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Study on physiochemical properties and the halalness of commercially marketed vinegar in Malaysia

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Abstract

Vinegar is very popular as traditional ingredient for cooking, pickling, and preservation. It is made from sugar or starch by an alcoholic and acetous fermentation and produces ethanol as a by-product. Alcohol is prohibited to be consumed for Muslim or used as ingredient if it is exceeding the allowable limit as stated by Islamic Council in Malaysia. According to Fatwa Committee National Council of Islamic Religious Affairs Malaysia, natural occurrences of ethanol in food products are acceptable if the ethanol contents are less than 1% in beverages and 0.5% for flavoring or coloring substances for the purpose of stabilization. On the other hand, for specific vinegar product, as stated by Malaysian Food Act and Regulation, acetic acid content must be at least 4%. According to FAO/WHO, a product is to be labelled as vinegar if the acetic acid content is 6% and with a maximum residual alcohol content of 0.5%v/v for wine vinegar and 1%v/v for other vinegars. This study investigated the physiochemical properties of the vinegar from different sources of raw materials. Total solubility (TA) by using Brix method, pH, and alcohol and acetic acid content by GC-TOF/MS of 12 commercial vinegars from Malaysia and abroad were studied. The result shown that for pH value of commercial vinegar are ranged from 2.51-3.14°Brix from 2.10-40.73°Brix, acetic acid is ranged from 0.0253-0.1276% and ethanol content from 0-0.5911%. Thus, this study will come out with the clear observation on ethanol content in fermented product which is vinegar in order to categories the halalness of the product that available in Malaysia market especially the ones that are produced internationally. Lastly, as shown by the profiling study, vinegar that are imported internationally may contain some amount of alcohol in contrast with the one that locally produced in Malaysia and has Halal certification.

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Introduction

The production of vinegar started as early as 5000 BC, where Babylonians used fruits of dates palm to make wine vinegar. Archaeologists have also found vinegar residues that can be traced back to 3000 BC. In China, the history of vinegar was recorded in the form of texts, dated back to 1200 BC. Since around 3000 BC, Asian, Europe and other traditional cuisines in the world have used a variety of agricultural sources to make vinegar. This shows that people, since ancient times, had already explored to diversify the raw material for vinegar production and this is also indicating that the use of vinegar as one of the additional ingredient and purposes are abundantly discovered. (Chen *et al*., 2015). Approximately 5000 years later, flavored vinegar has been produced and sold as a commercial product. Vinegars are mainly used as cooking ingredient for various purposes such as to enhance the taste and as preservatives in food. Furthermore, vinegar had also been used in industrial, medical and domestic uses because of its availability and it being a mild acid type.

In general, some dictionary has defined vinegar as sour wine. Basically vinegar is known as a condiment made from sugary or starchy materials by an alcoholic fermentation followed by and acetous fermentation. Alcoholic fermentation process occurs when yeasts change natural sugars to alcohol under controlled conditions. Then, in the acetous fermentation process a group of bacteria called as *Acetobacter* will convert the alcohol portion to acid. During the process, the proper bacteria culture, timing and the entire fermentation process should

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be carefully controlled. Vinegar consists mainly of acetic acid and water which is why sourness flavor is dominant in vinegar. The acetic acid content in vinegar is produced by the fermentation process of ethanol in the presence of acetic acid bacteria such as *Acidicaldus* and *Gluconobacter*. The main component that is needed to initiate the fermentation process is sugar content in the raw material where it will supply nutrient for the bacteria to starts its activity.

Commercial vinegar is produced either by slow or fast fermentation process. In a simple explanation, slow fermentation process proceeds slowly over the course of months or a year and commonly used for traditional vinegar. This process will allow the accumulation of a nontoxic slime composed of acetic acid bacteria that is caused by the longer fermentation period. On the other hand, for fast fermentation method, 'mother of vinegar' is added to the source liquid before adding air to oxygenate the mixture and promote the fastest fermentation. The term 'mother of vinegar' refers to the substance that composed of a form of cellulose and acetic acid bacteria that develops on fermenting alcoholic liquid. It takes about 20 hours up to 3 days in order to complete the fermentation process. Other than that, grape, apple, and other fruit juices are the examples of primary starting materials used for vinegar production (Adams, 1985) although rice vinegar, malt vinegar, and beer vinegar are also produced in some countries. (Budak *et al*., 2014)

In the local market focused in Malaysia, there are varieties of vinegar that can be found either locally produced or internationally imported from the foreign country. As stated by Ould El Hadj *et al.* (2001), vinegar can be produced from many kinds of sources like grapes, apples, potatoes, and some many other tropical fruits like pineapples, dates, oranges and bananas. One of the most popular commercial vinegar is apple cider vinegar. This vinegar is made from fermented cider or apple has a golden brown flavor and is used to make a variety of dressings, sauces and marinades. Apple cider also has an extremely versatile flavor. Another related topic is the distilled vinegar. Any type of vinegar is distilled to create clear, nondescript, acidic liquid. It is most often used to make pickles or dye eggs, where the acid content is more important than the flavor.

Other type of vinegar is rice wine vinegar. It has a very light, fresh flavor and is commonly used in Asian cuisine. This vinegar is less acidic and has a slightly sweet flavor compared to others. It is great for pairing with delicate flavors such as cucumber and fish. Malt vinegar is also familiar among the industries. Fermenting malted barley will produce malt vinegar. This vinegar has a fairly dark brown color, a pungent, tart flavor, and is a popular topping for fried potatoes. It is also a great pickling, due to its high acetic acid content, although the dark color can produce a visually unappealing product (Crisco Company, 2005).

Moreover, it is important to obtain a specific quality of vinegar in order to determine objectively the appropriate parameter that makes it possible to characterize and differentiate one vinegar sample from the other or the raw material used in the production. The study on physiochemical properties of vinegar is one of the ways to quantify the quality and status of the halalness of vinegar. In term of vinegar quality, sugar, density and dry matter positively influenced while other parameter such as acetic acid have a negative influence. (Masino *et al*., 2008). As mentioned earlier, vinegar will contain specific volatile and non-volatile compounds, including organic acids, sugars, amino acids and ester (Jang *et al.*, 2014). It will become a reference to study the physiochemical properties of a vinegar product. This study will be a screening stage in order to determine the four physiochemical parameter of vinegar product that locally marketed in Malaysia which are total solubility content (Brix), pH, acetic acid content and ethanol content. In the purpose of halal product, the amount of ethanol content also need to be considered because alcohol is considered as prohibited in Islam if the amount is exceeding from the allowable limit. Muslim should take in consideration when choosing the halal vinegar by identifying the process and the intention of vinegar production either it is from halal sources or haram sources other than the amount of ethanol content for example from wine or liquor.

Thus, the aim of this study is to determine the physiochemical properties of various types of vinegar sources that marketed in Malaysia neither locally being produced or internationally produced especially the ethanol content. It is because, there are varieties of vinegar product that widely available in Malaysia market however the issue on the status of its physiochemical properties and the halalness of vinegar is not clearly discovered. In Islam, fermented product is considered halal if it is from the halal sources and the process that has been used in the production is also not doubtful. Halal is clear and haram is clear, so, it is crucial for every Muslim to identify the halalness of a product before being consumed.

Materials and methods

Materials

Twelve commercial vinegar in Malaysia comprised of various raw materials which are apple vinegar, distilled vinegar, wine vinegar, chardonnay vinegar, fig vinegar, synthetic vinegar, malt vinegar, black vinegar, merlot vinegar, cheery vinegar and balsamic vinegar were purchased from a supermarket in Petaling Jaya, Selangor. Other chemical such as absolute ethanol, acetic acid standard, acetonitrile (ACN) were analytical grade and purchased from Sigma-Aldrich.

pH Analysis

A pH meter (Mettler Toledo Sevencompact) was used to measure the pH of the vinegar samples. Prior every run, pH meter need to be calibrated by using different buffer which are pH 4.01, pH 7.00 and pH 9.21 in order to check the accuracy of the instrument. Moreover, the linearity of the calibration curve of the pH calibration also recorded in Figure 1. 10 mL of vinegar sample was poured into a 25 mL beaker and was labelled accordingly. Then, the pH electrode was placed into the vinegar sample and the reading was recorded. All the result was recorded in triplicate.

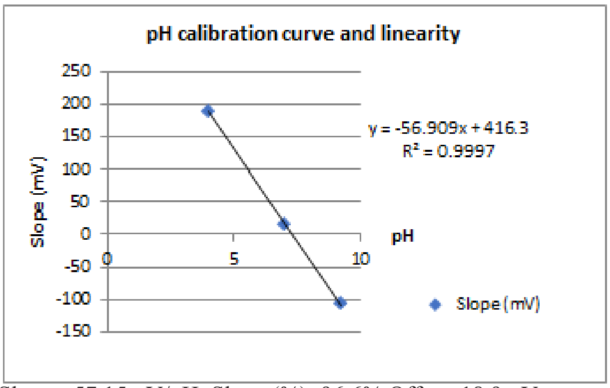
Brix Analysis

Brix of samples were measured by using digital refractometer (Brand Atago) at room temperature (25°C) and was reported as °Brix unit. It shows the sugar content of an aqueous solution or also known as total solubility (TA) in the sample. Hence, 1°Brix refer to 1g of sucrose in 100 g of sample whereby it is representing the strength of the sample as percentage by mass. Prior to every first run, the digital refractometer needed to be calibrated by using distilled water in order to check the exact reading which is 0.0°Brix. Then, approximately 1mL of vinegar sample was poured onto the sample hole of the digital refractometer. Then, the reading was measured once the start button is pressed. All the result was recorded in triplicate.

Acetic acid content analysis

Acetic acid calibration curve preparation

Calibration curve for acetic acid content was developed before the sample analysis. Different concentration of acetic acid standard which are 1ppm, 10 ppm, 30 ppm, 50 ppm and 70 ppm were prepared together with the internal standard (50 ppm) added in each prepared standard. Then, the standard prepared was pipetted approximately 500 uL in different vial prior to the standard injection in GC-TOF/MS. All results were recorded in triplicate and the mean was calculated. All results were then plotted into a graph that shows in Figure 2 by using Excel software in order to calculate the standard curve, gradient (m) and coefficient of determination (R^2). The result of



Slope: -57.15mV/pH, Slope (%): 96.6% Offset: 18.9mV

Figure 1. pH calibration curve and linearity

calibration curve is used to calculate the exact amount of acetic acid content in the commercial vinegar used by substituting in the formula.

Sample preparation

All samples were diluted from 100% to 0.5% (5000 ppm) concentration. 250 uL sample was pipetted into a 50 mL volumetric flask and mix with 1ml of 2500 ppm of acetonitrile as the internal standard to make the concentration of IS to be 50 ppm. Then, the mixture was top up with distilled water until the volume exactly 50 mL of the volumetric flask. Before the sample is ready to be injected, it needs to be mixed well by shaking the volumetric flask for approximately 10 second in order to completely homogenize the mixture. Pipette 500 uL 5000 ppm of sample in a vial and the sample was then injected into the GC-TOF/MS automatically by using auto sampler sample injection. All the results were recorded in triplicate.

GC-TOF/MS was performed using an Agilent time-of-flight mass spectrometer (Leco Software). Each sample (1uL) was injected into an Agilent 7890 Gas Chromatography system equipped to a Pegasus Time of Flight (tof) -Mass Spectrometer detector (Leco, St. Joseph, MI, USA). Compounds were separated on an Rtx-5MS capillary column (30 m x 0.25 mm; film thickness, 0.25 um). Helium carrier gas was used at a constant flow rate of 1.2 mL/min. The temperature program for metabolome analysis started with a 70°C for 1min followed by temperature ramping of 5°C/min to a final temperature of 100°C in split mode, which was maintained for 1 min. The acquisition rate was set to 30 spectra per second with the mass range of 20-1000m/z.

Ethanol Content Analysis

Ethanol calibration curve preparation

Calibration curve for ethanol content was

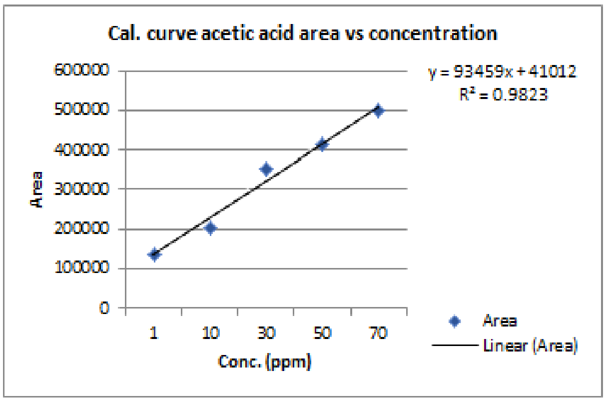


Figure 2. Calibration curve of acetic acid area vs concentration

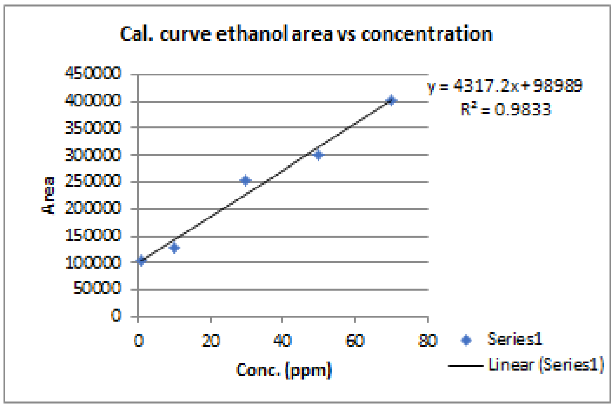


Figure 3. Calibration curve of ethanol area vs concentration

developed before the sample analysis. Different concentration of ethanol standard which are 1ppm, 10 ppm, 30 ppm, 50 ppm and 70 ppm were prepared together with the 50 ppm internal standard (ACN) added in every each standard. Then, the standard was pipetted approximately 5 mL into the headspace bottle and sealed with aluminium cap by using crimper. After that, the standard mixture was heated up to 100°C for 5 minutes to completely volatilize the compound in the standard prior standard injection in GC-TOF/MS. All the result was recorded in triplicate and the mean was calculated. Result then will be plotted into a graph that shows in Figure 3 by using Excel software in order to calculate the standard curve, gradient (m) and coefficient of determination (R^2). The result of calibration curve is used to calculate the exact amount of ethanol content in the commercial vinegar used by substituting in the formula.

Sample preparation

Pipette approximately 5 mL of 100% sample into 50mL volumetric flask to prepare 100000 ppm concentration and add with 1ml of 2500 ppm ACN as internal standard (IS). Then to pup with distilled water until the volume becomes 50 mL in the volumetric flask. Mix well the mixture by shaking gently for 10 second. The mixture of sample was then pipetted for approximately 5 mL into a 30 mL headspace bottle and tightly be sealed by crimper with an aluminium cap. The sample mixture was heated up to 100 °C for 5 min before the sample was injected to the GC-TOF/MS in order to allow the compound in the sample volatilised completely.

GC-TOF/MS was performed using an Agilent ion trap mass spectrometer (Leco Software). Each volatilized sample (6uL) was injected into an Agilent 7890 Gas Chromatography system equipped to a Pegasus Time of Flight (tof) -Mass Spectrometer detector (Leco, St. Joseph, MI, USA). Compounds were separated on an Rtx-5MS capillary column (30 m x 0.25 mm; film thickness, 0.25 um). Helium carrier gas was used at a constant flow rate of 1.2 mL/min. The temperature program for metabolome analysis started with a 70°C for 1min followed by temperature ramping of 5°C/min to a final temperature of 100°C in split mode, which was maintained for 1 min. The acquisition rate was set to 30 spectra per second with the mass range of 20-1000 m/z.

Results and discussion

pH values of the samples

pH value indicates a measure of the acid strength in food (Underhill, 1989). The lower the pH value shows the higher acidity. Based on the result recorded in Table 1, distilled vinegar recorded the highest acidity which is 2.51 while black vinegar showed the lowest acidity at pH 3.14. Synthetic vinegar is a vinegar that produced by using chemical where none of them came from natural sources so it is considered as synthetic acetic acid. So, based on the pH result reported, the vinegar that chemically produced is more acidic with the one that produced from the natural sources for example fruits. Mostly all vinegar samples are allocated in the right region of pH value for vinegar that is from 2.00 to 3.50. It is shown in Figure 4 *(http://chemistry.elmhurst.edu)*. Besides, the result also shown distilled vinegar is significantly different in compared with all other types of vinegar. Moreover, the result shows for same type of vinegar which is Wine A and Wine B are located at the same group. Hence, it is not significantly different between one another. Both samples are from same raw material. So, it is possible that vinegar of same sources and raw material will carry slightly same value of pH as clearly reported from the result. Moreover, pH value is also important information to categorise vinegar based on the acid strength. Research by Petsiou *et al.* (2015) stated that vinegar is good for increased glucose uptake, improved insulin sensitivity and also

Table 1. Analysis of pH value, total solubility (Brix), acetic acid and ethanol content in vinegar samples.

Sample	Mean ± σ			
	pH	Brix	A. acid (%)	Ethanol (%)
Apple Vinegar	3.08 ± 0.01^{abc}	3.60 ± 0.00^{h}	0.1042 ± 0.0237^{a}	0.0195 ± 0.0012^{b}
Distilled Vinegar	2.52 ± 0.01^{g}	2.90 ± 0.40^{i}	0.0972 ± 0.0135^{b}	0.0107 ± 0.0022^{b}
Wine Vinegar A	2.89 ± 0.04^{d}	4.10 ± 0.00^{g}	0.0424 ± 0.0070^{b}	0.0027 ± 0.0003^{b}
Chardonnay Vinegar	2.79 ± 0.01^{e}	25.20 ± 0.00^{d}	0.0792 ± 0.0169^{b}	0.0084 ± 0.0001^{b}
Fig Vinegar	2.77 ± 0.01^{ef}	2.10 ± 0.00^{j}	0.0537 ± 0.0023^{b}	0.0160 ± 0.0027^{b}
Wine Vinegar B	2.83 ± 0.01^{de}	4.40 ± 0.00^{g}	0.1276 ± 0.0264^{a}	0.0556 ± 0.0143^{b}
Synthetic Vinegar	2.70 ± 0.08^{f}	2.47 ± 0.06^{j}	0.0253 ± 0.0032^{b}	0.0000 ± 0.0000^{b}
Malt Vinegar	2.90 ± 0.01^{d}	5.57 ± 0.06^{f}	0.0646 ± 0.0162^{b}	0.0418 ± 0.0093^{b}
Black Vinegar	3.15 ± 0.02^{a}	40.73 ± 0.06^{a}	0.0395 ± 0.0012^{b}	0.0109 ± 0.0008^{b}
Merlot Vinegar	3.02 ± 0.01^{c}	27.07 ± 0.06^{b}	0.0397 ± 0.0031^{b}	0.5911 ± 0.0668^{a}
Cherry Vinegar	3.11 ± 0.01^{ab}	26.50 ± 0.00^{c}	0.0341 ± 0.0051^{b}	0.4699 ± 0.1100^{a}
Balsamic Vinegar	3.06 ± 0.01^{bc}	22.90 ± 0.00^{e}	0.0391 ± 0.0046^{b}	0.5768 ± 0.0993^{a}

Values expressed as mean ± standard deviation of samples analyzed in triplicate

lowered the triglycerides in the blood after a meal. (Petsiou *et al.*, 2015) Other than that, the impact of an acid on food flavour is much more determined by titratable acidity and also pH. In this study, only pH measurement was conducted because the aim is to know the acid strength rather than the titratable acidity. Titratable acidity is to measure the total acid concentration which means, as mentioned earlier; acetic acid is the major acid in vinegar. Hence, acetic acid content is already tested by using quantitative analysis by using GC-TOF/MS instrument. The constraints of various colour of samples are also contribute to the limitation to performed titratable acidity test. Colour diversity in the sample is due to the diversity of raw materials used during the vinegar production. Besides, research by Underhill (1989) also stated that the pH of vinegar gives an indication of its resistance to microbial attack. The total acidity of vinegar is expressed as acetic acid. Acetic acid is one of the organic acid and a monocarboxylic acid. According to Adam *et al.* (2004) organic acids are considered weak acids meaning the antimicrobial effect of organic acids is mainly caused by its undissociated forms. They passively diffuse through the bacteria cell wall and internalizing into neutral pH dissociating into anions and protons. Release of the protons causes the internal pH to decrease which exert inhibitory effects on the bacteria. Organic acids that are used to inhibit spoilage bacteria in meat are applied by spraying and dipping techniques. In a study conducted by Brandley, the addition of citric acid and acetic acid each reduced the growth of Enterobacteraceae. (Lingham, 2013). While, according to Hufnagel and Hagmann (2008), pH is strongly dependent on organic acids such as acetic acid, malic acid or lactic acid level.

Brix values of the samples

Brix value is referring to the total solubility content in vinegar. The total solids can be referred to refractive index (RI). According to Aurand *et al.* (1987), the refractive index of a sugar solution is a direct measure of its concentration. Different foods product will indicate different value of °Brix in various ranges, the higher the value the sweeter the product is. (Wong *et al.*, 2013). While study by Liu *et al.* (2007) also stated that Brix value is also used as an index for amount of sugar. Although it cannot quantify the total sugar of every single type of sugar but Brix can be used as indicator to determine the amount of total sugar in the sample based on the total soluble solid (TTS). Moreover, as stated by Sadler and Murphy (2010), Brix influences the flavour of food, but the best predictor of an acid flavour impact is the Brix/acidity ratio or taste index. Thus, different vinegar has different colour especially from

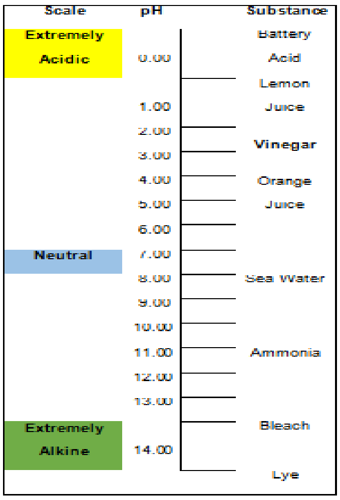


Figure 4. Scale for pH range of common substance.
Source: *(http://chemistry.elmhurst.edu/vchembook/184ph.html)*

the different raw material used in the production of vinegar. It is because there are some vinegar is clear in colour and there are also some is very opaque in colour. This factor will contribute to the different Brix value range. In general, Brix is a measurement of the amount of sugar in a solution per weight of total solution. Whereby, a solution that is 10 °Brix has 10 g of sugar per 100 g of solution or if the solution is simple sugar and water, it will be 90 g of water and 10 g of sugar. From the result in Table 1 shows, the highest Brix value is black vinegar which is 40.73°Brix while the lowest value is Fig vinegar which is only 2.10°Brix. Based on the physical observation, black vinegar is very dark in colour in compared with the others vinegar samples. Moreover, all the vinegar samples are significantly different between one another because the mean values are not allocated in the same group.

Acetic acid content of the samples

According to research by Jo *et al*. (2015) it was stated that, a product is considered as vinegar if acetic acid makes up <4.0% (w/v) of the total acid content. Acetic acid which is the main component in the vinegar will contribute to the sour taste of the vinegar. Commercial vinegars are classified into four groups, based on the total acid content, of low (4-5%), moderate (6-7%), double-strength (13-14%) and triple strength (18-19%). High acidity with a total acidity of >10% has several advantages, including the absence of an off-flavour and reduced amount used in food. High acidity vinegar is generally used in the food industries which consume large quantities of vinegar. Based on result recorded in Table 1, the lowest acetic acid amount is Synthetic vinegar which is only 0.0253% while the highest amount of acetic acid content is Wine B vinegar which is 0.1276%. Requirement from Malaysian Food Act and Regulation, acetic acid content must be at least 4%. Other than that, requirement stated by FAO/WHO, a product to be labelled as vinegar if the acetic acid content is 6% and with a maximum residual alcohol content of 0.5% v/v for wine vinegar and 1% v/v for other vinegars. So, from the result, study for amount of acetic acid content in commercial vinegar was conducted by using GC-TOF/MS instrument none of the sample recorded the percentage of acetic acid of at least 4% or more. It might be the sensitivity of the instrument and the method used for acetic acid determination is not high enough. This study was run more than once in order to make sure the result consistency and the result recorded is still the same. Thus in the related with pH value, the amount of acetic acid is not directly proportional with the pH value. As mentioned earlier, pH is to determine the acid strength while the acetic acid content is to determine the amount of specific acid content in the vinegar sample. GC-TOF/MS instrument is to study the quantification of specific compound in the vinegar sample included acetic acid. So it is cannot considered as comparable with the pH value of the vinegar sample.

Ethanol content of the samples

Alcohol which is known as ethanol in this study were analysed by using GC-TOF/MS instrument. In Islam, alcohol is considered as prohibited to be consumed by Muslim if the amount exceeds the allowable limit which is stated by Fatwa Committee National Council of Islamic Religious Affairs Malaysia to be less than 1% in beverages, and 0.5% for flavouring or colouring substances for the purpose of stabilization. Different country has different standard in determining the permissible amount of alcohol content. Whereby in ASEAN countries like Malaysia, Indonesia and Thailand the allowable amount of ethanol is only 1% while in Singapore the benchmarked level is 0.5% and other countries like Brunei, United Kingdom and Canada, alcohol content is totally prohibited to be present in Halal food (Jamaludin *et al*., 2016). Generally, vinegar is one of the examples of product that produced from two stages of fermentation process which is alcoholic fermentation and acetic acid fermentation. The issue on the halalness of vinegar product is either the alcohol content is completely been used during the first stage fermentation or there are still leftovers that stays until the end product. Thus, by focusing requirement by Fatwa Committee National Council of Islamic Religious Affairs Malaysia (FATWA), natural occurrences of ethanol in food products are acceptable, if ethanol contents are less than 1% for beverages while for flavouring or colouring substances is only 0.5% (FATWA Media Statement, 2011). From this research, the significant test conducted shows result in Table 1 the amount of ethanol content in all commercial vinegar samples are not exceeding the requirement as stated by the FATWA. Based on the result stated, the highest amount of ethanol content is in Merlot vinegar which is 0.5911% while the lowest amount is synthetic vinegar which is no (0%) amount of ethanol content appeared. Synthetic vinegar sample that has been used in this research is one of the vinegar that locally produced in Malaysia and already has halal certification by JAKIM. Hence, only Cherry vinegar was significantly different ($p<0.05$) from the others vinegar samples that have been used in the research in the amount of its ethanol content. On the other hand, eventhough the ethanol content for the samples are acceptable but in Islam the intention of making food is also considered when determining the halal status. This is especially emphasized in the production of vinegar; whether it came from haram sources e.g. wine (FATWA Media Statement, 2011). If the intention of producing the product was not towards haram, and the materials came from non-haram sources, the product is considered halal. However, if the product is from haram sources like wine or liquor then the product is considered as haram even if the end product has permissible amount of ethanol content. Besides, as stated by Solieri and Guidici (2008) the presence of ethanol in vinegar product will contribute to the aromatic profile.

Conclusion

To conclude with, vinegar is among the popular ingredient that has been used in cooking or even for industry. As one type of fermented product, vinegar is considered as crucial food ingredient to determine for the halalness. It is important to study the sources and process that has been used in the vinegar production. Based on this study, the amount of alcohol content in the commercial vinegar that has been analysed is consider followed the requirement. None of the samples are exceed from the allowable limit. Thus, the other factor that needs to take into consideration is the sources. The product is considered halal if the source is from the halal material and none of the inferences that can cause syubhah or even haram to the end product.

Acknowledgments

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