Original article

Amylase inhibitory and metal chelating effects of different layers of onion (Allium cepa L.) at two different stages of maturation in vitro

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Abstract

Researches on traditional medicinal plants are consolidating the belief that this system of medicine provides a viable alternative to conventional drugs for various free radical mediated diseases, e.g., diabetes. Onion (Allium cepa L.) is one of the oldest cultivated plants with multiple functional and biological properties. These are attributed to the presence of active phytomolecules, e.g., phenolic acids, flavonoids (especially quercetin), cepaenes, thiosulfinates and anthocyanins. In the present study, outer layers (transitional layer with the first living cells below the dry onion peel) and innermost layers from two different sizes of onion were studied for their \( \alpha \)-amylase inhibiting activity, flavonoid content, hydroxyl radical scavenging activity (HRSA) and metal chelating activity. Our results demonstrate that the outermost living layers had higher flavonoid content (ranging from 40.27 to 45.0 mg), followed by a continuous decrease towards the inner part of the bulb (ranging from 29.10 to 31.81 mg). Outermost living layers of the onion extracts were found most powerful hydroxyl radical scavenger and having higher ferrous ion chelating effects compared to the inner layers with higher HRSA of outer layers of smaller onion. Differences in % \( \alpha \)-amylase inhibitory activity were also observed among different layers and sizes of onions, ranging 52.0 to 44.8% in case of outer layers and 36.00 to 39.12% in case of inner layers, showing high inhibitory activity in outer layers of onion (\( p < 0.05 \)). Quercetin was a potent inhibitor with inhibitory activity ranging from 69.6 to 84.3% at different concentrations, respectively. The findings may explain the antioxidative and antidiabetic effect of onion and also foods containing quercetin.

Keywords: Allium cepa L., flavonoid content, hydroxyl radical scavenging activity, metal chelating activity, antidiabetic effect

1. Introduction

Reactive oxygen species (ROS) are produced in the cells by cellular metabolism and other exogenous environmental agents. They are generated by a process known as redox cycling and are catalysed by transition metals such as Fe\(^{2+}\) and Cu\(^{2+}\). Increased physiological burden of free radicals and over production of ROS causes an imbalance between oxidants and antioxidants in the body leading to a state of oxidative stress, thereby damaging cellular biomolecules and resulting in various degenerative diseases including diabetes (Halliwell and Gutteridge, 1999; Martim et al., 2003).

In recent years, researches on traditional medicinal plants are gradually emerging as viable alternatives to conventional drugs for various free radical mediated diseases. Due to their pleiotropic biological effects, flavonoids, a class of phenolic compounds widely distributed in plants have received special attention as dietary constituents during the last few years (Pandey and Rizvi, 2009; Del Rio et al., 2010). Recent nutritional investigations have reported that polyphenols may be able to modulate nutrient availability through the inhibition of digestive enzymes involved in lipid and starch breakdown, which could lead to beneficial effects on calorie intake, obesity (McDougall et al., 2009) and blood glucose control (Grussu et al., 2011). \( \alpha \)-amylase is one of the main enzymes in human that is responsible for the breakdown of starch to more simple sugars, thus the inhibitors of this enzyme can delay the carbohydrate digestion and reduce the rate of glucose absorption and obesity management by reducing sugar level in blood. The inhibition of \( \alpha \)-amylase has been suggested as a strategy for diabetes control as they can decrease the attenuated postprandial plasma glucose levels by ultimately decreasing glucose release from starch and improve the glucose tolerance in diabetic patients (Ali et al., 2006; Kwon et al., 2006). In recent years, natural sources of \( \alpha \)-amylase inhibitor have received a lot of interest due to search for alternative to synthetic enzyme inhibitors such as acarbose, metformin and orlistat, which have been found to exhibit adverse effects, mild efficacy and can cause gastrointestinal distress as a side effect (Randhir et al., 2008).

Onion (Allium cepa L.), one of the oldest cultivated plants used for culinary, medicinal and spiritual purposes, has recently been gaining attention for its multiple functional properties which include free radical scavenging activity, immune stimulation, cardioprotective effects, anticancer and anti-infectious property (Jaiswal and Rizvi, 2011).
2014; Jaiswal and Rizvi, 2012; Desjardins, 2008). The various biological and pharmacological properties associated with onions have been attributed to the presence of active phytochemicals, e.g., phenolic acids, flavonoids (especially quercetin), cepaenes, thiocyanates and anthocyanins (Corzo-Martínez et al., 2007). From 25 different flavonols characterised in onions, the most prominent are the quercetin and quercetin conjugates, mainly quercetin-3,4′-O-diglucoside (QDG) and quercetin-4′-O-monoglucoside (QMG) (Price and Rhodes, 1997; Bonaccorsi et al., 2005; Beesk et al., 2010). It has been reported by various investigators that the dry outer scale extract of onion containing large amounts of quercetin, quercetin glucoside, which is generally considered as waste, possesses radical scavenging property and is an effective antioxidant against nonenzymatic lipid peroxidation (Nuutila et al., 2003; Bonaccorsi et al., 2008; Lee et al., 2012).

Although there are citations of antihyperglycemic and antiadipic activity of some Allium species (Campos et al., 2003; Azuma et al., 2007; Nickavar and Yousefian, 2009), least attention was given on the activity of the different layers of onion extracts on in vitro α-amylase activity. The present study focuses on the study of amylase inhibition, antioxidant properties, antiadipic and metal chelating effects of different layers of the edible part of the onion and during different stages of development.

2. Materials and Methods

2.1 Plant material and sampling

The red variety onion (Pusa red cultivar) was purchased from local market of Allahabad, Uttar Pradesh, India. The selected plants were collected and the herbarium sheets were sent to the herbarium in Botany Department of Allahabad University, Allahabad, Uttar Pradesh, India and the voucher specimen number (Specimen No. 25840) were obtained. Onion with two different sizes (15-20 g and 25-60 g), were then sub-divided into two different parts: the innermost layers and the outermost layers (the transitional layer with the first living cells). Onion extracts were prepared by the method as described by Tundis et al. (2008), with some modifications. 5 g of onion (inner layers and outer layers) was ground with quartz sand in a cold mortar. The homogenized material was suspended in 10 ml solvent and centrifuged at 15,000 x g for 10 min at 4°C and the aliquots of the supernatants were collected. The solvent used was 100% aqueous methanol (pure methanol). Samples were re-extracted twice, the final volume made up to 100 ml with cold distilled water and stored at 4°C, before conducting experiments.

2.2 Chemicals and reagents

Alpha amylase from porcine pancreas and quercetin were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade procured from Merck, India or Himedia, India.

2.3 Determination of total flavonoid content

Total flavonoid content was determined by using a colorimetric method described previously (Dewanto et al., 2002; Jia et al., 1999). Briefly, 0.25 ml of diluted onion extracts was mixed with 1.25 ml of distilled water and, subsequently, with 0.075 ml of 5% sodium nitrite solution and allowed to react for 5 min. Then 0.15 ml of 10% aluminum chloride was added and allowed to further react for 6 min before 0.5 ml of 1 M sodium hydroxide was added. Distilled water was added to bring the final volume of the mixture to 3 ml. The absorbance of the mixture was immediately measured at 510 nm wavelength against a blank using a spectrophotometer. The flavonoid content was determined by a quercetin standard curve and expressed as mean (milligrams of quercetin equivalents per 100 g of fresh sample) ± SD for the triplicate extracts.

2.4 Metal (Fe²⁺) chelating activity assay

The chelating activity of onion extracts for ferrous ions Fe²⁺ was measured according to the method previously described (Dinis et al., 1994). Briefly, to 0.5 ml of extract in deionized water, 1.6 ml of deionized water and 0.05 ml of FeCl₂ (2 mM) were added. After 30s, 0.1 ml ferrozine (5 mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min at room temperature, the absorbance of the Fe²⁺-ferrozine complex was measured at wave length 562 nm. The chelating activity of the extract for Fe²⁺ was calculated using the following equation:

\[
\text{Chelating rate} = \frac{A_0 - A_1}{A_0} \times 100
\]

where \(A_0\) was the absorbance of the control (blank, without extract) and \(A_1\) was the absorbance in the presence of the extract.

2.5 α-amylase inhibitory activity

The α-amylase inhibition assay method was performed using the method previously described (Tundis et al., 2007). Briefly, a starch solution (0.5% w/v) was obtained by stirring 0.125 g of potato starch in 25 ml of 20 mM sodium phosphate buffer with 6.7 mM sodium chloride, pH 6.9 at 65°C for 15 min. The α-amylase (EC 3.2.1.1) solution was prepared by mixing 0.0253 g of α-amylase in 100 ml of cold distilled water. The colorimetric reagent was prepared mixing a sodium potassium tartrate solution (12.0 g of sodium potassium tartrate, tetrahydrate in 8.0 ml of 2 M NaOH) and 96 mM 3, 5-dinitrosalicylic acid solution. Control and onion extracts were added to starch solution and left to react with α-amylase solution at 25°C for 5 min. The reaction was measured over 3 min. The generation of maltose was quantified by the reduction of 3, 5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid, the product being detectable at 540 nm. In the presence of an α-amylase inhibitors, less maltose will be produced and the absorbance value would decrease. The inhibition percentage of α-amylase was assessed by the following formula:

\[
\text{Inhibition α-amylase %} = 100 \times \frac{(A_{\text{Control}} - A_{\text{Sample}})}{A_{\text{Control}}}
\]

2.6 Statistical analysis

Values were mean ± SD of 5-6 separate experiments done in triplicates. Statistical analyses were conducted using the software PRISM version 5.01 for Windows (Graph Pad Software, San Diego, California, USA). Statistical differences were analyzed with Student’s t test and the differences were considered to be significant when \(p < 0.05\).

3. Results and Discussion

The present study was undertaken to evaluate the antioxidant activity, antiadipic activity and also its correlation with flavonoid content, as a function of 1. Size of onion (big and small), 2. In inner layers and outer layers.
3.1 Total flavonoid content

Figure 1 shows the quantitative data of total flavonoid content of onion extracts expressed as mg quercetin/100 g fresh sample. Results showed a higher flavonoid content of outer living layers (ranging from 40.27 to 45.0 mg), followed by a continuous decrease towards the inner part of the bulb (ranging from 29.10 to 31.81 mg). Difference was also observed between flavonoid content of outer layers with respect to different stages of maturation, being higher in smaller onion \( (p < 0.05) \).

Our results are in good agreement with earlier reports, where difference in phenolic content in different layers and flavonoid content was observed in the different parts of the onion bulb (Jaiswal and Rizvi, 2012; Beesk \textit{et al}., 2010; Bilyk \textit{et al}., 1984; Prakash \textit{et al}., 2007). In general, a higher flavonoid content of onion extracts is mainly due to the presence of various phenolic compounds (quercetin, kaempferol and luteolin), among them, QDG and QMG are the main flavonoids, which make up to 80-85% of the total flavonoid content of onion extracts (Desjardins, 2008; Price and Rhodes, 1997; Rhodes and Price, 1996; Sellappan and Akoh, 2002). Quercetin and kaempferol and their glycosides have been reported to be the most abundant flavonoids in the acid hydrolysed samples of onion (Chu \textit{et al}., 2000; Price and Rhodes, 1997).

The difference in the distribution of flavonoids being higher in the outer layers, could be explained by the increased activity of light-induced enzyme phenylalanine ammonia lyase which catalyses the biosynthesis of flavonoids in the outermost localizing living cells of the whole onion bulb due to more exposure of sunlight than the outermost dry peel and innermost layers of the onion bulb portion (Friedman, 1997; Hirota \textit{et al}., 1999). The cells of outermost dead dried peel of onion are not exposed to sunlight, resulting in lesser biosynthesis of flavonoids, thus having flavonoid content lesser than the outermost living cells layer (Beesk \textit{et al}., 2010).

3.2 Hydroxyl radical scavenging activity (HRSA)

Hydroxyl radicals can be produced in cells by a variety of processes: such as phagocytosis, prostaglandin biosynthesis, ionizing, irradiation and decomposition of lipid hydroperoxides. Hydroxyl radicals, an extremely reactive in biological systems, generated by the Fenton reaction are known to cause oxidatively induced breaks in DNA strands to yield its open circular or relaxed forms and contribute to significant biological effects such as carcinogenesis, mutagenesis and cytotoxicity. They are reported to mediate the lethal cell injury in cultured hepatocytes (Halliwell \textit{et al}., 1999). All the onion extracts were studied for their HRSA and compared with quercetin of different concentrations \((10^{-4}\text{mol/l} \text{ and } 10^{-2}\text{mol/l})\).

Figure 2. Outermost living layers of the onion extracts were found to be most powerful hydroxyl radical scavenger compared to the inner layers with higher HRSA of outer layers of smaller onion. Quercetin also shows a high HRSA activity at different concentrations. The scavenging of hydrogen peroxide and reducing capacity of whole onion extracts has been recently documented by various investigators (Benkeblia, 2005; Shon \textit{et al}., 2004). Variation in free radical scavenging activity and \( \text{H}_2\text{O}_2 \) scavenging activity in different layers of onion has already been documented by Jaiswal and Rizvi (2012).

3.3 Metal chelating activity

Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However, iron is an extremely reactive metal and is known as effective lipid oxidation pro-oxidant, which catalyses various oxidation reactions in biological systems (Shon \textit{et al}., 2004). It causes lipid peroxidation through the Fenton and Haber-weiss reaction (Halliwell and Gutteridge, 1990) and decomposes the lipid hydroxide into peroxyl and alkoxy radicals that can perpetuate the chain reactions.
studies have proven that antioxidant activity (%) of outermost living layers and 4-α-metal chelating activity (%) of outermost living layers, 1999; Corzo-Martinez et al., 51x103} compounds have been reported in onion to show alkylperoxyl radical sulfoxide and S-methylcysteine sulfoxide and non-flavonoid propenylcysteine sulfoxide (major component), S-propylcysteine. Besides phenolic compounds, organosulfur compounds such as S-propenylcysteine sulfoxide (major component), S-propylcysteine sulfoxide and S-methylcysteine sulfoxide and non-flavonoid compounds have been reported in onion to show alkylperoxyl radical scavenging activity and also being responsible for most of its biological properties (Sawa et al., 1999; Corzo-Martinez et al., 2007). The triple synergistic action of phenols from onion in scavenging ROS, repairing DNA radicals and metal chelation has been reported (Prakash et al., 2007).

3.4 α-amylase inhibitory activity
In the present study, inhibitory activity of different sizes and layers of onion against porcine pancreatic α-amylase were studied and compared with quercetin (Figure 4). Results showed that quercetin was potent inhibitor against porcine pancreatic α-amylase, with inhibitory activity ranging from 69.6 to 84.3 % at different concentrations, respectively. Differences in % inhibitory activity were also observed among different layers and sizes of onions, ranging from 52 to 44.8 % in case of outer layers and 36 to 39.12 % in case of inner layers, showing high inhibitory activity in outer layers of onion (p < 0.05). Since, phenolics play a role in mediating amylase inhibition and have the potential for type II diabetes management (McCue and Shetty, 2004, Chethan et al., 2008).

Figure 3: Metal chelating activity (%) of outermost living layers and innermost layers of onion at two different stages of maturation, compared with quercetin (100 µg/ml) and EDTA (100 µg/ml). Values represent mean ±SD. Significant difference * (p < 0.05) and ** (p < 0.01) in the reducing power of outer layers with their respective inner layers of different sizes.

Phenolics are able to bind with the reactive sites of α-amylase and alter its catalytic effects, hence, the presence of phenolics and flavonoids in onion extracts would have contributed toward α-amylase inhibition. Our results corroborates with the report of Tadera et al., (2006), showing several flavonoid compounds especially quercetin for their inhibitory activity against α-amylase and that inhibitory activity increased appreciably with an increase in the number of hydroxyl group on the B ring. Epidemiological studies have also shown that the intake of certain types of flavonoids, including quercetin and myricetin is inversely associated with the risk of incident type II diabetes (Griffiths et al., 2002).
4. Conclusion

In conclusion, the outer living layer (the transitional layer with the first living cells below the dry onion peel) is expected to be a good resource for food ingredients and easily accessible source for nutraceutical compounds. However, difference in flavonoid content, metal chelating activity and HRSA of outer layers with respect to different sizes of onion could be explained on the basis of lesser moisture content in smaller onion as reported earlier by the same authors. We provide enough evidence for the strong biological antioxidant activity of the onion extracts and a good source of α - amylase inhibitors, therefore motivating the use of the natural products against oxidative stress and thereby against various degenerative diseases especially diabetes. We consider that this fraction deserves more intensive study, including its in vivo antioxidant activity in order to understand its potential as nutraceutical and for better extraction of flavonoids.

Conflict of interest

We declare that we have no conflict of interest.

References


