Comparison of α-amylase, α-glucosidase and lipase inhibitory activity of different types of vinegar

1Yasmin, F., 2Abdul Razak, K.N., 1Abdul Samad, N., 3Widyawati, T. and 1*Yusoff, N.A.

1Integrative Medicine Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Penang, Malaysia.
2School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia.
3Pharmacology Department, Medical Faculty, University of Sumatera Utara, 20155 Medan, Indonesia.

Abstract

Vinegar is regarded as a fine example of a traditional food that has several medicinal values. It is used as a home-remedy for the management of diabetes and obesity. We investigated how selected vinegar inhibit the digestive enzymes involved in carbohydrate and lipid metabolism. A total of seven types of vinegar were examined as follows: apple cider (ACV), balsamic (BV), brown rice (BRV), distilled white (DWV), malt (MV), nipah palm (NPV), and red wine (RWV) vinegar. In vitro enzyme inhibition tests were performed using α-amylase and lipase from porcine pancreas, and α-glucosidase from \textit{Saccharomyces cerevisiae}. In the α-amylase assay, NPV (IC$_{50}$ = 60.97±1.71 mg/mL) exhibited the strongest inhibitory effect, while RWV (IC$_{50}$ =786.7±0.96 mg/mL) showed the lowest inhibitory effect. RWV (IC$_{50}$ =92.49±1.51 mg/mL), by contrast, had the highest inhibitory effect against α-glucosidase and was followed by NPV (IC$_{50}$ = 227.5±1.06 mg/mL) and BRV (IC$_{50}$ = 565.1±2.65 mg/mL). All of the samples showed potent inhibitory effects against pancreatic lipase, with MV having the strongest and ACV the lowest effects, respectively. IC$_{50}$ values ranged from 48.45 mg/mL to 399.8 mg/mL. A concentration-dependent inhibitory effect was recorded for each of the vinegar against all three tested enzymes. None of the vinegar, however, exceeded the effects recorded for the standard drugs. Interestingly, a weak correlation was found between total acidity and enzyme inhibition, which asserted the presence of bioactive compounds in the vinegar. As a conclusion, vinegar can be incorporated into the diet to lower the meal’s glycaemic index and benefit those at risk of diabetes as well as diabetics.

1. Introduction

Diabetes mellitus (DM) is a heterogenous metabolic disorder characterized by persistent hyperglycaemia in fasting and/or postprandial states (Sperling et al., 2014). The prevalence of DM is increasing at an alarming rate. According to the International Diabetes Federation, 9.3% of adults worldwide were living with DM in 2019, with Type 2 DM accounting for nearly 90% of the cases (IDF, 2019). Obesity has been strongly linked to Type 2 DM (Schnurr et al., 2020). Research has shown obese adults to be seven times more likely to developing Type 2 DM than adults with healthy weights (Abdullah et al., 2010). DM can be managed by controlling postprandial glucose levels and reducing body weight through the suppression of carbohydrate and lipid hydrolysing enzymes (Wasai et al., 2018). Pancreatic α-amylase and intestinal α-glucosidase are exo-acting glycoside hydrolases that are essential for the metabolism of carbohydrates (Feng et al., 2015). Suppression of these enzymes effectively ameliorates postprandial hyperglycaemia by limiting enteral carbohydrate absorption. Pancreatic lipase, on the other hand, is a key enzyme for dietary fat metabolism. It hydrolyses up to 70% of the total dietary fats ingested to create readily absorbed forms in the small intestines (Liu et al., 2020). It can be hypothesized that by inhibiting the activity of α-amylase, α-glucosidase and pancreatic lipase, the onset of Type 2 DM and its complications may be delayed. A considerable number of functional foods have been shown to be potent inhibitors of digestive enzymes (Kwon et al., 2008; Venkatakrishnan et al., 2019; Kan et al., 2020). Vinegar is a functional food that is widely consumed. Over the past decades, the therapeutic effects of many kinds of vinegar have been thoroughly examined (Samad et al., 2016). Numerous clinical studies have highlighted the role of vinegar in
controlling blood glucose levels and normalizing the lipid profiles of diabetic patients (Liatis et al., 2010; Johnston et al., 2013; Petsiou et al., 2014). Preclinical animal studies have also reported the effects of different vinegar on metabolic parameters (Yusoff, Ahmad, Al Hindi et al., 2015; Seo et al., 2015; Yamashita, 2016). Despite the large number of studies demonstrating the therapeutic effects of various vinegar, the mechanisms underlying the anti-diabetic and anti-obesity activities of most vinegar remain elusive. This study was designed to test whether vinegar affects carbohydrate- and/or lipid-hydrolyzing enzymes as this could partly explain the observed therapeutic effects. Tests assessed the effects of these kinds of vinegar on α-amylase, α-glucosidase and pancreatic lipase. The correlations between enzyme inhibition and the total acid content were reported for each of the seven types of vinegar used.

2. Materials and methods

2.1 Chemicals

Alpha-glucosidase, porcine α-amylase, and pancreatic lipase were purchased from Sigma Aldrich Chemical (St. Louis, MO, USA). Acarbose, sodium hydroxide, and starch were supplied by Sigma Life Science (Waltham, MA, USA). Orlistat was purchased from J and K Scientific Ltd. All the reagents used were of analytical grade.

2.2 Vinegar samples and sample preparation

Seven types of vinegar were obtained from local retailers as follows: Apple cider vinegar (ACV), balsamic vinegar (BV), brown rice vinegar (BR), distilled white vinegar (DW), malt vinegar (MV), nipa palm vinegar (NPV) and red wine vinegar (RWV). These stock vinegar solutions were classified into grain vinegar based on the raw materials used in their respective production processes (Table 1). Vinegar samples were freshly prepared every time by diluting the stock solution with distilled water. Enzyme inhibition assays were conducted using samples at concentrations ranging from 4000 to 125 mg/mL.

Table 1. Vinegar samples

<table>
<thead>
<tr>
<th>Type of Vinegar</th>
<th>Content of vinegar (as per label)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple cider vinegar</td>
<td>Apple juice and water</td>
</tr>
<tr>
<td>Balsamic Vinegar</td>
<td>Concentrated grape must and sulphites</td>
</tr>
<tr>
<td>Brown Rice Vinegar</td>
<td>Organic brown rice, water and koji seed</td>
</tr>
<tr>
<td>Distilled White Vinegar</td>
<td>Distilled white vinegar and water</td>
</tr>
<tr>
<td>Malt Vinegar</td>
<td>Malted barley extract and water</td>
</tr>
<tr>
<td>Nipa Palm Vinegar</td>
<td>Nipa sap and water</td>
</tr>
<tr>
<td>Red Wine Vinegar</td>
<td>Red wine and water</td>
</tr>
</tbody>
</table>

2.3 pH and acetic acid measurement

The pH value of each vinegar was measured using a pH meter (Hanna HI221 pH) followed by titration (Bakir et al., 2017). To perform the measurement, 5 mL of vinegar mixed with 1% of phenolphthalein as the indicator; were titrated using 0.1 M of NaOH. Acidity values were presented as acetic acid equivalent percentages calculated using the following equation:

\[
\text{Percentage of acetic acid} = \left( \frac{V \times E \times 100}{M} \right) 
\]

Where \( V \) is the volume used of the titrant (NaOH) in litres, \( E \) is the vinegar equivalent (determined to be 0.006005), and \( M \) is the amount of the titrated vinegar (5 mL).

2.4 α-amylase inhibitory activity

The α-amylase inhibitory activity of each sample was evaluated according to Safamansouri et al. (2014). A 20 mM sodium phosphate buffer solution (pH = 6.9) containing 6.7 mM sodium chloride; was used. A starch solution (1% w/v) was prepared by mixing 1 g of soluble potato starch in 1 mL of the buffer solution. The α-amylase solution (50 unit/1 mL) was prepared by adding 0.01 g of α-amylase to 10 mL of the buffer. A colour reagent solution was obtained by mixing 0.1 g of 3,5-dinitro salicylic acid, 2.99 g of sodium potassium tartrate, and 0.161 g of sodium hydroxide in 10 mL of the buffer solution. Next, 50 μL of the vinegar sample was mixed with 150 μL of the starch solution and 10 μL of the enzyme solution in a 96-well plate. The plate was incubated for 30 mins at 37°C. The reaction was terminated by adding 20 μL of the colour reagent. The plate was further incubated in boiling water for 20 min. After cooling down to room temperature, the absorbance value of the reaction mixture was measured at a wavelength of 570 nm using a microplate reader (Bio Tek). Acarbose (20-0.313 mg/mL), an α-amylase inhibitor, was used as a standard reference. Alpha-amylase inhibition was presented as a percentage as follows:

\[
\% \text{ Inhibition} = 100 \times \left( \frac{A_{\text{sample}} - A_{\text{control}}}{A_{\text{control}}} \right) 
\]

Where \( A_{\text{control}} \) is the absorbance value of the control solution, and \( A_{\text{sample}} \) is the absorbance value of the sample. The half-maximal inhibitory concentration (IC₅₀) values indicated the concentration required of an α-amylase inhibitor to inhibit 50% of the enzymatic activity.

2.5 α-glucosidase inhibitory activity

The α-glucosidase inhibition activity of each sample was evaluated as previously demonstrated by Yusoff, Yam, Beh et al. (2015). A 0.1 M phosphate buffer (pH =
6.9) was used as the working buffer in this experiment. A yeast α-glucosidase (0.5 U/mL) solution and a 5 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) solution were prepared in the working buffer and acted as the enzyme and substrate solutions, respectively. To perform the measurements, 50 μL vinegar aliquots were mixed with 100 μL of the α-glucosidase enzymatic solution in a 96-well plate. After a 10-min incubation period at 37°C, each well was topped up with 50 μL of the pNPG solution. The plate was incubated for 5 min at 37°C. Alpha-glucosidase activity was determined by measuring the absorbance value of the 4-nitrophenol released from the pNPG solution at a wavelength of 405 nm. Acarbose (20-0.313 mg/mL), α-glucosidase inhibitor, served as a standard reference. Alpha-glucosidase inhibition was expressed as a percentage value calculated as follows:

$$%\text{Inhibition} = 100 \times \frac{(\Delta A_{\text{control}} - \Delta A_{\text{sample}})}{\Delta A_{\text{control}}} \quad (3)$$

Where $A_{\text{control}}$ is the absorbance of the control, and $A_{\text{sample}}$ is the absorbance of the sample. IC$_{50}$ values indicated the concentration required of an α-glucosidase inhibitor to inhibit 50% of the enzymatic activity.

2.6 Pancreatic lipase inhibitory activity

The porcine pancreatic lipase (PPL) activity of each sample was measured as previously described by Jo et al. (2017). A PPL enzymatic stock solution (0.1 mg/mL) was prepared in a 0.1 M Tris-HCl buffer (pH = 8.0). A 10 mM p-nitrophenyl butyrate (p-NPB) solution in acetonitrile served as the substrate solution. In a 96-well plate, 5 μL vinegar samples at different concentrations were mixed with 90 μL of the enzyme solution and incubated for 10 mins at 37°C. Then, 5 μL of the p-NPB solution was added to the reaction mixture. After a 15-min incubation period, the lipase inhibitory activity was determined by measuring the absorbance value of the formed p-nitrophenol at a wavelength of 405 nm. The inhibitory activities of the vinegar against pancreatic lipase were expressed as the percentage of inhibition and IC$_{50}$ values. The following formula was used:

$$%\text{Inhibition} = 100 \times \frac{(\Delta A_{\text{control}} - \Delta A_{\text{sample}})}{\Delta A_{\text{control}}} \quad (4)$$

Where $A_{\text{control}}$ is the absorbance of the control, and $A_{\text{sample}}$ is the absorbance of the sample. All samples were run in triplicate. Orlistat (10-3.13 mg/mL), an inhibitor of pancreatic lipase, was used as a standard reference.

2.7 Statistical analysis

The data were expressed as the mean±standard mean error (SEM). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey HSD as a post-hoc test. P values below 0.05 indicated statistical significance. The data was analysed using the IBM® SPSS statistical software.

3. Results and discussion

3.1 pH and total acidity

Table 2 shows the values of the pH and total acidity of the vinegar samples. The samples had pH values ranging from 2.62 to 3.23. RWV had the lowest pH, while ACV had the highest. The percentages of acetic acid in the samples were between 2.30±0.01% and 5.17±0.01%, with NPV exhibiting the lowest acetic acid content, and RWV showing the highest reading. These findings are in agreement with Bakir et al. (2017) as they reported pH values of eighteen vinegar ranging from 2.8 and 3.7 and total acidity values between 0.7±0.1% and 5.02±0.03%. According to Bakir et al. (2017), the pH value and the total acid content of vinegar are crucial determinants of its antimicrobial activity. The findings did not show a significant correlation between pH level or the total acid content of vinegar and its enzyme inhibitory effect.

<table>
<thead>
<tr>
<th>Type of Vinegar</th>
<th>pH</th>
<th>Acidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple Cider Vinegar</td>
<td>3.23</td>
<td>4.83±0.02</td>
</tr>
<tr>
<td>Balsamic Vinegar</td>
<td>3.2</td>
<td>3.06±0.06</td>
</tr>
<tr>
<td>Brown Rice Vinegar</td>
<td>2.82</td>
<td>4.09±0.01</td>
</tr>
<tr>
<td>Distilled White Vinegar</td>
<td>2.62</td>
<td>4.96±0.01</td>
</tr>
<tr>
<td>Malt Vinegar</td>
<td>2.93</td>
<td>4.80±0.01</td>
</tr>
<tr>
<td>Nipah Palm Vinegar</td>
<td>3.04</td>
<td>2.30±0.01</td>
</tr>
<tr>
<td>Red Wine Vinegar</td>
<td>2.62</td>
<td>5.17±0.01</td>
</tr>
</tbody>
</table>

3.2 Inhibitory effects of vinegar against carbohydrate-hydrolyzing enzymes

This assay assessed the α-amylase enzymatic inhibitory activity of the seven selected vinegar against acarbose. Table 3 shows the IC$_{50}$ values obtained for each of the vinegar and acarbose. All of the vinegar inhibited α-amylase, with the IC$_{50}$ values being in the range of 60.97±1.71 mg/mL and 786.7±0.96 mg/mL. NPV had the strongest inhibitory effect, while RWV showed the lowest. However, the effect of NPV was minimal compared with that of acarbose as the latter was greater by 16.8 folds. Yet, enzyme inhibition was found to increase with increasing vinegar concentration for each of the samples (Figure 1A).

Likewise, the α-glucosidase inhibitory activity of each vinegar was observed to be concentration-dependent (Figure 1B). RWV caused the greatest α-glucosidase inhibitory effect (IC$_{50}$ = 92.49±1.51 mg/mL) among the vinegar used. This was followed by NPV (IC$_{50}$ = 227.5±1.06 mg/mL) and BRV (IC$_{50}$ = 565.1±2.65 mg/mL). The lowest α-glucosidase inhibition was observed with MV (IC$_{50}$ = 2030±0.83 mg/mL). Considering that the IC$_{50}$ value of acarbose was found to be 17.02±7.26 mg/mL, acarbose surpassed the inhibitory
The effect of RWV by 5.4 folds. Both assays showed similar patterns of inhibition, except for red wine vinegar. Overall, our data suggested that the vinegar tested possessed moderate inhibitory activities against carbohydrate-hydrolyzing enzymes. Similar findings were reported by Johnston et al. (2010) in healthy adults. The ingestion of two teaspoons of vinegar with a meal rich in complex carbohydrates was observed to reduce postprandial glucose levels by almost 20%. Our findings also agree with a report by Ogawa et al. (2000) which looked into the inhibitory effects of acetic acid, a major compound of vinegar, on disaccharidase activity in Caco-2 cells.

### 3.3 Pancreatic lipase inhibitory activity

All vinegar samples exerted moderate inhibitory effects against pancreatic lipase in a concentration-dependent manner (Figure 2). Table 3 shows the IC<sub>50</sub> values of the vinegar samples to be in the range of 48.45 - 399.8 mg/mL. The samples inhibited lipase to varying extents as follows: MV > DWV > NPV > BRV > RWV > BV > ACV. The IC<sub>50</sub> value of orlistat, the reference, was determined to be 5.01±0.03 mg/mL, which demonstrated that orlistat inhibited lipase by 9.7 folds as compared with MV, the most potent inhibitor among the tested samples. Pancreatic lipase is responsible for the digestion of dietary fat. Lipase inhibition due to vinegar intake may, thus, slow down the storage of fat and inhibit weight gain, which can be beneficial in terms of controlling obesity and delaying the onset of Type 2 DM (Dechakhamphu and Wongchum, 2015). Previous reports have highlighted the therapeutic effects of vinegar in obesity. Halima et al. (2016) showed that

### Table 3. IC<sub>50</sub> values for α-amylase, α-glucosidase and lipase assays of seven types of vinegar

<table>
<thead>
<tr>
<th>Type of Vinegar</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (mg/mL±SEM)</th>
<th>α-amylase</th>
<th>α-glucosidase</th>
<th>Lipase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple Cider Vinegar</td>
<td>665±0.29</td>
<td>866.7±1.52</td>
<td>399.8±0.08</td>
<td></td>
</tr>
<tr>
<td>Balsamic Vinegar</td>
<td>262.7±2.22</td>
<td>784.5±1.50</td>
<td>138.5±0.08</td>
<td></td>
</tr>
<tr>
<td>Brown Rice Vinegar</td>
<td>192.7±0.03</td>
<td>565.1±2.65</td>
<td>105.9±0.06</td>
<td></td>
</tr>
<tr>
<td>Distilled White Vinegar</td>
<td>359±0.03</td>
<td>430±1.62</td>
<td>58.25±0.08</td>
<td></td>
</tr>
<tr>
<td>Malt Vinegar</td>
<td>187.4±0.06</td>
<td>2030±0.83</td>
<td>48.45±0.14</td>
<td></td>
</tr>
<tr>
<td>Nipah Palm Vinegar</td>
<td>60.97±1.71</td>
<td>227.5±1.06</td>
<td>83.11±0.17</td>
<td></td>
</tr>
<tr>
<td>Red Wine Vinegar</td>
<td>786.7±0.96</td>
<td>92.49±1.51</td>
<td>112.5±0.04</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>3.64±1.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.02±7.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.01±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Standard error of the mean (n=3), <sup>b</sup>Acarbose, <sup>c</sup>Orlistat

Figure 1. Dose dependent changes in percentage inhibitory activity of seven types of vinegar against A) α-amylase and B) α-glucosidase. Results are expressed as means±SEM for triplicates of each concentration. Different letters above error bars indicate significant differences (p < 0.05) among groups as analysed using Tukey’s post-hoc test. ACV: apple cider vinegar, BV: balsamic vinegar, BR: brown rice vinegar; DW: distilled white vinegar, MV: malt vinegar, NPV: nipah palm vinegar, RV: red wine vinegar

Figure 2. Dose dependent changes in percentage inhibitory activity of seven types of vinegar against lipase. Results are expressed as means±SEM for triplicates of each concentration. Different letters above error bars indicate significant differences (p < 0.05) among groups as analysed using Tukey’s post-hoc test. ACV: apple cider vinegar, BV: balsamic vinegar, BR: brown rice vinegar; DW: distilled white vinegar, MV: malt vinegar, NPV: nipah palm vinegar, RV: red wine vinegar
treatment with apple cider vinegar significantly improved lipid homeostasis in diabetic rats by suppressing the activity of lipid-metabolizing enzymes. Additionally, Chen et al. (2012) and Chatatikun and Kwanhian (2020) respectively reported that Nipah palm vinegar and black vinegar powder attenuated the activity of pancreatic lipase.

3.4 Correlation of acetic acid content with α-amylase, α-glucosidase and lipase inhibitory effect

To determine whether enzymatic inhibition was subject to the acetic acid content of vinegar, a correlation analysis was performed (Figure 3). A negative correlation was observed between the acetic acid content and the α-amylase inhibitory activity of the samples. By contrast, positive correlations were observed between the acetic acid content, and α-glucosidase and lipase inhibitory activities, respectively. Overall, there was only a weak correlation between the acetic acid content of vinegar and its inhibitory activities against α-amylase, α-glucosidase and lipase ($R^2 = 0.040, 0.101$ and $0.356$, respectively). The findings indicated possible inhibitory effects of some of the bioactive compounds present in the vinegar. Acetic acid is the major organic acid in vinegar, but other bioactive compounds have been documented in the past in different vinegar preparations. Those functional compounds include non-volatile organic acids, phenolic acids, and tetramethylpyrazine (TMP) (Xia et al., 2020). Work conducted on persimmon vinegar showed that citric acid and lactic acid worked with acetic acid to promote the appetite and stimulate the use of fatty acids (Moon et al., 2010). TMP was identified as a major compound in Chinese black vinegar, in which it exerted antioxidant and hypolipidemic effects (Chen et al., 2017). Phenolic acids, such as gallic acid, ferulic acid, caffeic acid and salicylic acid; have been identified in both grain- and fruit-vinegar, and have been shown to have promising antioxidant (Kelebek et al., 2017; Xie et al., 2017; Zhao et al., 2018) and antihyperglycemic effects (Yusoff, Yam, Beh et al., 2015).

4. Conclusion

The results presented here and those from previous studies suggest that vinegar have favourable effects in both healthy individuals and Type 2 diabetic patients. Vinegar can be incorporated into the diet to lower the meal’s glycemic index and benefit those at risk of diabetes as well as diabetics.

Conflict of interest

The authors declare that there is no conflict of interest.

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