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Research article

Production of Antioxidant Rich Tomato Vinegar: An Alternative to Coconut Vinegar in Culinary Production

Perumpuli Arachchige Buddhika Niroshie Perumpuli[1]*, Mirihana Arachchige Amal Buddhika[1] and Migelhewa Nidarhsa Kaumal[2]

[1]*Department of Food Science and Technology, Faculty of Agriculture, University of Ruhuna, Kamburupitiya, Sri Lanka*
[2]*Department of Chemistry, Faculty of Science, University of Colombo, Sri Lanka*

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Abstract

Tomato is an antioxidant-rich highly perishable fruit, and it is a crop with high postharvest losses due to its tender appearance and surplus production. Thus, to allow value addition and to reduce its postharvest losses, tomato vinegar, a novel type of vinegar to Sri Lanka was developed. During the study, locally available tomato was utilized as the major raw material, and tomato pulp with 10.7°Brix was prepared. Alcoholic and acetous fermentation was performed using Baker's yeast and *Acetobacter pasteurianus* PP21 respectively, at 30 and 36°C. Total acidity, total sugar content, total soluble solids, alcohol content, total phenols, total antioxidant activity (EC_{50} value), and total flavonoid contents of the developed tomato vinegar were examined, and the results were compared with those of commercially available coconut vinegar. The results revealed that the total acetic acid content of vinegar produced at 30 and 36°C was 5.139±0.145 (w/v) and 4.12±0.32 (w/v), respectively. The EC_{50} value of the tomato vinegar produced at 36°C (13.87±0.63) was found to be significantly lower than that of commercial coconut vinegar (101.5±1.66), indicating a high antioxidant activity. Moreover, compared to coconut vinegar, a higher phenolic content, 365.020±5.53 mg GAE/l, was found in the developed tomato vinegar. The results of the sensory evaluation revealed that the developed tomato vinegar was significantly better than the commercial coconut vinegar, either as its raw form or as used in the production of Sri Lankan traditional dishes. Thus, the developed tomato vinegar is a good alternative to coconut vinegar in Sri Lankan cuisine.

*Corresponding author: Tel.: +94-712424485
E-mail: buddhikap@agri.ruh.ac.lk

1. Introduction

Vinegar is a popular acidulous condiment that is extensively used in many countries due to its wide availability in different varieties. The history of vinegar goes back for more than 10,000 years, and it has been used as a preservative, flavoring agent, condiment, beverage, and folk medicine [1]. Vinegar is a rich source of many bioactive compounds such as organic acids, melanoidins, polyphenols, tryptophol, ligustrazine, and caffeoylsophorose [2]. The presence of such bioactive compounds gives vinegar a range of therapeutic effects including antioxidative, antidiabetic, antimicrobial, antiobesity, antitumor, antihypertensive, and cholesterol lowering properties [2-4].

Vinegar is widely produced from rice, malt, apples, wine, coconut and various other raw materials that contain sugar or starch via a two-stage fermentation process involving an initial phase of ethanol production by yeast and subsequently the production of acetic acid by acetic acid bacteria [5]. Besides, simultaneous vinegar fermentation is widely used in traditional vinegar production, where yeast and acetic acid bacteria (AAB) are inoculated simultaneously into the fermenting media [6]. Further, the production of vinegar is an exothermic process and the acetic acid yield and the quality of the vinegar are highly dependent on the temperature of the fermenting media. Therefore, production of vinegar using thermophillic AAB strains that can grow and produce acetic acid affect the quality of the final product whilst reducing cooling expenses in industrial vinegar production [7].

Nowadays, there is a growing demand for vinegars made from different types of fruits and vegetables due to increasing health concerns. This has led to the development of new types of vinegar from various sources including onion [8], pineapple [6], strawberry [9], and sweet potato [10].

Tomato (*Lycopersicon esculentum*), a freshly consumed popular vegetable among many people living all over the world, is an excellent source of vitamin C and different types of antioxidants such as carotenoids particularly lycopene, ascorbic acid, vitamin E, and phenol compounds as flavonoids. These compounds help in combating formation of free radicals which are known to cause cancers and many other disease conditions. Tomato contains all four types of carotenoids, and it is a rich source of lycopene which has the highest antioxidant activity of all the carotenoids [11]. Moreover, tomato is a rich source of trace elements such as selenium, copper, magnesium and zinc. Tomato is utilized either as a fresh fruit or is processed into products like tomato sauce, ketchup, soup, paste, juice, and etc. However, there are few studies on vinegar developed from tomato fermentation that can act as tomato-based functional foods and acetic acid and tomato-derived functional compounds [12, 13].

Even though tomato is used in many ways, there is still a large quantity of tomato being wasted due to surplus production and poor postharvest handling. Thus, the introduction of novel value-added products, such as tomato powder, instant tomato soup, ready-to-eat tomato products, tomato juice, and tomato vinegar from tomatoes, is vital. Under such circumstances, the production of vinegar from tomatoes is a worthy alternative that can extend the shelf life of the fruit while adding some value.

Due to its potential health benefits, such as lowering blood pressure, acting as an antioxidant, alleviating the effects of diabetes, preventing cardiovascular diseases, and providing refreshment after exercise, fruit vinegar has become popular with people in many Western countries as well as in Asian countries such as China and Japan [14-16]. Conversely, in Sri Lanka, vinegar made from coconut water or the sap of coconut palm inflorescence has been used extensively as a preservative and a flavoring agent in pickles and many dishes, and the use of vinegar made from any other source is novel [1, 17]. Thus, the current study was done to develop antioxidant rich tomato vinegar that could be used for culinary purposes as an alternative to coconut vinegar.

Furthermore, the best fermentation temperature in the production of tomato vinegar was also examined.

2. Materials and Methods

2.1 Materials

Overripe and damaged tomatoes that were not suitable for consumption in fresh form due to poor post-harvest handling were collected from a local market. Baker's yeast (*Saccharomyces cerevisiae*) was also purchased from the local market, and *Acetobacter pasteurianus* PP21 isolated from papaya during a previous study (paper under review) were used as the relevant yeast and acetic acid bacterial strains in the fermentation process.

2.2 Preparation of tomato juice

Over ripened tomatoes were washed with running tap water, and then they were blanched at 60°C for two min, and the peels were removed. Then, the fruits were cut into small pieces and blended with added water in a 1:1 ratio. The blended juice was filtered using a clean cheesecloth, and the extracted juice was centrifuged at 5,500 rpm for 7 min to remove all unwanted particles. Afterwards, the clarified tomato juice was heated at 80°C for 10 min, and the brix value of the tomato juice was adjusted to 10.7°Brix by adding sugar. Finally, the pH of the extracted fruit juice was adjusted to 4.0 by adding food grade citric acid.

2.3 Preparation of the inoculum of yeast and acetic acid

Both yeast and AAB inoculums were prepared as described by Konate *et al*. [18] with some modifications. Yeast inoculum was prepared by incubating 0.5 g of yeast powder in 100 ml of 10% sucrose (w/v) solution for 1 h at 30°C, and 20 ml of the yeast inoculum was added to inoculate 1 l of tomato juice. *Acetobacter pasteurianus* PP21 was pre-cultured in YPGD medium (where a loopful of AAB was inoculated to 10 ml of the culture medium) at 30ºC for 72 h, and 50 ml of the culture was used to inoculate 1 l of alcoholic juice.

2.4 Alcoholic and acetous fermentation

Prepared tomato juice was inoculated with revitalized yeast and allowed to ferment for 72 h [14] at room temperature in a 2 l conical flask under static conditions. Afterward, 200 ml of alcoholic juice 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 contaminations. Fermentation was carried out at ambient temperature (30°C) and 36°C to examine the optimum temperature conditions for producing tomato vinegar. Total carbohydrates, brix value, titratable acidity, and pH value were measured daily during fermentation.

After reaching the expected acidity level (around 4.5% w/v), the obtained tomato vinegar was centrifuged at 5,500 rpm for 10 min to remove all the residues and yeast cell mass. Finally, the harvested vinegar samples were pasteurized in a shaking water bath at 72ºC for 20 min to stop further acetification process.

2.5 Sample analysis

2.5.1 Total soluble solid content and acidity of tomato vinegar

The total sugar content of the samples was measured using a digital pocket refractometer (Atago PAL-22S Japan). Titratable acidity was measured by titrating the samples with 0.1N NaOH using phenolphthalein as an indicator and the total acidity was expressed as the quantity of acetic acid.

2.5.2 Determination of total sugar content

Total sugar was measured by the phenol sulphuric method using glucose as the standard [19]. All the analysis was done in triplicate.

2.5.3. Determination of alcohol content

The change in the ethanol content of the samples were measured enzymatically using membrane fractions obtained from *Gluconobacter frateurii* with ferricyanide as the electron acceptor [20].

2.5.4 Determination of total antioxidant capacity

Samples were analyzed for total antioxidant content using DPPH assay as originally described by Brand-Williams *et al*. [21], with some modifications. Samples were serially diluted with methanol to obtain 5, 10, 15, 20 mg/ml dilution solutions. 250 µl of sample or control (methanol) was added to 2.75 ml of 50 µM DPPH solution (1.97 mg in 100 ml of methanol) separately, and the absorbance at the steady state was measured at 517 nm (HACH, DR3900, Germany). Percentage of inhibition was calculated, and the inhibition percentage was plotted against sample concentration. The amount of sample needed to inhibit 50% of the initial DPPH (EC_{50} value) was calculated from a calibration curve determined by linear regression.

2.5.5 Determination of total phenolic content

Total phenolic content was measured using Folin-Ciocalteu reagent described by Singleton *et al*. [22], with some modifications. Appropriately diluted 400 µl of sample was mixed with 2 ml of Folin-Ciocalteu reagent (previously diluted with 10-fold distilled water), and one minute later and within an eight minute period, 2 ml of 7.5% sodium bicarbonate solution was added to stop the reaction. Then the mixture was made up to 10 ml with distilled water. This mixture was kept in the dark for 120 min, and the absorbance was measured at 760 nm. Results were expressed as mg gallic acid equivalents per litre (mg GAE/l) using gallic acid as the standard.

2.5.6 Determination of total flavonoid content

The total flavonoid content was measured by colorimetric assay in accordance with the method described by Zhishen *et al*. [23].

2.6 Sensory evaluation

Two different sensory evaluations were carried out to evaluate the acceptance of the prepared tomato vinegar over traditional coconut water vinegar using 30 semi trained sensory panelists. The prepared tomato vinegar sample and commercial coconut vinegar sample were presented to the sensory

panelists in transparent glasses at room temperature under normal lighting conditions with random three digit numbers. The colour, aroma, appearance, and overall acceptance of both vinegar samples were compared using a five point hedonic scale. Thereafter, a secondary sensory evaluation was carried out with two different *Sinhala pickels* prepared using both vinegar types separately, and the *pickels* were served at room temperature under normal lighting conditions with random three digit numbers. The colour, aroma, taste, appearance, and overall acceptability of both *pickels* made from both vinegar types were evaluated using a five point hedonic scale.

2.7 Statistical analysis

Friedman test was used as an inferential analysis method to analyze sensory data, and Tukey's test was used to analyze the results on bioactivity data using Mini tab statistical software for Windows (Version 14). All the experiments were conducted in triplicate to draw statistically valid conclusions and a probability value 5% ($p = 0.05$) was used in statistical analysis.

3. Results and Discussion

3.1 Tomato vinegar production with *A. pasteurianus* PP21

The use of *A. pasteurianus* PP21 and baker's yeast in production of tomato vinegar was examined at ambient temperature and 36°C for 10 days under laboratory conditions, and the obtained results are summarized in Figure 1. When considering the initial ethanol production during simultaneous tomato vinegar production, the highest ethanol production of 5.41±0.09% (v/v) was obtained at 30°C. Accordingly, the highest amount of acetic acid production was also observed at 30°C where the mean acetic acid production was found to be 5.139±0.145% (w/v). On the other hand, ethanol production at 36°C was recorded to be 4.934±0.024 % (v/v), and according to the Figure 1B, the ethanol production at 36°C was found to be significantly less than that of 30°C ($p<0.05$). Furthermore, even though less than the acetic acid production observed at 30°C, an acetic acid production of 4.12±0.32% (w/v) was also observed at 36°C. However, the peak acetic acid level produced at 30°C was found to be significantly higher than the peak acetic acid production at 36°C ($p<0.05$). This indicates that tomato vinegar can be successfully produced even at 36°C using *A. pasteurianus* PP21 strain. Moreover, these results are compatible with the acetic acid production of *A. pasteurianus* PP21 strain on YPGD medium with 6% (w/v) initial acetic acid at 36°C, which was reported previously (manuscript under review). Furthermore, as shown in Figure 1, both the sugar content and the brix value of the used tomato juice were found to be reduced drastically during the first day of the vinegar production, which was mainly due to the production of alcohol through the yeast fermentation. Subsequently, the alcohol produced was utilized by the added acetic acid bacteria strain to produce acetic acid as the major beneficial end product in tomato vinegar.

However, after 7 and 4 days of fermentation at 30°C and 36°C respectively, acetic acid consumption, which is an unfavorable phenomenon in the fermentation industry, was observed. This is mainly due to the unavailability of ethanol at the time of maximum acetic acid production, and subsequently the produced acetic acid provides the required carbon source for the growth of the AAB in the media. Along with the increment in the produced acetic acid level, the ethanol level in the tomato wine started to gradually reduce, and it reached a concentration that was less than 0.5% (v/v) at the point of maximum acetic acid production (Figure 1). Moreover, these results are compatible with the standards imposed by the Food Act for vinegar, which specify that the reduced ethyl alcohol level of vinegar should be less than 1% (v/v) in the final product [24]. Due to its

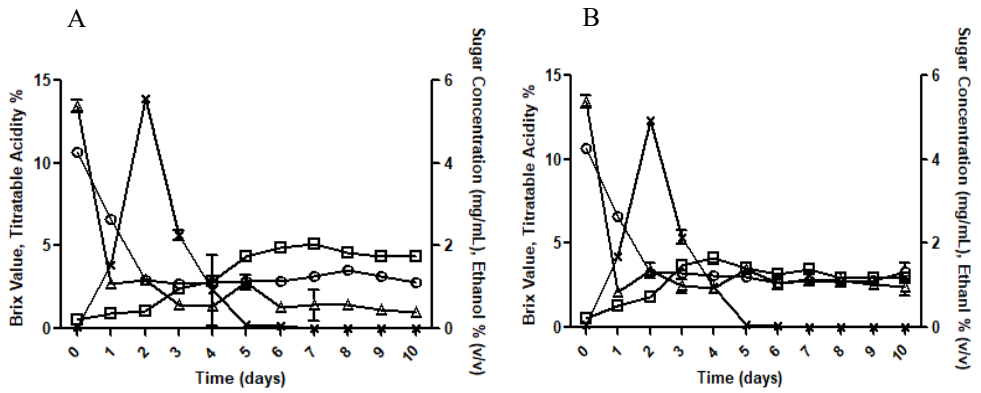

Figure1. Changes in Brix value (circle), acidity percentage (squares), sugar concentration (triangles), and ethanol content (crosses) in simultaneous tomato vinegar production using Baker's yeast and *A. pasteurianus* PP21 at 30°C (A), and 36°C (B)

unfavorable nature of acetate overoxidation, the tomato vinegar production should be terminated by inactivating AAB as soon as the maximum acetic acid production level is reached.

Moreover, Mounir *et al*. [25] compared thermotolerance and bioconversion abilities between two thermotolerant reference AAB strains and two wild type thermotolerant and mesophilic strains at 30, 34, 38 and 41°C under agitation in GYEA/Mg^{2+} medium. They observed that the wild type strains could perform considerable acetic acid production only at 30°C, and also no wild type mesophilic strains showed acetic acid production beyond 34°C. Likewise, Ndoye *et al*. [26] compared acid production among wild type mesophilic *Acetobacter pasteurianus* strain and two selected thermotolerant strains at 28, 30, 35, 38, 40, and 42°C and they also found that wild type mesophilic strains could not perform any acetic acid production in temperatures beyond 30°C. In contrast, the wild type *A. pasteurianus* PP21 used in the current study performed considerable thermotolerant activity since it performed a substantial acetic acid production at 36°C in static culture with very short lag phase

As is well known, industrial vinegar production is performed at ambient room temperature. However, during fermentation, heat is generated due to the respiration of acetic acid bacteria, and this will badly affect the efficiency of the fermentation process; thus, ability to perform acetic acid production at higher temperatures is advantageous for the fermentation industry, especially in countries like Sri Lanka. Consequently, the used strain can be effectively used in industrial vinegar production either at ambient temperature or at higher temperatures.

Similarly, Lee *et al*. [12] reported that they were able to produce tomato vinegar with 5.6% (56 g/l) acetic acid content at 30°C under laboratory conditions using tomato juice with 10-13°Brix. As was reported by them, 10-13°Brix fruit juice was found to be sufficient to obtain an alcohol content of 4-6% (v/v) within 2-4 days of alcoholic fermentation, and subsequently, 5.6% (56 g/l) acetic acid content in shaking culture condition (200 rpm) at 30°C within 8 days of an acetic acid fermentation cycle where the 12 days of total fermentation occurred. Moreover, according to Kaur *et al*. [27], tea vinegar with 47 g/l acetic acid content (4.7%) was successfully produced using 14°Brix tea extract at 30°C. They further mentioned that the highest acidity level was obtained within 36 h of acetic acid fermentation, which was followed by 48-96 h of alcoholic fermentation. Comparatively, *A. pasteurianus* PP21 used in the current study can be successfully utilized in production of tomato vinegar even at 36°C, giving an acetic acid production of 41.2 g/l within 3-5

days of simultaneous alcohol and vinegar production. Furthermore, it is evident that higher amount of acetic acid production in both studies is mainly due to the effective aeration conditions that were facilitated by shaking culture. However, when compared to the reported past work, it can be concluded that *A. pasteurianus* PP21 strain can produce acetic acid at a higher rate even at static culture condition, and thus, it would be a potential candidate to be used in industrial vinegar production. However, further studies should be done using an industrial fermenter or bioreactor to identify the performance of the strain under industrial conditions.

3.2 Examining the bioactivity of produced vinegar

The antioxidant activity and the total phenolic and total flavonoid contents of the produced tomato vinegar were examined, and the obtained results were compared with those of coconut water vinegar produced under laboratory conditions and commercially available coconut vinegar purchased from the local market (Table 1). As per the results summarized in Table 1, tomato juice is a rich source of total flavonoids, polyphenols and antioxidant activity, and the obtained values are significantly higher than those of other tested tomato and coconut vinegar products. Moreover, when compared to the commercially available coconut vinegar, the developed tomato vinegar was found to be a rich source of total phenolic content. Furthermore, the antioxidant activity of the developed tomato vinegar was found to be significantly higher than that of commercial vinegar, which could be owing to the higher polyphenol and flavonoid content of the fresh juice used in production of vinegar. However, the antioxidant activity of the tomato vinegar was slightly less than that of coconut vinegar that was produced under laboratory conditions, and no significant difference in antioxidant activity was observed.

Table 1. Total phenolic, DPPH antioxidant activity and total flavonoids content of tomato and coconut vinegar

	DPPH activity EC_{50}	**Total phenolic content mg GA eq/l**	**Total flavonoid content Tomato vinegar mg catechin eq/100 ml of Sample**
Tomato Juice	2.94 ± 0.86^{a}	924.22 ± 31.9^{c}	20.59 ± 2.66^{c}
Tomato Vinegar produced at 30°C	14.91 ± 2.7^{b}	333.80 ± 2.28^{b}	8.78 ± 1.277^{a}
Tomato Vinegar produced at 36°C	13.87 ± 0.63^{b}	365.020 ± 5.53^{b}	8.11 ± 0.793^{a}
Coconut water vinegar	12.72 ± 0.155^{b}	155.35 ± 10.01^{a}	18.82 ± 0.859^{c}
Coconut vinegar from the market	101.50 ± 1.66^{c}	132.26 ± 11.47^{a}	13.54 ± 0.555^{b}

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

According to Rice-evans *et al*. [28], polyphenols are considered as the most abundant antioxidant in the human diet that has the ability to quench free radicals. Among the group of polyphenols, flavonoids and their derivatives are the largest group that possess a strong antioxidant,

anticancer, anti-inflammatory and hepatoprotective activity due to their ability to scavenge reactive oxygen species. Moreover, as was reported by Nishidai *et al*. [29], vinegar produced by different types of acetic acid bacteria strains may have different levels of antioxidant activities. According to a study done by Mohamed *et al*. [30], the total phenolic content of coconut water vinegar was found to be 106.45±0.01 µg GAE/ml, and the results obtained in the current study are also in accordance with the reported value. Moreover, as was reported by Bakir *et al*. [31], vinegar produced from grapes and apples was found to have a total phenolic content of 220 mg GAE/L and 160 mg GAE/l, respectively. Comparatively, the developed tomato vinegar was found to be a rich source of phenolic compounds where the total phenolic contents of the tomato vinegar were found to be 333.5±2.28 mg GAE/l and 365.02±5.53 mg GAE/l when incubated at 30°C and 36°C, respectively. Moreover, there was no significant difference in antioxidant activity, total phenolic content and total flavonoid content of the tomato vinegar produced at 30°C and 36°C. These results suggest that tomato vinegar can be produced either at 30°C or 36°C without affecting its bioactive compounds. Additionally, in research done by Bakir *et al*. [31], they had observed a strong loss of antioxidant phenolic compounds during the acetic acid fermentation of both grape and apple vinegar, and the results of the current study on tomato vinegar are in agreement with that. Furthermore, reduction percentage of the total phenol content of the produced tomato vinegar was calculated, and when compared to the fresh tomato juice, it was found to vary within 60.5-63.88%.

3.3 Sensory evaluation of the produced vinegar

Acetobacter pasteurianus is one of the most commonly used AAB in the fermentation industry [32]. Moreover, according to US Food and Drug Administration (FDA), acetic acid produced by microbial fermentation is considered as a Generally Recognized as Safe (GRAS) substance [33]. Thus, the consumer acceptance of the developed tomato vinegar over commercially available coconut vinegar was examined using two different sensory evaluations. First, the acceptance of the raw tomato vinegar over commercial coconut vinegar was tested, and secondly the consumer preference for *Sinhala* pickle made using both vinegar types was examined. The obtained results are summarized in Tables 2 and 3, respectively.

As it is summarized in the Table 2, when compared to the raw coconut water vinegar, the developed raw tomato vinegar was found to be significantly higher in all the tested sensory attributes except for clarity. However, even though there were no significant differences, the sum of the ranks for clarity for the raw tomato vinegar was found to be higher than that for commercial coconut vinegar. The consumer acceptance of the clarity of the tomato vinegar could be affected by the turbidity of the tomato pulp, and thus, this could negatively affect the consumer preference of clarity of the tomato vinegar. Conversely, the clarity of the prepared tomato vinegar can be improved by using centrifugation or leaving the fermented vinegar for several months for aging without making any disturbance. This could improve the clarity of the final product.

Table 2. Sensory evaluation of the developed tomato vinegar with commercially available vinegar

Sensory attribute	Sum of ranks		P value (adjusted for ties)
	Tomato vinegar produced at 30°C	Commercial coconut vinegar purchased from market	
Colour	52.5	37.5	0.001
Clarity	48.0	42.0	0.201
Aroma	53.5	36.5	0.001
Taste	50.0	40.0	0.025
Overall acceptability	54.5	35.5	0.000

Table 3. Sensory evaluation of *Sinhala* pickle prepared using the developed tomato vinegar and commercial coconut vinegar

Sensory attribute	Sum of ranks		P value (adjusted for ties)
	Tomato vinegar produced at 30°C	Commercial coconut vinegar purchased from market	
Colour	47.5	42.5	0.197
Aroma	46.5	43.5	0.532
Taste	46.0	44.0	0.695
appearance	45.0	45.0	1.000
Overall acceptability	45.0	45.0	1.000

Moreover, according to the sensory results summarized in Table 3, there is no significant difference observed between the *Sinhala* pickle samples prepared using either tomato vinegar or commercial coconut vinegar. However, the pickle prepared using the tomato vinegar showed higher consumer preference for colour, aroma, and flavor. These results are also in accordance with the sensory results of the comparison of the raw vinegar types. Moreover, the results of the sensory evaluation show that both vinegar types have similar levels of acceptance for both appearance and for overall acceptability. These results suggest that just as is the case for commercially available coconut vinegar, the developed tomato vinegar can be successfully used in the preparation of traditional Sri Lankan dishes.

4. Conclusions

During the current study, the authors were able to produce antioxidant rich tomato vinegar successfully, using locally available tomatoes. Moreover, the results confirmed that the tomato vinegar with 4.12% (w/v) acetic acid was effectively produced within 3-5 days of simultaneous fermentation at 36°C. Furthermore, the results of the sensory evaluation reveal that the developed tomato vinegar can be used as an alternative to commercial coconut vinegar with significantly higher sensory acceptance. As a whole, the ability to produce vinegar at a higher temperature without any damage to acid production ability, antioxidant capacity and sensory properties would be advantageous to a tropical country like Sri Lanka.

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