academicJournals

Vol. 7(22), pp. 2770-2775, 28 May, 2013
DOI: 10.5897/AJMR12.2322
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Biological treatment for enhancement of the antioxidant capacity in agro-biomass as animal feed

Mohammad Faseleh Jahromi[1], Juan Boo Liang[1]*, Mohammad Yahaghi[2] and Parisa Shokryazdan[3]

[1]Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[2]Northeast Animal Breeding Station, Mashhad, Iran.
[3] Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

Accepted 22 May, 2013

Rice straw was fermented by two strains of *Aspergillus terreus* (ATCC 74135 and ATCC 20542*)* to enhance the antioxidant content of this agro-biomass as ruminant feed. After 8 days of fermentation, antioxidant capacity of samples was determined using Ferric reducing antioxidant power (FRAP), 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and 1,1-diphenyl-2-picryl- hydrazyl (DPPH) methods. Results of all three methods showed that treatment by both strains of *A. terreus* significantly (P<0.01) improved the antioxidant capacity of rice straw with higher enhancement for *A. terreus* ATCC 74135 than *A. terreus* ATCC 20542. *In vitro* gas production method was used to examine the stability of antioxidant components of the fermented samples under rumen microbial influence. Our results showed that rumen liquid sample containing fermented rice straw have higher antioxidant capacity than the control (untreated rice straw) after 48 h incubation, indicating that antioxidant capacity of the fermented rice straw remains stable during ruminal fermentation. These results show that biological treatment using *A. terreus* can be a practical method for production of high quality agro-biomass as ruminant feed with natural antioxidant capacity.

Key words: Antioxidant capacity, biological treatment, agro-biomass, *Aspergillus terreus*.

INTRODUCTION

Fat content and its quality are among the most important factors determining the quality of meat in the market. Free radical oxidation of fatty acid content in animal fat is the primary factor that causes the deterioration of the quality of stored meat and meat products (Gokalp et al., 1983; Karami, 2010). Antioxidants are compounds or substances that are able to inhibit or interfere with the free-radical oxidation reaction fundamental to glyceride oxidation (Sherwin, 1990). Vitamin E is the major lipid-soluble antioxidant in animal tissues which delay oxidative deterioration of meat. Asghar et al. (1991) reported that vitamin E not only improves the oxidative stability of pork lipids, it also maintains the redness of the muscle and decreases drip loss. Addition of vitamin E to feed increases α-tocopherol concentration in sarcosomes and thus, significantly increased the stability of the lipids against oxidation and improved the quality of farm animal products (Armstrong, 2002; Pokorny et al., 2001)

Lipid oxidation can also be reduced by supplementing antioxidants to the diet of the animals (Coronado et al., 2002; Karimi et al., 2010). Natural antioxidants are found in almost all plants, microorganisms, fungi and even in

*Corresponding author. E-mail address: jbliang@upm.edu.my; Tel: +60 3-8947 2132. Fax: +60 3 8947 2213.

Abbreviations: FRAP, Ferric reducing antioxidant power; **ABTS**, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid; **DPPH**, 1,1-diphenyl-2-picryl- hydrazyl; methods; **RS**, rice straw; **FRS**, fermented rice straw.

animal tissues (Akarpat et al., 2008). Although some synthetic antioxidants are available, toxicity and carcinogenicity of these components (Juntachote et al., 2006) and their low water solubility (Bekhit et al., 2003) reduce the usage of these antioxidants and thus natural antioxidants are preferred. Furthermore, consumers generally perceive natural antioxidants which are better than their synthetic counterparts.

Studies have shown that polysaccharides of fungal mycelia have high antioxidant capacity and different polysaccharides and polysaccharide-proteins have been isolated from fungi with high antioxidant capacity (Li and Tsim, 2003; Leung et al., 2009). In addition, biological treatment is a well-known method for enhancement of the quality of agro-biomass as animal feed. However, there is limited published research concerning the use of *A. terreus* to enhance antioxidant content of agro-biomass, including rice straw (RS). The present study focuses on the effect of biological treatment on enhancement of antioxidant capacity of RS using two strains of *A. terreus (*ATCC 74135 and ATCC 20542) and the stability of antioxidant capacity of the fermented rice straw (FRS) to rumen microbial degradation.

MATERIALS AND METHODS

Chemicals

Unless otherwise stated, all chemicals used in this study were obtained from Nacalai Tesque, Kyoto, Japan.

Fungi, Substrate and Fermentation

Rice straw (RS) collected locally were dried at 60°C for 24 h and ground to uniform size (mesh size 6) and the two strains of *A. terrus (*ATCC 20542 and ATCC 74135*)* were obtained from American Type Culture Collection (ATCC). Spore suspensions of the fungal strains were prepared by washing 7 day old culture slants with sterilized 0.1% Tween-80 solution. Spores were counted whit a hemocytometer (Biirker-Tiirk;Schreck, Hofheim, Germany), adjusted to approximately 10^7 spores/ml, and were used as inocula in the study. Solid state fermentation of RS was carried out in 500 ml Erlenmeyer flasks. Twenty grams of each substrate was placed in individual flasks and moisture content was adjusted to 50% by addition of 20 ml water. The flasks were plugged with cotton and autoclaved at 121°C for 15 min. Each flask was inoculated with 10% (V/W) inoculums. The flasks were incubated at 25°C for 8 days in triplicate for each treatment. At the end of fermentation, flasks were removed from the incubator and the fermented rice straw samples were dried and stored at -20°C for antioxidant capacity study and *in vitro* gas production study.

In vitro gas production

In vitro gas production method as described by Menke and Steingass (1988) was used for studying the effect of microbial fermentation of feed on antioxidant capacity of rumen liquid and stability of antioxidant capacity of feed during the microbial fermentation in the rumen. Five hundred milligrams of each treated sample (RS and FRS) were transferred into a 100 ml syringes. Buffered and mineral solution (Menke and Steingass, 1988) was prepared and placed in a 39°C water-bath under continuous flushing of CO_2. Rumen fluid was collected before the morning feeding from two ruminally fistulated male local Kedah-Kelantan cattle that were fed with an equal weight rations (approximately 4 kg dry matter) containing 40% concentrates and 60% grass hay, twice daily at 08:00 and 18:00. Rumen fluid, collected using a manually operated vacuum pump, was transferred into a pre-warmed bottle and immediately transported back to the laboratory, filtered through eight layers of cheesecloth and flushed with CO_2. Rumen fluid was added to the buffered mineral solution with constant stirring, while maintaining the temperature in a water bath at 39°C. About 30 ml of buffered rumen fluid was dispensed into individual syringes, each containing the 500 mg of treated sample and the syringes were incubated at 39°C for 48 h. Incubation was performed in triplicate within each of the two runs. At the end of the incubation, liquid layer of each syringe was collected for subsequent antioxidant capacity analysis.

Sample Preparation for Determination of Antioxidant Capacity

For determination of antioxidant capacity of the RS and FRS, 10 ml of methanol were added to 1 g of the dried sample in 50 ml tube. The mixture was shaken continuously on an orbital shaker for 1 h. The extract was then centrifuged at 2500 ×g for 10 min using ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA) and stored at -20°C for further analysis. The liquid component from the *in vitro* gas production study (previous section) was centrifuged at 2500 ×g for 10 min and the supernatant was used for determination of antioxidant capacity in rumen liquor.

DPPH Radical Scavenging Activity

Antioxidant capacity of the samples was measured on the basis of scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Tagashira and Ohtake, 1998). In a test tube containing 150 µl of samples, 5 ml of DPPH methanol solution (100 mg/l) was added. After 30 min of incubation in the dark at room temperature, the absorbance was measured against a blank (methanol) at 517 nm using spectrophotometer (Barnstead Turner SM110255, USA). Inhibition of DPPH radical was calculated as a percentage (%) using the formula:

$$\text{Percentage inhibition (\%)} = [(A_{control} - A_{sample})/A_{control}]*100$$

Where, $A_{control}$ is the absorbance of the control reaction (containing all reagents except test compound), and A_{sample} is the absorbance of the test compound. α-tocopherol was used as standard (Figure 1).

Ferric reducing antioxidant power (FRAP) assay

In the FRAP method, the yellow Fe^{3+}-TPTZ complex is reduced to the blue Fe^{2+}-TPTZ complex by electron-donating substances under acidic conditions (pH less than 7). Any electron donating substance with a half reaction of lower redox potential than Fe^{3+}/Fe^{2+}-TPTZ will drive the reaction, resulting in the formation of the blue complex. To prepare the FRAP reagent, a mixture of 300 mmol/l acetate buffer pH 3.6 (6.4 ml 2 M sodium acetate solution and 93.6 ml 2 M acetic acid solution (1 l)), 10 mmol/l TPTZ (in 40 mmol/l HCl) and 20 mmol/l ferric chloride (10:1:1, v:v:v) was made. One hundred and fifty microliters of sample was mixed with 4.5 ml of FRAP reagent. After 5 min, the absorbance was read at 593 nm using a spectrophotometer (Barnstead, USA). The FRAP reagent was used as blank and the final absorbance of each sample was compared with those obtained from the standard curve made from 0 to 1000 µmol/l ferric sulphate ($FeSO_4$-7H2O) (Figure 2). The results

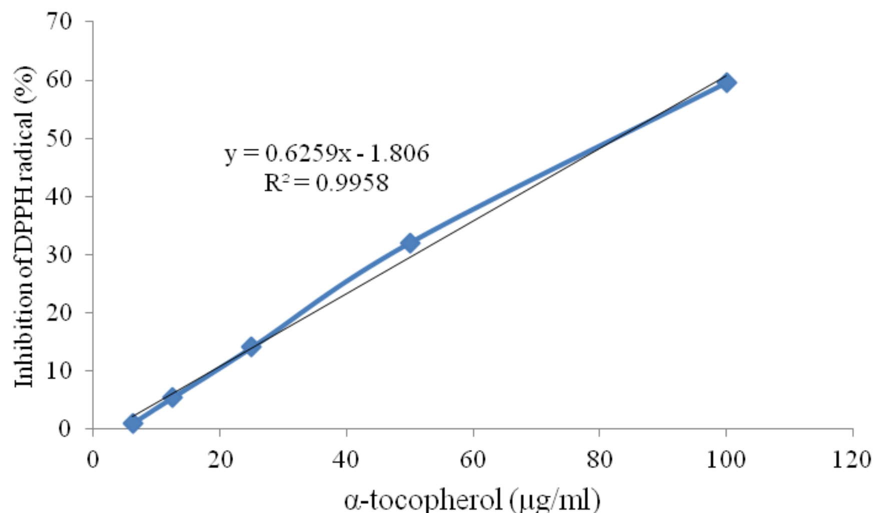

Figure 1. Inhibition of DPPH radical (%) using different concentration of α-tocopherol.

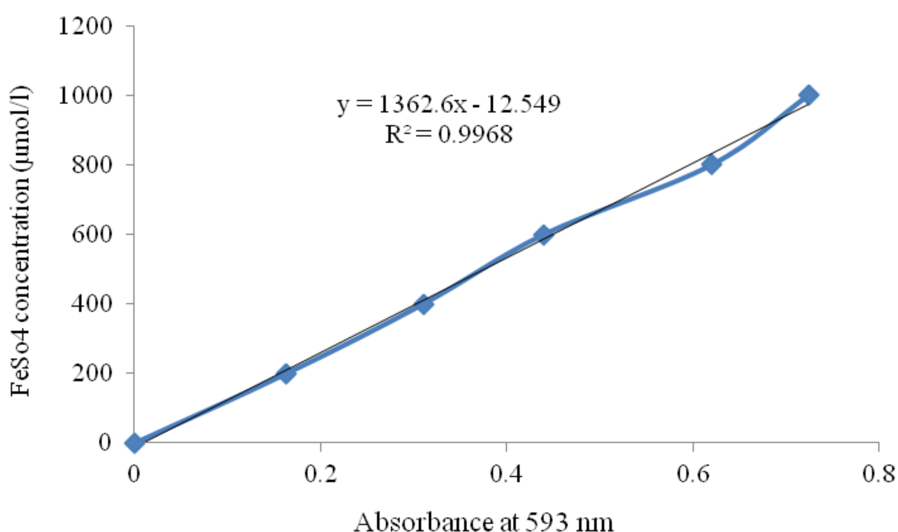

Figure 2. Standard carve of $FeSO_4$ for used in FRAP method.

were expressed in µmol Fe2+/l sample (Szollosi and Szollosi Varga, 2002).

ABTS Assay

For ABTS assay, the procedure described by Arnao et al. (2001) was used with some modifications. The stock solutions contained 7.4 mM $ABTS^{\bullet+}$ solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing $ABTS^{\bullet+}$ solution with methanol to obtain an absorbance of 1.1±0.02 units at 734 nm using the spectrophotometer. Fresh $ABTS^{\bullet+}$ solution was prepared for each assay. Each sample (150 µl) was allowed to react with 2850 µl of the $ABTS^{\bullet+}$ solution for 20 min in the dark condition before the absorbance was read at 734 nm. The capacity to scavenge the $ABTS^{\bullet+}$ radical was calculated using the following equation:

$$ABTS^{\bullet+} \text{ scavenging capacity } (\%) = [(A_{Control} - A_{Sample}) / A_{Control}] \times 100$$

Where, $A_{Control}$ is the initial absorption of the $ABTS^{\bullet+}$ and A_{Sample} is absorbance of $ABTS^{\bullet+}$ in the presence of samples. α-tocopherol was used as standard (Figure 3).

Statistical analysis

Data were analysed using the general linear model (GLM) procedure of SAS version 9.1 (2008). All multiple comparisons among means were performed using Duncan's new multiple range test. Data were expressed as mean ± standard deviation.

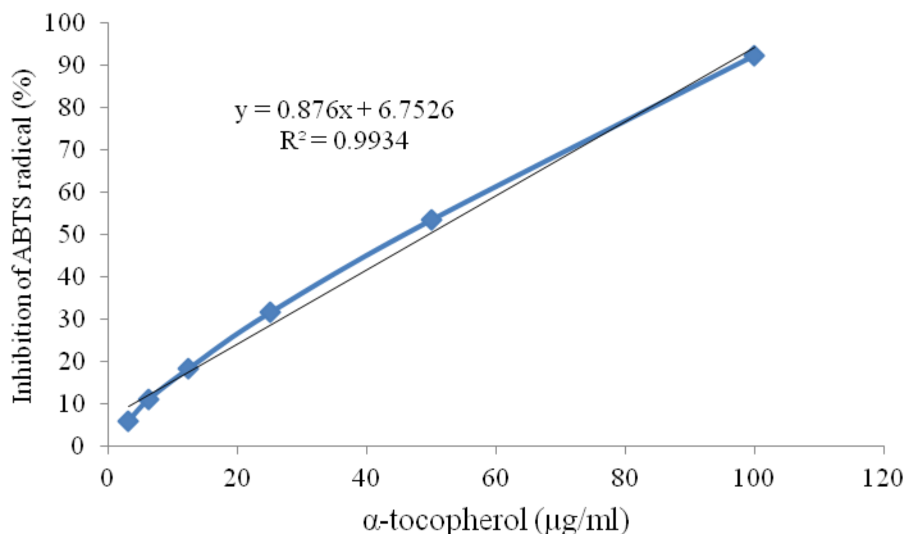

Figure 3. Inhibition of ABTS radical (%) using different concentration of α-tocopherol.

Table 1. Effect of biological treatment on antioxidant activity of rice straw (RS) and fermented rice straw (FRS).

Treatment	FRAP (µM Fe^{2+}/l extract)	Inhibition of ABTS radical (%)	Inhibition of DPPH radical (%)
RS	102.36±12.66^c	32.90±1.76^c	15.35±4.05^c
FRS by *A. terreus* ATCC 74135	501.61±17.78^a	76.33±3.24^a	44.52±1.62^a
FRS by *A. terreus* ATCC 20542	301.30±20.63^b	65.37±1.76^b	25.77±1.88^b
Significant	**	**	**

Data are Mean ± SD. **, Significantly different at 1% level; a, b and c: indicating means within column differed significantly.

RESULTS AND DISCUSSION

Effects of biological treatment of RS using two strains of *A. terreus* (ATCC 20542 and ATCC 74135) on its antioxidant capacity are shown in Table 1. The results showed that antioxidant capacity of FRS treated using the two strains of *A. terreus* significantly increased (P<0.01) the antioxidant capacity of the FRS in all the three antioxidant test procedures adopted in this study (FRAP, ABTS and DPPH).

In the FRAP essay, antioxidant capacity was measured as the ability of the antioxidant components in the test sample to reduce ferric (III) to ferrous (II) in a redox-linked colorimetric reaction (Li et al., 2006). Our results showed that biological treatment of rice straw significantly increased (P<0.01) its capability on reduction of Fe^{3+} to Fe^{2+} in the FRAP reagent (Table 1). ABTS scavenging capacity denotes the ability of antioxidant components in the test sample to remove the $ABTS^{\bullet+}$ radicals, which were generated from the oxidation of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) by potassium persulphate ($K_2S_2O_8$). The ABTS scavenging activity of RS increased (P<0.01) from 32.90 to 76.33% and 65.37%, respectively, for FRS treated by ATCC 74135 and ATCC 20542 (Table 1). Likewise, DPPH is a compound that contains a nitrogen free radical which is readily destroyed by a free radical scavenger. This assay is a test on the ability of the antioxidant compounds functioning as proton radical scavengers or hydrogen donors (Singh and Rajini, 2004). Our data (Table 1) indicated that treatment of RS with the two strains of *A. terreus* resulted in higher DPPH scavenging activity than that of untreated RS. The overall results of this study showed that treatment RS by ATCC 74135 resulted in higher enhancement in antioxidant capacity than that by ATCC 20542.

The higher antioxidant capacity recorded in the FRS is assumed to be due to the polysaccharides of mycelial biomass that were produced during the growth of fungi on this substrate as suggested by data from the following studies. Li and Tsim (2003) reported strong antioxidant capacity of a polysaccharide (with a molecular composition of 10 glucose: 60 mannose: 75 galactose) which they isolated from the mycelia biomass of a *Cordyceps anamorph* fungus. Similarly, Chang et al. (2007) reported that polysaccharides isolated from biomass of *Phellinus baumii Pilat* (*basidiomycete* fungus belonging to the genus *Phellinus*) also exhibited antioxidant capability. Natural antioxidants isolated from plants, fungi and marine

Table 2. Antioxidant activity of rumen fluid after 48 h incubation of rice straw (RS) and fermented rice straw (FRS).

Treatment	FRAP (µM Fe^{2+}/l rumen fluid)	Inhibition of ABTS radical (%)	Inhibition of DPPH radical (%)
RS	617.43±45.08^{b}	68.60±1.11^{b}	10.56±1.07^{b}
FRS by *A. terreus* ATCC 74135	738.70±43.01^{a}	75.33±1.97^{a}	13.27±0.95^{a}
FRS by *A. terreus* ATCC 20542	683.29±26.01^{ab}	73.03±1.25^{a}	12.80±1.06^{a}
Significant	*	**	*

Data are Mean ± SD. *, significantly different at 5% level; **, significantly different at 1% level; a, b and c: indicating means within column differed significantly

algae have been suggested to be the most useful nutraceuticals and functional foods constituents in health and disease prevention (Gutteridge and Halliwell, 1994). Leung et al. (2006) reported that polysaccharides produced from biomass of fungi are potential natural antioxidants. It has been suggested that antioxidant capacity of polysaccharides is due to the hydroxyl group of its molecules (Lindsay, 1996). However, SH, COOH, C=O, –S– and –O– functional groups in some polysaccharides and most polysaccharide-protein complex molecules can also confer a radical scavenging effect (Leung et al., 2009).

It is important that the antioxidant ability of the FRS to be stable to ruminal microbial degradation, if it is to be of use as an antioxidant agent to the host animals. To examine the above property of the antioxidant components, RS and FRS samples were separately incubated for 48 h under *in vitro* condition by inoculating rumen fluid into the buffer and mineral solution. The results showed that FRS significantly improved the FRAP (P<0.05), DPPH (P<0.05) and ABTS (P<0.01) scavenging activities in the rumen fluid (Table 2). The above results thus showed that the antioxidant capacity of the FRS is stable to ruminal microbial activity and therefore suggesting that biological treatment of rice straw using *A. terreus* offers a practical method to enhance the nutritive value of this agro-biomass as feed for ruminant animals.

Conclusion

Results of the present study showed that solid state fermentation using *A. terreus* is a viable method to enhance feeding value of agro-biomass, such as rice straw, by increasing its antioxidant capacity. Our results further showed that the antioxidant components in the FRS were stable in the rumen ecosystem and not degraded by the microorganisms in the rumen. Since antioxidant is beneficial to the health of the animal and can also improve the quality of their products (meat and milk), antioxidant enriched FRS, produced using the present method, can be a high quality roughage source for ruminant production.

ACKNOWLEDGEMENT

This study was supported by the Fundamental Research Grant Scheme (FRGS 1/2010 UPM) of the Department of Higher Education, Malaysia.

REFERENCES

Akarpat A, Turhan S, Ustun NS (2008). Effect of hot-water extracts from mytle, rosmary, nettle and lemon balm leaves on lipid oxidation and color of beed patties during frozen storage. J. Food Process Preserv. 32: 117-132.

Armstrong D (2002). Oxidative Stress Biomarkers and Antioxidant Protocols. Humana Press, Totowa, New Jersey. p. 322.

Arnao MB, Cano A, Acosta M (2001). The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem. 73: 239-244.

Asghar A, Gray JL, Booren AM, Gomaa EA, Abouzied MM, Miller ER (1991). Effects of supranutritional dietary vitamin E levels on subcellular deposition of α-tocopherol in the muscle and on pork quality. J. Sci. Food and Agric. 57: 3141.

Bekhit AED, Geesing GH, Ilian MA, Morton JD, Bickerstaffe R (2003). The effects of natural antioxidants on oxidative processes and methmyoglobin reducing activity in beef patties. Food Chem. 81:175-187.

Chang ZQ, Oh BC, Rhee MH, Kim JC, Lee SP, Park SC (2007). Polysaccharides isolated from *Phellinus baumii* stimulate murine splenocyte proliferation and inhibit the lipopolysaccharide-induced nitric oxide production in RAW264.7 murine macrophages. W. J. Mic. Biotech. 23:723-727.

Coronado SA, Trout GR, Dunshea FR, Shah NP (2002). Antioxidant effects of rosemary extract and whey powder on the oxidative stability of wiener sausages during 10 months frozen storage. Meat Sci. 62: 217-224.

Ge Q, Zhang A, Sun P (2009). Structural investigation of a novel water-soluble heteropolysaccharide from the fruting bodies of *Phellinus baumii* Pilát. Food Chem. 114: 391-395.

Gokalp HY, Ockerman HW, Plimpton RF, Harper WJ (1983). Fatty acid of neutral and phospholipids, rancidity scores and TBA values as influenced by packaging and storage. J. Food Sci. 48: 82-834.

Gutteridge JMC and Halliwell B (1994). Antioxidants in nutrition, health, and disease. Oxford, New York, NY: Oxford University Press.

Juntachote T, Berghofer E, Siebenhandl S, Bauer F (2006). The oxidative properties of Holy basil and Galangal in cooked ground pork. Meat Sci., 72: 446-456.

Karami M, Alimon AR, Sazili AQ, Goh YM (2010). Meat Quality and Lipid Oxidation of Infraspinatus Muscle and Blood Plasma of Goats under Dietary Supplementation of Herbal Antioxidants. J. Anim. Vet. Adv. 9(22): 2839-2847

Leung PH, Zhao SN, Ho KP, Wu JY (2009). Chemical properties and antioxidant activity of exopolysaccharides from mycelial culture of *Cordyceps sinensis* fungus Cs-HK1. Food Chem. 114: 1251–1256.

Leung PH, Zhang QX, Wu JY (2006). Mycelium cultivation, chemical composition and antitumour activity of a Tolypocladium sp. fungus isolated from wild Cordyceps sinensis. J. Appl. Microbiol. 101: 275-283.

Li YF, Guo CJ, Yang JJ, Wei JY, Xu J, Cheng S (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chem. 96:254-260.

Lindsay RC (1996). Food additives. In Fennema OR (Ed.), Food Chemistry (pp. 778–780). New York: Marcel Dekker Inc.

Menke KH and Steingass H (1988). Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim. Res. Dev. 28:7-55.

Pokorny J, Yanishlieva N, Gordon M (2001). Antioxidants in Food

Practical Applications. Wood head Publishing Limited, Cambridge. pp: 381- 388.
SAS Institute Inc., (2008). STAT User's Guide, Release 9.1. SAS Institute Inc., Cary, NC.
Singh N and Rajini PS (2004). Free radical scavenging activity of an aqueous extract of potato peel. Food Chem. 85: 611-616.
Sherwin ER (1990). Antioxidants. In Food Additives, 1st Ed. (Branen AL, Davidson PM, Salminen S, eds.) pp. 139–191, Marcel Dekker, Inc., New York, NY.
Szollosi R and Szollosi I (2002). Total antioxidant power in some species of Labiate (Adaptation of FRAP method). Acta Biologica Szegediensis. 46(3-4):125-127.
Tagashira M, Ohtake Y (1998). A new antioxidative 1,3-benzodioxole from *Melissa officinalis*. Planta Medica. 64: 555-558.