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Physicochemical properties, antioxidant activities, and sensory evaluation of pineapple peel biovinegar

Y Selvanathan¹ and N Masngut^{1,2*}

¹Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia.

²Centre of Excellence for Advanced Research in Fluid Flow (CARIFF), Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Pahang, Malaysia.

*Corresponding author: nasratun@ump.edu.my

Abstract. The objective of this study was to characterize a biovinegar (OPPB) produced from natural fermentation of pineapple peel. Physicochemical properties, antioxidant activities and sensory evaluation were investigated to compare its quality with apple cider vinegar (ACV) and dates vinegar (DV). The vinegars were evaluated for their scavenging activity towards 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical for the antioxidant activities. Physicochemical properties such as ascorbic acid, reducing sugar, acidity, acetic acid, ethanol, total soluble solid, pH and sucrose content were also determined. Consumer preferences and perception towards the biovinegar were carried out through a survey. The OPPB shows a comparable radical scavenging of 82 % and physicochemical properties such as acidity of 3.03 %, ascorbic acid of 1.43 mg equi. AA/100 mL sample, pH of 3.16, acetic acid of 0.61 %, ethanol of 1.03 %, sucrose of 4.0 g/100 g, reducing sugar of 3.18 % and total soluble solid content of 8.0°Brix with commercial vinegar (ACV and DV). Descriptive statistical test indicates that there were no significant differences in color intensity, sourness, sweetness, flavor, and aroma with the vinegars tested but OPPB was 56 % more preferred than ACV and DV by the respondents.

1. Introduction

Biovinegar is produced from a fermentation of variety of cereals and fruits which has been used for foods for preservation, flavoring and pickling [1]. It can be made from a suitable raw material of agricultural origin containing starch, sugars, or both, by the process of double fermentation, first alcoholic followed by acetous [2]. Biovinegar natural fermentation means utilizing the naturally occurred of mixed microorganism strains on the raw materials itself such as yeast and bacteria to conduct the fermentation. On the other hand, biovinegar fermentation also utilizing specific single microorganism strain for the fermentation to occur.

The aroma of single strain fermentation is weaker than that the mixed strains. The latter can increase the complexity of the aroma as proved by Liu et al. [3] in the study of apple vinegar produced from single strain was lacking in flavor and aroma than that the mixed strains. The mixed strains in fermentation produces an exquisite taste of vinegar due to the different characteristic of various microorganism. Fermentation is an effective way of harnessing the medicinal richness of a fruit. The



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fermentation of mixed strains has shown to produce vinegar with more health benefit than the single one. Chen et al. [4] reported higher antioxidant activity in mixed strains of citrus vinegar than its single strain by 38.3 % DPPH free radical scavenging assay.

In the present study, the action of OPPB from the previous work of optimizing the pineapple peel using response surface methodology was evaluated together with other selected commercial vinegars (ACV and DV) against DPPH radical scavenging activity and their physicochemical properties. The physicochemical properties evaluated were acid, acetic acid, pH, and ethanol, sucrose, reducing sugar, ascorbic acid and total soluble solid content. In addition, this study also assessed the consumer preferences and perceptions on three different vinegars (OPPB, ACV and DV) through two measures that were survey and sensory test.

2. Materials and methods

2.1. Substrate for aerobic fermentation

OPPB was made from the optimized condition analysed by the Central Composite Design (CCD) which made using pineapple peel juice with mixed strains, 7 % w/v addition of glucose at 27 °C temperature for five days aerobic incubation which was quite similar to Raji et al. [5] with modification using mixed strains and carried out the study using Response Surface Methodology (RSM). ACV (mfg: March 2019) and DV (mfg: 2 October 2018) were obtained from the supermarket in Kuantan city, Pahang, Malaysia.

2.2. DPPH radical scavenging activity and ascorbic acid content

The sample ratio with reagent was 1:19 which the assay mixture contained 2.85 mL of 0.024 % DPPH radical solution and 0.15 mL of the sample. The solution was rapidly mixed and after standing for 30 min at room temperature the absorbance of the mixture was measured at 515 nm [6]. The result was calculated as the percentage inhibition according to the following formula:

$$\% \text{DPPH inhibition} = \left(\frac{C-S}{C} \right) \times 100 \% \quad (1)$$

where C and S are the absorbance of the control and sample, respectively. A known antioxidant concentration, i.e. ascorbic acid was used to develop the free radical scavenging calibration graph as shown in Eq. (2) with the coefficient of determination 0.9988. The concentration of ascorbic acid (mg equi. AA/100 mL) was determined by using radical scavenging calibration graph.

$$\% \text{DPPH inhibition} = (5.8095 \times \text{Concentration of ascorbic acid mg equi. AA/100 mL}) - 0.7575 \quad (2)$$

2.3. Determination of acetic acid concentration

The concentration was quantified using HPLC (Agilent technologies, Palo Alto, CA, USA) adopted method by Zhang et al. [7] with modification of detection wavelength and flowrate of mobile phase. Samples were filtrated with a 0.45 µm membrane filter and injected at 10 µL for acetic acid analysis using Synergy Hydro C18 250 organic acids column (300 × 4.6 mm, Japan) with sulphuric acid as mobile phase at 0.5 mL/min, acetic acid as standard and measured with a UV detector at 221 nm (1260 VWD, 1200 series; Agilent Technologies). Calibration curve for acetic acid concentration up to 5 % was constructed as shown in Eq. (3) with the coefficient of determination of 0.9955.

2.4. Physicochemical testing

2.4.1. Determination of pH and acid content. A pH meter (Mettler Toledo) used for all pH value measurements. The total acidity was estimated using 1.0 mL vinegar sample, phenolphthalein and a neutralizing agent of 0.1 M NaOH, which yielded total acid content (%). This method was adopted from [5].

2.4.2. Determination of reducing sugar concentration. The reducing sugar was estimated using Dinitrosalicylic acid (DNS) method of Teixeira et al. [8]. Thus, 1.5 mL of vinegar sample was added into 3 mL of 0.4 M DNS reagent and the mixture was heated at 100°C for 5 min. After cooling to room temperatures, 0.2 mL of the mixture was withdrawn and diluted with 1.8 mL of 0.05 M citrate buffer. Each sample was scanned with UV/VIS spectrophotometer at wavelength 540 nm to obtain the OD values and compared with the glucose calibration curve. Glucose calibration curve was developed earlier at concentration range between 0 – 10 % using the same procedure as the sample with the coefficient of determination of 0.9988.

2.4.3. Determination of total soluble solid, sucrose and ethanol content. The total soluble solid [9], sucrose [10] and ethanol content [11] were estimated using refractometer (MASTER-KMW 2593, Atago, Japan).

2.5. Sensory Evaluation

A survey was carried out to determine consumer preference and perception of biovinegar made from waste with other commercial vinegars and also to explore consumer knowledge and insight of fruit biovinegar made from waste and likelihood to accept the products produced from this kind of raw material. Thirty volunteers composed of 20 females and 10 males among the university student and staff were taken part as the respondent. The age range of the respondent was between 21 and 48 years. The vinegars were labelled with A (OPPB), B (DV) and C (ACV) so that the panels would not be biased, this is to ensure minimization of error. The volunteers were asked to answer first part of the survey regarding the consumption rate and purpose of consuming vinegar. Then, the second part was for a sensory attribute. The sensory attributes assessed were color intensity, sourness, sweetness, taste/ flavor and aroma using Rank 1 to Rank 5 which was from the weakest to the strongest. Final part of the survey, the respondent had to choose which vinegar they preferred the most in terms of taste/ flavor. The questionnaire design and data collection conducted as follows.

2.5.1. Design of the questionnaire. The survey began with a brief description of the research study-consumer perceptions about biovinegar made from waste and mixed strains in which respondent was informed about this novel method for biovinegar production. The questionnaire consisted of five sections: (1) demographics (2) consumption rate (3) purpose (4) sensory attributes and (5) most preferred vinegar. In the first section, a series of demographic questions (gender, age and race) were asked. In the second section, the respondent was assessed with the consumption rate of the vinegar, which had to indicate either “few times in a year”, “once a month”, “once a week” or “2 – 5 times a week”. Next, the respondent was asked about their purpose of consuming vinegar. In the fourth section, the respondent's perception was assessed using five items and ranked their responses on a scale from 1 to 5. In the last section, the respondent was asked to choose their preferred vinegars in terms of the taste and flavor.

2.5.2. Statistical analysis. Microsoft Excel was used to analyse the median and significant differences between values using the descriptive statistic. The significant difference was determined at p less than

0.05 as significant items. A descriptive analysis was performed to the profile of respondent's demographics and purpose of consuming vinegar.

3. Results and discussion

3.1. DPPH radical-scavenging activity and ascorbic acid content

The DPPH free radical scavenging activity is shown in Figure 1. The OPPB was the second highest after ACV with 82 ± 0.60 % anti-free radical activity which is the efficiency to scavenge half of the radicals. Dates vinegar has the lowest radical scavenging activity with only 25 ± 0.68 %. Study by Hossain and Rahman [12] the radical scavenging activity of pineapple fruit is 95 %. It seems that the fermentation does reduce the scavenging activity of the OPPB by 13 % which may be caused by the oxidation of the antioxidant compound such as ascorbic acid and phenolic, happens during the period. The radical scavenging activity reduction may also be because of the reduction of anthocyanin which is a class of compounds with antioxidant effects. This assumption is proved by the study of Wu et al. [13] which has a reduction of radical scavenging activity as much as 46.06 % during the fermentation of purple potato vinegar. Furthermore, it is correlated with the anthocyanin reduction as fermentation happens. The study shows that reduction of anthocyanin directly affects the reduction of radical scavenging activity of purple potato vinegar. There were many possibilities studied in research carried by Wu et al. [13] which were hydrolyzation of microbial enzymes, ethanol accumulation may promote polymerization of anthocyanin and also glycosidase metabolism of yeast, acetic acid bacteria and other microorganisms may lead to anthocyanin degradation [13]. The result of the current study is comparable with previous works which Kim et al. [14] had reported the DPPH free radical scavenging activity of blackberry vinegar was 12 % while Keser et al. [15] reported the grape vinegar had 58.07 % activity. The result from the current study as depicted in Figure 1 shows that vinegars are also free radical-scavengers, particularly of the peroxy radicals, which are the major propagators of the oxidation chain of fat, thereby terminating the chain reaction [1].

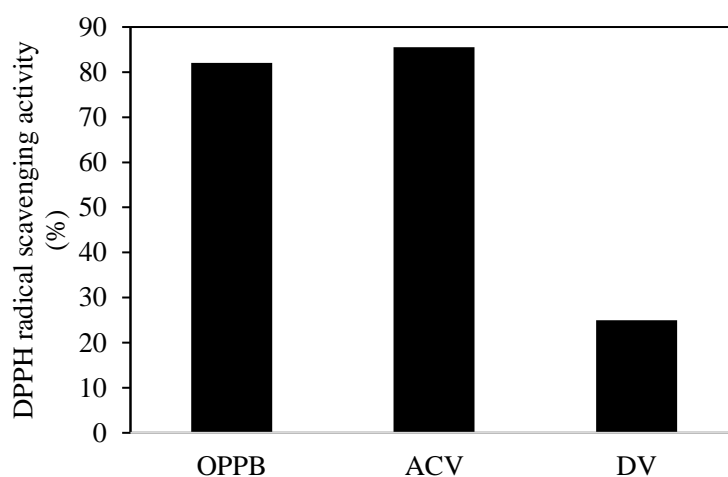


Figure 1. DPPH radical scavenging activity.

Antioxidant scavenges free radicals from the body cells, and prevents the damage caused by oxidation. Antioxidant activity can be determined from the ascorbic acid content. The ascorbic acid in current OPPB was 1.43 mg equi. AA/100 mL sample as shown in Figure 2. The OPPB stands as the second highest after ACV. ACV shows higher antioxidant activity because not only ascorbic acid was

higher but also other antioxidant compounds such as phenolic and flavonoids are higher as studied in Gopal et al. [16]. Ascorbic acid also known as vitamin C in common term. Such results was also been reported by Kong et al. [17] with 2.32 mg/100mL ascorbic acid produced by fermented papaya beverage. As ascorbic acid is heat sensitive; fermentation, pasteurization and sterilization had resulted in its reduction compare with the fresh juice although fermentation led to retention of ascorbic acid in the vinegar studied [18]. The dissolved oxygen in fermentation process increased the reaction between antioxidant compounds such as ascorbic acid thus decreases the ascorbic acid concentration. Ayub et al. [19] reported a maximum of 26 % decreased level of ascorbic acid during pasteurization of strawberry juice. Common shelf life of ascorbic acid is around two years [20], and to prolong the effectiveness is best to avoid heat and air flow.

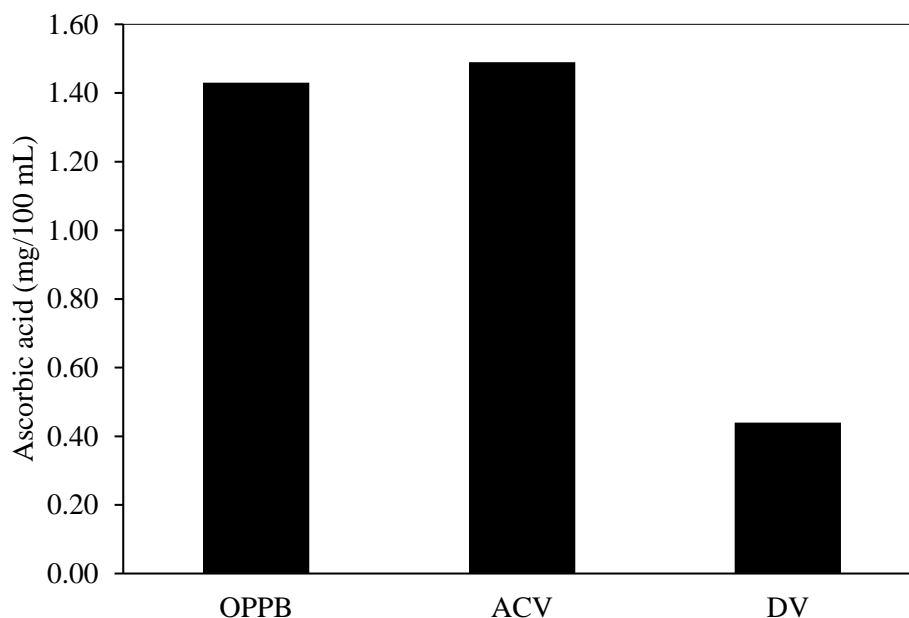


Figure 2. Ascorbic acid concentration.

3.2. Physicochemical properties of pineapple peel vinegar

The pH values of these vinegars were ranged between pH 2.73 and 3.16, with OPPB was the highest as shown in Table 1. Kim et al. [14] reported similar findings; pH of commercial vinegar in Korea were from pH 2.81 to 3.20 with acid content up to 2.41 %. Xia et al. [21] reported high acidity in Shanxi aged vinegar which was between 3.5 % and 8.0 %. Roda et al. [22] reported the total acids produced by pineapple peel and core was 5.0 %. Raji et al. [5] reported around 4.77 % of total acids content in vinegar made by thin strips of pineapple peel. It can be said that there were variety of pH range for these biovinegar and it is inversely proportional to its total acid content. As can be seen from Table 1, total acidity in OPPB was the lowest among all as it has the highest pH value.

Reducing sugar present in the vinegars is shown in Table 1. The OPPB contained the highest reducing sugar of 3.18 %. Higher reducing sugar concentration is benefited the taste of vinegar since vinegar is known for the sourness with pungent smell. Consumer likely prefers the food with less sour with slight of sweetness. Meanwhile, with food of higher reducing sugar content may increase the blood glucose level if consume in excess amount. Fructose and glucose are the highest sugar content in fruit

vinegar, with content varying based on the types of fruits used [14]. In this case, pineapple juice has reducing sugar in a range of 1.3 – 3.35 %. Meanwhile, reducing sugar content of dates and apple are 20 % [23] and 11.09 % [24]. High sugar content may lead to high ethanol and subsequently high acid content in the produced biovinegar. Even though, OPPB had the highest total sugar content in produced biovinegar but the produced acidity was the lowest compared to other biovinegar. This may be the result of mixed strains action that is not as efficient to use up the sugar as specific strain used in the production of other two commercial vinegar (ACV and dates vinegar).

Ethanol content of the OPPB was 1.03 % in which the lowest from other vinegars as shown in Table 1. Ethanol was an intermediate product during vinegar fermentation before it was oxidized into acetic acid. Residual ethanol was unavoidable in biovinegar as the conversion during acetification was around 70 % as studied in Roda et al. [22] and Patel and Pandya [25]. OPPB has the highest sucrose content of 4.0 g/100g with total soluble solid (TSS) of 8.0 °Brix. The TSS may include soluble solids such as sugar, organic acid, amino acids, and soluble pectin. Accumulation of TSS is strongly related to the ripening of the fruit [26], this also associated with increase concentration of soluble solids.

Vinegar also contains volatile and non-volatile acids. Acetic acid is commonly volatile acid in the vinegar. From Table 1, dates vinegar had the highest acetic acid content of 4.9 % and OPPB had the lowest of 0.6 %. Study by Zhang et al. [27] stated about 73 % of acid content in vinegar is volatile in which may present as acetic acid, oxalic acid, tartaric acid, succinic acid, malic acid and pyroglutamic acid. The rest 27 % is non-volatile acids presents significantly as lactic acid [27]

Table 1. Physicochemical properties of vinegars.

Biovinegar	pH	Acidity (%)	Acetic acid (%)	Reducing sugar (%)	Ethanol (%)	Sucrose (g/100g)	Total soluble solid (°Brix)
OPPB	3.16±0.09	3.03±0.03	0.61±0.05	3.18±0.50	1.03±0.15	4.00±0.25	8.0±0.25
ACV	3.12±0	5.34±0.76	3.91±0.05	0.31±0.07	1.70±0	1.75±0	3.5±0
DV	2.73±0	5.83±0.59	4.91±0.01	0.25±0.03	1.50±0	1.50±0	3.0±0

OPPB: Optimized pineapple peel biovinegar

ACV: Apple cider vinegar

DV: Dates vinegar

3.3. Sensory evaluation of pineapple peel vinegar

The questionnaire consisted of two sections with nine questions. In this evaluation, the majority of these respondents were females (66.66 %) with (33.33 %) males aged between 21 and 48 years; 50 % Malay, 3.33 % Chinese, 43.33 % Indian and 3.33 % Bangladesh. All respondents were staff (3.33 %) and students (96.67 %) of Universiti Malaysia Pahang (UMP). There were 30 participants involved as respondent. Three different vinegars were given for the ranking preference test, which one of them was OPPB and other two were Apple Cider vinegar (ACV) and Dates vinegar (DV). The outcomes of the survey were discussed in the subsequent sections.

3.3.1. Consumption rate against race, gender and age. Figure 3 shows the consumption rate based on different races, which are Malays, Chinese, Indians and others. Malays consumption was the highest; 50 % of the overall race on consuming vinegar. For the category of “few times in a year” Malay race stands the highest percentage of 40 % then, secondly goes to Indian race around 36.67 %. While, for the category “Once a month” Malay and Indian race were in equal amount of 6.67 %. This authenticate the

portion of Malays in Malaysia is the largest (62 %). Therefore, the market holds the strongest by Malay race in Malaysia. While, Chinese is the second largest portion (20.6 %) but the survey do not meet many Chinese respondent, thus, cannot conclude anything from this survey. Indian is the smallest portion in Malaysia (6.2 %), but the survey meets around 13 respondents out of 30 from the community.

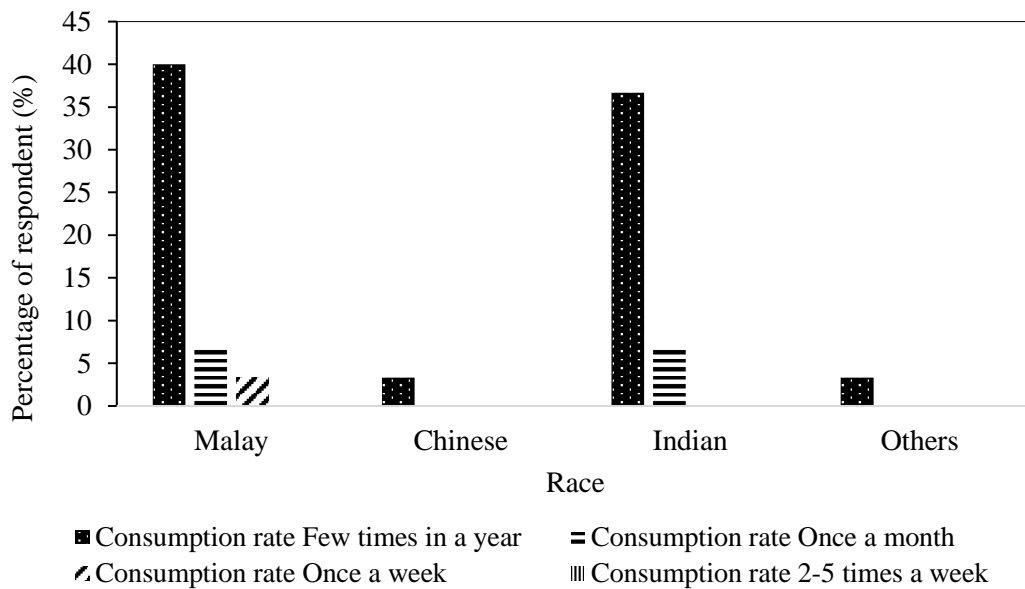


Figure 3. Consumption rate on race.

Table 2 exhibits the descriptive analysis of consumption rate on gender from these 30 participants. As shown in Table 2, 68 % with the frequency (17) were females whom consume vinegar few times in a year. While, there is no differences between male and female whom consume vinegar for once a month. Nevertheless, only a female consume vinegar for once in a week. From the survey, the marketability of vinegar is the highest within female compared to male, which endorse the vinegar industry since in Malaysia, females holding the average portion of 48.58 %.

Figure 4 shows the consumption rate on age. The age of respondents ranged from 21 and 48 years. The frequency of respondents age were 2 for 21 years, 8 for 22 years, 12 for 23 years, 1 for 24 years, 4 for 25 years, 1 for 26 years, 1 for 29 years and 1 for 48 years old, thus the majority was age of 23. Eighty-three percentage of with frequency of 25 of the participants consume vinegar few times in a year whereas age of early twenties (20 – 25) hold up the highest portion of 76.67 %. From this evaluation, the market of vinegar should target early twenties due to their highest consumption rate.

Table 2. Descriptive analysis of consumption rate on gender.

Consumption rate	Gender	Frequency (N)	Valid percent (%)
Few times in a year	Male	8	32
	Female	17	68
One a month	Male	2	50
	Female	2	50
Once a week	Male	0	0
	Female	1	100
2 – 5 times a week	Male	0	0
	Female	0	0

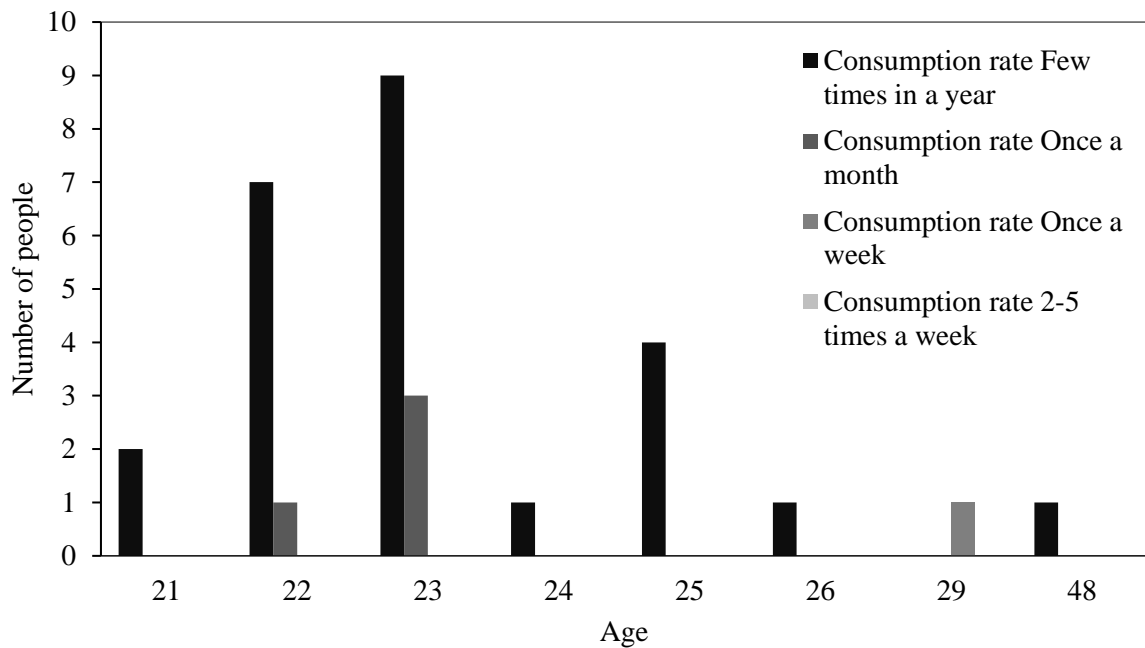


Figure 4. Consumption rate on age.

3.3.2. *Consumer purpose of using vinegar.* Vinegar been used for various foods for preservations and often used for flavoring food and pickling. It also known used for lowering blood sugar level, beauty, and weight loss and reduce cholesterol. Based on the survey, the participants mostly use vinegar for cooking (49 %) and few numbers of people used it for health benefit as shown in Figure 5.

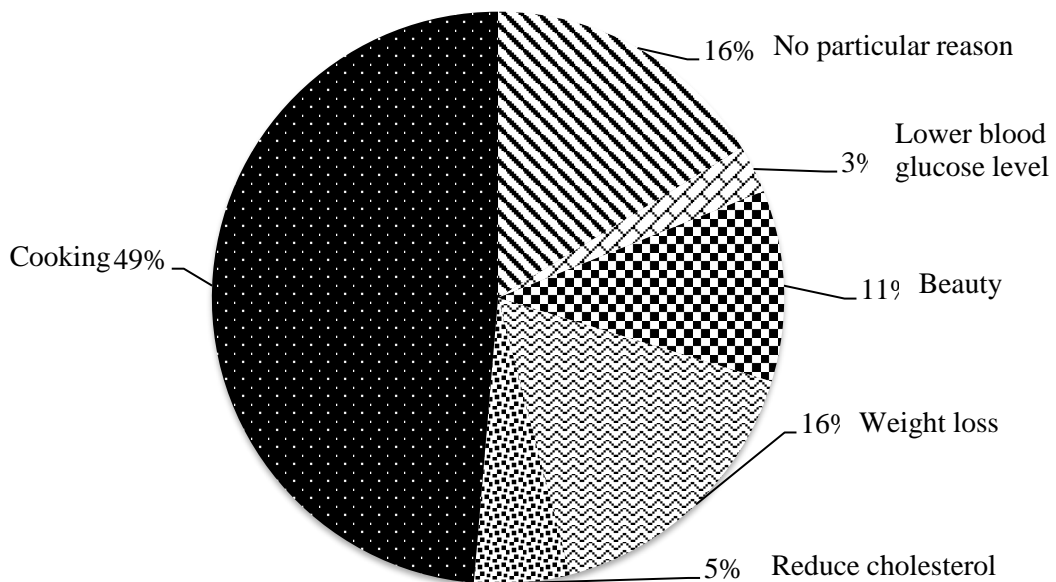


Figure 5. Distribution of the consumer purpose of using vinegar.

3.3.3. *Ranking for preference evaluation.* Table 3 presents an estimated median based on the descriptive statistic test and p -value for vinegars (OPP, ACV and DV) in an evaluation of ranking for preferences in terms of sweetness, sourness, aroma, color intensity and flavor.

Table 3. Recorded median and p – value of ranking of preferences based on descriptive statistic test on studied vinegars.

Ranking for preferences	Vinegars			p -value
	OPP	ACV	DV	
Sweetness	2.5	2	1	0.90
Sourness	3	4	5	0.98
Aroma	4	4	4	0.50
Color intensity	1	5	3	0.89
Flavor	3	4	5	0.98

Note. Ranking varied from 1 to 5 (1 is the weakest)

* $p < 0.05$ is significant

As shown in Table 3, there was no significant difference ($p > 0.05$) between the preferred sweetness of the vinegars, however, most participants favored the sweetness of OPP (2.5) compared to DV (1) and ACV (2). These results indicate that the participants mostly prefer the OPP due to its sweetness because of high reducing sugar content. There were no significant differences ($p > 0.05$) in the participants preferred sourness among the vinegars. Meanwhile, participants indicated the sourness is DV (5) as compared to OPP (3) and ACV (4). This result corroborates with the finding in Section 3.2, indicating that highest acidity and acetic acid content in DV. In this case, we found that the participants are likely to choose less sour vinegar. In terms of aroma, there was no significant differences ($p > 0.05$) since all these vinegars ranked the same. Therefore, aroma of vinegars does not affect the preferences of consumer. Other than that, it is also no significant differences ($p > 0.05$) for the flavor among the vinegars, yet, most participants favored the flavor of DV (5.0) compared to OPP (3) and ACV (4). This is maybe because of the preferences of the fruit, since DV made from dates, which is one of the most favorite fruit in Malaysia especially in Malay community. There were also no significant differences ($p > 0.05$) in the participants preferred color intensity among these vinegars. Nonetheless, participants indicated their preferences for the color intensity of ACV (5) as compared to OPP (1) and DV (3). This is maybe because of the cloudy appearance of any vinegar, which contains of the mother. Consumer likely prefer vinegar with the mother compared than that without.

3.3.4. *Overall preferences.* Figure 6, represent the overall preferable vinegar among the participants. Based on the pie chart of Figure 6, OPP (56 %) has the highest preference than that to DV (17 %) and ACV (27 %). Therefore, from the sensory evaluation, OPP able to compete with the commercial vinegar. The outcome from the sensorial experiment was that OPP provided a good impression as participants experienced the real product produced by this new method. This impression is an indication of a positive perception of a vinegar made by mixed strains and waste. The objective of this study was to get a preliminary understanding of vinegars made by waste which is not edible and also using mixed strain fermentation that were on pineapple waste which maybe of *Penicillium*, *Mucor*, *Rhizopus*, *Pseudomonas* and *Acetobacter* [28]. Whereas, the commercial vinegar made using edible part of fruit and single strain fermentation. Throughout the survey, the participants who have the experienced in vinegar consumption were considered.

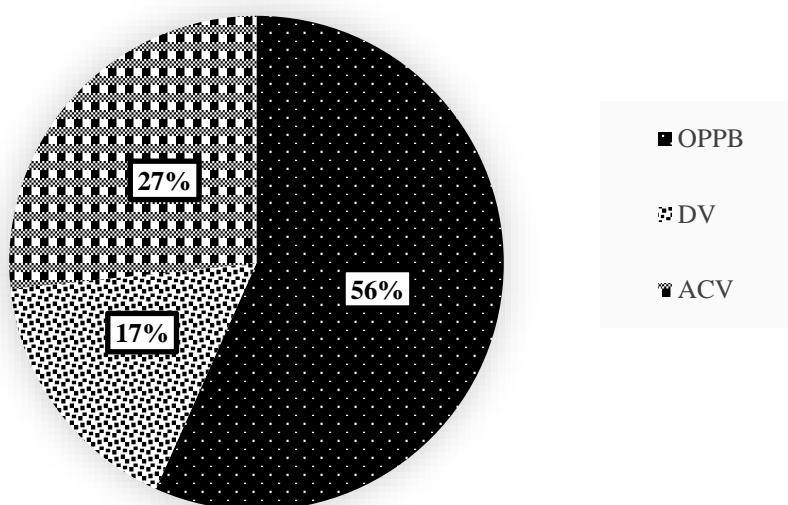


Figure 6. Distribution of the consumer preferences in vinegars (OPPB, ACV and DV).

4. Conclusion

It is concluded that OPPB was the second highest DPPH radical scavenging activity of 82 % and ascorbic acid content of 1.43 mg equi. AA/100 mL sample after ACV but has higher value than DV. Nevertheless, all tested vinegars were found to be an effective antioxidants even though OPPB had the lowest acidity than the rest of the commercial vinegars. While, DV has the highest acidity and acetic acid. Meanwhile, there was a general awareness of how commercial vinegar is made among these participants and also the respondent was informed how the OPPB was made differently than commercial vinegars. Comparing to the commercial vinegars ACV and DV, OPPB was 57 % more preferred by the respondents and also preferred the most in sensory attributes of sweetness and sourness. The results obtained from the sensory tests and consumer survey provided a useful insight into perception of vinegar made by waste with mixed strains versus the single strains fruit commercial vinegar. Thus the concept will be beneficial to promote this waste reduction alternative in the food industry.

Acknowledgments

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