

## Onion, a Potent Inhibitor of Xantine Oxidase

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### Abstract

Onion (*Allium Cepa*) contains high levels of flavonoids. Although there are many studies indicating the inhibitory effects of flavonoids on xanthine oxidase, there is no report on the effect of onion on this enzyme. Therefore, in the present study, the inhibitory effects of onion on xanthine oxidase are investigated.

Fresh filtered juice of onion was prepared and its inhibitory effect on guinea pig liver and bovine milk xanthine oxidase activity was assayed spectrophotometrically using xanthine as substrate. In addition, the effects of hydromethanolic extract of the powdered onion and its major flavonoid, quercetin, were also studied. The juice caused more than 80% inhibition on both guinea pig and bovine milk xanthine oxidase. The extract also resulted in a marked inhibition on guinea pig liver ( $IC_{50}=10 \mu\text{g/ml}$ ) and bovine milk ( $IC_{50}=13 \mu\text{g/ml}$ ) xanthine oxidase activities. Quercetin exerted its inhibitory effect on bovine milk xanthine oxidase through a linear mixed-type ( $K_i=0.06\pm 0.04$  and  $K_i=0.22\pm 0.16 \mu\text{M}$ ), whereas, the guinea pig enzyme was inhibited in a competitive manner ( $K_i=0.11\pm 0.02 \mu\text{M}$ ).

In conclusion, consumption of onion as a staple vegetable with a potent inhibitory effect on xanthine oxidase not only could be useful in some diseases such as gout, but also may result in some interactions with those drugs that are metabolized by xanthine oxidase.

**Keywords:** Onion; Xanthine oxidase; Flavonoids; Inhibition.

### Introduction

Onion (*Allium Cepa*) is a staple food with a high content of flavonoids. The major flavonoids in onion are two quercetin glycosides, quercetin 4'-O-beta-glucoside (Q4'G) and quercetin 3,4'-O-beta-diglucosides (Q3,4'G) and only trace amounts of its flavonoids are present as their aglycone form, quercetin (Figure 1) (1, 2). However, following consumption of an onion meal, both monoglucoside and diglucoside are efficiently hydrolyzed in the small intestine by beta-glucosidases to quercetin, most of which is then absorbed (2).

There are many reports indicating the

beneficial effects of these polyphenolic compounds such as antioxidative (3), anticarcinogenic (4) and enzyme-inhibiting (5) activities. However, the most of the therapeutic properties of flavonoids have been ascribed to their enzyme inhibitory and antioxidant activity (6, 7). One of the important enzymes affected by flavonoids is xanthine oxidase. Xanthine oxidase (Xanthine: O<sub>2</sub> oxidoreductase EC 1.2.3.2) is a molybdenum containing enzyme which has a key role in the formation of uric acid from xanthine so can lead to accumulation of uric acid and ultimately it is responsible for medical condition known as gout (8). This enzyme also serves as an important biological source of some reactive oxygen species that are involved in many pathological processes such as ischemia-reperfusion injuries, inflammation,

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3,5,7,3',4'-Pentahydroxyflavone (QUERCETIN)

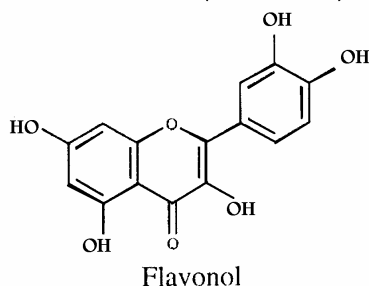


Figure 1. Structure of quercetin

atherosclerosis, cancer and aging (8, 9). There are also some important drugs (such as 6-mercaptopurine) that are metabolized by xanthine oxidase (10). Alongside of some flavonoids which act as inhibitor of xanthine oxidase, quercetin has been shown that is one of the most potent inhibitors of xanthine oxidase (9, 11, 12). Taking into account the high levels of flavonoids in onion, the inhibitory effects of flavonoids on xanthine oxidase and the importance of this enzyme in medicine, it is more likely that onion reduces xanthine oxidase activity. However, to our knowledge there is no report in the effect of onion on xanthine oxidase activity. The aim of this study is, therefore, to investigate the effects of onion on xanthine oxidase.

## Experimental

### Materials

Bovine milk xanthine oxidase (Grade I), quercetin, allopurinol and xanthine were purchased from Sigma (Poole, Dorset, England). Other chemicals were supplied by Merck (KgaA, Darmstadt, Germany).

### Methods

#### Juice preparation

Onion was peeled and the fresh filtered juice was prepared by crushing of the edible part of onion followed by filtration of the resultant crude and used at the same day after 30 fold dilution.

#### Extract preparation

The peeled onion was dried and powdered. The hydromethanolic (methanol/water, 70:30) crude extract of the powdered onion was prepared at room temperature. The extract was

then filtered and the filtrate was concentrated to dryness by rotatory evaporation at low pressure yielding the powdered material.

#### Isolation and structure identification of quercetin

The powdered onion was extracted by boiling distilled water. The aqueous extract was concentrated by rotatory evaporator and was defatted with diethylether twice. The defatted extract was kept in refrigerator at 4°C for crystallization. The crystals were hydrolyzed at 100°C by 5% H<sub>2</sub>SO<sub>4</sub> for 1 h and then the extract was allowed to cool. The precipitate was separated by centrifuge from hydrolyzed solution and its structure was determined by <sup>1</sup>HNMR and <sup>13</sup>CNMR as quercetin.

#### Preparation of hepatic guinea pig xanthine oxidase

Partially purified xanthine oxidase was prepared from mature male Dunkin-Hartley guinea pig liver (400-600g, Tabriz University of Medical Sciences, Tabriz, Iran) according to Johnson et al. method (13) as follows: The animal previously maintained on a standard laboratory diet, was killed between 9.00 am and 10.00 am by cervical dislocation. Liver was immediately perfused with ice-cold 1.15% isotonic KCl solution using Potters homogenizer. The homogenate, then, was heated on a steam bath at 55-57 C for 10 min and cooled at 4°C. The resulting crude was centrifuged at 15,000 g for 45 minutes at 4 C. The supernatant was treated by 50% saturated solution of ammonium sulphate (35.3 g/100 ml) at 4°C. The resulting suspension was re-centrifuged at 6,000 g for 20 minutes at 4 C. The precipitate was dissolved in a minimum volume of 0.1 mM EDTA solution and ultimately was kept at -86°C until use.

#### Spectrophotometric measurement of enzyme activity

The enzyme activity was measured spectrophotometrically using xanthine as the specific substrate of xanthine oxidase as described elsewhere (14). In brief, xanthine (50 μM) was separately incubated with partially purified guinea pig liver fraction or bovine milk

xanthine oxidase in Sorenson's phosphate buffer pH 7.0 containing 0.1 mM EDTA at 37°C, and the initial oxidation rates were measured up to 5 minutes at 295 nm. The reactions were also measured in the presence of onion juice, onion extract and quercetin (1-10  $\mu$ M), and the results were compared with the inhibitory effect of 100  $\mu$ M allopurinol (the standard inhibitor of xanthine oxidase).

#### Spectrophotometric determination of kinetic constants

$K_m$  (Michaelis-Menten constant) and  $V_{max}$  (maximum initial velocity) values for the oxidation of xanthine by guinea pig liver fraction and bovine milk xanthine oxidase were determined spectrophotometrically from a Lineweaver-Burke double reciprocal plot of  $1/v$  against  $1/[S]$  as described before (14, 15). The line of the best fit through the points on the plot was calculated using linear regression by the least square method.

The reactions were also studied in the presence of quercetin and the effect of this flavonoid on the  $K_m$  and  $V_{max}$  values on the Lineweaver-Burke plots was examined.

The  $IC_{50}$  values of onion juice, extract and quercetin were obtained from the inhibitor concentration-activity curve.

#### Protein determination

Protein concentrations of partially purified enzyme fractions were determined spectrophotometrically using a Pierce BCA Protein assay kit with bovine serum albumin as a protein standard (16).

### Results And Discussion

The inhibitory effects of onion juice, extract and quercetin have been tabulated in Table 1. Interestingly, the juice resulted in >80% inhibition on xanthine oxidation by bovine milk or guinea pig liver xanthine oxidases. The corresponding value for the extract was more than 95% inhibition which was comparable to that caused by 100  $\mu$ M allopurinol, the standard and potent inhibitor of xanthine oxidase (Table 1). The  $IC_{50}$  values of the extract for the inhibition of bovine milk and guinea pig liver

**Table 1.** The effects of onion juice, the extract, quercetin and allopurinol on bovine and guinea pig liver xanthine oxidases\*

	Bovine milk xanthine oxidase		Guinea pig liver xanthine oxidase	
	%Inhibition	$IC_{50}$ ( $\mu$ g/ml)	%Inhibition	$IC_{50}$ ( $\mu$ g/ml)
Juice	81 $\pm$ 7	—	86 $\pm$ 12	—
Extract	95 $\pm$ 3 <sup>a</sup>	13 $\pm$ 6	97 $\pm$ 3 <sup>a</sup>	10 $\pm$ 5
Quercetin	95 $\pm$ 3 <sup>b</sup>	0.12 $\pm$ 0.02	96 $\pm$ 10 <sup>b</sup>	0.18 $\pm$ 0.07
Allopurinol <sup>c</sup>	97 $\pm$ 6 <sup>c</sup>	0.82 <sup>d</sup>	99 $\pm$ 4 <sup>c</sup>	—

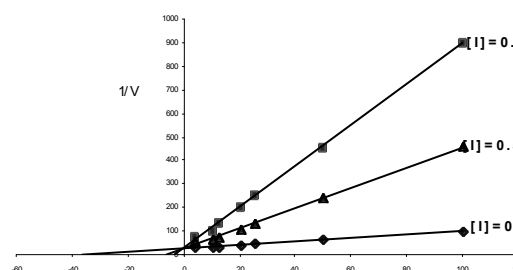
\* The results are expressed as mean  $\pm$  SD, n = 3.

a) Concentration of the extract used: 100  $\mu$ g/ml, b) Concentration of quercetin used: 10  $\mu$ M, c) Concentration of allopurinol used: 100  $\mu$ M, d) Ref. (16)

enzymes were found 13 and 10  $\mu$ g/ml, respectively.

The main flavonoid isolated from the extract after acid hydrolysis was identified as quercetin. Quercetin reduced markedly the initial oxidation rate of xanthine catalyzed by either bovine milk or guinea pig liver xanthine oxidases. The  $IC_{50}$  values obtained were similar to those reported by others. Chang et al (8) isolated quercetin from stems of Bougainvillea with an  $IC_{50}$  value of 7.2  $\mu$ M on xanthine oxidase. Comparing the  $IC_{50}$  values of quercetin and allopurinol indicates that this flavonoid is more potent inhibitor of xanthine oxidase than allopurinol, the standard inhibitor of xanthine oxidase.

The analysis of the kinetics of the reactions revealed that quercetin exerts its inhibitory activity on bovine milk xanthine oxidase through a linear mixed type ( $K_i = 0.06 \pm 0.04$  and  $K_{i'} = 0.22 \pm 0.16$   $\mu$ M), whereas the guinea pig liver enzyme was competitively inhibited by this flavonoid ( $K_i = 0.11 \pm 0.02$   $\mu$ M). The reported results concerning the manner of inhibition caused by quercetin on xanthine oxidase are not uniform. Bindoli et al (12) have reported that quercetin is a competitive inhibitor



**Figure 2.** Lineweaver-Burk plot of guinea pig liver xanthine oxidase inhibition by quercetin isolated from onion ( $K_m = 2.8$   $\mu$ M,  $V_{max} = 5.6 \pm 2.8$   $\mu$ mol/min/mg protein,  $K_i = 0.11 \pm 0.02$   $\mu$ M)

of xanthine oxidase, however, according to Nagao et al (17), this flavonoid exerts its inhibitory effects through mixed inhibition. In Figure 2, the Lineweaver-Burk plot of xanthine oxidase inhibition by quercetin with the guinea pig liver has been illustrated.

As it has been mentioned, quercetin usually exists in onion as its mono or diglucoside forms (1, 2). Following consumption of onion meal, the mono and diglucosides forms of quercetin are hydrolyzed to quercetin (1, 2, 18). According to Graefe et al. (18), the plasma level of quercetin after consumption of onion supplement is 2.3 µg/ml. As the IC<sub>50</sub> value of quercetin for xanthine oxidase is 0.4 µM, it is more likely that eating large amounts of onion reduces xanthine oxidase activity. Xanthine oxidase is capable of oxidizing some important drugs such as 6-mercaptopurine (10, 11). Therefore, some food-drug interactions may occur in those patients taking these drugs and onion at the same time. On the other hand, it has been well documented that xanthine oxidase plays an important role in ischemia-reperfusion injuries (19, 20). Thus, having onion in daily diet may have beneficial effects with this respect. In addition, inhibition of xanthine oxidase activity by allopurinol is an effective strategy in the treatment of gout. The IC<sub>50</sub> value of allopurinol for inhibition of xanthine oxidase is 5.4 µM (21) which is similar to that of quercetin.

In conclusion, taking into account the potent inhibitory effect of onion juice and quercetin on xanthine oxidase activity together with the high level of flavonol quercetin in onion, the consumption of this staple vegetable could be as effective as allopurinol in the treatment of gout. The possible usefulness of onion in these conditions is a subject for further studies. This is also possible to see some interactions between onion consumption and the action of those drugs that are metabolized by xanthine oxidase.

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