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*Short Communication*

# Increase in flavonoids content in red onion peel by mechanical shredding

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**Quercetin, a bioflavonoid extracted from red onion peel, has a marked inhibitory activity against phosphodiesterase 5A. Mechanical shredding of red onion peel increased its total flavonoid content two fold compared to control. Change in individual flavonoid content was studied using HPLC. Quercetin 4'-O-β-glucopyranoside showed the highest increase in content after shredding with slight change in quercetin content. Radical scavenging activity was assayed using DPPH. Extract of the shredded peel showed lesser activity (19.7%) compared to the control (31.2%).**

**Key words:** *Allium cepa*, Alliacea, red onion peel, flavonol, quercetin 4'-O-ß-glucopyranoside.

## INTRODUCTION

Recent study (Lines and Ono, 2006) has shown that quercetin, a bioflavonoid extracted from red onion peel, showed marked inhibitory activity against phosphodiesterase 5A, with an IC50 value of 1.9 µM. It is known that a potent selective phosphodiesterase 5A inhibitor, sildenafil, is used for treatment of sexual dysfunction (Junemann, 2003). Quercetin and quercetin 4'-O-β-glucopyranoside are the major flavonoids in red onion peel (*Allium cepa* L., liliaceae) (Fossen et al., 1998). Beside its aforementioned biological value, quercetin is known to reduce the carcinogenic activity of several cooked food mutagens, inhibit enzymatic activities associated with several types of tumor cells (Leighton et al., 1992) as well as to have anti-oxidative properties (Arai et al., 1995).

In this study, we demonstrate the relation between mechanical shredding of red onion peel and its flavonoid content. Increase in total flavonoid content is demonstrated. Quercetin and quercetin 4'-O-β-glucopyranoside contents were determined after mechanical shredding. Antioxidant activity, as specifically linked to flavonoid content, was investigated.

## MATERIALS AND METHODS

### Plant material

Red onion (*A. cepa* L.) was purchased from local market. A voucher specimen was deposited in the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, University of Beni-Sueif, Egypt. The outer red scales were air-dried, mechanically shredded then incubated in the dark at room temperature until analysis for 3 days. Control experiment was prepared by freeze-drying red onion peel after removal from bulbs.

### Assay of total flavonoid content

Total flavonoid content was measured by the aluminum chloride colorimetric assay (Zhishen et al., 1999). An aliquot (1 ml) of extracts (0.5 g dried shredded peel in 50 ml 80% aqueous MeOH) or standards solution of quercetin (3, 6, 14 mg/ml) was added to 10 ml volumetric flask containing 4 ml dd $H_2O$. To the flask 0.3 ml 5% $NaNO_2$ was added. After 5 min, 0.3 ml 10% $AlCl_3$ was added. At the 6th min, 2 ml 1M NaOH solution was added and the total volume was made up to 10 ml with dd $H_2O$. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content was expressed as mg quercetin equivalents (QE) / 1 g dry plant material. Samples were analyzed in triplicates.

### HPLC analysis

Two grams, of dried shredded red onion peel and from control were extracted (3 x 100 ml, 12 h each) with MeOH. The filtered extracts were combined, concentrated under reduced pressure and reconstituted in 100 ml MeOH. HPLC was carried out with a Shimadzu liquid chromatograph equipped with LC-10 AD pump, SPD-10A UV detector, Inertsil ODS-3 (5 µm, 4.6 x 250 mm) column for analytical purposes. An aliquot of 20 µL were injected. Flavonoids were quantified at 360 nm using peak area by comparison to a calibration curve derived from the quercetin.

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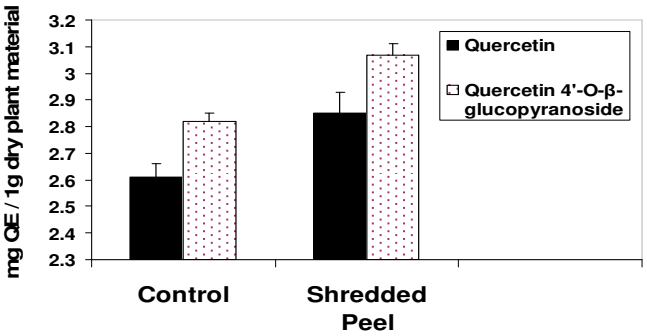


**Figure 1**. Results from HPLC analysis of flavonoid content in shredded red onion peel compared to control.

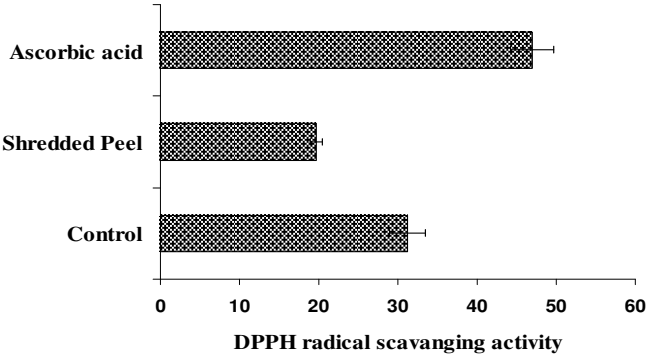


**Figure 2.** Results of DPPH radical scavenging assay of shredded red onion peel, control and ascorbic acid.

Ambient temperature was used. The flow rate was 1 ml/min. Isocratic elution with 0.5% ortho-phosphoric acid aqueous solution: acetonitrile (100:50) mixture afforded the following retention times: quercetin 21.38 min, quercetin 4'-O-β-glucopyranoside 8.49 min. The assay was done in triplicate for each case.

#### Identification of quercetin 4'-O-β-glucopyranoside

Dried onion scales (1 g) from dried shredded red onion peel and from control were extracted (2 x 25 ml, 3 h each) with MeOH. The solvent was evaporated to dryness and the extracts were dissolved in 1 ml MeOH. An aliquot (ca. 100 µl), from each case, was chromatographed on silica gel 60 F254 TLC plates using EtOAc/ HCOOH/G. AcOH/ $H_2O$ (10:1.1:1.1:3) as developing solvent. Band at Rf value = 0.67, in case of shredded peel, was localized by charring with 10% $H_2SO_4$. The TLC was repeated for shredded red onion peel and the elicited band was scraped off the plates re-extracted using MeOH. The MeOH extract was evaporated and reconstituted in 1 ml MeOH then analyzed by MS. EI MS was acquired at 30 e.v. using Jeol JMS- AX500 mass spectrometer. EI MS *m/z* 447 $[M]^+$; 301 $[M\text{-glu}]^+$; 323 $[M\text{-glu-C8H5O3}]^+$; 339 $[M\text{-glu-C8H5O4}]^+$; 351 $[M\text{-glu-C9H5O4}]^+$.

#### DPPH radical scavenging assay

Measurement of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was performed according to a method previously reported with some modification (Ichikawa et al., 2003). Thirty micro liters of sample solution was added into 970 µl of DPPH solution (100 µM in methanol). After incubation at 25ºC for 20 min. The absorbance at 515 nm was measured. The percentages of inhibition of DPPH radical activity was calculated according to the following equation:

% of inhibition of radical activity = $(A_0 - A_{20}) \times 100 / A_0$
$A_{20}$ = Absorbance after incubation for 20 min
$A_0$ = Initial Absorbance
Ascorbic acid was tested as a positive control.
The data are the mean of triplicate measurements.

## RESULTS AND DISCUSSION

Colorimetric determination of flavonoid content in plant material based on color reaction with $AlCl_3$ reagent is well-known method to determine total flavonoid content (Zhishen et al., 1999). The color produced is proportional to the concentration and obeying Beer's law. Assay of total flavonoid content showed 87% increase in shredded red onion peel (0.73 ± 0.04 mg quercetin equivalent/g dried peel) compared to control (0.39 ± 0.02 mg quercetin equivalent/g dried peel). This result emphasizes the importance of mechanical shredding that significantly changed the level of bioactive natural products in plant material. It is well documented that most of flavonoids are categorized into phytoalexin, which is produced under stress conditions to protect the host plant (Ebel, 1986; Shirley, 2002). There are many stress factors known to increase flavonoids, such as UV, cut, chemicals, heat etc. Quercetin is also a stress compound produced by oxidetive stress. It sounds predictable that mechanical shreding increases quercetin. Therefore, the present case is an example of this phenomenon.

An isocratic HPLC analysis method was used to determine quercetin and quercetin glycosides; representing the major flavonoids in red onion peel. Comparing the HPLC chromatograms from shredded red onion peel and control, the main difference was in peak eluted at 8.49 min. This peak showed marked increase in peak area in case of shredded peel. Online HPLC UV bands (366 nm band I and 253 nm band II) (Fossen et al., 1998) gave a hint to the chemical structure of this compound to be quercetin 4'-O-β-glucopyranoside. In order to confirm the chemical structure of the elicited compound, preparative TLC was performed on shredded peel extract. Band of the elicited peak on the TLC plates, corresponding to 8.49 min on the HPLC chromatogram, was scratched from the plates and extracted with MeOH. The extract after evaporation and reconstitution was analyzed using EI MS. A fragmentation pattern that corresponds to quercetin 4'-O-β-glucopyranoside confirmed the chemical structure of this compound. The results of HPLC analysis for quercetin and quercetin 4'-O-β-glucopyranoside is shown in Figure 1.

Flavonoids act as antioxidant agents by direct free radical scavenging, transition metal chelation and maintenance of endogenous antioxidants such as the glutathione and superoxide dismutase systems (Tourino et al., 2005). Extracts from shredded red onion peel and control were compared with their ability to scavenge DPPH free

radicals (Figure 2). Shredded peel showed a decrease (19.7%) in radical scavenging activity compared to control scales (31.2%). The reduction of DPPH free radical scavenging activity in shredded scales is contradictory with the increase in quercetin's content as well as its glycosides by mechanical shedding. This might be attributed to loss of other metabolites by shredding process that contributes to the radical scavenging activity.

Consequently, mechanical shredding can be used to increase level of flavonoids extracted from onion peel used in manufacturing of health promoting beverages (Lines and Ono, 2006).

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### REFERENCES

Arai H, Nagao A, Terao J, Suzuki T, Takama K (1995). Effect of d-alpha-tocopherol analogues on lipoxygenase-dependent peroxidation of phospholipid-bile salt micelles. Lipids 30:135-140.

Ebel J (1986). Phytoalexin Synthesis: The Biochemical Analysis of the Induction Process. Ann. Rev. Phytopathol. 24:235-264.

Fossen T, Pedersen AT, Andersen ØM (1998). Flavonoids from red onion (Allium cepa). Phytochem. 47:281-285.

Ichikawa M, Ryu K, Yoshida J, Ide N, kodera Y, Sasaoka T, Rosen RT (2003). Identification of Six Phenylpropanoids from Garlic Skin as Major Antioxidants. J. Agric. Food Chem. 51:7313-7317.

Junemann KP (2003). How effective are PDE-5 inhibitors? Urol. A 42:553–558.

Leighton T, Ginther C, Fluss L, Harter WK, Cansado J, Notario V (1992). In Phenolic Compounds in Foods and Their Effects on Health II, ed. Huang MT, Lee CY. American Chemical Society Symposium Series 507, Washington DC.

Lines TC, Ono M (2006). FRS 1000, an extract of red onion peel, strongly inhibits phosphodiesterase 5A (PDE 5A). Phytomed. 13:236–239.

Shirley BW (2002). Biosynthesis of flavonoids and effects of stress. Current Opinion in Plant Biology 5(3):218-223.

Tourino S, Selga A, Jimenez A, Julia L, Lozano C, Lizarraga D, Cascante M, Torres JL (2005). Procyanidin Fraction from Pine (Pinus pinaster) Bark: Radical Scavenging Power in Solution, Antioxidant Activity in Emulsion, and Antiproliferative Effect in Melanoma cells. J. Agric. Food Chem. 53(12):4728-4735.

Zhishen J, Mengcheng T, Jianming W (1999). Research on antioxidant activity of flavonoids from natural materials. Food Chem. 64:555-559.