

RESEARCH ARTICLE

FUNCTIONAL CONSTITUENTS OF FRUIT JUICES AFTER ACETIC ACID FERMENTATION

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Received: 12 August 2021; Accepted: 02 December 2021

ABSTRACT

Fruits are dense sources of nutrients with many functional properties. Acetification of fruit juices generates different types of fruit vinegar with a variety of flavors and uses. In this study, Alcoholic juices fermented by employing *Saccharomyces cerevisiae* were subjected to acetification by pre-cultured *Acetobacter pasteurianus* PP21 at both 30 °C and 36 °C temperatures and they were tested for their Brix value, titratable acidity, alcohol content, antioxidant activity, phenolic content and flavonoid contents. Compared to the acetic acid production at 36 °C, both mango and jackfruit juices showed their peak acetic acid production of $4.5 \pm 0.015\%$ (w/v) and $4.2 \pm 0.04\%$ (w/v) respectively, at 30 °C. The final Brix value of mango and jackfruit juices subjected to acetification at 30 °C and 36 °C was found to be constant at 3.6, 5.7, and 3.1, 5.7 °Bx respectively. Furthermore, the final alcohol level of all the vinegar samples was found to be less than 0.5% (v/v). Moreover, the antioxidant activity, phenolic content and flavonoid contents of both mango and jackfruit juices after acetification were significantly higher ($p < 0.05$) at 36 °C compared to vinegar produced at 30 °C. As per the results, both mango and jackfruit juices can be successfully used as a potential source of functional constituents in vinegar production at both 30 °C and 36 °C.

Keywords: Acetic acid bacteria, Bioactivity, Fermentation, Fruit vinegar, Jackfruit, Mango

INTRODUCTION

Mango (*Mangifera indica*) and Jackfruit (*Artocarpus heterophyllus*) are popular tropical fruits having higher nutritional significance, a delicious taste and aroma, and benefits to human health (Masibo and He, 2008). Major carbohydrates found in ripen mango flesh are sucrose, glucose and fructose (Quintana *et al.* 2021; Torres-León, 2016). Additionally, it contains dietary fiber such as cellulose, hemicellulose and lignin, and pectin in smaller amounts (Bello-Pérez *et al.* 2007). Functional constituents of mango flesh include phenolic acids such as gallic, vanillic, syringic, protocatechuic like hydroxybenzoic acid derivatives, and p-coumaric, chlorogenic, ferulic, and caffeic acids like hydroxycinnamic acid derivatives (Burton-Freeman *et al.* 2017); Flavonoids and other polyphenolic compounds such as catechins, kaempferol, anthocyanins, and tannic acid (Haytowitz *et al.* 2018;

Ediriweera *et al.* 2017); and pigments such as chlorophylls, carotenoids, and flavonoids (Nelson and Cox, 2017; Choo, 2018; Ellong *et al.* 2015). The variety of mango and their ripening stage plays an important role in the amounts of carbohydrates present in the flesh, for the generation of aroma compounds and the ethylene production (Maldonado-Celis *et al.* 2019).

The nutritional and functional significance of jackfruit flesh is associated with the carbohydrates, proteins, lipids, vitamins and minerals, and the phytochemicals present in it. The fruit contains starch and dietary fiber which are increased with the fruit maturity (Ranasinghe *et al.* 2019). It is a rich source of many phytochemicals including phenolic compounds, carotenoids, flavonoids, volatile acids, sterols, and tannins (Chandrika *et al.* 2004; Amadi *et al.* 2018). Different types of carotene present include β -carotene, α -

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carotene, β -zeacarotene, α -zeacarotene and β -carotene-5,6 α -epoxide and a dicarboxylic carotenoid and crocetin (Chandrika *et al.* 2004).

Fermentation of alcoholic juices of fruits into fruit vinegar like products have become prominent worldwide. Different types of fruit vinegar are being produced with the variety of raw materials. Acetification of fruit juices enables the retention of functional characteristics of the raw materials compared to other processing techniques applied on fruit product development and also can be used as a way of treating excessive fruit surplus. *Acetobacter* and *Gluconacetobacter* are the most popular genera of Acetic acid bacteria (AAB) used in vinegar fermentation. They have been identified as powerful in oxidizing ethanol and in tolerating the accumulated acetic acid concentration in the medium (Lynch *et al.* 2019). Usually, the industrial acetic fermentation is performed around 30 °C using mesophilic strains that are capable of growing and performing oxidative fermentation better at this temperature. Increasing atmospheric temperatures have possess some practical problems to the fermentation industries, as they require huge cooling systems to maintain the fermentation temperatures for the optimum functioning of acetic acid bacteria. Instead, recent studies have identified the potential of some acetic acid bacterial strains to perform acetic acid production at higher temperatures.

There are many studies conducted on the development and compositional analysis related to various fruit vinegar (cider vinegar, grape vinegar, strawberry vinegar) based on the acetic acid fermentation (Yunyang 2005; Budak *et al.* 2015; Pure and Pure 2016). However, the presence of the functional constituents of mango and jackfruit juice after fermentation are limited in the previous literature. Therefore, this study identifies the functional constituents present in mango and jackfruit juice after subjecting to alcoholic and acetic acid fermentation using *Acetobacter pasteurianus* PP21 at both 30 °C and 36 °C.

MATERIAL AND METHODS

Substrates

Fresh quality, fully ripen (ripen stage 04) mangoes of *siini amba* variety and fully ripen jackfruit were collected from the local market, Matara, Sri Lanka. All fruits were peeled, cleaned well with tap water, and cut into small pieces. Then they were blended with water (1:1 ratio) until a homogenous mixture was obtained. Unwanted particles were removed by filtering the blended juice using a clean cheesecloth and centrifuging the filtrate at 5,500 rpm for 7 min. The initial Brix value of both mango and jackfruit juice was adjusted to 12 °Bx by adding table sugar.

Preparation of the inoculum

Both yeast and AAB inoculums were prepared according to a procedure described by Konate *et al.* (2015) with some modifications. For the preparation of yeast inoculum, 0.5 g of yeast powder was added to a 100 mL of 10% sucrose (w/v) solution, incubated for one hour at 30 °C, and 20 mL of the yeast inoculum was added to inoculate one liter of fruit juice. *Acetobacter pasteurianus* PP21 was pre-cultured in YPGD medium (0.5 g of yeast extract, 0.5 g of polypeptone, 0.5 g of glycerol, and 0.5 g of glucose per 100 mL tap water) at 30 °C for 72 hrs, and 50 mL of the culture was used to inoculate one liter of the alcoholic juice.

Alcoholic and acetous fermentation

Fruit juices were separately inoculated with revitalized yeast and allowed to ferment for 72 hrs (Konate *et al.* 2015) at room temperature in a 2 L conical flask under static conditions. Afterwards, 200 mL of alcoholic juices; mango (4.9 °Bx), and jackfruit (5.7 °Bx) was transferred into a 500 mL Erlenmeyer flask, and 10 mL of pre-cultured AAB inoculum was added. Flasks were sealed with a cotton plug to prevent any contaminations. At both 30 °C and 36 °C fermentation was carried out to identify the optimum temperature conditions to produce mango and jackfruit vinegar. Total sugar content, Brix value, and titratable acidity of each sample were measured daily during fermentation.

After reaching the expected acidity level (around 4.0% w/v) the obtained vinegar samples were centrifuged at 5,500 rpm for 10 min to remove all the residues and yeast cell mass. Finally, the acetification process was stopped by pasteurizing the harvested vinegar samples in a shaking water bath at 72 °C for 20 min.

Analysis of vinegar samples

Brix value, titratable acidity, and Alcohol content

The Brix value of each sample was measured using a digital pocket refractometer (Atago, PAL-22S, Japan). As described by Chun *et al.* (2014) acid-base titration method was used to measure the Acetic acid concentration, by titrating with 0.1N NaOH using phenolphthalein as the indicator and the total acidity was expressed as the amount of acetic acid present. The alcohol content of the samples was measured using an alcohol hydrometer (Fisherbrand, 11-590, US). All the analysis was done in triplicates.

Determination of antioxidant capacity

The method given by Brand-Williams *et al.* (1995) was used to identify the antioxidant activity of vinegar samples using DPPH assay, with few modifications. Initially, the samples were serially diluted with methanol to obtain 5, 10, 15, 20 mg/mL dilution solutions. Two hundred fifty microliters of sample or control (methanol) were added to 2.75 mL of 50 µmol DPPH solution (1.97 mg in 100 mL of methanol) separately, and the absorbance at the steady-state was measured with UV visible spectrophotometer (HACH, DR3900, Germany) at 517 nm. The inhibition percentage of the samples was calculated according to equation 01, and the inhibition percentage was plotted against sample concentration. The amount of sample required to inhibit 50% of the initial DPPH (EC50 value) was calculated from a calibration curve determined by linear regression.

Percentage inhibition =

$$100 - \left[\frac{\text{Abs blank} - \text{Abs sample}}{\text{Abs blank}} \right] \times 100 \quad \text{---Eqn. 1}$$

Determination of total phenolic content

Folin-Ciocalteu reagent was used to determine the total phenol content of samples using the methodology given by Singleton *et al.* (1999) with some modifications. Two milliliters of Folin-Ciocalteu reagent which has been diluted previously with 10-fold distilled water was mixed with an accurately diluted 400 µL of the sample. After one minute and within eight minutes period, 2 mL of 7.5% sodium bicarbonate solution was added to stop the reaction, and then volumed up to 10 mL using distilled water. This mixture was placed at dark for 120 minutes, and the absorbance was measured at 760 nm. Results were expressed as mg of gallic acid equivalents per liter (mg GAE/l) using the equation obtained by linear regression of gallic acid standard curve prepared by serially diluted gallic acid solution (250, 125, 62.5, 31.25, 15.625, 7.812, 3.906, 1.953 µg/mL)

Determination of total flavonoid content

A colorimetric assay was used to determine the total flavonoid content according to a method described by Zhishen *et al.* (1999). One milliliter from properly diluted aqueous or methanolic fractions of the samples or catechin standard solutions or (water or methanol) the blank solution was added to 10 mL volumetric flask, and 4 mL of distilled water was added. At the beginning, 0.3 mL of 5% NaNO₂ was added, and 5 min later, 0.3 mL of 10% AlCl₃ was added to the mixture. At the 6th min, 2 mL of 1 M NaOH was added, and the solution was volumed up to 10 mL with distilled water and the mixture was mixed well. After 15 min period, the absorbance was determined at 510 nm. The total flavonoid content was expressed as milligrams of catechin equivalent per 100 mL of the sample by equation obtained by linear regression of catechin standard curve prepared by serially diluted catechin solution (250, 125, 62.5, 31.25 µg/mL).

RESULTS AND DISCUSSION

Production of mango and jackfruit vinegar at 30 °C and 36 °C

Changes in the chemical composition of the developed mango and jackfruit vinegar were measured in terms of Brix value, alcohol

content, and titratable acidity at 30 °C and 36 °C, and the results are summarized in fig. 01. Simultaneous vinegar production consists of two sequential fermentation steps where the initial alcoholic fermentation happens with the yeast action, and subsequently, the acetification occurs by the action of AAB. As per the results in Fig. 1, initially, the ethanol production has occurred, and both mango and jackfruit wines gave more than 4% (v/v) alcohol production at both tested temperatures. However, the highest alcohol production was observed in mango wine produced at 30 °C, and it was significantly higher ($p < 0.05$) than the other wine types produced. Furthermore, both mango and jackfruit wines gave a comparatively higher amount of alcohol production at 30 °C. This is mainly due to the favorable temperature conditions for the fermentation of *Saccharomyces cerevisiae* at 30 °C. According to a study by Li *et al.* (2012), the ethanol content of three different mango wines produced from three types of mango juice (where initial Brix varied between 13.25 ± 0.00 to 16.82 ± 0.00 °Bx) was found to be ranged from 6.33 ± 0.96 to $8.05 \pm 1.15\%$ (v/v). Accordingly, in the current study, a similar pattern of alcohol production by *S. cerevisiae* was observed at both tested temperature levels. Expected alcohol level when a traditional substrate is being used in the production process is therefore deviated to a higher magnitude in case of fruit vinegar production.

Brix value represents the level of soluble solids present in a solution. Fruit juices primarily contain sugars as their soluble solids and thus, the Brix value shows the estimates of sugar in a particular fruit juice sample. As it is shown in Fig. 1, with the progression of the alcohol production, Brix value of all the vinegar samples was started to decline. Reduction of the Brix value with the fermentation time indicates that more sugar is being utilized by yeast to convert them into alcohols (Saha and Banerjee 2013). However, the available sugar samples in all four vinegar samples were not completely oxidized, and thus the Brix value was found to remain constant after the peak alcohol production of

mango and jackfruit vinegar produced either at 30 °C or 36 °C, thus giving a sweet taste to the final product. As it is shown in Fig. 1, the Brix value of mango and jackfruit vinegar produced at 30 °C was found to remain as 3.6 °Bx and 5.7 °Bx respectively. Furthermore, that of mango and jackfruit vinegar samples produced at 36 °C was also found to be constant at 3.1 °Bx and 5.7 °Bx respectively. Additionally, the Chinese National Standard (2005) stated that fruit vinegar should contain not less than 1.2% salt-excluded soluble solids, and as per the results shown in Fig.1, the remaining total soluble solids of all the vinegar samples were found to be within the standard level. Thus, the results highlighted that a higher amount of sugar is converted efficiently into alcohol in mango juice fermentation compared to jackfruit juice. Moreover, according to Kim *et al.* (2012), the readily available sugar in fruit vinegar is glucose while fructose, sucrose, maltose, and other sugars affecting the sweetness exist in smaller quantities.

Conferring to the results, the total titratable acidity of the alcoholic juice of mango and jackfruit at the time of acetic acid bacteria inoculation was found to be 0.51 g/L and 0.86 g/L respectively. During the progression of fermentation at 30 °C, the mango juice showed its peak acetic acid production of $4.5 \pm 0.015\%$ (w/v) on the 7th day of the fermentation. Comparatively, at the same temperature level, the peak acetic acid production in jackfruit vinegar ($4.2 \pm 0.04\%$ (w/v)) was observed on the 6th day of the fermentation cycle. The increase in the acetic acid level in all four vinegar types is due to the oxidation of available ethanol by AAB under aerobic conditions. Thus, ethanol has become the main source of carbon for AAB, and this leads to a drastic reduction in ethanol level in the vinegar media with the progression of acetic acid fermentation (Fig.1). However, as per the results shown in Fig. 1, the residual alcohol level of all four vinegar types was found to be less than 0.5% (Joint FAO/WHO Food Standards Programme 2000).

Furthermore, the increase in titratable acidity during the acetification is mainly due to the accumulation of organic acids, particularly acetic acid produced by the AAB. However, according to Chidi *et al.* (2015), a small amount of acetic acid could be produced by yeast as a by-product of alcoholic fermentation. Nevertheless, with the continuation of the fermentation process, a reduction of the acetic acid level was found especially at 30 °C which is due to the overoxidation of the produced acetic acid (Gullo *et al.* 2006). In contrast, compared to the acetic acid production at 30 °C, both mango and jackfruit vinegar showed significantly low ($p < 0.05$) amount of acetic acid production at 36 °C, and it was ranged between 3.65 ± 0.162 to 3.74 ± 0.00 % (w/v). Moreover, according to a study done by Bouatenin (2021), the titratable acidity of mango and papaya vinegar was reported as $6.12 \pm 0.14\%$ and $5.88 \pm 0.42\%$ (w/v). According to the Chinese National Standard (2005), the acidity level of fruit vinegar should be not less than 4.5% (calculated as acetic acid). However, as per the obtained results, only the mango vinegar produced at 30 °C could be able to reach the standard level of acetic acid.

Bioactivity of the developed vinegar samples

Total phenolic content, flavonoid content, and the antioxidant activity of both mango and jackfruit vinegar produced at 30 °C and 36 °C are summarized in Table 1.

Total phenolic content

As per the results shown in Table 1, compared to the vinegar produced at 30 °C, and 36 °C, the unfermented fruit juice of mango and jackfruit are having a significantly ($p < 0.05$) higher level of total phenols. Moreover, the total phenol content of unfermented mango juice (1590.826 ± 11.161 mg GAE/L) was found to be three times greater than that of unfermented jackfruit juice (590.645 ± 16.623 mg GAE/L). Furthermore, the phenol content of mango was regarded as high when compared with other phenol rich fruits such as pomegranate (1387 mg GAE/L) (Ordoudi *et al.* 2014) and

blueberries (86.7 ± 1.56 mg GAE/100 mL) (Su and Chien 2007) that have been utilized as raw materials in the production process of vinegar. However, in the current study, the total phenolic content was found to be significantly less than their unfermented fruit juices where a five times reduction is being observed in mango vinegar and the reduction in jackfruit vinegar was only two times.

There are many types of research done on the phenolic content of alcoholic fermentation. However, little is known about the variation in phenolic content in the acetification process. According to the study by Su and Chien (2007) the total phenolic content of the blueberry vinegar (98.1 ± 1.66 mg GAE/100 mL) was found to be significantly higher than that of blueberry juice and blueberry wine (86.7 ± 1.56 and 85.8 ± 1.54 mg GAE/100 mL respectively). Furthermore, a study done by Ubada *et al.* (2013) has shown that the type of container used in the acetification process affects the total phenolic content produced during acetification. According to them, the use of glass vessels reported the lowest values for total phenolic content, and the use of wooden barrels has increased the tested parameters. Moreover, they have found that the vinegar produced using cherry barrels showing a higher antioxidant activity while the vinegar produced by oak barrels showing a higher total phenol content. According to them, the difference in the bioactivity is mainly due to the difference in porosity wood that facilitates the oxygen permeation. However, in the current study, acetification of fruit vinegar was done using glass vessels, and thus, as reported by Ubada *et al.* (2013), the acetic acid production will be badly affected by the less porous nature in glass vessels, and concurrently a reduction in phenol content during acetous fermentation was observed. Moreover, according to Ubada *et al.* (2013), centrifugation and pasteurization of vinegar samples will also reduce the antioxidant compounds, and thus, the reduction in total phenolic content in the current study could be also due to centrifugation and pasteurization of the produced vinegar samples.

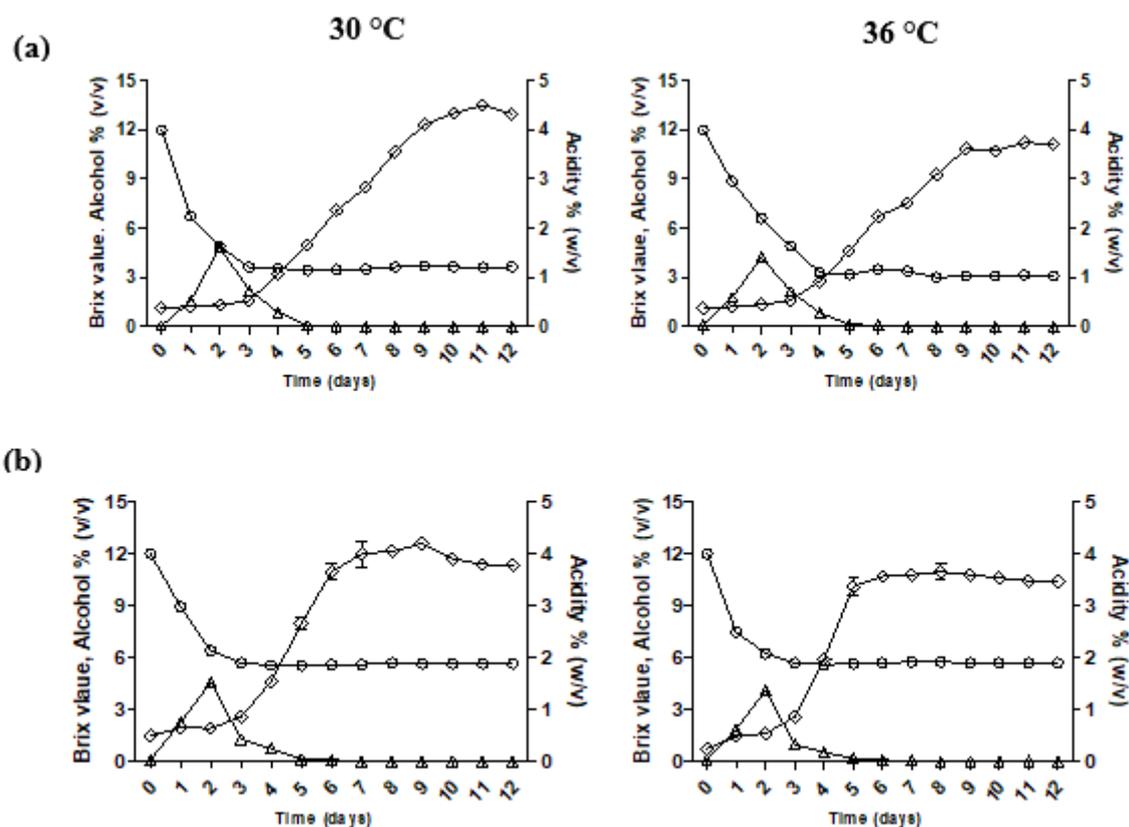


Figure 1: Changes of Brix value (circle), alcohol (triangle) content, and titratable acidity (square) during alcoholic and acetous fermentation of mango (a) jackfruit (b) at 30 °C, and 36 °C

As per the results of the current study (Table 1), mango vinegar produced at 36 °C is having a significantly higher ($p < 0.05$) level of total phenols compared to the production at 30 °C. Moreover, when considering the jackfruit vinegar, when compared to the total phenol content at 36 °C, a significantly higher level of total phenols content was observed at 30 °C (333.869 ± 19.657 mg GAE/L). Higher total phenol content is an indication of well-fermented vinegar (García-Parrilla, 1997) and thus, effective vinegar fermentation with desirable phenolic content could be reached at 36 °C for mango vinegar and 30 °C for jackfruit vinegar.

Total antioxidant activity

DPPH radical scavenging activity is measured as the inhibition percentage of the free radicals by antioxidants, and thus, the EC₅₀ value indicates the concentration required to have a 50% antioxidant effect (Chen *et al.*

2013) was calculated. The obtained results are summarized in Table 01. As per the results, compared to the vinegar samples, the unfermented mango juice and jackfruit juice showed a higher DPPH radical scavenging activity. Moreover, the DPPH radical scavenging activity (EC₅₀ value of 2.24) was highest in unfermented mango juice. Furthermore, the results demonstrate that the antioxidant activity of all four types of vinegar is being significantly reduced with the fermentation process. Larrauri *et al.*, (1996) has stated that the antioxidant activity is depending on the structural constitution of the phenolic compounds available. As it is clearly shown in Table 1, the total phenol content of all the vinegar samples was found to be significantly reduced with the acetification process. Thus, the reduction in total phenol content during acetic acid fermentation has led to the reduction in the radical scavenging activity of the produced vinegar. However, in

Table 1: Antioxidant activity, total phenolic content, total flavonoid content of Mango and Jackfruit; raw juice, vinegar at 30 °C and 36 °C

		EC ₅₀	Total phenols mg GAE/L	Total FC mg CE/100 mL of sample
Mango	Raw juice	2.24 ^c	1590.826 ^a ± 11.161	46.210 ^a ± 3.481
	Vinegar at 30 °C	10.96 ^b	340.826 ^c ± 6.907	19.515 ^c ± 6.577
	Vinegar at 36 °C	11.73 ^a	362.997 ^b ± 7.807	20.727 ^b ± 2.362
Jackfruit	Raw juice	3.09 ^c	590.645 ^a ± 16.623	48.879 ^a ± 4.001
	Vinegar at 30 °C	18.02 ^a	333.869 ^b ± 19.657	14.818 ^c ± 2.405
	Vinegar at 36 °C	15.97 ^b	314.602 ^c ± 32.071	19.818 ^b ± 3.721

Data values are represented as mean ± SE. Different lowercase letters under the same column under each vinegar type indicate a statistically significant difference ($p < 0.05$).

contrast, a study by Su and Chien (2007) has reported that the antioxidant and/or antiradical activity of a substance will be enhancing during acetification through the production of phenolic dimers and oligomers through oxidative phenolic reactions. Conversely, a study by Ordoudi *et al.* (2014) on pomegranate juice on alcoholic and acetic acid production, and found that the DPPH radical scavenging activity of pomegranate vinegar was 55% lower than the fresh juice. They further describe that strong radical scavengers could be partially degraded during both alcoholic and acetic acid fermentation processes. However, during the current study, such oxidative reactions could be affected due to the use of glass vessels that determines the aeration condition, where eventually a reduction in antioxidant activity is been occurred.

Total flavonoid content

The total flavonoid content of the unfermented mango and jackfruit juice and the vinegar produced at the tested temperature levels were also found to be behaving in a similar profile to the total phenolic content and the DPPH radical scavenging activity. According to the findings of Bakir *et al.* (2016), the total flavonoid content of both grape vinegar and apple vinegar was found to be decreased with the acetification, and the results of the current study are in accordance with it. Further, a study by Wu *et al.* (2017) on vinegar production using sweet potato, also showed that the total phenolic content,

total flavonoid content, and DPPH radical scavenging activities were reducing over the progression of fermentation. They further describe that the difference in DPPH radical scavenging activity may be due to the production of acetic acid during acetification, as well as the antioxidants activity found in acetic acid itself.

CONCLUSION

The results of the study highlights that the possibility of mango and jackfruit juices as ideal substrates to perform acetic acid fermentation with sufficient composition of functional constituents; the polyphenols, flavonoids and antioxidants at both 30 °C and 36 °C. Studies on the sensory parameters, and the microbiological aspects of fermented fruit juices are needed to be carry out; specially to produce marketable products such as vinegar, with satisfactory organoleptic parameters, health benefits and shelf life.

ACKNOWLEDGEMENT

This work was financially supported by a grant from the National Research Council of Sri Lanka (NRC 16-025).

AUTHOR CONTRIBUTION

AB and ND carried out the experiment. BNP designed the research work, and both BNP and ND wrote the manuscript.

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