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Quantification of Quercetin in Different Parts of Onion and Its DPPH Radical Scavenging and Antibacterial Activity

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Abstract Levels of quercetin in different parts of onion were investigated using high performance liquid chromatography (HPLC) and liquid chromatography mass spectrometry (LC/MS) suitable for use with functional food material. Two main peaks were observed on HPLC chromatograms from the extracts of the skin, and the outer, middle, and core parts of onion. Using LC/MS, peak 1 was tentatively identified as quercetin monoglucoside at m/z 466.4, and peak 2 as quercetin with [M]+ at m/z 303.3. The levels of quercetin in the skin, and the outer, middle and core parts of the plant were 16.83, 2.67, 0.95, and 0.35 mg/g, respectively. In the study of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, skin, the nonedible part, contained the highest amount of quercetin, compared to the other edible parts, and showed the highest DPPH radical scavenging activity. Levels of quercetin and DPPH radical scavenging activity increased from core to skin. All parts of onion exhibited the strongest antibacterial activity only against *Staphylococcus aureus* and *Vibro parahaemolyticus*. Antibacterial activities of onion exhibited that *S. aureus* was more sensitive than *V. parahaemolyticus*. Among the four onion extracts, the middle part showed the strongest inhibitory activity against *S. aureus* but all onion extracts showed similar antibacterial activities against *V. parahaemolyticus*.

Keywords: onion, quercetin, DPPH radical scavenging activity, antibacterial activity.

Introduction

Onion (*Allium cepa*) is one of the most widely used vegetables as medicinal and edible plants in the world. Several studies have shown that onion contains very high level of flavonoids, especially quercetin and its glycosides, and is one of the major sources of flavonoids in western diets (1, 2). Flavonoids occur mainly in the leaves and outer parts of plants. Among flavonoids, quercetin and its glycosides are mainly presented in vegetables, except for those glycosides of kaempferol, luteolin, and apigenin (1, 3).

Quercetin is found in many foods including red wine, tea (1), onions, apples, and kales (4). The glycosides of quercetin in onion are absorbed more easily than those found in tea (5). Epidemiological studies in the US have estimated the average human daily intake of quercetin to be approximately 25 mg per person (6). Initially, dietary quercetin was known to be mutagenic, and caused an increase in the incidence of tumors (7-9). However, it has recently been reported to possess anti-thrombotic (10), antiviral (11, 12), anti-inflammatory (13), antiangiogenic (14), antimicrobial (15), and antiproliferative activities (16, 17). In addition, the potentiality of quercetin as a natural anticarcinogen (18) and its strong ability to retard hydroperoxide formation as an antioxidant (19) have been reported.

Quantitative determination of individual glycosides of flavonoid is difficult in plant materials, as they are so numerous. Therefore, the glycosides are normally hydrolyzed and the resulting aglycones are identified and quantified (20). Hydrolysis of flavonoid glycosides involves high concentrations (1-2 M) of mineral acids under refluxing conditions (2, 21). In order to determine the quantity of quercetin in onion, this study followed the methods for acid hydrolysis (20, 22, 23). Most research on onion has been done using the methodology of Justesen *et al.* (22). However, this study was conducted to analyze the amount of quercetin in different parts of onion with the developed methods using high performance liquid chromatography (HPLC) and liquid chromatography mass spectrometry (LC/MS).

The objective of this study is to determine the quantitation and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and antibacterial activity of quercetin at the core, middle, and outer sections for edible parts, and the skin for nonedible part, and to provide scientific information for a functional food material from onion.

Materials and Methods

Sample preparation Onion was obtained from a wholesale market in January 2003, and divided into edible parts (outer, middle, core) and nonedible part (skin). All parts were lyophilized for 48-72 hr after storage at -55°C. Freeze-dried samples were pulverized with a blender (Hanil, Korea).

Extraction and hydrolysis Each 0.5 g sample was mixed with 40 mL of 62.5% aqueous methanol containing butylated hydroxyanisole (BHA) (2 g/L), to which 10 mL of 6M HCl was added carefully to bring the total volume to 50 mL. The extract consisted of 1.2 M HCl in 50% aqueous methanol. The extract was heated to 90°C in a water bath, refluxed for 2 hr, and then allowed to cool in a refrigerator. Approximately 3 mL of the extract was filtered through a 0.45 μm filter prior to 20 μL injection into the HPLC system.

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High performance liquid chromatography separations Hydrolyzed samples were analyzed using Hewlett Packard model 1100 series. A Phenomenex (Torrance, USA) Luna C_{18} column (250×4.6 mm, 5 μm) protected by a guard column (LC_{18}) was used. The mobile phase consisted of methanol-water (30:70, v/v) with 1% formic acid (A) and 100% methanol (B). The gradient was 25-86% B over 50 min at a flow rate of 0.5 mL/min. UV spectra was monitored at 280 and 380 nm.

Identification by liquid chromatography/mass spectrometry LC/MS was conducted on a QUATTRO LC triple quadrupole tandem mass spectrometer that was connected with an HP-1100 HPLC. The eluents used were methanol-water (30:70, v/v) with 1% formic acid (A) and 100% methanol (B). At a flow-rate of 0.5 mL/min, the gradient was 25-86% B over 50 min. The source block temperature was 200°C and desolvation temperature was 70°C. LC-scan limit was from 70°C to 450 a.m.u. at a scan rate of 1.0 scan/sec.

Quantitative analysis Quantitation was conducted on external standardization under HPLC condition. The quercetin standard was supplied by Sigma (St. Louis, MO, USA). Calibration curves of standards ranging from 0.1 to 10 mg/mL (5 levels) revealed good linearity with R^2 values exceeding 0.99.

Measurement of DPPH radical scavenging activity Following a modified version of the method of Yoshida *et al.* (24), different parts of the onion extracts on DPPH radicals were measured. Four milliliters of aliquot methanolic extract from onion was added to 1 mL of 0.5 mM DPPH in methanol. Discoloration was detected with a Spectronic Unicam spectrophotometer (Model Genesis 10vis, USA) at 520 nm. The scavenging activity of DPPH radicals was expressed as:

$$\text{DPPH scavenging activity (\%)} = \left(1 - \frac{\text{Absorbance at 520 nm in presence of sample}}{\text{Absorbance at 520 nm in absence of sample}}\right) \times 100$$

Investigation of antibacterial activity Antibacterial activity was investigated by disc diffusion test (25). Six reference bacterial strains (*V. parahaemolyticus* KCCM 11965, *S. choleraesuis* KCCM 41038, *Clostridium perfringens* KCCM 12098, *Clostridium butyricum* KCCM 35433, *S. aureus* KCCM 11593, *Bacillus cereus* KCCM 11204) which had been poisoned by food were purchased from the Korean culture center of microorganisms. Twin-layer petri dishes were formed with 1% lower agar (Nutrient agar, Difco Laboratories, Detroit, MI, USA) previously poured into the petri dishes, followed by the poured addition of 0.8% upper agar (Nutrient agar and Nutrient broth, Difco) which had been inoculated with individual microorganisms. The powder samples (0.5 g) were added to 200 mL of 50% ethanol and the solutions were extracted by refluxing at 80°C for 1 hr. The hydro-alcoholic extracts were evaporated by a rotary evaporator until 5 mL of resides remained. Sterile filter papers were impregnated with 5 μL extracts and placed on the culture medium. The control disc was impregnated with sterilized water and put it in the middle of the culture medium. After 24 hr of incubation at 37°C, the diameter of the clear zone around the disc was measured in millimeters. All tests were performed in triplicate.

Results and Discussion

Analysis and identification by HPLC and LC/MS The main peaks in the skin, and the outer, middle and core parts of onion were detected at retention times of 18-19 min (peak 1) and 26-27 min (peak 2) (Fig. 1). Quercetin, which is one of the flavonoid standards, was matched with peak 2 at a retention time of 26 min. Therefore, peak 2 was potentially considered as the quercetin. However, peak 1, which was measured at a retention time of 17-18 min, was obscure. An examination of the relationship between peaks 1 and 2 using acid hydrolysis during extraction demonstrated that the area of peak 1 was inversely proportional to the area of peak 2. Thus, peak 1 was considered as unhydrolyzed quercetin.

The mass spectrum at peak 1 was evaluated by LC/MS as m/e 466.4 and was found to be quercetin monoglucoside (Fig. 2). Peak 2 was evaluated as quercetin with [M]+ at m/e 303.3 (Fig. 3). Fossen *et al.* (26) and Price and Rhodes (27) reported that the major glycosides are quercetin-4'-glucoside and quercetin-3',4'-diglucoside

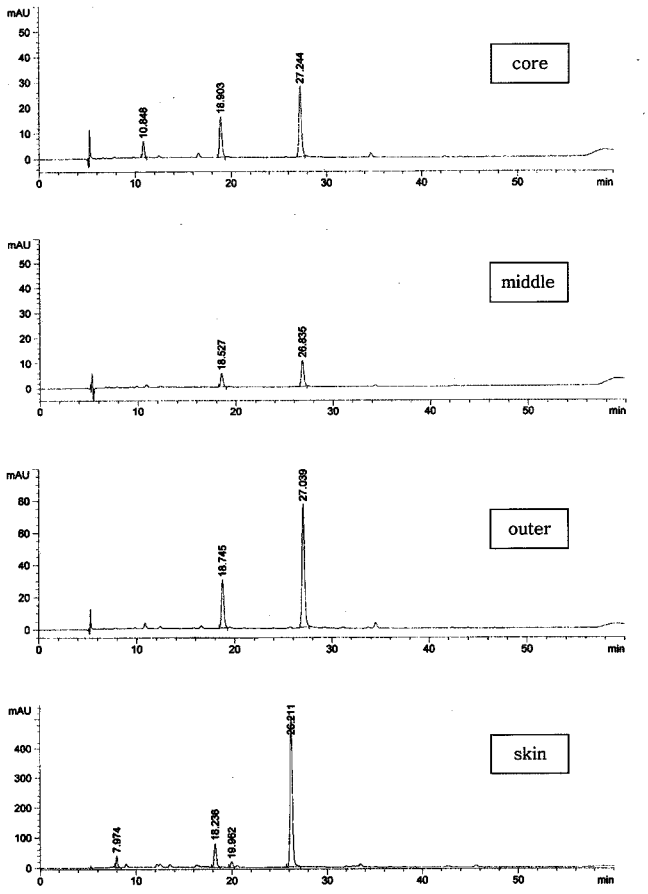

Fig. 1. HPLC chromatograms in different parts (core, middle, outer, skin) of onion.

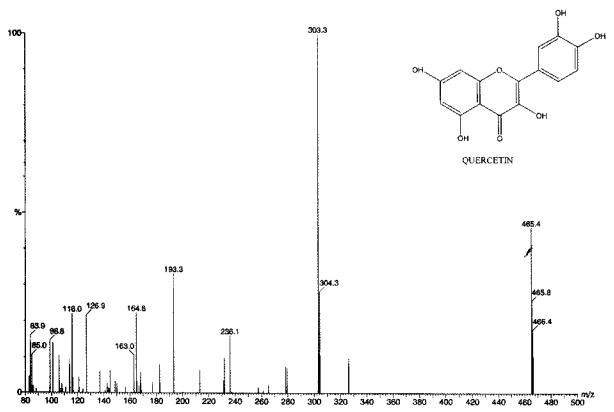

Fig. 2. Mass spectrum of peak 1 in onion.

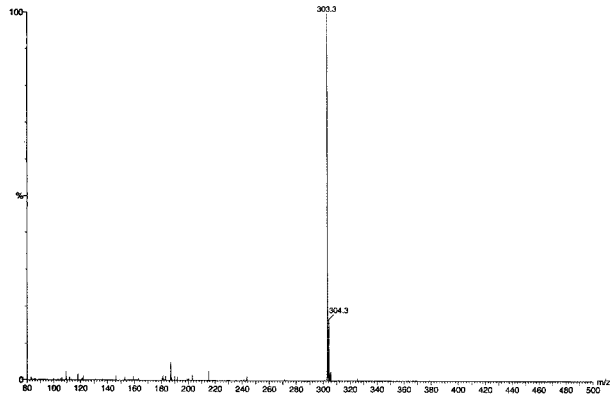

Fig. 3. Mass spectrum of peak 2 in onion.

in onion. In addition, Park and Lee (28) separated, isolated, and identified seven flavonoids (quercetin, quercetin monoglucoside, quercetin diglucoside, isorhamnetin glycoside, rutin, kaempferol) with Sephadex LH-20 chromatography, HPLC, thin layer chromatography, mass spectroscopy, and nuclear magnetic resonance spectroscopy. These differences might have arisen from the use of different hydrolysis methods and different wavelength during analysis by HPLC.

Quantitative analysis of quercetin in different parts of onion The amounts of quercetin in the skin, and the outer, middle, and core parts were 16.83, 2.67, 0.95, and 0.35 mg/g dried weight, respectively, in our study (Table 1). This showed that the level of quercetin was increased with greater distance from the core. Skin, the nonedible part, showed outstanding levels of quercetin comparing to the other edible parts.

Previous studies reported that the levels of quercetin were commonly below 10 mg/kg, except for onions (280-490 mg/kg), kale (110 mg/kg), broccoli (30 mg/kg), and green beans (45-60 mg/kg) among vegetables (Hollman and Arts, 29). Hollman *et al.* (30) reported that onion contained over 50 mg/kg of flavonoids and belonged to the high flavonoid group. Justesen *et al.* (22) found significant amounts of quercetin, with an average of 34.8± 1.0 mg/100 g fresh weight in onion. This result was accordance that of Chu *et al.* (31) and Mizuno *et al.* (32), and quercetin was located in the first and second layers of onion. The quercetin levels were higher than those reported in the literature, in which the quercetin content was only 26.09 mg/kg in the interior of onion and was 258.85 mg/kg in the outer leaves of onion, according to Chu *et al.* (31).

Measurement of DPPH radical scavenging activity DPPH, which creates stable free radicals, loses its color when DPPH radicals capture radicals (RO·, ·OH). Blois (33) conducted research on DPPH radical scavenging activity by using the level of DPPH discoloration. In this study, DPPH radical scavenging activity was investigated on aqueous methanolic extracts of the different parts of onion by measuring the absorbance at 520 nm.

In Table 1, all parts of the onion extracts exhibited more than 85% DPPH scavenging activities. Especially, the nonedible skin (91.96%) showed the strongest DPPH radical scavenging activities against hydroxyl radicals, followed by the core (86.25%), middle (86.48%), and outer (88.03%) parts (Fig. 4). The DPPH radical scavenging activity showed the same trend as the quercetin level in increasing from the core to the skin according to the different parts of onion. This result is in agreement with the findings of Chu *et al.* (31). Therefore it was assumed that DPPH radical scavenging activity was affected by the quercetin level of onion.

Investigation of antibacterial activity Different parts of the onion extracts exhibited different inhibitory activities against only *V. parahaemolyticus* and *S. aureus*, as shown in Table 2. No antibacterial activities were shown against *S. choleraesuis*, *C. perfringens*, *C. butyricum* and *B. cereus* in this study. However, Benkeblia (34) reported that essential oil extracts of onions had the strongest antibacterial activities against *S. Enteritidis* and *S. aureus*, whereas the inhibitory activity of the extracts from onion and *Allium* plants has not yet been widely investigated.

The antibacterial activities of onion showed that *S. aureus* was more sensitive than *V. parahaemolyticus*. Especially, the middle part showed the strongest inhibition against *S. aureus*. All parts of onion exhibited similar

Table 1. Quercetin levels and free radical scavenging activity (%) in different parts of freeze dried onion

Samples		Peak area	Amount (μg/mL)	Quercetin level (mg/g)	DPPH radical scavenging activity (%)
Edible parts	Outer	1421.51	13.37	1.34	88.025±0.022
	Middle	505.98	4.72	0.47	86.481±0.041
	Core	192.81	1.74	0.17	86.253±0.017
Nonedible parts	Skin	9016.13	85.91	8.59	91.957±0.011

Table 2. Antibacterial activity for extracts of different parts of onion against six main pathogens causing food poisoning

	Zone of inhibition (mm)[1]			
	Skin	Outer	Middle	Core
Vibrio parahaemolyticus	13	16	15	15
Salmonella choleraesuis	-[1)]	-	-	-
Clostridium perfringens	-	-	-	-
Clostridium butyricum	-	-	-	-
Staphylococcus aureus	15	26	31	27
Bacillus cereus	-	-	-	-

[1)]Not detected.

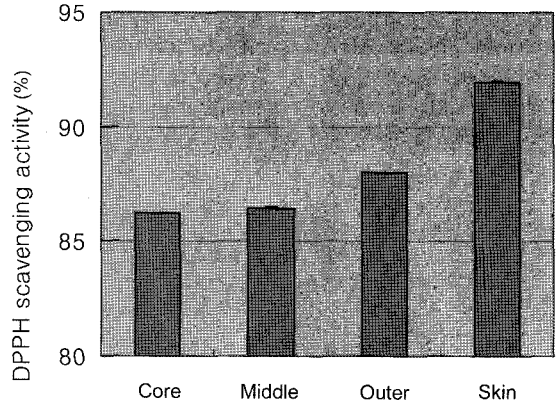

Fig. 4. DPPH radical scavenging activity of different parts of onion.

antibacterial activities against *V. parahaemolyticus*. Contrary to its highest quercetin levels, the skin part demonstrated the weakest antibacterial activities. This result confirmed the variation of antibacterial activities among the different parts of onion.

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