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AUTHORS: Mehmet AKYÜZ,Sevda KIRBAG,Sevda KIRBAG,Mustafa KARATEPE,Mustafa
KARATEPE,Mehmet GÜVENÇ,Fikriye ZENGİN

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Research Article

Vitamin and fatty acid composition of *P. eryngii* var. *eryngii*

Mehmet AKYÜZ ^{a,*}, Sevda KIRBAĞ ^b, Mustafa KARATEPE ^c, Mehmet GÜVENÇ ^d, Fikriye ZENGİN ^e

^a Bitlis Eren University, Faculty of Science and Arts, Department of Biology, 13000 Bitlis-TURKEY

^b Firat University, Faculty of Science, Department of Biology, 23119 Elazığ-TURKEY

^c Firat University, Faculty of Science, Department of Chemistry, 23119 Elazığ-TURKEY

^d Adiyaman University, Faculty Science and Arts, Department of Biology, 02040 Adiyaman-TURKEY

^e Firat University, Faculty of Education, Department of Primary School Teaching, 23119 Elazığ-TURKEY

* Corresponding author: mehmetaky210@hotmail.com & mehmetaky210@mynet.com

Abstract

In this study, fatty acid and vitamin contents of *P. eryngii* var. *eryngii* grown on various agro-residue were determined. The distribution of oil acid in 100 g lipid extracted from samples were found to be 0.00-0.59 g C_{14:0}, 0.00-1.68 g C_{15:0}, 13.56-20.09 g C_{16:0}, 0.00-4.98 g C_{16:1}, 0.00-0.22 g C_{17:1}, 1.35-5.41 g C_{18:0}, 10.77-34.80 g C_{18:1}, 33.06-70.39 g C_{18:2}, 0.00-1.17 g C_{18:3}, 0.00-0.53 g C_{20:0}, 0.00-0.59 g C_{20:2}, 0.00-0.25 g C_{20:1}, 0.00-4.14 g C_{20:3}, 0.00-0.51 g C_{20:5}, 0.00-1.15 g C_{22:0}, 0.00-0.30 g C_{22:1}, 0.00-3.54 g C_{22:2}, 0.00-0.33 g C_{22:4}, 0.00-1.15 g C_{22:5}, 0.00-3.93 g C_{22:6} and 0.00-2.07 g C_{24:0}, of wet weight. The contents of vitamin were 0.014-0.064 A, 0.869-3.565 E, 55.980-473.405 C and 0.087-5.619 MDA mg/kg of wet weight. It has been determined that *P. eryngii* var. *eryngii* is rich from the point of view of unsaturated fatty acids such as linoleic acid, oleic acid and palmitic acid, and especially vitamins C, also antioxidants vitamins such as vitamin A, E and MDA.

Keywords: Fatty acid, mushroom, *P. eryngii* var. *eryngii*, vitamin

1. Introduction

There are at least 12.000 species of fungi that can be considered to be mushrooms, with at least 2.000 species showing various degrees of edibility (Chang 1999). They have been eaten and appreciated for their flavor, economic and ecological values, and medicinal properties for many years, but only approximately 22 species are intensively cultivated (Manzi et al. 1999). In most countries there is a significant consumer acceptance of cultivated mushrooms, such as *A. bisporus*, *Pleurotus* spp., *L. edodes*, *V. volvacea*, and *Auricularia* spp. (Diez & Alvarez 2001).

Pleurotus spp. represents the third largest group of cultivated mushrooms in the world, grown on a variety of plant residues, and they have been found to be nutritionally and gastronomically important (Cohen et al. 2002). Several studies have been carried out on the chemical composition and nutritional quality of different culture mushrooms (Ragunathan et al. 1996; Manzi et al. 1999; Gong et al. 2003; Ragunathan & Swaminathan 2003; Guo et al. 2007 etc.). In general, fruit bodies contain 90% water and 10% dry matter. The protein content varies between 27% and 48%, carbohydrates are less than 60%, and lipids 2-8% (Crisan & Sands 1978; Ranzani & Sturion 1998; Morais et al. 2000).

Mushrooms are excellent sources of linoleic, oleic and palmitic acid, and also thiamine, riboflavin, nicotinic acid, biotin and ascorbic acid (Esselen & Fellers 1946; Watt & Merrill 1963; Duda et al. 1988; Breene 1990; Alan & Padem 1991; Oei 1996; Pauli 1998; Longvah & Deosthale

1998; Diez & Alvarez 2001; Mattila et al. 2001; Wang et al. 2001; Çağlarırnak et al. 2002; Qi et al. 2004; Agrahar-Murugkar & Subbulakshmi 2005; Barros et al. 2007; Çağlarırnak 2007; Furlani & Godoy 2007; Barros et al. 2008a; Barros et al. 2008b; Furlani & Godoy 2008; Kalac 2009; Ribeiro et al. 2009).

There are numerous past studies indicating the need for the examination of other specific edible mushrooms. However, it is not clear to understand especially their contents of fatty acids, vitamin A, C, E and MDA. The objective of the present study is to examine the fatty acid and vitamin contents of *P. eryngii* var. *eryngii* grown on various agro-residue.

2. Material and Methods

2.1. Mushroom Samples

The samples used in this study were obtained from the culture work of Kirbag & Akyüz (2008). Wheat-cotton straw (W-P), wheat-millet straw (W-M), wheat-soybean straw (W-S), wheat-corn stalk (W-C), wheat-bean stalk (W-B) and wheat straw (W) at a ratio of 1:1 were used in preparing six different types of compost. All composts were supplemented with 10% and 20% of rice bran (RB), therefore, 18 types of composts were prepared. The samples obtained after culture were harvested in sterile conditions and labeled. These fresh samples were used in this study.

2.2. Sample Preparation and Fatty Acid Assays

2.2.1. Fatty Acid Analysis

2 g fresh mushroom materials for fatty acid analyses were finely grounded in a mill and were then extracted with hexane/isopropanol (3:2 v/v) (Hara & Radin, 1978). The lipid extracts were centrifuged at 10.000 g for 5 minutes and filtered, and the solvent was then removed on a rotary evaporator at 40°C. The extracted lipids were stored under -25°C until further analysis. Fatty acids in the lipid extracts were converted into methyl esters by means of 2% sulphuric acid (v/v) in methanol (Christie 1990). The fatty acid methyl esters were extracted with n-hexane. The methyl esters were then separated and quantified by gas chromatography and flame-ionization detection (Shimadzu GC 17 Ver. 3) coupled to a Glass GC 10 software computer software. Chromatography was performed with a capillary column (25 m in length and 0.25 mm in diameter, Permabound 25 (Macherey-Nagel, Germany) using nitrogen as a carrier gas (flow rate 0.8 ml/min). The temperatures of the column, detector and injection valve were 130-220, 240 and 280°C, respectively. Identification of the individual methyl esters was performed by frequent comparison with authentic standard mixtures analysed under the same conditions.

2.3. Sample Preparation and Vitamin Assays

2.3.1. Determination of mushrooms Vitamin A and E levels

Homogenized mushrooms samples (1 g) were transferred into polyethylene tubes and 2 ml ethanol was added to the tubes. After 0.3 ml n-hexane was filled into tubes for vitamins extractions, they were centrifuged. This step was repeated two times. N-hexane in tubes was evaporated using the nitrogen. Then the residues were solved in mobile phase (methanol: acetonitrile: chloroform; 47: 42: 11, v/v). Chromatograms were monitored at 326 and 296 (vitamin A and E, respectively) and injection volume was set 50 µL. Techsphere ODS-2 packed column (5 µm particle, 250 × 4.6 ID) was used and flow rate was 1.0 ml min⁻¹ (Miller et al. 1984).

2.3.2. Determination of mushrooms Vitamin C and MDA levels

0.5 ml of HClO₄ (0.5 M) and 4.5 ml distilled water were added onto an aliquot portion of (1.0 g) mushrooms samples (Cerhata et al. 1994). Then, the samples were centrifuged at 4500 rpm for 5 min and supernatants were injected into HPLC system. Addition of acid was necessary to precipitate proteins and release the MDA bound to the amino groups of proteins and other amino compounds. Acid addition was also needed to maintain the stability of vitamin C. The mobile phase was 30 mM KH₂PO₄ -Methanol (82.5+ 17.5, v/v %, pH 3.6) and the flow rate was 1.2 mL min⁻¹. Chromatograms were monitored at 250 nm and injection volume was 20 µL. A Wakosil II 5C18 RS 5µm (150 × 4.6 mm SS, SGE, AUS) column was used at room temperature (Karatepe 2004).

3. Statistical Analysis

Experimental values are given as means ± standard deviation (SD). Statistical significance was determined by one way variance analysis (ANOVA). Differences at P<0.05 were considered to be significant. A LSD (Least Significant Difference) multiple comparison test for comparison of multiple means was used with SPSS 13.0 computer programs (SPSS, Chicago, Illinois, USA) (n = 3).

4. Results and Discussion

The effect of some lignocellulosic wastes on the fatty acid and vitamin contents of *P. eryngii* var. *eryngii* is shown in Table 1-2.

In 100 g of wet *P. eryngii* var. *eryngii* grown on agro-residues was found as 0.00-0.59 g C_{14:0}, 0.00-1.68 g C_{15:0}, 13.56-20.09 g C_{16:0}, 0.00-4.98 g C_{16:1}, 0.00-0.22 g C_{17:1}, 1.35-5.41 g C_{18:0}, 10.77-34.80 g C_{18:1}, 33.06-70.39 g C_{18:2}, 0.00-1.17 g C_{18:3}, 0.00-0.53 g C_{20:0}, 0.00-0.59 g C_{20:2}, 0.00-0.25 g C_{20:1}, 0.00-4.14 g C_{20:3}, 0.00-0.51 g C_{20:5}, 0.00-1.15 g C_{22:0}, 0.00-0.30 g C_{22:1}, 0.00-3.54 g C_{22:2}, 0.00-0.33 g C_{22:4}, 0.00-1.15 g C_{22:5}, 0.00-3.93 g C_{22:6} and 0.00-2.07 g C_{24:0}, depending on the type of material used and the rate of additive matter as seen in Table 1. The major fatty acid found in the studied species was linoleic (C_{18:2}), followed by oleic (C_{18:1}) and palmitic acid (C_{16:0}) as seen in Table 1, and also other fatty acids were only found in very small amounts as shown in Table 1. C_{18:2} contents of *P. eryngii* var. *eryngii* increased as RB ratios in W-C, W-M and W-S increased, but led to the change of content for W and W-P as seen in Table 1. Generally, C_{18:1} and C_{18:0} contents of *P. eryngii* var. *eryngii* which was obtained from W, W-C (1:1), W-M (1:1), W-P (1:1) and W-S (1:1), observed to be very high, but increase in RB led to the change of C_{18:1} and C_{18:0} contents for W, W-C (1:1), W-M (1:1), W-P (1:1) and W-S (1:1) as shown in Table 1. The differences between the fatty acid concentrations of samples may be attributed to a number of factors, such as mushroom strain/type, cultivated with different substrates, growth region, time of harvest, handling conditions.

A deficient intake of essential fatty acids can be responsible for many problems, such as dermatitis, immunosuppression and cardiac disfunctions as stated by Burtis & Ashwood (1996). Our data are changeable to those reported by other researchers (Longvah & Deosthale, 1998; Diez & Alvarez 2001; Barros et al. 2007; Barros et al. 2008a; Barros et al. 2008b; Kalac 2009; Ribeiro et al. 2009). Large quantitative differences, probably due to the heterogeneity of the samples analysed, were found to be those in the cited studies. The fatty acid profiles of the different mushroom species appeared to be distinct. Oleic and linoleic acids were present in all of them as the most abundant compounds, linoleic acids was the major compound in *P. eryngii* var. *eryngii*. Based on Table 1, we can see that the presence of these fatty acids occurs only in certain species and there are great quantitative differences on these compounds among the mushroom species. Genes may be responsible for the synthesis of the enzymes participating in the biosynthetic pathways for the formation of these fatty acids which could be distinct (Qi et al. 2004; Riberio et al. 2009).

Table 1. Fatty acid composition of "King Oyster Mushroom" (*P. eryngii* var. *eryngii*) grown on various agro-wastes (%).

Materials	Fatty acids													
	C _{14:0}	C _{15:0}	C _{16:0}	C _{16:1}	C _{17:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}	C _{20:0}	C _{20:1}	C _{20:2}	C _{20:3}	C _{20:5}
W**	*	1.68	16.47	*	*	3.78	26.57	49.54	1.17	*	*	*	*	*
W + 10 % RB	*	1.29	15.58	0.89	*	1.43	14.95	61.04	*	*	*	0.59	*	0.51
W + 20 % RB	*	1.31	17.84	0.65	*	2.07	19.04	56.24	*	0.50	*	*	*	*
W-C (1:1)	*	*	16.75	*	*	2.46	22.02	58.77	*	*	*	*	*	*
W-C (1:1)+10 % RB	*	1.30	17.00	*	*	1.66	17.34	61.85	*	*	*	*	*	*
W-C (1:1)+20 % RB	*	1.33	17.10	*	*	1.70	13.62	65.07	*	*	*	*	*	*
W-M (1:1)	*	*	17.70	*	*	3.98	30.47	39.03	*	*	*	*	1.55	*
W-M (1:1)+10 % RB	*	1.45	15.39	*	*	2.13	21.44	56.52	*	*	*	*	*	*
W-M (1:1)+20 % RB	*	1.29	17.08	*	*	2.05	18.61	60.09	*	*	*	*	*	*
W-P (1:1)	*	1.04	18.26	0.31	*	3.91	34.80	40.21	*	0.53	*	*	*	*
W-P (1:1)+10 % RB	*	1.26	15.71	0.69	*	1.99	17.99	55.70	0.61	*	*	*	*	*
W-P (1:1)+20 % RB	*	1.46	13.56	0.73	*	2.53	27.99	51.37	*	*	*	*	*	*
W-S (1:1)	0.59	0.56	20.09	4.98	0.22	5.41	24.56	33.06	0.80	0.29	0.25	0.55	4.14	*
W-S (1:1)+10 % RB	*	1.27	16.20	0.72	*	2.65	23.21	53.00	*	*	*	*	*	*
W-S (1:1)+20 % RB	*	1.17	16.29	*	*	1.35	10.77	70.39	*	*	*	*	*	*

** : control medium, *: not detected., W: Wheat Straw, C: Corn Stalk, M: Millet Straw, S: Soybean Straw, B: Bean Straw, P: Cotton Stalk, RB: Rice Bran

A lack of vitamins in the diet causes deficiency diseases. Many of these diseases such as xerophthalmia, scurvy, beri-beri and pellagra are the most common all over the world particularly in the developing countries. The dietary vitamins requirement are necessary to prevent deficiency disorders (Winichagoon 1992). Mushrooms are excellent sources of thiamine, riboflavin, nicotinic acid, biotin and ascorbic acid (Breene 1990; Mattila et al. 2001; Furlani & Godoy 2008).

There has been lack of information on vitamin A, E, C and MDA contents of mushrooms. The contents of vitamins were 0.014-0.064 A, 0.869-3.565 E, 55.980-473.405 C and 0.087-5.619 MDA mg/kg wet weight in *P. eryngii* var. *eryngii* as seen in Table 2. This difference probably was due to the use of mushroom samples obtained from different grown medium. These data are changeable to those reported by other researchers (Barros et al. 2008a; Barros et al. 2008b; Mattila et al. 2001).

The highest vitamin A contents were 0.064 mg/kg on W-B (1:1), 0.063 mg/kg on W-M (1:1) + % 20 RB, 0.059

mg/kg on W-B (1:1) + % 20 RB, whereas the lowest were 0.014 mg/kg on W-M (1:1), 0.018 mg/kg on W and W + % 20 RB, 0.019 mg/kg on W-C (1:1). No statistical difference was found with the vitamin A contents of *P. eryngii* var. *eryngii* which was obtained from W, W + % 10 RB, W + % 20 RB, W-C (1:1), W-C (1:1) + % 10 RB, W-C (1:1) + % 20 RB, W-M (1:1), W-M (1:1) + % 10 RB, W-P (1:1) + % 10 RB, W-P (1:1) + % 20 RB, W-S (1:1), W-S (1:1) + % 10 RB, W-S (1:1) + % 20 RB. Besides, difference in W-M (1:1) + % 20 RB, W-P (1:1), W-B (1:1), W-B (1:1) + % 10 RB and W-B (1:1) + % 20 RB was not considered statistically important (Table 2). The lowest MDA content was 0.087 mg/kg on W-B (1:1) + % 10 RB, whereas the highest was 5.619 mg/kg on W-M (1:1) + % 10 RB as seen in Table 2. This difference probably was due to the use of mushroom samples obtained from different grown region.

The lowest vitamin E contents was 0.869 mg/kg on W-C (1:1), whereas the highest was 3.565 mg/kg on W-P (1:1) + % 10 RB in Table 2. No statistical difference was found with the vitamin E content of mushroom samples

which was obtained from W-C (1:1), W-M (1:1), W-M (1:1), W-S (1:1), W-S (1:1) + % 10 RB, W-B (1:1) + % 10 RB, W-B (1:1) + % 20 RB, W-B (1:1) + % 10 RB and W-B (1:1) + % 20 RB was not considered statistically important (Table 2). The lowest MDA content was 0.087 mg/kg on W-B (1:1) + % 10 RB, whereas the highest was 5.619 mg/kg on W-M (1:1) + % 10 RB as seen in Table 2. This difference probably was due to the use of mushroom samples obtained from different grown region.

The lowest vitamin E contents were 0.869 mg/kg on W-C (1:1), whereas the highest was 3.565 mg/kg on W-P (1:1) + % 10 RB in Table 2. No statistical difference was found with the vitamin E content of mushroom samples

Table 2. Vitamin contents of “King Oyster Mushroom” (*P. eryngii* var. *eryngii*) grown on various agro-wastes (mg/kg).

Materials	Vitamins			
	A	E	C	MDA
W**	0.018±0.002 ^a	1.014±0.284 ^{ac}	309.664±14.652 ^{afg}	1.104±0.426 ^{agh}
W + 10 % RB	0.042±0.006 ^{ab}	0.961±0.353 ^a	81.915±15.686 ^b	0.244±0.167 ^{ag}
W + 20 % RB	0.018±0.008 ^a	1.004±0.024 ^{ac}	114.483±43.547 ^{bd}	2.770±0.223 ^{bef}
W-C (1:1)	0.019±0.007 ^{ad}	0.869±0.151 ^a	79.014±8.511 ^b	1.275±0.568 ^{af}
W-C (1:1)+10 % RB	0.024±0.008 ^{ad}	1.152±0.381 ^{ac}	100.421±9.040 ^b	1.332±0.390 ^{af}
W-C (1:1)+20 % RB	0.035±0.007 ^{ab}	2.795±2.468 ^{ab}	226.070±13.734 ^{cg}	4.588±0.155 ^{cd}
W-M (1:1)	0.014±0.005 ^a	2.037±0.414 ^{ab}	225.310±17.231 ^{cg}	1.047±0.461 ^{agh}
W-M (1:1)+10 % RB	0.029±0.012 ^{ac}	2.003±0.591 ^{ab}	343.899±11.862 ^{af}	5.619±0.637 ^c
W-M (1:1)+20 % RB	0.063±0.013 ^b	1.206±0.339 ^{ac}	214.535±5.476 ^{cg}	0.436±0.041 ^{ag}
W-P (1:1)	0.053±0.019 ^{bc}	0.975±0.116 ^a	55.980±8.079 ^b	1.108±0.658 ^{agh}
W-P (1:1)+10 % RB	0.028±0.016 ^{ac}	3.565±2.061 ^b	175.004±2.024 ^{cd}	4.029±0.510 ^{de}
W-P (1:1)+20 % RB	0.029±0.011 ^{ac}	2.914±2.240 ^{bc}	369.248±9.920 ^a	2.992±0.213 ^{ef}
W-S (1:1)	0.036±0.007 ^{ab}	1.245±0.141 ^{ac}	473.405±25.732 ^e	0.782±0.165 ^{ag}
W-S (1:1)+10 % RB	0.020±0.003 ^{ad}	1.631±0.629 ^{ac}	323.853±31.005 ^{af}	0.358±0.023 ^{ag}
W-S (1:1)+20 % RB	0.029±0.006 ^{ac}	3.471±2.714 ^b	363.010±42.809 ^a	3.621±0.496 ^{bde}
W-B (1:1)	0.064±0.007 ^b	1.370±0.334 ^{ac}	219.796±4.745 ^{cg}	2.273±0.138 ^f
W-B (1:1)+10 % RB	0.048±0.004 ^{bcd}	1.140±0.451 ^{ac}	290.586±22.722 ^{fg}	0.087±0.006 ^g
W-B (1:1)+20 % RB	0.059±0.005 ^b	1.379±0.168 ^{ac}	247.797±5.118 ^g	2.010±0.177 ^{hf}

** : control medium, W: Wheat Straw, RB: Rice Bran, C: Corn Stalk, M: Millet Straw, S: Soybean Straw, B: Bean Straw, P: Cotton Stalk,

Each value is expressed as mean ± SD of three replicate analyses.

Value with different small letters in the same column is significantly different at the level of 0.05 (P<0.05),

The reported vitamin C contents were 9.90 mg/100 g in *P. eryngii* var. *eryngii* (Alan & Padem 1991), 6.30-7.19 mg/100 g in *Pleurotus* spp., *A. bisporus* ve *L. edodes* (Furlani & Godoy 2007), 3.38-16.01 mg in *P. ostreatus*, *P. sajor-caju* ve *L. edodes* (Çağlarırnak 2007), 81.9 mg/100 g in *A. bisporus*, 46.3 mg/100 g in *F. velutipes* and 20.0 mg/100 g in *V. volvacea* (Oei 1996; Pauli 1998), 4.21-6.05 mg/100 g in *C. cibarius*, *L. piperatus* and *B. edulis* (Çağlarırnak et al. 2002), 1.6-8.6 mg in *A. bisporus* (Esselen & Fellers, 1946; Watt & Merrill 1963), 0.18-0.24 mg/g in *L. deliciosus*, 0.19-0.50 mg/g in *S. imbricatus* and 0.22-0.52 mg/g in *T. portentosum* (Barros et al. 2007) and 5.22-7.36 mg/100 g in *L. deliciosus* (Duda et al. 1988).

Vitamin C contents (55.980-473.405 mg/kg) are similar to that reported in similar studies (Esselen & Fellers 1946; Watt & Merrill 1963; Duda et al. 1988; Alan & Padem 1991; Agrahar-Murugkar & Subbulakshmi 2005; Barros et al. 2007), lower than that reported earlier (Alan & Padem 1991; Oei 1996; Pauli 1998; Agrahar-Murugkar & Subbulakshmi 2005; Barros et al. 2007; Furlani & Godoy, 2007), and higher than previously reported (Esselen & Fellers, 1946; Watt & Merrill, 1963; Alan & Padem 1991; Oei 1996; Pauli 1998; Çağlarırnak et al. 2002; Agrahar-Murugkar & Subbulakshmi 2005; Barros et al. 2007; Çağlarırnak 2007; Furlani & Godoy 2007).

In conclusion, *P. eryngii* var. *eryngii* is good source of unsaturated fatty acids including linoleic acid, oleic acid and palmitic acid (Table 1). The studied mushroom can be regarded as healthy foods. The high content of polyunsaturated fatty acids, such as linoleic acid, contribute to the recommendations of mushrooms in diets of people with high blood cholesterol. In additions, it is rich from the point of view of vitamins C, also antioxidants vitamins such as vitamin A, E and MDA. Furthermore, as it is a source of important antioxidant, it can be used in the diet as nutraceuticals or functional foods maintaining and promotion of health, longevity and life quality.

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