tested against HepG-2 and HT-29 colon cancer cells for its antiproliferative effects. The results indicated a dose- and timed-dependent effect of MRSO on human colon cancer cells as presented in **Fig. 4** . MRSO at final concentration of 7 mg/mL of media suppressed cell proliferation during the treatment on HepG-2 and HT-29 especially the latter, whereas the low concentration of MRSO had no significant antiproliferative effect on the cancer cells. The seed oil showed the greatest effects on inhibiting the growth of HepG-2 and HT-29 by as much as 46% and 57% at 96 h, respectively and had more significant antiproliferative effect on HT-29 than HepG-2.

Conclusions

Overall utilization of agro-industrially processing waste is a good way to add value to primary products, with the advantage of reducing waste disposal problems. The study showed that MRSO maintained lower free fatty acids content in comparison with the other vegetable oils, and was rich in unsaturated fatty acids and contained 2.48% of $C_{22:2}$, which was not found in other vegetable oils. MRSO oxidized when the temperature exceeded 60 . The effects of antioxidants on MRSO were in order of PG > TBHQ > BHT >BHA, additionally Fe^{2+} and Cu^{2+} accelerated the oxidation of MRSO.MRSO had the greatest effects on inhibiting the growth of HepG-2 and HT-29 by as much as 46% and 57% at 96 h. Thus MRSO may play a significant role in food quality and human health in the future, however further study is required to explore the mechanisms.

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