Chapter 14
Taiwan Fruit Vinegar

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14.1 Introduction

Vinegar has been used as a seasoning in cooking since ancient times. However, recent research has shown that, in addition to its well-known anti-bacterial activity, vinegar (when consumed as a drink) confers considerable health benefits, including lowering blood pressure, acting as an anti-oxidant, alleviating the effects of diabetes, preventing cardiovascular diseases, providing refreshment after exercise, etc. (Nishidai et al., 2000; Ogawa et al., 2000; Fushimi et al., 2001, 2002; Kondo et al., 2001; Shimoji et al., 2002; Sugiyama et al., 2003; Johnston et al., 2004). Consumers are now beginning to appreciate the health benefits of drinking vinegar. Therefore, in addition to the traditional use of vinegar products such as rice vinegar, wine vinegar and cider vinegar as a food flavouring, there is a growing demand for fruit vinegar products that are sold as a health food. The emergence of these new products has resulted in name changes from wine or cider vinegar to grape or apple vinegar, with these being categorized as fruit vinegars. Thus, there are two types of fruit vinegar products on the market: one with a high content of acetic acid which is either used as a seasoning or is diluted with 4-8 times as much water as a health drink, and another that is ready to drink as a beverage.

There are only a few reports in the literature on research into fruit vinegar products made from fruits other than grapes or apples. Most research has studied the acetic acid yield from fermentation using fruit peel and other wastes as the raw materials (Richardson, 1967; Anon., 1973; Adams, 1978; Grewal et al., 1988), and there has been little research work on the quality aspects of fruit vinegars. Koizumi et al. (1987) evaluated the general composition, amino acids and organic acids of some high-priced special vinegars in Japan, and found that they were not of high quality. This tells us that, if fruit vinegar products are good for health and for drinking, much work needs to be done in figuring out the important criteria for manufacturing and marketing healthy and palatable fruit vinegar products. In general, many consumers, especially the younger generation (under 20 years old), do not like fruit vinegar products. This chapter discusses the definition of fruit vinegar and

L. Solieri, P. Giudici (eds), *Vinegars of the World*

Table 14.1 The Chinese National Standard (CNS) quality standards for edible vinegar (Chinese National Standard, 2005)

Variety \ Composition		Acidity (%, calculated as acetic acid)	Salt-excluded soluble solids (%)	Non-volatile acidity (%, calculated as acetic acid)	Total nitrogen(%)
Brewing vinegar	Grain vinegar	Not less than 4.2	Not less than 1.3	–	–
	Fruit vinegar	Not less than 4.5	Not less than 1.2	–	–
Other brewing vinegar		Not less than 4.0	Not less than 1.2	–	–
High-acidity vinegar		Not less than 9.0	Not less than 1.5	–	–
Condiment vinegar		Not less than 1.0	Not less than 6.0	–	–
Artificial vinegar		Not less than 4.0	Not less than 1.2	Not more than 1.0	Not more than 0.2

Table 14.2 The regulation for composition of vinegar by FAO/WHO (Joint FAO/WHO Food Standards Programme, 2000)

	Wine vinegar	Vinegars other than wine vinegar
Total acid content	Total acid not less than 60 $g \cdot L^{-1}$ (calculated as acetic acid) and not more than the amount detainable through the use of biological fermentation	Total acid not less than 50 $g \cdot L^{-1}$ (calculated as acetic acid) and not more than the amount detainable through the use of biological fermentation
Residual alcohol content	Residual alcohol (v/v) not more than 0.5%	Residual alcohol (v/v) not more than 1%
Soluble solids (exclusive of added sugars or salt)	Soluble solids not less than 1.3 $g \cdot L^{-1}$, 1% acetic acid	Soluble solids not less than 2.0 $g \cdot L^{-1}$, 1% acetic acid

describes the types of fruit vinegar products available in Taiwan. It then examines the manufacture of fruit vinegar using pineapple as the raw material, and shows the results of analysis using near infrared spectroscopy to determine its physico-chemical properties.

14.2 Definitions of Fruit Vinegar for Taiwan and the FAO/WHO

Edible vinegar is classified into brewing vinegar and artificial vinegar according to the Chinese National Standard (CNS) definitions CNS14834 and N5239 (Chinese National Standard, 2005). The difference between the two vinegars is due to the addition of glacial acetic acid (or acetic acid). The definition of a fruit vinegar, such as cider, wine or orange vinegar, according to the CNS brewing vinegar standards, is that it must have been fermented from at least one fruit, and each litre of raw material must contain more than 300 g of fruit juice. The quality specification for edible vinegar by the CNS mainly concerns the acidity and salt-excluded soluble solids, as shown in Table 14.1. The acidity of grain vinegar and fruit vinegar is above 4.2 % and 4.5%, respectively. The contents of salt-excluded soluble solids in grain vinegar and fruit vinegar must be above 1.3 % and 1.2%, respectively.

As defined by FAO/WHO, vinegar is a liquid, fit for human consumption, produced exclusively from suitable products containing starch and/or sugars by the process of double fermentation; first alcoholic and then acetous: and fruit vinegar is a vinegar obtained by acetous fermentation from wine of fruit (Joint FAO/WHO Food Standards Programme, 2000). Their composition specification not only includes the acidity and salt/sugar-excluded soluble solids, but also includes the residual alcohol content, food additives and food impurity (Table 14.2). Regarding the name of a vinegar, it is named ‘X vinegar’ if the raw material used is X only. If there is more than one raw material, it is named either X or Y vinegar depending on whether X or Y is the predominant material.

To compare the two above-mentioned specifications, it must be remembered that the FAO/WHO guidelines refer to soluble solids in the fruit vinegar exclusive of sugar and salt added during the manufacturing process, while the Taiwanese CNS standard excludes salt but does not mention the sugar content, or consider the influence that added sugars may have on soluble solids calculated for different types of vinegar. This is because, in the past, edible vinegars used for seasoning were made exclusively from grains with no added sugar. It is obvious that the regulations for edible vinegar in Taiwan need to be updated, especially for the various types of fruit vinegar as well as the sugar-added vinegars that are already available.

14.3 Fruit Vinegar in Taiwan

Taiwan is located in the subtropical and tropical zones and its climate is very suitable for growing fruit, so a wide variety of fruits are available in all seasons. Traditionally, commercially produced vinegar is made from rice or unpolished rice.

However, because of a lack of the appropriate fermentation technology and facilities for fruit vinegar production in Taiwan, many fruit vinegars are not made by alcoholic and acetic fermentations. Many of them are simply made by adding fruit or fruit juice and sugar into the rice vinegar and leaving it for more than 3 months. The rice vinegar is used as a solvent to extract the nutrients and aroma of the fruit. There are only a few fruit vinegar products obtained from real alcoholic and acetous fermentation. These are made either by going through a natural fermentation process or by using manufacturing methods and microorganisms that have been kept secret.

In 2002-2003, we surveyed the fruit vinegar products sold in Taiwan to examine their label information and physico-chemical properties (Chang et al., 2005). In total, 66 fruit vinegar products, comprising 12 mei (also known as Japanese apricot), 17 cider, 3 mulberry, 4 lemon, 8 blended, 15 wine, 2 orange and 5 other (starfruit, blueberry, pineapple, grapefruit and passion fruit) vinegars were collected, as listed in Table 14.3. Among these, 8 cider and 14 wine vinegar were imported. For the domestic vinegars, apart from the wine vinegar which is used solely as a seasoning, all the other products can be diluted with 4-8 parts of water before drinking as a beverage.

Two samples, acquired from organic food stores, had no label except for the product name. Of the 64 samples with labels, 29 had nutrients listed on the label, while 35 did not. Production methods were classified into five categories, according to information on the labels. They were brewed from juice by alcoholic and vinegar fermentation (F); brewed from juice and alcohol (FA); F mixed with grain vinegar (FG); juice mixed with grain vinegar (JG); and F mixed with juice (FJ). From Table 14.3, it can be seen that 28 samples were F, while 26 were JG. Those categorized as FG, FA and FJ had 6, 3 and 1 samples, respectively. Domestic products, such as mei, cider, mulberry, lemon and blended vinegars, were mostly made with juice mixed with rice vinegar or sorghum vinegar, while most imported products were produced by alcoholic and acetous fermentation. The average price of wine vinegars, per 100 mL, was the highest of all the fruit vinegars.

Total sugar content of vinegar, with no extra sugar added, was less than 3%, while those with sugar added ranged from 8% to 64%. Most imported cider and wine vinegars had no sugar added, with the acidity being about 5-7%. Most domestic products with sugar added had an average acidity of less than 3%. Besides acetic acid, the major organic acids found in fruit vinegars were malic, lactic and citric acids. Mulberry vinegar was found to be higher in lactic and succinic acids than the others. Red wine vinegars were rich in tartaric, malic and lactic acids.

Twenty-six out of 44 Taiwan local fruit vinegar samples belonged to this type, while only nine samples were made purely from fruit juice fermentation. In addition, for healthy drinking and to increase palatability and consumer acceptance, sugar is normally added to balance the sourness of acetic acid produced. By so doing, the acidity of resultant vinegar usually cannot meet the regulations of either CNS or FAO/WHO. In addition, the proportion of salt-excluded soluble solids becomes higher than it should be due to the addition of sugar. This clearly shows that the regulation by CNS is re-adjustable. The soluble solids should be measured by exclusion of salt and sugar, rather than salt only.

Table 14.3 Label analysis and classification of fruit vinegars available in the Taiwan marketplace

	Domestic								Imported		
Samples	**Total**	**Mei**	**Cider**	**Mulberry**	**Orange**	**Lemon**	**Blended**	**Others**	**Total**	**Cider**	**Wine**
No. of samples	44	12	9	3	2	4	8	6	22	8	14
Classification[a]											
F	9	2	3	2	–	–	2		19	6	13
JG	26	5	4	1	2	4	6	4	–	–	–
FG	6	3	2	–	–	–	–	1	–	–	–
FA	–	–	–	–	–	–	–	–	3	2	1
FJ	1	1	–	–	–	–	–	–	–	–	–
Unknown	2	1	–	–	–	–	–	1	–	–	–
Labelled	42	11	9	3	2	4	8	5	22	8	14
Without NL[b]	16	6	1	3	0	0	4	2	19	5	14
With NL[b]	26	5	8	0	2	4	4	3	3	3	0
Incorrectly labelled[c]	14	2	5	–	2	2	2	1	2	2	–

[a] F, vinegar brewed from juice by alcoholic and vinegar fermentation. FA, vinegar brewed from juice and alcohol. FG, F mixed with grain vinegar. JG, juice mixed with grain vinegar. FJ, F mixed with juice.

[b] NL, nutrient label.

[c] Total sugar contents are >20% greater than the amount stated on the label.

In terms of the names for fruit vinegars, according to the regulation of CNS, edible vinegar is classified by the raw material used at the alcoholic fermentation stage; while by FAO/WHO regulations it is classified by the raw material used at the acetous fermentation stage, such as wine vinegar or cider vinegar. Any other substance added in the product should have some suitable descriptor on the label. According to the classification standards by both CNS and FAO/WHO, most of the fruit vinegar products available on the Taiwan market should have the name of either rice or sorghum vinegar.

Furthermore, since the consumer treats fruit vinegar as a health drink, the daily consumption of fruit vinegar should not be overlooked. The inspection of labels for accuracy of information needs to be taken seriously in order to protect the consumer. Our survey revealed that 61.9% of domestic fruit vinegar products had nutrition information on the label, but only 13.6% of imported ones had nutrition labelling. In addition, the total sugar content in 55.2% of labelled products was found to be 20% higher than the values stated. These results show that the quality of fruit vinegar sold in the Taiwan market is not well controlled.

14.4 The Brewing of Pineapple Vinegar

The flavour and mouth-feel of edible vinegar are influenced by the acetic acid bacteria strains used (Lin and Chen, 2002), base wine for brewing (Ciani, 1998), brewing method (Lin and Chen, 2002) and storage (Okumura, 1995; Tesfaye et al., 2002). Along with the increase in standard of living, people gradually now care more about the natural characteristics and health benefits of foods than they did in the past, and the demand for high-quality foods is increasing. Manufacturing a good product with genuine high quality is the only way to attain a substantial market share. Pineapple is a major economic crop in Taiwan. As well as canned pineapple or pineapple juice, pineapple vinegar is also available in the Taiwan market. However, it is mainly produced by mixing pineapple juice with grain vinegar. Only a few come from brewing, and the quality needs to be improved before they are widely accepted by the consumer. The following sections describe the complete procedure for the brewing of high-quality pineapple vinegar in terms of the raw materials used, the brewing of pineapple base wine, the selection of acetic acid bacteria, and the effects of additional nutrients and storage on the quality of pineapple vinegar.

14.4.1 Raw Materials and Brewing of Pineapple Base Wine

High-quality raw materials are essential for manufacturing high-quality processed foods. Pineapple vinegar is no exception. The ‘Smooth Cayenne’ cultivar of pineapple was selected as the raw material because of its unique flavour. After cutting off the head, tail and peel, the fruit was further cut and blended into pulp. To every kilogram of pulp was added 0.2 mL Pectinex Ultra SP-L (Novo Nordisk

Ferment Ltd., Japan), and the pulp was then left for 30 minutes at room temperature to obtain better juice yield and clarity. The pineapple juice was centrifuged and heated at 60-70 °C for 10 min to get rid of spoilage bacteria. After cooling, the juice was stored at –20 °C before use (Chang, 2007).

For brewing the pineapple base wine to be used for acetous fermentation, the optimum fermentation conditions for brewing pineapple wine, as established in our laboratory (Wen, 2001), were used. After thawing, sugar was added to the pineapple juice to increase the total soluble solids (TTS) to 26 °Brix and pH was adjusted to 3.5. The 50 mL starter (*Saccharomyces* spp.) was inoculated into 830 mL juice and the fermentation took place at 12-14 °C. The end-point of brewing was chosen as the point when the TTS no longer changed. After brewing, the wine was centrifuged (15,400 × g) for 30 min, ready for further acetous fermentation.

14.4.2 Selection of Acetic Acid Bacteria

Pineapple base wines with different pH and alcohol concentrations were used as materials to compare the volatile compounds and sensory quality of fermented pineapple vinegar in order to select the most suitable acetic acid bacteria (AAB) for brewing pineapple vinegar. Five different AAB strains: *Acetobacter aceti* BCRC 12324, *A. aceti* BCRC 14156, *A. aceti* BCRC 11569, *A. aceti* 3012 and *Acetobacter* sp., were investigated (Chang et al., 2006).

For examining their ethanol tolerance and fermentation efficiency (FE, percentage of acetic acid produced from alcohol), these five AAB were inoculated into the base wine with ethanol content ranges of 3-9% and the brewing of pineapple vinegar took place at 30 °C. The results showed that the first three AAB had a FE of up to 90%, which was much higher than the other two AAB when the ethanol content is around 5-7% (Table 14.4).

Using pineapple base wine with 6% ethanol content as the substrate, these five AAB were inoculated for acetous fermentation. The major volatiles, including 3-methyl-1-butanyl acetate, 2-phenylethyl acetate, 3-methyl-1-butanol, 2-methyl-1-butanol, 2-phenylethanol, 3-methyl butanoic acid, hexanoic acid, octanoic acid, *n*-decanoic acid and benzaldehyde (Table 14.5), were found in the headspace of the pineapple vinegar bottle.

After being diluted with 6% acetic acid solution to a concentration of 50 ppm, the ten volatiles listed above were smelt by 25-35 panelists and the odour characteristics were identified. Esters and alcohols were recognized as a ripened-fruit aroma or floral aroma while the acids were more related to oily, aged or mouldy odours. These results are in agreement with those of Lin (1994) in wines and Charles et al. (1992) in red wine vinegar. The volatiles of esters and alcohols in the pineapple vinegar brewed by *A. aceti* BCRC 12324, *A. aceti* BCRC 14156 and *A. aceti* BCRC 11569 were much higher than those by *A. aceti* 3012 and *Acetobacter* sp., and the acids with an unpleasant smell were much lower. Therefore, combining the FE and sensory data, it was shown that AAB of *A. aceti* BCRC 11569 and *A. aceti* BCRC 14156 are suitable for brewing pineapple vinegar.

Table 14.4 Effects of different alcohol contents in pineapple wine on acetic acid production and fermentation efficiency by five acetic acid bacteria[a]

		Alcohol (%, v/v)						
Strain	**Time (weeks)**[b]	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**	**8.0**	**9.0**
A. aceti BCRC 12324	3.0		3.09 (74.03b)	4.08 (78.20ab)	4.93 (78.74ab)	6.07 (83.10a)	5.25 (62.89c)	3.33 (35.46d)
A. aceti BCRC 14156	2.5			4.78 (91.62b)	5.94 (94.87a)	6.69 (91.59b)	7.35 (88.05c)	8.17 (87.00c)
A. aceti BCRC 11569	2.5			4.69 (89.89a)	5.50 (87.85a)	6.59 (90.22a)	6.62 (79.30b)	8.20 (87.31a)
A. aceti 3012	4.0		4.29 (102.78a)	5.13 (98.32ab)	5.68 (90.72b)	6.72 (92.00b)	5.35 (64.09c)	
Acetobacter sp.	3.0	2.08 (66.44ab)		3.08 (59.03b)	5.08 (81.14a)	5.88 (80.05a)	6.59 (78.94a)	6.72 (71.56ab)

[a] The data (n=2) are expressed as the difference in acidity after and before acetous fermentation, and the values in parentheses are the fermentation efficiency. The means in each row bearing different letters are significantly different at $P<0.05$.

[b] Fermentation period.

Table 14.5 Analysis of volatile compounds (ppm) in pineapple vinegar fermented by five acetic acid bacteria[a]

Volatile compounds	*A. aceti* BCRC 12324	*A. aceti* BCRC 14156	*A. aceti* BCRC 11569	*A. aceti* 3012	*Acetobacter* sp.	Descriptor[b]
Esters	**9.20**	**10.79**	**12.70**	**5.40**	**4.53**	
2-propenyl acetate	0.04a	0.11a	0.18a	0.03a	0.08a	
3-methyl-1-butyl acetate	5.90ab	6.38ab	11.54a	1.71b	1.29b	Banana and apple-like
2-phenylethyl acetate	3.26b	4.30b	5.48a	3.66b	3.16b	Rose-like
Alcohols	**37.70**	**37.97**	**36.76**	**19.43**	**16.88**	
3-methyl-1-butanol	20.64a	20.02a	22.01a	6.35b	4.93b	Banana-like
2-methyl-1-butanol	1.46ab	1.68a	1.82a	1.04b	1.23b	
2-phenylethanol	15.60ab	16.27ab	16.93a	12.04bc	10.72c	Rose-like
Acids	**57.24**	**52.34**	**56.78**	**78.16**	**72.60**	
propanoic acid	3.40a	1.42b	1.67b	2.03b	3.84a	
2-methylpropanoic acid	2.46a	2.64a	3.52a	3.22a	2.99a	
3-methyl butanoic acid	12.94b	13.43b	13.12b	34.30a	27.86a	Stink of feet
hexanoic acid	3.11a	3.06a	3.29a	3.74a	3.04a	Slightly rancid
octanoic acid	26.50a	22.26a	23.98a	25.94a	24.28a	Rancid and musty
n-decanoic acid	8.83a	9.53a	11.20a	8.93a	10.59a	Rancid and stuffy closet
Others						
benzaldehyde	137.67a	122.55a	52.85b	80.52b	54.28b	Almond
3-methylpentane	–	–	0.61a	0.04a	–	
Sum	**241.81**	**223.65**	**159.70**	**183.55**	**148.29**	

[a] The means (n=3) with different letters for each compound differ significantly at $P<0.05$.

[b] Descriptor for each compound with concentration of 50 ppm in 6% acetic acid.

–, undetectable. BCRC, Bioresource Collection and Research Centre.

In addition, a further comparison on the quality of pineapple vinegar fermented by these two AAB, *A. aceti* BCRC 11569 and *A. aceti* BCRC 14156, was carried out. These two starters with five different ratios of 1:0, 2:1, 1:1, 1:2 and 0:1 were used for brewing the pineapple vinegar. The results showed that the pineapple vinegar fermented by the starter of *A. aceti* BCRC 14156 alone (0:1) was the best, due to its low content of acids and significantly high values of ethyl acetate and benzaldehyde, with a little ester and almond aroma, respectively. It is concluded the *A. aceti* BCRC 14156 is better than *A. aceti* BCRC 11569 for pineapple vinegar fermentation.

14.4.3 The Effect of Pineapple Juice with and without Peel on the Quality of Pineapple Vinegar

The pineapple juice used for brewing pineapple vinegar was obtained by the juice-making process. It is of interest to know whether the peel makes any contribution to the flavour of pineapple vinegar.

In order to examine the effect of pineapple juice with or without peel on the quality of vinegar, the following three different juice treatments were tested (Chang, 2007).

Table 14.6 The analysis of volatile compounds in different pineapple vinegars[a]

Volatile compounds (ppm)	PVA	PVB	PVC
Esters	**113.76**	**151.57**	**51.67**
ethyl acetate	94.15a	112.53a	25.42b
2-propenyl acetate	1.03b	2.28a	2.76a
3-methyl-1-butyl acetate	13.58b	26.01a	14.79b
2-phenylethyl acetate	5.00b	10.75a	8.70ab
Alcohols	**32.83**	**63.70**	**41.41**
3-methyl-1-butanol	22.51b	40.94a	21.67b
2-phenylethanol	10.32b	22.76a	19.74a
Acids	**26.27**	**43.14**	**46.30**
propanoic acid	0.31a	0.39a	0.04a
2-methylpropanoic acid	1.65a	2.01a	1.08b
3-methyl butanoic acid	4.38a	4.01b	3.53c
hexanoic acid	1.91b	5.08a	5.36a
octanoic acid	11.94b	25.31a	27.80a
n-decanoic acid	6.08b	6.34b	8.49a
Others			
benzaldehyde	95.44a	43.85b	56.52ab
acetoin	2.10a	1.07b	1.97a
3-methylpentane	–	0.36a	0.31a
Sum	**270.04**	**303.69**	**198.18**

[a] Means (n=3) with different letters for each compound differ significantly at $P<0.05$.
–, undetectable.

Table 14.7 The sensory evaluation of different pineapple vinegars[a]

	Aroma				Flavor			
	Fruity	Wine	Pungency	Ester	Sour	Wine	Pungency	Overall mouth-feel
PVA	2.87c	2.46a	2.00a	3.35a	2.81a	2.84a	2.39a	2.92c
PVB	3.35b	2.50a	1.77b	2.46b	2.34a	2.31ab	1.89b	3.19b
PVC	4.31a	2.08b	1.96a	2.00b	3.27a	2.11b	2.73a	3.46a

[a] Means (n=12) of 5-point scaling: weak (1), moderate (3), strong (5). Means in each column with different letters are significantly different at $P<0.05$.

- *Pineapple base wine A (PWA)*: Pineapple juice obtained without peel was used and sugar was added to increase the total soluble solids (TTS) to 26 °Brix. After adjusting the pH to 3.5, the 50 mL starter (*Saccharomyces* sp.) was inoculated into 830 mL juice and fermentation took place at 12-14 °C. The end-point of brewing was when the TTS no longer changed. After brewing, the wine was centrifuged (15,400 × g) for 30 min, ready for further acetous fermentation.
- *Pineapple base wine B (PWB)*: Pineapple juice obtained without peel was used and no sugar was added. The following steps were as for PWA.
- *Pineapple base wine C (PWC)*: Pineapple juice obtained with peel was used and again no sugar added. The following steps were all the same as for PWA.

The alcohol contents of the three pineapple base wines (PWA, PWB and PWC) were 15.35%, 7.53% and 6.25%, respectively. Using the three pineapple base wines for acetous fermentation, the first step was to adjust the ethanol content down to 6% (v/v). Then 10% starter of *A. aceti* BCRC 14156 was added and brewing process took place at 30 °C until the acidity no longer increased. After centrifugation, the pineapple vinegars (PVA, PVB and PVC) were produced.

From the results of the volatile analysis (Table 14.6) and sensory test (Table 14.7), it was found that the PVA (no peel and high in sugar) was higher in benzaldehyde but lower in esters, alcohols and acids than PVB. The sensory characteristics of PVA, such as fruity aroma and mouth-feel, were much weaker than that of PVB (no peel and low sugar) and PVC (with peel and low sugar); only pungency was stronger.

On the other hand, there were many more volatiles, such as 3-methyl-1-butyl acetate, 3-methyl-1-butanol, 2-propenyl acetate, 2-phenylethyl acetate, 2-phenylethanol, hexanoic acid, octanoic acid and *n*-decanoic acid, found in PVB and PVC. Although the concentration of the first two volatiles (3-methyl-1-butyl acetate and 3-methyl-1-butanol), was lower in PVC than in PVB, the fruity aroma was much higher in PVC than in PVB and PVA.

The peel of pineapple might contain some undetectable trace volatile compounds which conferred a significant fruity aroma to the finished vinegar. Although the concentrations of most volatile compounds of PVC were no higher than those of PVB, the fruity aroma of PVC was significantly higher than PVB and PVA.

14.4.4 The Effect of Additional Nutrients on the Quality of Pineapple Vinegar

There are several studies showing the beneficial effects of nutrients added during the brewing of vinegar (Nanba and Kato, 1985a, 1985b; Lai, 1989). Nanba and Kato (1985b) reported that total amino acid content decreased gradually, and glutamic acid, aspartic acid and serine contents decreased considerably in the early stages of acetic acid fermentation. Our study showed that the final acidity of pineapple vinegar was raised by adding 0.05% ammonium sulphate, 0.2% peptone, 0.1% L-proline, 0.1% L-aspartic acid or 0.5% yeast extract in PWA before vinegar fermentation. As the protein content was only 0.14-0.21%, these five added nutrients could be supplied as a nitrogen source and would stimulate the acetous fermentation.

After L-aspartic acid addition, the pineapple vinegar was higher in fruitiness and mouth-feel and less pungent than the control without L-aspartic acid. Furthermore, the influence of the addition of 0.1% L-aspartic acid on the volatile compounds and sensory quality of PVA, PVB and PVC were investigated. The analysis of volatile compounds revealed that there was no significant difference for most volatile compounds in PVB and PVC with or without L-aspartic acid added. For PVA with no L-aspartic acid added, the volatile compounds such as 2-propenyl acetate, 3-methyl-1-butyl acetate, 2-phenylethyl acetate, 3-methyl-1-butanol, 2-phenylethanol, 3-methyl butanoic acid and octanoic acid were significantly lower in concentration than for PVB and PVC. With L-aspartic acid addition, the above-mentioned volatile compounds in PVA were substantially higher than those of the control, and there was no obvious difference from PVB and PVC, except that the concentrations of 3-methyl-1-butyl acetate and 2-phenylethanol were still lower. In terms of the results of sensory evaluation, this indicated that sensory characteristics of PVB and PVC, which were more flavourful, did not change significantly after L-aspartic acid added, but that PVA was more fruity-scented than the control.

14.4.5 The Effects of Storage on the Quality of Pineapple Vinegar

After brewing, the pineapple vinegar (PVA, PVB and PVC) was subjected to the storage test of 8-12 months at room temperature. The appearance of all the vinegars gradually became darker due to the Maillard reaction or the polymerization reaction of polyphenols. During the first 6 months storage of PVA, owing to the existence of some previously undetected secondary products of acetic acid bacteria in the pineapple vinegar, the acetic acid and these secondary products were able to participate in further reactions in the pineapple vinegar. As a result, alcohol volatile compounds such as 3-methyl-1-butanol and 2-phenylethanol continued to undergo esterification with acetic acid, which in turn led to an increase in the concentration of esters such as 3-methyl-1-butyl acetate and 2-phenylethyl acetate in the headspace of the pineapple vinegar (Table 14.8). This result is in agreement with the change in phenylethyl acetate of sherry wine vinegar during the early

Table 14.8 Changes in volatile compounds (ppm) in pineapple vinegars with different base wine during storage[a]

Vinegar	PVA			PVB			PVC	
Storage time (months)	**0**	**6**	**12**	**0**	**6**	**12**	**0**	**8**
Esters	**16.45**	**26.61**	**26.91**	**44.74**	**77.34**	**25.64**	**153.22**	**120.90**
ethyl acetate	2.51a	2.64a	2.40a	8.59a	4.93b	2.64c	127.07a	83.68b
2-propenyl acetate	0.20a	0.59a	0.96a	1.18a	4.44a	1.81a	2.76a	3.50a
3-methyl-1-butyl acetate	3.26b	8.50a	10.05a	12.55b	49.04a	9.87b	14.79a	9.93b
2-phenylethyl acetate	10.48a	14.88a	13.50a	22.42a	18.97ab	11.32b	8.70b	23.79a
Alcohols	**22.93**	**29.25**	**25.03**	**60.83**	**40.45**	**28.88**	**41.41**	**41.46**
3-methyl-1-butanol	7.95b	15.29a	13.29a	26.09a	24.07a	15.29b	21.67a	20.19a
2-phenylethanol	14.98a	13.96a	11.74a	34.74a	16.38b	13.59b	19.74a	21.27a
Acids	**32.45**	**30.94**	**25.58**	**84.42**	**42.05**	**42.18**	**46.31**	**40.04**
propanoic acid	0.96ab	–	1.80a	2.30a	–	1.59b	0.04	–
2-methylpropanoic acid	2.51a	–	0.96b	3.87a	–	2.14b	1.08b	8.80a
3-methyl butanoic acid	5.53c	12.55a	10.00b	10.62b	16.23a	9.95b	3.53a	2.52b
hexanoic acid	1.94	–	–	6.17	–	–	5.37	–
octanoic acid	13.99a	12.77a	9.38a	44.41a	19.41b	14.99b	27.80a	25.00a
n-decanoic acid	7.52a	5.62ab	3.44b	15.05a	6.41b	13.51a	8.49a	3.72b
Others								
benzaldehyde	19.49b	38.31a	34.11a	8.95c	93.35a	55.72b	56.52b	163.21a
acetoin	1.38a	1.29a	1.70a	2.22a	0.97b	1.21b	1.97a	2.43a
3-methylpentane	–	0.16	–	0.55a	0.37a	–	–	–
SUM	**92.70**	**127.56**	**113.33**	**201.71**	**254.53**	**153.63**	**299.53**	**268.04**

[a] Means (n=3) with different letters for the same vinegar differ significantly at $P<0.05$ during storage.

Table 14.9 Changes in sensory quality of pineapple vinegars fermented from different base pineapple wine during storage[a]

Vinegars	Storage (months)	Aroma				Flavour			
		Fruity	Wine	Pungency	Ester	Sour	Wine	Pungency	Overall mouth-feel
PVA	0	2.83a	2.03a	2.46a	2.25a	3.00a	1.38b	2.88a	2.13b
	6	2.88a	2.53a	1.88ab	2.12a	2.54b	2.23a	2.13b	2.54ab
	12	2.39a	2.42a	1.48^{b}	2.65a	2.59b	2.59a	2.50ab	3.07a
PVB	0	3.50a	1.63b	1.75a	2.42a	2.67a	1.34b	2.26a	2.79b
	6	3.33a	2.56a	2.17a	2.38a	2.39b	2.27a	2.11a	3.50a
	12	2.42b	3.15a	2.27a	2.54a	2.42b	2.79a	2.05a	3.15ab
PVC	0	4.31a	2.08b	1.96b	2.00a	3.27a	2.11a	2.73a	3.46a
	8	2.56b	3.00a	2.60a	2.20a	2.85a	2.33a	2.52a	2.24b

[a] Means (n=13) of 5-point scaling: weak (1), moderate (3), strong (5). Means with different letters for the same vinegar differ significantly at $P<0.05$ during storage.

stages of storage (Morales et al., 2002; Palacio et al., 2002). Meanwhile, compounds such as 3-methyl-1-butanol and 2-phenylethanol would be further synthesized because the secondary products from acetic acid bacteria would continue to react, and thus the concentration of these two compounds would not decrease even though both are consumed by the esterification process; the same result was also found in the above-mentioned studies (Morales et al., 2002; Palacio et al., 2002). However, the changes in concentration of these compounds were not significant for PVA after 6 months storage. The fruity aroma of PVA was slightly reduced after 1 year of storage, while the wine flavour and overall mouth-feel were significantly enhanced over time (Table 14.9).

In the first 6-month storage period, the volatile compounds in the headspace of PVB, such as 2-propenyl acetate, 3-methyl-1-butyl acetate, 2-phenylethyl acetate, 3-methyl-1-butanoic acid and benzaldehyde, continued to increase, while the volatile compounds of high concentration before storage, such as 2-phenylethanol and octanoic acid, decreased instead of increasing further, because the concentration in the headspace of the pineapple vinegar was already saturated (Table 14.8). After 1 year of storage, the decrease in the volatile compounds of PVB was clearly pronounced. After 6 months storage, the fruity aroma of PVB did not show any obvious difference from that before storage, but the fruity aroma clearly became weaker after 1 year of storage. Similar to PVA, the aroma and flavour of wine, as well as the overall mouth-feel of the PVB was significantly enhanced over time (Table 14.9).

The fruity aroma, which was originally strong in PVC, became noticeably weaker after 8 months of storage. One reason might be that the concentration of 3-methyl-1-butyl acetate had dropped significantly (Table 14.8), or there were undetected changes in the minuscule amount of volatile compounds in the pineapple peel that helped increase the fruity odour in vinegar. Consequently, the scent of the PVC showed no discernible difference from that of the PVA or PVB after 8 month storages, and the overall mouth-feel of the PVC also weakened substantially due to the considerable attenuation of its fruity aroma (Table 14.9).

Okumura (1995) and Tesfaye et al. (2002) have reported that overall impression or aroma intensity of sherry wine vinegar, rice or grain vinegar were clearly increased with ageing (or storage). However, fruity volatile compounds with high volatility would be gradually lost from the cap of the storage bottle over time, and this would cause the reduction in fruity odour and overall mouth-feel of PVC, which originally had the most fruit scent and good overall mouth-feel. To prevent the loss of fruity aroma during storage, the pineapple vinegar should be stored at low temperature or consumed within a short period of time.

On the other hand, the pineapple base wine brewed from peeled pineapple with sugar added to 26 °Brix (PWA) had a higher alcohol content (15-16%, v/v), and could be used for vinegar fermentation after 2-3 times dilution. Although this would result in vinegar with less fruity aroma and overall mouth-feel, the addition of 0.1% L-aspartic acid in PWA before the vinegar fermentation could enhance the fruity aroma in the final product.

14.5 Monitoring the Changes in Physico-Chemical Properties During Brewing of Pineapple Wine and Vinegar Using Near Infrared Spectroscopy

Traditionally, food analysis has been laborious and time-consuming, but recently, near infrared spectroscopy (NIRS) has become the preferred tool for analysing foods, especially major quality-related components, in the food industry. NIRS is not only fast, easy, and does not require the use of chemicals, but is also highly precise. Because the edible vinegar available in Taiwan is not regulated adequately, this study aims to establish NIRS calibration models for the soluble solids, total sugar content, alcohol content, acidity and acetic acid content in vinegar. In addition, changes in the brewing methods for pineapple wine or vinegar could be monitored and the results could be used for quality control (Chang, 2007).

A total of 130 samples were randomly selected from pineapple juice, the fermentation intermediate and products of pineapple wine and vinegar. All samples were divided into two parts – one for physico-chemical analyses and the other for scanning with a near infrared spectrometer at wavelengths between 400 and 2500 nm. For NIRS, ten samples were first selected randomly as the unknown set. After eliminating samples with variations that were too large or with an analytical value of zero from the remaining 120 samples, the rest of the samples were divided into the calibration set and the validation set in a ratio of 3:1. The calibration set was used to establish the calibration models, and the validation set was used to check the closeness between the NIRS predicted and experimental values. Finally, the unknown set was used to compare the difference between the experimental values and NIRS predicted values using a paired Student's *t*-test.

Table 14.10 shows the calibration values of soluble solids, total sugar content, alcohol content, acidity and acetic acid content for the calibration set as established by the NIRS. The explanation ability (R^2) of the five models was 0.975, 0.951, 0.972, 0.983 and 0.948, respectively. Subsequently, the spectra of the validation set were placed into the five calibration models to evaluate the predictability of the calibration curves. The outcome indicated that the correlation coefficient (r) between the NIRS predicted values and the experimental values for soluble solids, total sugar content, alcohol content, acidity and acetic acid were 0.987, 0.983, 0.985, 0.986 and 0.965, respectively. RPD (residual predictive deviation) – the ratio of standard deviation (SD) of the experimental data to the SEP (standard error of prediction) – was the statistical standard used to check the validation of the calibration models. It has been suggested that $RPD > 3.0$ indicates that the calibration model should perform well for quantitative analyses (Williams and Sobering, 1996). The RPD values for these five calibration models were 5.93, 5.47, 5.25, 4.50 and 3.20, respectively. This clearly indicates these calibration models should be applicable in actual situations. Finally, the ten randomly selected samples of unknown set were placed into the above calibration models, and the results showed there was no significant difference between the NIRS predicted values and the experimental values of soluble solids, total sugar content, alcohol content, acidity and acetic acid.

Table 14.10 Statistical descriptors for NIRS calibration and validation sets for physico-chemical properties of vinegar samples[a]

	Calibration set				Validation set				
Descriptors	*N*	**Range**	**SEC**	R^2	*N*	**Range**	**SEP**	*r*	**RPD**
Soluble solids (°Brix)	82	3.1-28.0	0.913	0.975	28	3.0-22.0	0.920	0.987	5.93
Total sugars (g · 100 mL^{-1})	81	0.30-29.55	1.577	0.951	27	0.10-17.63	0.679	0.983	5.47
Ethanol (%, v/v)	72	0.04-17.17	0.947	0.972	26	0.02-16.25	0.970	0.985	5.25
Acidity (%, w/v)	81	0.25-7.16	0.260	0.983	27	0.39-6.32	0.424	0.986	4.50
Acetic acid (mg · 100 mL^{-1})	59	0.04-76.17	5.931	0.948	18	0.14-47.30	6.139	0.965	3.20

[a] N, sample number. SEC, standard error of calibration. SEP, standard error of prediction. RPD, the ratio of the standard deviation (SD) of the sample to the SEP.

It can be observed from the outcome that the five calibration models for soluble solids, total sugar content, alcohol content, acidity and acetic acid could be used for monitoring the changes in these five constituents during fermentation of pineapple wine and vinegar and the products as well. It is concluded that the NIRS technique is a suitable tool for monitoring the quality of pineapple wine and vinegar during the fermentation processes.

14.6 Conclusions

Human beings have been consuming vinegar as a seasoning for thousands of years. Recently, with the appearance of reports showing that the consumption of vinegar is associated with health benefits, many fruit vinegar products have become available in the Taiwan market. Most Taiwanese people understand the term ‘fruit vinegar’ to mean rice vinegar that has been infused with fruit and sugar, and this is obviously different from the definition of fruit vinegar given by CNS and FAO/WHO. Therefore, there is a need to distinguish this kind of product, which usually has large amounts of sugar added, from the healthier brewed fruit vinegar. In addition, there is an urgent need to revise or update the regulations for edible vinegar products and to improve the technique for manufacturing healthy and palatable fruit vinegar products. We believe that these finding are not only applicable to Taiwan but to other places as well.

Our study also showed that the quality of fruit vinegar is greatly affected by the raw material of the fruit with regard to variety, processing with or without peel, the acetic acid bacteria chosen, the addition of nutrients, and storage time and temperature. The effects of the above-mentioned factors on the quality of the end-product should be evaluated not only by the fermentation efficiency, but also by sensory tests.

For acetous fermentation, we found that *A. aceti* BCRC 14156 was the best strain for brewing pineapple vinegar because it resulted not only in higher in fermentation efficiency, but also in higher volatile compounds with floral and fruity aromas. Because the alcohol tolerance of these acetic acid bacteria is around 5-7%, the pineapple base wine (PWA) with 15% alcohol was diluted before brewing vinegar. This resulted in lower ester, alcohol and acid contents and in a weaker fruity aroma and mouth-feel. However, with the addition of nutrients such as L-aspartic acid, the volatile compounds in PVA were substantially increased, so that there was then no obvious difference between PVA and the other two samples: PVB and PVC. On the other hand, the pineapple base wine with peel and no added sugar (PWC) was directly brewed without dilution. With many more constituents from the peel, its favourable sensory characteristics were clearly derived. Therefore, we conclude that using the pineapple base wine from the juice with peel but no added sugar is the best option. Another observed benefit is that the peeling step is thus eliminated, saving labour and time. Although storage test results indicated that PVC was less stable than the other vinegars, its quality should be improved when it is stored at low temperature instead of room temperature.

Besides the sensory evaluation for examining the sensory quality, monitoring the important physico-chemical properties such as the contents of ethanol, acetic acid, sugar, volatiles and acidity, pH, etc. in all the steps during alcoholic and acetous fermentation and storage is an important task for manufacturing a genuinely high-quality product. Our work also indicates that using NIRS is a feasible way of monitoring changes occurring at all stages of the brewing and storage of pineapple vinegar.

References

Adams MR (1978) Small-scale vinegar production from bananas. Trop Sci 20:11–19

Anon (1973) Converting pineapple waste to vinegar. Food Eng 45:79

Chang RC (2007) Investigation of the factors affecting the quality of pineapple vinegar. PhD thesis, National Chung-Hsing University, Taichung, Taiwan, Republic of China

Chang RC, Lee HC, Ou ASM (2005) Investigation of the physicochemical properties of concentrated fruit vinegar. J Food Drug Anal 13:348–356

Chang RC, Tai CY, Dent DF, Ou ASM (2006) Selection of acetic acid bacteria for fermenting pineapple vinegar. Taiwan. J Agric Chem Food Sci 44:351–359

Charles M, Martin B, Ginies C, Etievant P, Coste G, Guichard E (1992) Potent aroma compounds of two red wine vinegars. J Agric Food Chem 48:70–77

Chinese National Standard (2005) Edible Vinegar (CNS 14834, N5239). Ministry of Economic Affairs, Taiwan, Republic of China

Ciani M (1998) Wine vinegar production using base wines made with different yeast species. J Sci Food Agric 78:290–294

Fushimi T, Tayama K, Fukaya M, Kitakoshi K, Nakai N, Tsukamoto Y, Sato Y (2001) Acetic acid feeding enhances glycogen repletion in liver and skeletal muscle of rats. J Nutr 131:1973–1977

Fushimi T, Tayama K, Fukaya M, Kitakoshi K, Nakai N, Tsukamoto Y, Sato Y (2002) The efficacy of acetic acid for glycogen repletion in rat skeletal muscle after exercise. Int J Sports Med 123:218–222

Grewal HS, Tewari HK, Kalra KL (1988) Vinegar production from substandard fruits. Biol Wastes 26:9–14

Johnston CS, Kim CM, Buller AJ (2004) Vinegar improves insulin sensitivity to a high-carbohydrate meal in subjects with insulin resistance or type 2 diabetes. Diabetes Care 27:281–282

Joint FAO/WHO Food Standards Programme (2000) Proposed Draft Revised Regional Standard for Vinegar. In: Codex Coordinating Committee for Europe

Koizumi Y, Uehara Y, Yanagida F (1987) The general composition, inorganic cations, free amino acids and organic acids of special vinegars. Nippon Shokuhin Kogyo Gakkaishi 34:592–597

Kondo S, Tayama K, Tsukamoto Y, Ikeda K, Yamori Y (2001) Antihypertensive effects of acetic acid and vinegar on spontaneous hypertensive rats. Biosci Biotechnol Biochem 65:2690–2694

Lai MF (1989) Studies on the opened-type fermentation of wine vinegar. Master's thesis, National Chung-Hsing University, Taichung, Taiwan, Republic of China

Lin SB, Chen LC (2002) The preliminary studies for the selection of yeasts and acetobacters in brewing mead and honey vinegar. J Ilan Inst Technol 9:297–304

Lin TF (1994) Contribution of yeast to flavor development in wine. Spec Top Sci Technol Alcohol Bev 16:1–24

Morales ML, Tesfaye W, Garcia-Parrilla MC, Casas JA, Troncoso AM (2002) Evolution of the aroma profile of sherry wine vinegars during an experimental aging in wood. J Agric Food Chem 50:3173–3178

Nanba T, Kato H (1985a) Stimulative effects of the various compounds on the acetic acid production by Acetobacter pasteurianus No. 2. Nippon Shokuhin Kogyo Gakkaishi 30:331–337

Nanba T, Kato H (1985b) Changes in several components during acetic acid fermentation. Nippon Shokuhin Kogyo Gakkaishi 30:646–654

Nishidai S, Nakamura Y, Torikai K, Yamamoto M, Ishihara N, Mori H, Ohigashi H (2000) Kurosu, a traditional vinegar produced from unpolished rice, suppresses lipid peroxidation in vitro and in mouse skin. Biosci Biotechnol Biochem 64:1909–1914

Ogawa N, Satsu H, Watanabe H, Fukaya M, Tsukamato Y, Miyamoto Y, Shimizu Y (2000) Acetic acid suppresses the increase in disaccharidase activity that occurs during culture of Caco-2 cells. J Nutr 130:507–513

Okumura H (1995) Stabilizing the quality of vinegar. J Brew Soc Jpn 90:410–415

Palacio V, Valcarcel M, Caro I, Perez L (2002) Chemical and biochemical transformations during the industrial process of sherry vinegar aging. J Agric Food Chem 50:4221–4225

Richardson KC (1967) Submerged acetification of a vinegar base produced from waste pineapple juice. Biotechnol Bioeng 9:171–186

Shimoji Y, Tamura Y, Nakamura Y, Nanda K, Nishidai S, Nishikawa Y, Ishihara N, Uenakai K, Ohigashi H (2002) Isolation and identification of DPPH radical scavenging compounds in Kurosu (Japanese unpolished rice vinegar). J Agric Food Chem 50:6501–6503

Sugiyama A, Saitoh M, Takahara A, Satoh Y, Hashimoto K (2003) Acute cardiovascular effects of a new beverage made of wine vinegar and grape juice, assessed using an in vivo rat. Nutr Res 23:1291–1296

Tesfaye W, Garcia-Parrilla MC, Troncoso AM (2002) Sensory evolution of sherry wine vinegar. J Sensory Stud 17:133–144

Wen WS (2001) Studies on the fermenting conditions of pineapple wine using response surface methodology. Master's Thesis, National Chung-Hsing University, Taichung, Taiwan, Republic of China

Williams PC, Sobering D (1996) How do we do it: a brief summary of the methods we use in developing near infrared calibrations. In: Davies AMC, Williams PC (eds) Near Infrared Spectroscopy: The Future Waves. NIR Publications, Chichester, UK, pp. 185–188