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Total Antioxidant Activity and Total Phenolic Contents in Yemeni Smoked Cheese

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ABSTRACT

The antioxidant activity and total phenolic content of smoked cheese subjected to smoke treatment by different types of wood materials (*Dodonia viscosa*, *Zizyphus spin christi*, and *Acacia asak*) are reported in this study. The total phenolic contents were determined according to the Folin-Ciocalteu method and were significantly higher (3-fold) in the smoked cheese (ranged from 59.9 to 62.1 mg /100g fresh weight of cheese, as Catechin equivalent) compared to the non-smoked cheese. However, the three types of smoked cheese did not significantly differ from each other. The total phenolic content in commercially smoked cheese was approximately 2-times higher than the three treated cheese samples. Antioxidant activity of smoked cheese samples were assayed *in vitro* by the inhibition of liver homogenate oxidation using $FeSO_4$ /ascorbate system. The addition of different levels (25 µl, 50 µl, 100 µl and 200 µl) of cheese suspension equivalent to 2.5mg, 5mg, 10mg and 20mg of smoked cheese respectively to the reaction mixture showed that the antioxidant activity increased with increasing levels of smoked cheese suspension. The total antioxidant activity obtained from different levels (30.9 - 46.3%) of the cheese treated by *Zizyphus spina christi* was the highest and was significantly different in comparison with the other two treatments. The total antioxidant activity of commercially smoked cheese are similar to that of *Zizyphus spina christi* at the lower levels and higher at the higher levels.

INTRODUCTION

At present, there is overwhelming evidence to indicate that free radicals cause oxidative damage to lipid, protein, and nucleic acids, where active oxygen species such as $O^{\cdot}_2$, $HO^{\cdot}$, or lipid peroxyl radical $LOO^{\cdot}$ may lead to many biological complications, including carcinogenesis, mutagenesis, aging, and atherosclerosis (Halliwell and Gutteridge, 1989). For example, the accumulation of cholesterol esters is caused by oxidation of blood plasma lipids (Retsky, Freeman & Frei, 1993). Therefore, the oxidation of blood plasma lipids is strongly associated with atherosclerosis and endothelial dysfunction (Schmidt *et al.*, 1994; Tesfamariam, 1994). Polyunsaturated fatty acids or fatty acyl side chains in biological membranes can be peroxidised in the presence of enzymes or in their absence by exposure to reactive

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oxygen species and to transition metal ions in a free radical chain reaction. This results in lipid peroxidation which can be deleterious for membrane permeability and can produce toxic compounds in humans, such as malonaldehyde (MA) and acetaldehyde, where they produce abnormal adducts with biological substances, including DNA and RNA (Feinman, 1988; Esterbauer, Schaur & Zollner, 1991).

Natural antioxidants can be phenolic compounds (tocopherol, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyl derivatives, amino acids, and amines), carotenoids as well as ascorbic acid (Larson, 1988; Hudson, 1990; Hall and Cuppett, 1997). Phenols, because of their molecular structure, which include an aromatic ring with hydroxyl groups containing mobile hydrogens, are very efficient scavengers of peroxyl radicals (Halliwell, 1990; Aruoma, 1994). Moreover, the action of phenolic compounds can be related to their capacity to reduce and chelate ferric ion, which catalyses lipid peroxidation (Gazzanie *et al.*, 1998). Despite the identification of some phenolic compounds in smoked cheese (Knowles, Gilbert & McWeeny, 1974), it appears that there is no data for their antioxidant activities. A significant proportion of the smoked flavour has been associated with the phenolic fraction of the smoke and hence most liquid smoke preparations are largely composed of this fraction (Knowles, Gilbert & McWeeny, 1974 & 1975a,b; Cadwallader, 1996). The aim of the present study was therefore to determine the total water-soluble phenolic compounds and total antioxidant activity in Yemeni smoked cheeses.

MATERIAL AND METHODS

Samples Preparation

The smoking process

Smoked cheese samples were prepared in a Smoking Unit in the laboratory of Food Sciences Department, Faculty of Agriculture. Three types of local wood material most commonly used in cheese-smoking, namely *Zizyphus spina christi*, *Acacia asak*, and *Dodonia viscosa*, were used in this study. The smoking was continued until the surface of the cheese sample had a nice brown colour all over and imparted a characteristic aroma and flavour.

The preparation of cheese samples extracts

For total antioxidant activity, about 2 g of each sample was homogenised with about 10ml of distilled water. The homogenate was transferred quantitatively to a test tube containing 5 ml of distilled water and was boiled for 5 minutes and then cooled. The resultant solution was filtered through a cheese-cloth and the volume was adjusted to 20 ml with distilled water. Each sample was extracted in triplicate. These sample solutions were used to measure the total antioxidant activity, by using different levels of the solution (25 μl, 50 μl, 100 μl, and 200 μl).

To measure the total phenolic content in smoked cheese, about 2 g of the sample was homogenised and extracted for 30 minutes with 100ml of 80% methanol containing 1% HCl at about 80ºC on an orbital shaker at 200rpm. The mixture was cooled, filtered and the residue was washed with the same solvent. Each sample was prepared in triplicate.

Preparation of liver homogenate

Adult male guinea pigs were obtained from the Experimental farm of the Faculty of Agriculture, Sana'a

University. The animals were maintained on a formulated diet to fulfill their requirement. Feed and water were provided *ad libitum*. The animals were fasted overnight prior to sacrifice. The liver from each one was dissected and a 20% homogenate was prepared in an ice-cold phosphate buffer, pH 7.4 and centrifuged at 20000 g (Sorval RC-5B Refrigerated Superspeed Centrifuge) for 15 minutes in a refrigerated centrifuge to remove the cell debris. The supernatant was used for the *in vitro* studies.

Antioxidant activity towards lipid peroxidation in liver homogenate

The effect of different extracts of smoked cheeses on $FeSO_4$ / ascorbate - induced peroxidation in liver homogenate was assessed using an incubation mixture, containing 0.4 ml of tissue homogenate, 4 ml of the oxidising solution (50 μmol/ L $FeSO_4$; 1 mmol/ L $KH2PO_4$; 0.2 mmol/ L ascorbic acid in 0.15M Tris-HCl buffer, pH 7.4), and different volumes of smoked cheese extracts (25 μl, 50 μl, 100 μl, 200 μl) equivalent to 5, 10, 20 and 40 mg of extracted cheese respectively. Incubations were carried out in a water bath at 37°C for 20 minutes. The tubes were shaken every 5 minutes. The reaction was stopped by the addition of 1ml of 10% trichloroacetic acid (TCA) after the incubation period. The tubes were shaken well and 1.5 ml of thiobarbituric acid (TBA) (1% in 0.05N NaOH) reagent was added and the tubes were heated at 80°C for 30 minutes. The tubes were centrifuged at 5000 g for 10 minutes and the colour developed in the supernatant was read (Milton Roy Spectronic 1001 plus) at 532 nm (TBARS1). As the control, the homogenate was peroxidised by $FeSO_4$ / ascorbate without the antioxidants (TBARS2). The reactions without $FeSO_4$ / ascorbate were carried out for each of the test substance as the blank (TBARS3 and TBARS4, respectively). The antioxidant potential of the sample was calculated by the following equation:

Antioxidant activity (%) = (1 - TBARS1 - TBARS3 / (TBARS2 - TBARS4) x 100

All of the tests were carried out in duplicate, and the results were averaged.

Determination of the total phenolic contents

The concentration of total phenolic content in smoked cheese was determined by Folin-Ciocalteu procedure (Singleton and Rossi, 1965) and expressed as mg / 100g of smoked cheese as catechin equivalent (CE).

Statistical Analysis

Differences in antioxidant activity and correlations between phenolic content in smoked cheese and their antioxidant activity were tested by one-way analysis of variance using SPSS computer programme. Significant level was taken at $P < 0.05$ unless otherwise indicated.

RESULTS AND DISCUSSION

Since volatile phenolic compounds in wood materials used in cheese smoking may be absorbed on the cheese surface and consequently may confer some antioxidant activity, the present study was aimed at determining the total phenolic compounds and total antioxidant activity in cheese smoked by three different wood materials.

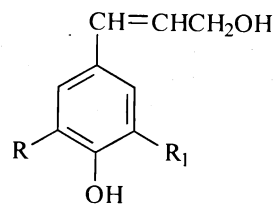
Table 1 shows the total phenolic contents and total antioxidant activity in extracts of cheese smoked by three different wood materials (*Dodonia viscosa*, *Zizypus spina christi*, and *Acacia asak*) and commercially smoked cheese. The total phenolic contents in the smoked cheese were similar in all the three treatments and were approximately 3 times higher than the non-smoked cheese. However, in comparison with the commercially smoked

Table 1. Total antioxidant activity and total phenolic content in smoked cheese treated by different types of wood materials

Wood Material	*Total Antioxidant Activity (%)*				*T.Ph.(mg/100 g FW)*
	2.5 mg	*5 mg*	*10 mg*	*20 mg*	
Non-smoked cheese	11.62±0.34	10.41±0.24	8.33±0.74	4.52±1.48	20.87±1.70
Zizyphous spina christi	30.92±1.04a	35.32±0.53a	39.99±2.38a	46.25±0.12a	60.66±2.32a
Acacia asak	16.35±1.44b	20.31±1.22b	25.32±0.69b	30.92±1.88c	62.14±2.24a
Dodonia viscosa	10.72± 0.89c	13.39±1.35c	23.63±1.74b	40.58±2.31b	59.86±3.66a
Commercial smoked Cheese	33.34±0.98	41.64±1.36	54.81±2.10	76.82±4.42	115.91±9.60

Values (± SD) in the same column followed by the same letter are not significantly different at $P < 0.05$.

cheese, the treated cheese samples contain 52% of the total phenolic compounds. The phenolic compounds may have resulted from the wood combustion since it was found that a significant proportion of the smoke flavour produced from burned wood materials has been associated with the phenolic fraction of the smoke (Knowles, Gilbert & McWeeny, 1975a,b). Moreover, earlier studies on phenols nitrosation (Knowles, Gilbert & McWeeny, 1974 & 1975a,b) showed that the smoking of bacon resulted in the deposition of phenols in the meat matrix, mainly methyl phenols (Cresols), 4-substituted-2-methoxyphenols (Guaiacols) and 4-substituted-2,6-dimethoxyphenols (Syringols). The chemical composition of wood smoke was found to vary with the type of wood, the degree of combustion and the accessibility of air (Arseculeratne, Samarajeewa & Welianga, 1976). Since wood materials are rich in lignin, the phenolic compounds may result from the heat degradation during smoking, where lignin is a polymer that originates from three derivatives of phenylpropane (p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol). The very high total phenolic content measured in the commercially smoked cheese (5.52 times that of the non-smoked cheese) might be explained by the long period of smoking as is apparent from its much darker brown colour (Riha and Wedorff, 1993). Moreover, the presence of phenolic compounds in the non-smoked cheese might be attributed to the pasture, animal metabolism and amino acid catabolism or microbial activity (Ha and Lindsay, 1991a,b; Lopez and Lindsay, 1993).



(1) p-Coumaryl alcohol, where $R = R_1 = H$
(2) Coniferyl alcohol, where $R = H$, $R_1 = -OCH_3$
(3) Sinapyl alcohol, where $R = R_1 = -OCH_3$

The percentage total antioxidant activity was observed to be related to the amount of cheese samples (2.5 mg, 5 mg, 10 mg and 20 mg) which were equivalent to the level of volume extracts that were added to the reaction mixtures. Of the three treatments, cheese treated by *Zizyphus spina christi* gave the highest total antioxidant activity with all levels and was significantly different from the other two treatments. The antioxidant activity obtained from the first level of cheese

treated by *Zizyphus spina christi* was about three times higher than that treated by *Dodonia viscosa* and about two times higher than that treated by *Acacia asak*. Comparison of the percentage antioxidant activity obtained from cheese samples treated by *Dodonia viscosa* and *Acacia asak* smoke shows the antioxidant activity to be significantly higher in those treated by *Acacia asak* at the lower amount of cheese samples used. At the higher levels, however, the total antioxidant activity was observed to be higher in the cheese samples smoked by *Dodonia viscos*. The antioxidant activity observed in the three treatments are very much higher than that in the non-smoked cheese, particularly at the higher levels, which is in line with the higher total phenolics in the treated samples. Along the same line, the antioxidant activity of the commercially smoked cheese was higher than the non-smoked cheese and similar to that of *Zizyphus spina christi* at the lower levels of 2.5 and 5 mg. However, the antioxidant activity is much higher than that of *Zizyphus spina christi* at the higher levels of 10 and 20 mg.

The discrepancies in antioxidant activity could be attributed to the type of wood materials since the chemical composition of wood smoke varies with the type of wood, and different phenolic compounds are found in the lignin in wood material (Aseculeratne, Samarajeewa & Welianga, 1976). Other studies have shown that the differences in antioxidant activity may depend on structural dissimilarities, primarily the degree of hydroxylation and methylation of the compound (Meyer *et al.*, 1988). In addition, some phenolic compounds, as antioxidants, may react faster than others under the same conditions (Gazzani *et al.*, 1998).

CONCLUSION

Smoked cheese has higher phenolic content and antioxidant activity as compared to non-smoked cheese due to the wood combustion. Differences in the antioxidant activity is accounted for by the different wood materials and possibly the different phenolic compounds present rather than the total phenolic contents *per se*.

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