SHORT REPORT

A C G publications

Rec. Nat. Prod. 8:2 (2014) 189-194

records of natural products

The Cooking Effect on Two Edible Mushrooms in Anatolia: Fatty Acid Composition, Total Bioactive Compounds, Antioxidant and Anticholinesterase Activities

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(Received April 01, 2013; Revised September 12, 2013, Accepted December 13, 2013)

Abstract: The effects of baking practices on the fatty acid and bioactivity of edible mushrooms; namely, *Lactarius delicious* and *Ramaria flava*, naturally growing in Anatolia were investigated. Each mushroom species was divided into two parts and one of the parts was baked. Both baked and unbaked materials were extracted with hexane and methanol, successively. The fatty acid contents of baked and unbaked extracts of both species were carried out by GC and GC-MS analytical techniques. In the unbaked extracts palmitic acid (9.7-14.43%), stearic acid (41.41-6.68%), oleic acid (25.94-47.12%) and linoleic acid (22.85-9.78%) were identified as major fatty acids, respectively. In the baked extracts, however, palmitic acid (7.92-19.12%), stearic acid (49.94-6.23%), oleic acid (18.07-45.13%) and linoleic acid (23.36-9.25%) were identified as major fatty acids, as well. The antioxidative effect of the extracts of baked and unbaked mushroom species was also determined by using four complimentary assays. In addition, the extracts and the major fatty acids were also evaluated for anticholinesterase activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) which are the chief enzymes of Alzheimer's disease. The baked methanol extract of *R. flava* showed the highest activity in DPPH scavenging, ABTS scavenging and BChE assays, while the unbaked hexane extract of *R. flava* exhibited the best lipid peroxidation inhibition activity. In conclusion, baking proved to have influence in nutritional values and bioactivity properties of *L. delicious* and *R. flava*. The nutrient concentration and bioactivities of *L. delicious* were decreased when baked; however, baked *R. flava* proved to have higher nutrient concentrations and higher bioactivities than unbaked samples.

Keywords: *Lactarius delicious*; *Ramaria flava*; Fatty acids; Antioxidant activity; Anticholinesterase activity; cooking effect; Edible mushrooms.

1. Mushroom Source

The mushrooms are low in calories and fats as delicious foods, having rich source of vitamins, proteins and minerals, especially in potassium and phosphorus. Therefore, various mushrooms species have been the focus of researchers' interest. Hitherto, lectins, polysaccharides, polysaccharide-peptides, polysaccharide-protein complexes, lanostane-type triterpenoids, phenolics and flavonoids have been isolated from some edible mushroom species [1]. Furthermore, various biological activities such as antioxidant, antibacterial, antifungal [2], anti-immunomodulatory, antitumor [1], anti-

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 03/19/2014EISSN: 1307-6167

inflammatory [3], cytotoxic [1] and anticholinesterase [4] activities of the isolated compounds and/or complexes were investigated.

Lactarius delicious (L. : Fr.) Gray and *Ramaria flava* (Schaeff.: Fr.) Quél. which are consumed mushroom species were obtained from a local market in Muğla-Turkey in December 2009 and identified by Professor Aziz Türkoğlu, Department of Biology, Faculty of Science, Mugla Sıtkı Koçman University. Voucher specimens were deposited in the Fungarium of the same Department of the University, and coded with fungarium numbers AT 1152 and AT 1136, respectively.

2. Previous Studies

There are reports about the changes in the chemical composition and antioxidant activities of several mushroom species after cooking, such as *Lactarius delicious*, *Macrolepiota mastoidea* (Fr. : Fr.) Singer, *Macrolepiota procera* (Scop. : Fr.) Singer, *Sarcodon imbricatus* (L. : Fr.) P. Karst. [5].

Previous studies on *L. delicious.* Up to date, various biological activities such as antioxidant [6,7], acetylcholinesterase inhibitory [7], trypsin inhibitory [8], antimicrobial, [9], immunostimulant, anti-inflammatory [10] activities of the extracts of *Lactarius delicious* have been investigated. From the mushroom, proteoglycans [11], guaiane sesquiterpenoids [12], polysaccharides [13], terpenoids, phenolics and nitrogen containing compounds [14] have been isolated. Antitumor activity of the compounds isolated by Ding et al. and Liu has been studied [13,14]. Moreover, the fatty acid composition by GC-MS [15] and phenolic and organic acid profile [16], and sugar composition [15] by HPLC of *L. delicious* were also studied.

Previous studies on *R. flava*. During previous studies various biological activities such as antioxidant [9, 17], antimicrobial, [9,17], α-amilase and α-glucosidase inhibitory [18] activities of the extracts of *R. flava* have been investigated. From the mushroom, adenine, 5*α*,8*α*-epidioxyergosta-6,22-diene-3*β*-ol, 5*α*,8*α*-epidioxyergosta-6,9(11),22-triene-3*β*-ol, phenylacetic acid, bis(2-ethylhexyl) phthalate, 5*α*,8*α*-epidioxyergosta-6,9(11)-diene-3*β*-ol, stigmasterol, D-allitol, *α*-D-(+)-glucose, 3-*O*-*β*-D-glucopyranosyl-22*E*,24*R*-5*α*,8*α*-epidioxy-ergosta-6,22-diene and 3-*O*-*β*-D-glucopyranosyl-22*E*,24*R*-5*α*,8*α*-epidioxyergosta-6,9,22-triene have been isolated and elucidated [19]. The fatty acid composition by GC-MS [20] of *R. flava* was also studied.

3. Present Study

In this research, the fatty acid composition, antioxidant and anticholinesterase activity together with bioactive compounds were studied in both baked and unbaked materials of *L. delicious* and *R. flava*. Following procedures were carried out.

Mushroom materials and preparation of the extracts: Mushroom samples (1.0 Kg each) were divided into two parts. One part of mushroom was baked for two hours at 200ºC by using an oven. Unbaked and baked samples were extracted with 1 L hexane three times at room temperature, filtered and evaporated to dryness in vacuum. Similarly, the residues of both unbaked and baked mushroom samples were extracted with 1 L methanol three times at room temperature and the solvent was evaporated under vacuum. The yields of the eight extracts are given in Table 1.

Derivatization of fatty acids and GC and GC/MS analyses: The hexane extracts of both unbaked and baked mushroom samples (100 mg) were methylated by using BF_3: MeOH reagent as previously mentioned [21]. Qualitative and quantitative analysis of the fatty acids were performed using GC (Shimadzu GC-17 AAF, V3, 230V series gas chromatography, Japan) and GC/MS (Varian Saturn 2100T, USA). The library search was carried out using NIST and Wiley 2005, GC-MS libraries. Supelco™ 37 components of (Fatty acid Methyl ester) FAME mixture (Catalog no: 47885-U) was used for the comparison of the GC chromatograms [21].

Antioxidant and anticholinesterase activities: Antioxidant activity were used by β-carotene/linoleic acid bleaching assay [21], free radical scavenging activity by DPPH assay [21], cation radical scavenging activity by $ABTS^{\bullet+}$ assay [21] and superoxide anion radical scavenging assay [22]. BHT and α-tocopherol were used as antioxidant standards for comparison of the activity. Acetylcholinesterase and butyrylcholinesterase enzymes inhibitory activities were assayed by the Ellman method *in vitro* [23]. Galantamine was used as a reference compound. The data on all antioxidant activity tests were triplicated. Significant differences between means were determined by

Student's-*t* test, $p<0.05$ were regarded as significant. IC_{50} values were calculated from the concentration-effect linear regression curve.

Total Bioactive compounds: The total phenolic content of the mushrooms was determined based on the method previously described [21], as pyrocatechol equivalents (PEs). Standart pyrocatechol curve (y=0.0132[PEs]+0.015; r^2: 0.9972) was used to calculate total phenolic content.

As for total flavonoid content of the mushrooms were determined based on the aluminium chloride method [21], as quercetin equivalents (QEs). Standard quercetin curve (y=0.0153[QEs]-0.0142; r^2: 0.9993) was used to calculate total flavonoid contents.

Ascorbic acid content of 100 mg the crude methanol extract was determined as ascorbic acid equivalents (AAEs) as previously mentioned [24]. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic ascorbic acid (y=-0.0537[AAEs]-0.9581; r^2: 0.9988) and the results were expressed as mg of ascorbic acid/g of extract.

β-carotene and lycopene content in 100 mg crude methanol extract was determined according to the previous method [24]. Contents of β-carotene and lycopene were calculated according to the following equations: lycopene (mg/100 mL) = -0.0458 x A_{663} + 0.372 x A_{505} - 0.0806 x A_{453}; β-carotene (mg/100 mL) = 0.216 x A_{663} - 0.304 x A_{505} + 0.452 x A_{453}. The results were expressed as µg of carotenoid/g of extract.

Antioxidant activity of extracts of baked and unbaked mushroom species is given in Table 1 by four complimentary tests. The antioxidant activity was compared with those of BHT and α-tocopherol used as standards in food and pharmaceutical industry. In β-carotene-linoleic acid assay, unbaked hexane extract of *R. flava* demonstrated the best lipid peroxidation inhibition activity with an IC_{50} of 60.30±0.62 µg/mL, followed by unbaked methanol extract of *R. flava* (IC_{50}: 80.19±0.63 µg/mL), and baked methanol extract of *R. flava* (IC_{50}: 89.73±0.62 µg/mL). In general the baked extracts for both mushroom species showed less activity than the unbaked extracts. In contrast, in DPPH assay, the baked extracts indicated higher activity than the unbaked extracts for both mushroom species. In general the DPPH free radical scavenging activity of the *R. flava* was better than to that of *L. delicious*. The best activity was found to be in baked methanol extract of *R. flava* indicating 0.17±0.00 mg/mL, IC_{50} value, and followed by the baked hexane extract of *R. flava* (IC_{50}: 1.16±0.02 mg/mL).The superoxide anion radical scavenging activity supports the lipid peroxidation inhibition activity by the β-carotene-linoleic acid assay. Similarly, in $O^{2\bullet-}$ scavenging assay the unbaked extracts of both mushrooms demonstrated better activity than those of baked extracts. The best activity, however, was found in the unbaked hexane extract of *L. delicious* (58.99±2.46%) at 25 µg/mL. In $ABTS^{\bullet+}$ scavenging activity, however, the activity of *R. flava* was similar to that of DPPH assay while the activity of *L. delicious* resemble that of lipid peroxidation inhibition activity. In other words, the baked extracts of *R. flava* were better than the unbaked extracts, while the unbaked extracts of *L. delicious* showed better activity than the baked extracts. The best $ABTS^{\bullet+}$ scavenging activity was found in baked methanol extract of *R. flava* with an IC_{50} of 0.12±0.09 mg/mL.

Table 1. Antioxidant activity of the extracts of *L. delicious* and *R. flava* by the β-carotene-linoleic acid, $DPPH^{\bullet}$, $ABTS^{\bullet+}$, and Superoxide assays [a].

Mushrooms		Extracts	Yields (%)	β-carotene-linoleic acid assay	$DPPH^{\bullet}$ assay	$ABTS^{\bullet+}$ assay	$O_2^{\bullet-}$ assay
				IC_{50} (µg/mL)	IC_{50} (mg/mL)	IC_{50} (mg/mL)	Inhibition (%) (at 25 µg/mL)
L. delicious	Unbaked	Hexane	0.27	146.3±0.12	2.41±0.61	0.44±0.00	58.99±2.46
		Methanol	2.04	148.0±0.53	6.43±0.25	0.56±0.01	41.66±0.69
	Baked	Hexane	0.08	298.3±1.23	2.13±0.16	0.70±0.03	10.87±1.85
		Methanol	1.32	236.4±1.00	5.58±0.11	0.62±0.06	18.83±0.45
R. flava	Unbaked	Hexane	0.01	60.30±0.62	1.56±0.02	0.41±0.80	41.52±2.88
		Methanol	1.04	80.19±0.63	4.63±0.03	2.77±0.53	28.65±3.48
	Baked	Hexane	0.01	470.0±0.33	1.16±0.02	0.40±0.46	40.28±0.97
		Methanol	0.85	89.73±0.62	0.17±0.00	0.12±0.09	7.94±0.98
	α-Tocopherol [b]			2.10±0.08	7.31±0.17 [c]	4.31±0.10 [c]	44.60±0.19
	BHT [b]			1.34±0.04	45.37±0.47 [c]	4.10±0.06 [c]	87.59±0.00

[a] IC_{50} and Inhibition % values represent the means ± standard deviation of three parallel measurements ($p<0.05$).
[b] Reference compounds.
[c] Reference compounds that are given in µg/mL concentration.

As expected total phenol content, total flavonoid content, ascorbic acid content, β-carotene content and lycopene content of *L. delicious* were decreased when the mushroom species was baked. On the contrary, except ascorbic acid content, total phenol content, total flavonoid content and β-carotene content of baked *R. flava* mushroom were found to be higher than unbaked *R. flava*.

Table 2. Total bioactive compounds of methanol extract of *L. delicious* and *R. flava* [a].

Mushroom sample		Total Phenols (mg/g)	Total Flavonoids (mg/g)	Ascorbic acid (mg/g)	β-carotene (mg/g)	Lycopene (mg/g)
L. delicious	**Unbaked**	6.21±0.15	4.29±0.04	4.58±0.05	0.07±0.00	0.04±0.00
	Baked	2.80±0.11	0.60±0.00	4.69±0.08	0.01±0.00	0.01±0.00
R. flava	**Unbaked**	5.90±0.09	0.86±0.09	5.13±0.04	0.04±0.00	*nd*
	Baked	9.84±0.21	0.92±0.28	4.86±0.37	0.14±0.01	0.06±0.02

[a] Values expressed are means ± standard deviation of three parallel measurements (p<0.05).

The fatty acid composition of both baked and unbaked mushrooms species are given in Table 3. In both species palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$) and linoleic acid ($C_{18:2}$) were identified as major fatty acids. The content of the unbaked and baked materials of both mushroom species were found to be similar to each other qualitatively, except four compounds. But these compounds are in low quantity. The palmitic acid and oleic acid percentages of *L. delicious* decreased when the mushroom species were baked; on the other hand, stearic acid and linoleic acid percentages increased. Only the palmitic acid percentage of *R. flava* increased after baking. Conversely, the percentage composition of stearic acid, oleic acid and linoleic acid of *R. flava* decreased.

Table 3. Fatty acid composition of *Lactarius delicious* and *Ramaria flava*.

Fatty acids	*Lactarius delicious*		*Ramaria flava*	
	Unbaked (%)	**Baked (%)**	**Unbaked (%)**	**Baked (%)**
Caprylic acid ($C_{8:0}$)	-	-	0.27	-
Lauric acid ($C_{9:0}$)	-	-	0.07	0.09
Capric acid ($C_{10:0}$)	0.01	0.7	1.05	0.37
Undecanoic acid ($C_{11:0}$)	*t*[b]	*t*[b]	-	-
Lauric acid ($C_{12:0}$)	-	-	2.24	3.24
Tridecanoic acid ($C_{13:0}$)	-	-	0.81	1.98
Myristic acid ($C_{14:0}$)	-	-	3.63	3.75
Pentadecanoic acid ($C_{15:0}$)	*t*[b]	*t*[b]	0.89	1.26
Palmitic acid ($C_{16:0}$)	**9.7**	**7.92**	**14.43**	**19.12**
Palmitoleic acid ($C_{16:1,}\ \Delta^{9}$)	0.01	*trace*	0.78	1.3
Margaric acid ($C_{17:0}$)	-	-	-	0.06
Stearic acid ($C_{18:0}$)	**41.41**	**49.94**	**6.68**	**6.23**
Oleic acid ($C_{18:1,}\ \Delta^{9}$)	**25.94**	**18.07**	**47.12**	**45.13**
Linoleic acid ($C_{18:2,}\ \Delta^{9,12}$)	**22.85**	**23.36**	**9.78**	**9.25**
Arachidic acid ($C_{20:0}$)	0.08	-	1.42	1.22
cis-11,14-eicosadienoic acid ($C_{20:2,}\ \Delta^{11,14}$)	-	-	0.89	0.64
Heneicosanoic acid ($C_{21:0}$)	-	-	3.63	2.53
Behenic acid ($C_{22:0}$)	-	-	3.95	2.95
11-Docosenoic acid ($C_{22:1,}\ \Delta^{11}$)	-	-	0.25	-
[*Z*,*Z*,*Z*]-8,11,14-Docosatrienoic acid ($C_{22:3,}\ \Delta^{8,11,14}$)	-	-	2.06	0.84
Tricosanoic acid ($C_{23:0}$)	-	-	0.05	0.04
Total saturation	51.20	58.56	39.12	42.84
Total unsaturation	48.80	41.43	60.88	57.16
L/*O*[a]	0.88	1.29	0.21	0.20

[a] *L*/*O*, linoleic acid-oleic acid ratio; [b] *t, trace which is less than* <0.01%.

Table 4 shows the *in vitro* acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of the extracts of baked and unbaked studied mushroom species and their major fatty acids. In general the extracts of baked mushroom species demonstrated better activity than those of unbaked extracts. Among the all extracts, methanol extracts obtained from both baked and unbaked mushroom species were inactive against AChE. The acetylcholinesterase inhibitory activity of the hexane extracts, however, increased after baking. Among the hexane extracts the best inhibitory activity was found in baked *R. flava* mushroom indicating 1.05±0.19 mg/mL IC_{50} value. At the same conditions linoleic acid and oleic acid demonstrated 0.267±0.05 mg/mL and 0.127±0.03 mg/mL, respectively (Table 4).

As for BChE inhibitory activity, except hexane extracts of *L. delicious*, the butyrylcholinesterase inhibitory activity of the extracts of baked mushroom species were found to be higher than the unbaked ones. In contrast, the hexane extract of unbaked *L. delicious* showed better activity than the

hexane extract of baked. At the same conditions the best BChE inhibitory activity was demonstrated by the hexane extract of baked *R. flava* (IC_{50}: 0.113±0.08 mg/mL), followed by methanol extract of baked *R. flava* (IC_{50}: 0.17±0.10 mg/mL). Among the major fatty acids, however, linoleic acid and oleic acid demonstrated 0.126±0.04 mg/mL and 0.142±0.03 mg/mL, respectively (Table 4). Since unsaturated fatty acids inhibited the AChE and BChE enzymes according to the results, unsaturated fatty acids may be responsible for the inhibition of both AChE and BChE enzymes.

Table 4. *In vitro* anticholinesterase activity of the extracts and major fatty acids of the mushroom species[a].

Mushrooms	Extracts		AChE assay IC_{50} (mg/mL)	BChE assay IC_{50} (mg/mL)
L. delicious	**Unbaked**	Hexane	3.81±0.66	0.18±0.11
		Methanol	>4.00	0.57±0.21
	Baked	Hexane	2.02±0.56	0.28±0.13
		Methanol	>4.00	0.26±0.12
R. flava	**Unbaked**	Hexane	1.41±0.69	0.21±0.09
		Methanol	>4.00	0.89±0.33
	Baked	Hexane	1.05±0.19	0.13±0.08
		Methanol	>4.00	0.17±0.10
Major fatty acids	Palmitic acid ($C_{16:0}$)		>4.00	>1.00
	Stearic acid ($C_{18:0}$)		>4.00	>1.00
	Oleic acid ($C_{18:1}$, Δ^9)		0.127±0.03	0.142±0.03
	Lineloic acid ($C_{18:2}$, $\Delta^{9,12}$)		0.267±0.05	0.126±0.04
Standard	Galantamine [b]		0.005±0.00	0.050±0.00

[a] IC_{50} values represent the means ± standard deviation of three parallel measurements (p<0.05).
[b] Standard drug.

In conclusion, baking process to have influence in *L. delicious* and *R. flava* nutritional values and their bioactivity properties. Baked *L. delicious* proved to have lower nutrient concentrations and lower bioactivities than unbaked samples. Nevertheless, the baked one has also bioactivity and nutrition. However, baked *R. flava* proved to have higher nutrient concentrations and higher bioactivities than unbaked samples. Therefore, *R. flava* is strongly advised to be eaten after cooked.

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