Effect of inulin addition in cucumber drinks on urease inhibitory, antioxidant activity and its consumer acceptability

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Abstract. Cucumber (Cucumis sativus) juice is the best rejuvenation tonic used in the folk medicine. However, during juicing, all the fibre content of the juice will be removed. Addition of inulin to the juice not only help to replace the fibre content but it also acts as prebiotic which may help to stimulate the growth of beneficial bacteria in guts. Therefore, this study focused on the effect of inulin addition in the cucumber drink on urease inhibitory and antioxidant activities, as well as on consumer acceptability. Cucumber drink was prepared using a domestic juicer and the different amounts of inulin added to the drink were based on the nutritional conditions for dietary fibre claims of a food product as a ‘source of fibre’ and ‘high fibre’ (i.e. 3 g/100 mL and 6 g/100 mL, respectively). Urease inhibitory activity was determined using phenol-hypochlorite reaction and antioxidant activity was determined using DPPH radical scavenging assay. The results show that non-fortified cucumber juice (100% without inulin) had the highest urease inhibitory and DPPH radical scavenging activities (95.1% and 38.3%, respectively). The results show that the addition of inulin to the cucumber drink does not have any impact on the urease inhibitory and DPPH radical scavenging activities. On the other hand, consumer acceptance test shows that cucumber drinks fortified with 6 g of inulin were more acceptable to the consumer based on consumers’ 9-point hedonic scale distribution.

1. Introduction
Cucumber (Cucumis sativus) belongs to the Cucurbitaceae family. Cucumber is classified as a fruit because it develops from a flower and has an enclosed dicotyledonous seed, but is always confused as a vegetable [1]. Cucumber contains 95% of water and flavonoids that can act as anti-inflammatory agents. Cucumber has soothing properties that ease an upset digestion and protect the stomach, most probably due to its urease inhibitory and antimicrobial activities. Cucumber has been used in folk medicines as an antioxidant [2]. The total antioxidant activity and total phenolic content of whole fresh cucumbers have previously been determined through an extensive screening on common vegetables [3]. Thus, drinking cucumber juice will help the consumers to gain the health benefits.
Inulin is a type of soluble dietary fibre known as fructan, which is a form of fructose molecules that are linked together. Inulin cannot be digested in the small intestine, but it travels to the lower gastrointestinal tract and acts as a prebiotic by promoting the growth of beneficial bacteria in the gut. Inulin is converted by the bacteria into a short-chain fatty acid that help nourishing colonic microbes [4]. Therefore, inulin fortification in cucumber juice can increase fibre content of the drink. On top of that, inulin can help promote the healthy balance between ‘good’ and ‘bad’ bacteria, by stimulating the growth of good bacteria that can improve digestion and relieve constipation. Furthermore, inulin can reduce the symptoms of inflammatory bowel disease.

Inulin is considered as a functional food and has been added to many foods to improve health [5]. However, effect of inulin in cucumber drink and its formulation has not been well studied. Therefore, this project was carried out to determine the effect of the cucumber drink fortified with inulin on urease inhibitory activity, antioxidant activity, and consumer acceptance of the inulin-fortified cucumber juice.

2. Methodology

2.1. Chemicals

Inulin were bought from local shop at Kangar, Perlis. 2,2-diphenyl-1-picrylhydrazyl (DPPH), urease (Jack bean), phosphate, sulphuric acid (H2SO4), sodium hydroxide, sodium nitroprusside, sodium hypochlorite, nutrient broth (NB), and hydrochloric acid (HCl) was purchased from Sigma-Aldrich Co., Malaysia.

2.2. Plant Material and Juice Preparation

Fresh whole cucumbers were purchased from the local market in Kangar, Perlis. The cucumbers were washed and rinsed thoroughly under running tap water to remove dirt and unwanted particles. The cucumbers were then dried using tissue paper, and juice was extracted using a domestic juicer (Breville BJE200XL Juicer, Australia). The juice was then readily available for the formulation of inulin-fortified cucumber drink at different concentrations.

2.3. Cucumber Drink Preparation

The cucumber juice prepared in Section 2.2 was used to formulate different concentrations of inulin-fortified cucumber drink, as shown in Table 1. The inulin concentration was added based on nutritional values for ‘source’ and ‘high’ content of dietary fibre supplementation in a food product i.e. 3 g/100 mL and 6 g/100 mL [6]. Non-fortified cucumber drink was used as a control. All drinks were stored in 200 mL glass bottle, and pasteurized at 95 °C for 3 min in order to kill unwanted microorganisms [7]. These bottles were tightly closed and turned upside down to remove oxygen from the headspace to ensure longer protection from undesirable colour and flavour changes. Prior to pasteurization, glass bottles were blanched at 100 °C for 5 min, and then left to dry and cool in an oven (Cornell COR-CEOSE561SL USA). The drinks were kept in a refrigerator (Samsung RT35K5562SL/ME, South Korea) at 7 °C and analysed within seven days of the drink production.
Table 1. Formulation of inulin-fortified cucumber drinks at different concentrations.

| Percentage cucumber juice (%) | Percentage of water (%) | Amount of inulin (g) |
|-------------------------------|-------------------------|----------------------|
| 20                            | 80                      | Without inulin       |
| 40                            | 60                      |                      |
| 60                            | 40                      | 3 g                  |
| 80                            | 20                      |                      |
| 100                           | 0                       |                      |
| 20                            | 80                      | 6 g                  |
| 40                            | 60                      |                      |
| 60                            | 40                      |                      |
| 80                            | 20                      |                      |
| 100                           | 0                       |                      |
| 20                            | 80                      |                      |
| 40                            | 60                      |                      |
| 60                            | 40                      |                      |
| 80                            | 20                      |                      |
| 100                           | 0                       |                      |

2.4. Urease Inhibitory Activity

About 0.02 g of urease (Jack bean) was mixed together in 10 mL phosphate buffer to obtain a concentration of 2 mg/mL (standard assay mixture) [8]. Urease inhibitory activity of the cucumber drinks was determined based on the phenol-hypochlorite reaction. Briefly, 100 µL of cucumber drinks were mixed with 100 µL of 2 mg/mL urease (Jack bean) solution and 0.2 mL of 100 mM phosphate buffer pH 7.0 containing 25 mM urea. The mixture was then incubated in a water bath (Julabo TW12, Germany) at 37 ºC for 30 min. Then, 500 µL of solution A (containing 5.0 g phenol and 25 mg of sodium nitroprusside in 500 mL distilled water) and 500 µL of solution B (containing 2.5 mg sodium hydroxide and 4.2 mL of sodium hypochlorite in 500 mL distilled water) were added to the mixture and incubated again in water bath at 37 ºC for another 30 min. The absorbance was measured at 625 nm using a spectrophotometer (Hitachi U-1100, Japan). All measurements were performed in triplicate. Clarithromycin (Merck, Germany) was used as a positive control. The percentage inhibitions of urease were calculated using an Equation 1.

\[
% \text{Urease Inhibition} = \left(1 - \frac{\text{Absorbance sample at 625 nm}}{\text{Absorbance blank at 625 nm}}\right) \times 100
\]  

(1)

2.5. Antioxidant Activity

Antioxidant activity of the inulin-fortified cucumber drink was measured using DPPH radical scavenging assay [9]. One mL of cucumber drinks was allowed to react with one mL of 0.1 mM DPPH in methanol, and incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using a spectrophotometer (Hitachi U-1100, Japan). The absorbance of cucumber drink was measured against control incubation containing distilled water. Ascorbic acid was used as a reference of standard compound. The percentage of DPPH radical scavenging activity was calculated using an equation (2).
\[ \text{% of inhibition} = \frac{(\text{Control}-\text{Sample})}{\text{Control}} \times 100 \quad (2) \]

2.6. Consumer Acceptance Test
Consumer acceptance test was conducted using only three different concentrations of the cucumber drinks in order to avoid excessive daily intake of inulin by the research subjects. Twenty-four consumers participated in the test. The participants were recruited from students and staffs of the School of Bioprocess Engineering, Universiti Malaysia Perlis. The drink samples were prepared one day prior to test and stored in refrigerator at 4 °C. All drinks were served at room temperature on the day of the test.

Three mL of each of the cucumber drinks were poured into 5 mL opaque white plastic cup labeled with randomly three digits code. The cups were then placed on a tray and presented to the study participants in an isolated sensory tasting room. Study participants evaluated the colour, odour, flavour, aftertaste and overall liking attributes on a 9-point hedonic scale. After each evaluation session, participants cleanse their palate with plain water before they were allowed to test the next drink sample.

2.7. Statistical analysis
Statistical analyses were performed using Minitab® software Version 17 (Minitab, LLC). One-way analysis of variance (ANOVA) was used to test for significance between group means and Tukey’s post-hoc analysis was used for pair-wise multiple comparisons of the group means, where P<0.05 considered as significant. The correlation between consumer acceptability and the effects of inulin-fortified cucumber drink were determined by using Principle Component Analysis (PCA) by Minitab.

3. Results and Discussion
3.1. Urease Inhibition Activity
Urease inhibitory activity of cucumber drink fortified with inulin are shown in Figure 1. The results showed that the inhibition percentage of urease was found to be within the range of 47% to 95%, which increased proportionately with higher percentage of juice concentration. In inulin-fortified cucumber drinks at juice concentrations of 60% and lower, percentage inhibition of urease showed no difference among the various juice concentrations following Tukey’s test. In addition, no significant difference was also found in the percentage of urease inhibition between inulin-fortified drinks and control drinks (i.e. non-fortified cucumber drink) at juice concentrations of 60% and lower.

![Figure 1](image_url)  
**Figure 1.** Effect of inulin addition in cucumber drinks on urease inhibitory. Data are mean ± SEM. Different letters indicate significant differences between groups within the same juice percentage.
However, the percentage of urease inhibition at juice concentrations of 80% and 100% was significantly higher in non-fortified control drinks than the inulin-fortified cucumber drinks. This result suggests that there was no synergistic effect between inulin and cucumber juice that may influence urease inhibitory activity. Neither inulin nor cucumber juice have effect on urease inhibitory activity at low percentage. To the best of our knowledge, no scientific studies have been reported concerning the potential of cucumber as a urease inhibitor. A study performed by Makkar et al. [10] indicated that *Cucumis melo* showed urease inhibitory activity when tested against ruminal fluid urease [10]. Thus, this study suggested that plant in the genus Cucumis may have the potential to be exploited as urease inhibitor.

3.2. Antioxidant Activity

In accordance with the results obtained from the urease inhibitory activity, the antioxidant activity values in inulin-fortified cucumber drinks were also proportionate to the increased percentage of juice concentration, as depicted in Figure 2. The antioxidant activity between inulin-fortified cucumber drinks and control drinks were statistically significant, whereby non-fortified control drinks at 100% juice concentration demonstrated the highest antioxidant capacity among all juice concentrations. However, no significant differences were found in the antioxidant capacity between 2 different concentrations of inulin (3 g/100 mL and 6 g/100 mL) that were added to the cucumber drinks. This result shows that inulin may not effective as an antioxidant agent. It is also suggested that antioxidant activity of the cucumber drinks could be due to the effect solely exerted by the cucumber. Furthermore, overall antioxidant capacity of the analyzed cucumber drinks were generally low (Figure 2). This finding is in agreement with the study conducted by Yunusa et al. [11], who reported that low percent inhibition of DPPH radical scavenging activity was observed among the different parts of cucumber analyzed in their study.

![Figure 2](image_url)

**Figure 2.** Effect of inulin addition to cucumber drinks on antioxidant activity. Data are mean ± SEM. Different letters indicate significant differences between groups within the same juice percentage.
3.3. Consumer Acceptance Test

Only three formulated cucumber drinks (100% juice with and without inulin) were chosen for the consumer acceptance testing, in order to avoid excessive daily intake of inulin. The means score of consumers’ sensory acceptability were summarized in Table 2. High score of means indicates the consumer’s high acceptance level of the drink. Cucumber drinks fortified with 6 g inulin are well accepted by the consumers as the means score were greater than 5 for all attributes tested. To interpret the consumers’ preferences, all the means score of consumer acceptance attributes were mapped using principal component analysis (PCA) to obtain preference drink. The score plot (Figure 3) demonstrates that cucumber drinks fortified with 6 g inulin were well liked by consumers as all attributes have similar strong vectors emphasizing that drink fortified with 6 g inulin were more acceptable for every attribute and no one attribute has a particularly large influence on the distribution.

Inulin may influence the colour of the drinks. It has been observed that drinks fortified with inulin were more stable in term of colour and not easily oxidized during storage. The addition of inulin may also mask the original taste and flavor of cucumber drink by increasing its sweetness. Therefore, cucumber drink has become more preferable and accepted by the consumers.

| Drinks     | Colour     | Odour      | Flavour    | Aftertaste   | Overall liking |
|------------|------------|------------|------------|--------------|----------------|
| Without inulin | 6.08 ± 1.06<sup>b</sup> | 5.08 ± 1.14<sup>a</sup> | 5.33 ± 1.4<sup>ab</sup> | 5.42 ± 1.14<sup>ab</sup> | 5.50 ± 1.06<sup>ab</sup> |
| 3g inulin  | 7.33 ± 1.2<sup>a</sup> | 5.67 ± 1.34<sup>a</sup> | 4.50 ± 1.35<sup>b</sup> | 4.75 ± 0.74<sup>b</sup> | 5.17 ± 0.56<sup>b</sup> |
| 6g inulin  | 7.83 ± 0.92<sup>a</sup> | 6.00 ± 1.67<sup>a</sup> | 6.17 ± 1.17<sup>a</sup> | 5.58 ± 1.14<sup>a</sup> | 6.0 ± 0.83<sup>a</sup> |

<sup>Note:</sup> Data represent mean ± standard deviation (N=24). Values that are followed by different letter within each row are significantly different (P<0.05) using Tukey’s Honest Significant Different.

![Figure 3. Principal Component Analysis (PCA) of Consumer Acceptance Test Attributes.](image-url)
3.4. Correlation of consumer acceptance test with urease inhibitory and antioxidant activity

The correlation between consumer acceptance test with urease inhibitory and antioxidant activity were also determined using the PCA. Means of all data were plotted against the first two components (first component horizontally, second component vertically) (Figure 4). The first component contributed for 63.7% associated with the amount of inulin added in the drinks. The second component contributed for 36.3% associated with the activities tested for the drinks. It was clearly shown that drinks without inulin are more effective toward urease inhibitory and antioxidant activity and drinks fortified with 6 g inulin are well accepted by the consumers compared to drinks fortified with 3 g inulin.

Figure 4. Correlation between consumer acceptance test with urease inhibitory and antioxidant activity.

4. Conclusion

The effect of inulin addition in cucumber drinks on urease inhibitory, antioxidant activity and its consumer acceptability have been determined in this study. The results show that cucumber drink fortified with inulin has less effect in urease inhibitory and antioxidant activity. 100% juice without inulin shows the highest urease inhibitory and antioxidant activity. This result concluded that addition of inulin in cucumber drink did not have any significant impact on the urease inhibitory and antioxidant activity. However, 100% juice fortified with 6 g of inulin was more preferable by the consumers. Optimization study should be carried out to maximize the urease inhibitory and antioxidant activity of the cucumber drinks fortified with inulin with its consumer acceptability.

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