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Nutrition Research 26 (2006) 659–663
NUTRITION RESEARCH
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Comparative analysis of the in vitro antioxidant activity of white and black pepper

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Received 3 May 2006; revised 23 September 2006; accepted 26 September 2006

Abstract

As a result of normal metabolic processes, the human body produces reactive oxygen species capable of oxidizing biomolecules that can damage DNA, cells, and contribute to chronic disease. This process can be attenuated or perhaps reversed by diets containing spices that have the ability to scavenge reactive oxygen species. The present study measured the concentration of polyphenols in peppercorn (black and white) using the Folin-Ciocalteu method and investigated the radical scavenging activities of hydrolyzed and nonhydrolyzed pepper extracts using 1,1-diphenyl-2-picrylhydrazyl, the superoxide radical, and the hydroxyl radical as substrates. The hydrolyzed and nonhydrolyzed extracts of black pepper contained significantly ($P < .001$) more polyphenols compared with those of white pepper. For either of these peppercorns, the hydrolyzed extract contained significantly ($P < .001$) more polyphenols compared with the nonhydrolyzed extract. A dose-dependent effect was observed in the free radical and reactive oxygen species scavenging activities of all the extracts, with the black pepper extracts being the most effective. Peppercorns especially black pepper, which constitutes an important component in the diet of many sub-Saharan and oriental countries, can therefore be promoted for their nutritional importance as antioxidants and radical scavengers.

Keywords: Polyphenols; Antioxidants; In vitro; Peppercorn; Free radicals

1. Introduction

The implication of oxidative and free radical–mediated reactions in degenerative processes related to aging and chronic disease conditions is important. Free radicals in the form of reactive oxygen species (ROS) and reactive nitrogen species are implicated in numerous diseases such as inflammation, metabolic disorders, reperfusion damage, atherosclerosis, and carcinogenesis [1,2]. Aerobic respiration, stimulated polymorphonuclear leukocytes, macrophages, and peroxisomes are the main endogenous sources of most of the oxidants produced by cells [3,4]. Thus, compounds that can scavenge free radicals can play a role in improving health in oxidative stress-related disorders.

In recent years, phytochemicals are increasingly purported to exert potent beneficial actions to support health and may play a role in reducing synthetic drug use for the treatment of metabolic complications. To this effect, research has focused on the identification and isolation of compounds from natural products with high antioxidant capacities [5-7]. One of such natural products is peppercorn, which has culinary applications as well as health benefits. Peppercorns are the berries of *Piper nigrum* and *Piper*

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0271-5317/$ – see front matter © 2006 Published by Elsevier Inc.
doi:10.1016/j.nutres.2006.09.019

guineense and are also known as African black pepper or Ashanti pepper. They are used as spices and preservatives; they also have applications as insecticides and are used in herbal medicine and in the cosmetic industry [8-10]. Peppercorns are usually white or black depending on the time of harvest [11,12]. The white peppercorn is produced from fully ripe berries, whereas the black peppercorn is produced from unripe but fully developed berries. Chemically, peppercorn contains lignans, alkaloids, flavonoids, aromatic compounds, and amides [13,14].

The purpose of the present study was to measure the amount of compounds with antioxidant capacity in the 2 different types of peppercorn available in the Cameroonian market and to compare the antioxidant activity of the pepper extracts relative to other compounds in vitro.

2. Methods and materials

2.1. Sample preparation and determination of polyphenol concentrations

White and black peppers (peppercorn) were purchased from a local market in Yaoundé, Cameroon. Sample preparation and lyophylization were carried out as previously described [15]. Lipids were then extracted from the lyophyliate using hexane, and free and total polyphenols were extracted as described by Vinson et al [16,17]. The concentration of polyphenols present in the extracts was determined using a 5-fold diluted Folin-Ciocalteu reagent, with catechin as the reference standard.

2.2. Radical scavenging effect of peppercorn extracts

The acidic pH of hydrolyzed extracts was neutralized by diluting with 1 N sodium bicarbonate (2.2 times) before use.

2.2.1. Free radical scavenging activity

The percentage (%) free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of extracts was assessed as previously described by Hatano et al [18] and was calculated as follows:

$$\%\ \text{radical scavenging effect} = [(\text{Abs}_1 - \text{Abs}_2)/\text{Abs}_1] \times 100$$

where Abs_1 is the absorbance of the control, and Abs_2 is the absorbance of the plant extract.

2.2.2. Scavenging of superoxide radical

The method of Nishikimi et al [19] was used to measure superoxide radical scavenging activity of the extracts. The superoxide radical was generated in a phenazine methosulfate–NADPH system by oxidation of NADPH and assayed by the reduction of nitroblue tetrazolium.

2.2.3. Scavenging of hydroxyl radical

The hydroxyl radical scavenging activity of extracts was determined using a modification of the method of Halliwell et al [20]. The reaction mixture consisted of $FeCl_3$ (300 μmol), EDTA (780 μmol), 2-deoxiribose (2.8 mmol), ascorbic acid (300μmol), and H_2O_2 (4 mmol) in potassium phosphate buffer (20 mmol, pH 7.4). The final reaction volume (1 mL), which included the peppercorn extract, was incubated at 37°C for 1 hour. After incubation, 1 mL of trichloroacetic acid (2.8% wt/vol) and 1 mL of thiobarbituric acid (1% wt/vol) were added and further incubated at 100°C for 20 minutes. The reaction mixture was then allowed to cool at room temperature, and the absorbance read at 532 nm.

2.3. Statistical analysis

Analyses were carried out in triplicate, and the mean ± standard deviations are reported. Data were subjected to a 1-way analysis of variance (Kruskal-Wallis), and Student Newman-Keuls test was applied to determine significant difference between groups and concentrations ($P < .001$) using SigmaStat version 3.01.

3. Results and discussion

3.1. Phenolic concentration

Phenolic compounds are the principal antioxidant constituents of natural products and are composed of phenolic acids and flavonoids, which are potent radical terminators [21,22]. They donate hydrogen to radicals and break the reaction of lipid oxidation at the initiation step [23]. Caffeic acid, ferulic acid, and vanillic acid are examples of phenolic acids isolated as natural antioxidants in fruits, vegetables, and other plants [24,25]. The high potential of polyphenols to scavenge free radicals may be because of their many phenolic hydroxyl groups [26]. In the present study, the

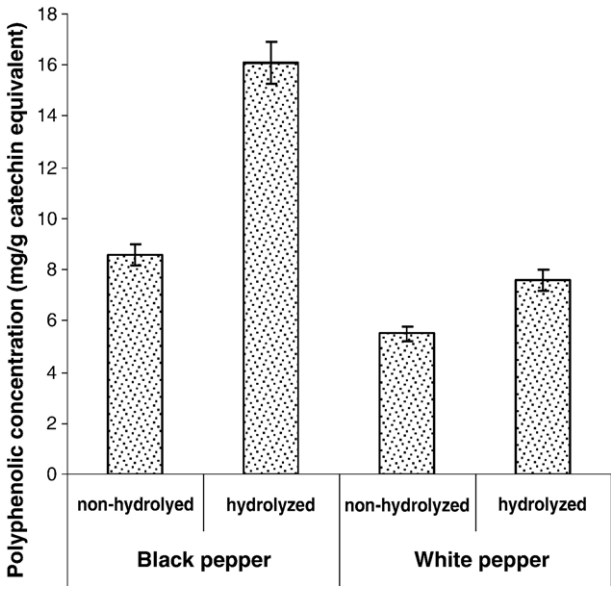

Fig. 1. Polyphenol concentration of peppercorn extracts (milligram per gram catechin equivalent). Values are means ± SD of analyses done in triplicate.