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### Recommended Citation

Siddique, A., Suleman, M., Bukhari, I. H., Khan, M. H., Aujla, M. I., & Faiz, A. u. (2016). Anti-Enzymatic Potential of Two Different Varieties of *Malus domestica*, (Malus Communis Desf, Ripe and Unripe Fruits) and their Constituent's Analysis by Gas Chromatography and Mass Spectrometry (GC-MS), *Journal of Bioresource Management*, 3 (1).

DOI: <https://doi.org/10.35691/JBM.6102.0042>

ISSN: 2309-3854 online

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## Anti-enzymatic potential of two different varieties of *Malus domestica*, (*Malus Communis Desf*, ripe and unripe fruits) and their constituent's analysis by gas chromatography and Mass Spectrometry (GC-MS)

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### Abstract:

The paper presents comparative study of urease and alpha-amylase inhibitory activities in two varieties of *Malus species*, i.e. unripered apple and gold coloured ripe apple. It is very important to know the anti enzymatic potential of these two varieties because the enzyme inhibition can be therapeutic and cure illness caused by malfunctioning of some enzymes. Objectives of this study was to determine the presence of chemical constituents, their role and inhibition activities of urease and alpha-amylase in selected apple varieties. Gas Chromatograph-Mass Spectrometric (GC-MS) was used to identify their chemical constituents. Both varieties exhibited profound activities of urease and alpha-amylase that increased by the increase in the sample's concentration. The IC<sub>50</sub> (µg/mL) were 19.77, 19.60, 19.95 and 99.92 for red delicious and golden delicious respectively, whereas, thiourea standard was at 107.90 µg/mL. The IC<sub>50</sub> of unripe and ripe fruits were respectively 0.063, 0.051, 0.06 and 0.065 µg/mL comparing with acarbose standard, i.e. 0.044 µg/mL. GC-MS confirmed the presence of 1-ethyl-3-methylbenzene, 1,2,3-trimethylbenzene, 4-methyl-2-furancarboxaldehyde, cymene, glyceric acid, 1-deoxy-2,4, 3,5-dimethylene-d-xylitol, 1,2-butanediol, 1-(2-furyl)-2,3-dimethyl, 2,4-dihydroxy-2,5-dimethyl, 1-3-furan-3-one 2,4-dihydroxy-2,5-dimethyl-3(2*H*)-furan-3-one and 2,3-dihydro-3,5-dihydroxy-6-methyl(4*H*)-pyran-4-one in the selected samples.

**Keywords:** *Malus domestica*, Urease, alpha-amylase, thiourea and acarbose standards.

### INTRODUCTION

Enzyme inhibition can be therapeutic because  $\alpha$ -amylase and urease inhibitors play a significant role in treating illness caused by these two enzymes (Fishbein *et al.*, 1965; Stefano *et al.*, 1999). Urease is a metallo-enzyme (Loggan *et al.*, 2001) which is virulent for both humans and animals, causing infections such as gastrointestinal ulcers, kidney stones, catheter disease and pyelonephritis. Urease inhibitors are therefore considered to be the most potent anti-ulcer drugs (Arfan *et al.*, 2010). In the metabolic system,  $\alpha$ -

amylase controls the blood glucose level and converts the large starch polymers into smaller units which ultimately gives glucose (Mecewan *et al.*, 2010). Acarbose ( $\alpha$ -amylase inhibitor) reduces glucose bioavailability (Jerald *et al.*, 2008). Flavonoids and phenolic contents present in apples act as an excellent source of the anti-cancer agent (Jose *et al.*, 2009). Apple pulp contains more phenolic contents, ~10% greater, than its peel (Robert *et al.*, 2005). Many aromatic compounds in apples along with some other flavoured compounds were detected by SPME and GC/MS (Izeo *et al.*, 2000; Bracewell *et al.*, 1930). Many alcoholic and ester precursors were detected in apples after putting them under specific conditions. Some of their enzymatic activities were reported (Beborah *et al.*, 1994; Song *et al.*, 1997)

## **Materials and Methods:**

### **Plant materials**

*Malus domestica* (extracts) of two varieties, i.e. 184 grams of red delicious and 102 grams of golden delicious, were collected in the fall of 2011 from Lahore, Punjab, Pakistan. The samples were kept at -4°C until the completion of the work.

### **Urease Inhibition Assay:**

The principle method used in this inhibition was based on urease catalysis (Ghous, 2010). Methanolic extracts were dissolved in a phosphate buffer (pH 7.0) and stored at 4°C, followed by the preparation of further dilutions of this stock solution. Exact amounts: 1 ml of sample/standard, 15  $\mu$ l of urea, 485  $\mu$ l of phosphate buffer. 2.5 ml of reagent 1 were added to a test tube and kept at 37°C for 5 min. 2.5 ml of reagent 2 was also added to the test tube. The reaction mixture was incubated at 25°C for 10 min and the absorbance on UVD at 625nm was measured.

Blank solution consisted of 500  $\mu$ l of phosphate buffer and 2.5 ml of reagent 2 and 1 added respectively. Samples and standard (thiourea) were prepared by the same procedure. The percentage inhibition was calculated by using the following formula:

$$\% \text{ inhibition} = \frac{\text{Control (activity)} - \text{Treatment}}{\text{Control (activity)}} \times 100$$

### **$\alpha$ - amylase Inhibition Assay:**

*Aspergillus oryzae* and  $\alpha$ -amylase of fungal origins were used for this assay (Geethalakshmi *et al.*, 2010). Similarly, 0.5 % (w/v) of starch solution was used as a substrate. Further dilutions of the stock solution were prepared in DMSO (colouring reagent). The test sample (1ml) and the enzyme mixture (1ml) were added in a test tube and kept for half an hour at 25°C. This mixture (1ml) was added to 1.0 ml of the 0.5% starch solution and incubated for 3 min at 25°C. After adding the 1ml DNS reagent, the mixture was incubated at 85°C for 15min in a water bath. The mixture was cooled by adding 9ml of distilled water. Absorbance was noted on UVD at 540nm by running the test sample and blank parallel, and then compared with standard (acarbose).

## Gas Chromatography – Mass Spectrometry analysis

Samples of red delicious and golden delicious (apple extracts) were washed and stored at 18°C. 2.0 g of the fresh fruit samples were cut, crushed, and poured into a 250ml beaker 20cm<sup>3</sup> of diethyl ether was carefully added. It was covered with aluminium foil and kept for 24hrs. It was then filtered and the filtrate was taken for GC-MS. Oven temperature was set at 60°C for 5min, followed by an increase in the temperature that became 280°C/10min. FID and MS temperature was set as 250°C and 200°C respectively.

Chemical constituents were identified by the comparison of their mass spectra with those of the online computer library NIST 05a L and Agilent USA, MS Agilent Technologies 5975 C MSD and GC Agilent Technologies 7890 A.

Sr #	Concentration (µg/5ml)	Red Delicious <i>Malus domestica</i> (unripe) % inhibition	Red Delicious <i>Malus domestica</i> (ripe) % inhibition	Golden Delicious <i>Malus domestica</i> (unripe) % inhibition	Golden Delicious <i>Malus domestica</i> (ripe) % inhibition	Thiourea (standard) % inhibition
1.	05	12.96±0.05	20.96±0.99	10.21±1.14	0.50±0.03	01.99±0.05
2.	10	22.01±0.00	22.01±0.56	22.56±0.32	1.21±0.12	02.19±0.89
3.	15	38.99±0.21	38.99±1.76	38.90±0.48	1.69±0.00	02.87±0.60
4.	20	55.4±0.23	58.04±0.00	53.60±0.22	1.81±0.00	03.87±3.56
5.	40	57.34±0.40	59.61±0.22	61.06±0.23	3.63±0.00	19.45±1.84
6.	60	59.66±0.46	66.43±1.06	65.03±0.00	9.63±0.62	28.68±1.39
7.	80	62.93±0.00	72.59±1.05	69.46±0.23	30.9±1.04	39.83±1.76
8.	100	69.92±0.80	81.11±0.40	72.72±0.00	47.87±0.60	49.09±1.05
9.	120	71.7±0.23	86.47±0.17	77.15±0.92	66.05±0.115	51.04±2.54
10	140	76.6±0.61	92.3± 0.00	81.34±0.39	79.39±0.60	55.21±1.53
11	160	85.0±0.46	93.0± 0.00	88.34±0.83	81.81±0.00	59.45±0.26
12	180	87.64±0.89	93.70±0.00	92.76±0.22	81.81±0.00	64.23±0.33

13	200	92.53±0.60	93.93±0.23	93.46±0.46	83.63±0.00	70.23±1.14
<b>IC<sub>50</sub> (µg/ml)</b>		19.77	19.60	19.95	99.92	107.90

## RESULTS AND DISCUSSION

Two varieties of *Malus domestica*, i.e. red delicious and golden delicious apples, were chosen for the determination of biological activities and the presence of chemical constituents in them. Three basic enzymatic activities were analyzed in these two selected varieties of *Malus domestica* and results are shown in the below text and tables.

Table 1: Urease Inhibition Activity of Fruit Extracts.

### Urease inhibition activity:

Table 1 shows the different concentrations according to their inhibition properties. Lower concentration values of red delicious gave 20 % inhibition, which was even higher than the standard sample values. Low inhibition was given by golden delicious at low concentrations of the sample. Red delicious showed 93.93 % of inhibitory activity among all the selected samples, which was again higher than the standard sample value (Table1).

### $\alpha$ -amylase inhibition activity:

Analysing the anti-diabetic activity of  $\alpha$ -amylase (Table2), it is observed that lower concentrations of red delicious showed greater inhibition than golden delicious. Percentage inhibition for unripe and ripe was 77.3% and 67.6% respectively when compared with the acarbose standard.

Table 2. % inhibition of ripe & unripe fruits of *Malus Domestica*.

Sr. No	Concentration (µg/ml)	Red Delicious <i>Malus domestica</i> (unripe) % inhibition	Red Delicious <i>Malus domestica</i> (ripe) % inhibition	Golden Delicious <i>Malus domestica</i> (unripe) % inhibition	Golden Delicious <i>Malus domestica</i> (ripe) % inhibition	Acarbose Standard % inhibition
01	0.04	18.33±0.03	34 ± 0.01	20.0 ± 0.03	21.0 ± 0.04	45.78
02	0.05	34.30 ±0.02	47.67 ± 0.1	27.32 ± 0.00	36.62 ± 0.6	58.86
03	0.06	56.0 ± 0.05	67.33 ± 0.03	51.0 ± 0.06	43.33 ± 0.09	67.80
04	0.08	70.2 ± 0.00	79.50 ± 0.05	67.6 ± 0.002	77.33 ± 0.01	80.3
	<b>IC<sub>50</sub> values</b>	0.063	0.051	0.06	0.065	0.044

### GC-MS Analysis:

Most of the chemical constituents were common in both types of fruits, e.g. 1-ethyl-3-methylbenzene, cymene and 1,2,3-trimethyl benzene (Table3). However, some of the chemical constituents were specific in pulp and peel. 2,3-dihydro-3,5-dihydroxy-6-methyl,4*H*-pyran-4-one were found in golden delicious. Similarly, glyceric acid and 1-deoxy-2,4:3,5-dimethylene-d-xylitol were only found in red delicious, while furancarboxaldehyde, 3-hexanone,4-methyl were common in both fruit types.

**Table 3.** GC-MS analysis of ripe and unripe fruits of *Malus domestica*.

Sr. No	Compounds	Red Delicious (unripe)	Red Delicious (ripe)	Golden Delicious (unripe)	Golden Delicious (ripe)	Reported as
01	1-Ethyl-3-methylbenzene	+	+	+	+	
02	1-Methyl-4-(1-methylethyl)benzene/ cymene	+	+	+	+	<i>Rapparini et al. (2001)</i>
03	1,2,3-Trimethyl benzene	+	+	+	+	
04	2,3-Dihydro-3,5-dihydroxy-6-methyl,4 <i>H</i> -pyran-4-one	—	—	+	—	
05	Glyceric acid	+	—	—	—	<i>Rudell et al. (2008)</i>
06	1-Deoxy-2,4:3,5-dimethylene-d-xylitol	+	—	—	—	<i>Yamaguchi et al. (1994)</i>
07	1,2-Butanediol,1-(2-furyl)-2,3-	+	—	—	—	

	dimethyl					
<b>08</b>	2,4-Dihydroxy-2,5-dimethyl-3(2 <i>H</i> )-furan-3-one	+	—	—	—	<i>Bruno et al. (2009)</i>
<b>09</b>	Furancarboxaldehyde,3-Hexanone,4-methyl	+	—	+	+	<i>Kondo et al. (2004)</i>

## CONCLUSIONS

By comparing both varieties of *Malus domestica*, we concluded that red delicious (ripe) had more markedly appreciable urease and  $\alpha$ -amylase activity than golden delicious. We also calculated the SEM (standard error of mean) and IC<sub>50</sub> values for the authenticity of our data. Most of the chemical constituents were common in all fruit samples, but not all, as some of them were specified by their presence in some fruit samples.

## ACKNOWLEDGEMENT

The research work was conducted in the research laboratories of Forman Christian College/University Lahore, Punjab, Pakistan. Authors acknowledge the support of the management of Forman Christian College University, Lahore and HEC Pakistan, in this regard.

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