Impact of the Ripening Stage of Wax Apples on Chemical Profiles of Juice and Cider
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ABSTRACT: Wax apple fruit (Syzygium aqueum Alston cv. Taaptimjan) is a tropical fruit with many nutritional bioactive compounds and high economic value. However, when fully ripe, the thin-skinned fruit is highly susceptible to physical damage and microbial spoilage that significantly reduce its commercial value. The present study was aimed to find a suitable ripening stage between the ripe and overripe wax apple fruits for developing a value-added beverage specifically cider. The chemical profiles of ripe and overripe wax apple juice and cider were determined by measuring sugar, acid, alcohol, mineral, proximate, ascorbic acid, amino acid, antioxidant, and volatile levels. Overall, the results showed significant variations. The physicochemical and proximate analysis showed highest values for ripe fruit juice than overripe fruit juice and the cider samples. The amino acids in the samples were found almost at similar levels. Polyphenolic content and antioxidant activities were higher in ciders than in unfermented juices. In addition, the overripe fruit cider had more volatile compounds than ripe fruit cider and unfermented juices. Overall, the overripe fruit is suitable for producing cider, whereas the ripe fruit is more suitable for the unfermented beverages.

INTRODUCTION
Fruits and vegetables produced in Thailand have good reputation for extraordinary quality and nutritional properties because of the uniqueness of the tropical conditions. Tropical fruit crops are one a major component contributing to the Thai economic growth, and the export value has trended upward in the recent decades.1 Wax apple is an important fruit grown in all parts of Thailand, with a high yield of 64 954 tons valued at 2431 million baht in 2016. Wax apple belongs to the genus of Syzygium in the Myrtaceae family. This fruit has many unique organoleptic characteristics of being glossy, colorful, tender, juicy, with sweet low-acidity taste, and with a pleasant aroma of roses. Depending on the variety, the color of wax apple varies from green to dark red.2 The fruit is widely grown in the tropical region of Asia, in which Thailand, Taiwan, Malaysia, and Indonesia are the major producers. In general, these fruits are pear or bell-shaped, about 3.4–6 cm long and 4–5 cm wide, and weigh from 70 to 200 g.

These are rich sources of nutrition with water, carbohydrate, protein, vitamins, and minerals, along with some medicinal properties such as antidiabetes, anti-inflammatory, anticonvulsant, antihypertensive, antimicrobial, antituberculosis, and anti diarrheal activities.1,2 However, this fruit is highly perishable because of its thin skin and can be easily damaged. Furthermore, wax apple has a plentiful water content in the flesh, inducing cracks, rot, and perishing.2 In addition, it is also sensitive to low temperatures (0–10 °C) that induce chilling injuries.2 The short shelf life and susceptibility to damage during transportation limits economic expansion of the wax apple market. Therefore, novel fruit processing techniques are needed to take full advantage of the quality and nutritional benefits. There have been several studies on extending the storage life of fresh fruits. However, wax apples are not widely considered in terms of processing and new product development, especially in the field of beverages. Thus, the processing of both wax apple juice and cider could enhance the value of the fruit and contribute to international commercialization.

Cider is a fermented low alcoholic beverage made from unfiltered fruit juice, mostly from apples. The alcohol content in cider depends on the sugar level of fruit juice and varies from 1 to 12%.4 During fermentation, microorganisms (mainly yeast) convert sugar to ethanol.4 Yeasts belong to the class of Saccharomyces in the fungal phylum Ascomycota. Generally, the species Saccharomyces, Kloekera, Candida, and Pichia are widely used in cider fermentation. High tolerance of acidity enables the yeast to survive and grow in fruit juices. It also has a significant role in the creation of the aroma profile in cider. The maximal tolerance of ethanol by yeast during fermentation is about 65% ethanol yield or 53 g/L ethanol, and after that, the yeast cell growth and the ethanol production slow down gradually.5 The efficiency and rate of fermentation are influenced by several factors, such as sugar content, temper-
nature, storage time, and pH. One key factor for the cider making industry is the choice of fruits, and traditionally apples are widely used because of natural flavor, level of sugars, water content, and richness in phytochemicals.\textsuperscript{6} Besides apples, there are many other fruits such as pear, currants, gooseberries, mulberries, elderberries, cherries, oranges, dates, pineapples, raspberries, and strawberries, which are likewise applicable to making ciders.\textsuperscript{6} However, no prior study has attempted to produce cider from the wax apple.

Fruit ripening is an irreversible developmental process that involves specific biochemical and physiological attributes. Wax apple is a non-climacteric fruit, which produces low ethylene and has a low respiration rate during ripening. Because it follows a non-climacteric pattern, the fruit will not further develop off the tree and will not respond to ethylene for ripening. Therefore, the development of wax apples until the harvest plays a crucial role in the fruit characteristics, such as color development, acid and sugar contents, and phytochemicals. To establish a high-quality standard of cider beverage, it is important to evaluate the impact of the fruit variety at different ripening stages on the various important compounds. Therefore, the purpose of this study was to determine whether the ripening stage has an effect on the juice and cider quality, and to generate new information about the physicochemical profiles of wax apple juices and ciders, when the apples are used at different stages of maturation. Parameters were quantified in samples including chemical profile (sugars, acids, alcohols, proximate, ascorbic acid), minerals, amino acid profile, phytochemicals, antioxidants, and flavor.

### RESULTS AND DISCUSSION

The physical qualities of ripe and overripe wax apple juices and their ciders are presented in Table 1. The color characteristics lightness (L), chroma (C\(^*\)) and hue (H\(^*\)) varied significantly among the samples. Ripe fruit juice exhibited higher L values than the other samples. Furthermore, fermentation slightly decreased the L values of wax apple juice to lower values for the overripe fruit juice than for the other samples. Additionally, fermentation slightly increased yellowness of food. The changes in color were due to interactions of anthocyanin degradation, which could be from degradation of anthocyanins during fermentation.

### Table 1. Physicochemical Profiles of Ripe and Overripe Wax Apple Juices and Ciders\textsuperscript{a}

| Analysis                     | Wax Apple Juice | Wax Apple Cider |
|------------------------------|-----------------|-----------------|
| **Lightness (L)**            | 44.4 ± 0.1      | 37.4 ± 0.3      |
| **Chroma (C\(^*\))**         | 24.8 ± 0.2      | 21.12 ± 0.4     |
| **Hue (H\(^*\))**            | 37.74 ± 0.1     | 58.15 ± 0.1     |
| **Viscosity (cP)**           | 6.58 ± 0.3      | 5.23 ± 0.3      |
| **Density (g/cm\(^3\))**     | 1.033 ± 0.1     | 1.002 ± 0.0     |
| **Electrical Conductivity**  | 2.62 ± 0.1      | 3.24 ± 0.1      |

\textsuperscript{a}(-) indicates no detection of an amino acid. Data presented are mean and standard error of the mean (SEM) from six replications.
Table 2. Amino Acid Profiles of Ripe and Overripe Wax Apple Juices and Ciders

| Amino acid analysis (mg/100 mL) | Wax apple juice | Overripe wax apple juice | Wax apple cider | Overripe wax apple cider |
|--------------------------------|-----------------|--------------------------|----------------|--------------------------|
| Alanine                        | 73.27 ± 3.50    | 70.06 ± 1.8              | 12.36 ± 0.7    | 9.77 ± 1.1               |
| Arginine                       | 3089.34 ± 5.0   | 2875.15 ± 5.0            | 2031.76 ± 2.0  | 1897.65 ± 7.0            |
| Aspartic acid                  | 205.02 ± 4.0    | 176.45 ± 2.0             | 76.01 ± 1.8    | 68.79 ± 5.5              |
| Cystine                        | -               | -                        | -              | -                        |
| Glutamic acid                  | 154.37 ± 5.6    | 78.45 ± 2.2              | 96.53 ± 1.0    | 74.38 ± 1.1              |
| Glycine                        | 50.04 ± 2.76    | 48.76 ± 1.3              | 43.47 ± 1.1    | 44.71 ± 1.0              |
| Histidine                      | 22.55 ± 1.50    | 17.89 ± 0.9              | 20.54 ± 0.5    | 15.51 ± 0.4              |
| Hydroxylysine                  | -               | -                        | -              | -                        |
| Hydroxyproline                 | 30.68 ± 3.0     | 25.74 ± 1.4              | 24.49 ± 0.7    | 22.41 ± 0.5              |
| Isoleucine                     | 5.46 ± 1.10     | 3.21 ± 0.5               | 4.75 ± 0.1     | 3.31 ± 0.1               |
| Leucine                        | 19.24 ± 1.7     | 10.11 ± 2.0              | 14.27 ± 1.0    | 8.10 ± 0.5               |
| Lysine                         | 292.32 ± 1.5    | 245.56 ± 2.0             | 250.74 ± 1.0   | 231.50 ± 4.0             |
| Methionine                     | 4367.81 ± 7.5   | 4389.12 ± 5.5            | 4396.66 ± 4.0  | 4371.56 ± 2.0            |
| Phenylalanine                  | 17.23 ± 1.2     | 25.44 ± 0.8              | 11.77 ± 1.0    | 19.47 ± 0.7              |
| Proline                        | 66.67 ± 4.1     | 61.75 ± 1.0              | 202.54 ± 1.5   | 217.13 ± 3.0             |
| Serine                         | 69.18 ± 1.1     | 70.19 ± 0.5              | 43.88 ± 0.5    | 48.95 ± 1.0              |
| Threonine                      | 242.38 ± 1.1    | 247.86 ± 2.0             | 212.88 ± 1.5   | 232.78 ± 1.0             |
| Tryptophan                     | 11.14 ± 0.5     | 9.07 ± 0.8               | -              | -                        |
| Tyrosine                       | 9.62 ± 0.7      | 7.24 ± 0.1               | 10.56 ± 0.5    | 9.84 ± 0.4               |
| Valine                         | 1.07 ± 0.2      | 0.97 ± 0.1               | 0.74 ± 0.1     | 0.67 ± 0.1               |
| Asparagine                     | -               | -                        | -              | -                        |
| Cysteine                       | -               | -                        | -              | -                        |
| Glutamine                      | 774.94 ± 2.4    | 815.26 ± 3.0             | 841.95 ± 1.0   | 917.14 ± 1.4             |
| **Sum of amino acid**          | **9502.33 ± 1.96** | **9178.28 ± 1.46**       | **8295.9 ± 0.89** | **8193.5 ± 1.95**        |

(-) indicates no detection of an amino acid. Data presented are mean and SEM from six replications.

and polyphenol oxidase during the fermentation. The ripe fruit juice had slightly higher viscosity than the other samples; the overripe fruit juice and the cider samples did not significantly differ. The density was slightly higher for the overripe fruit juice and the cider samples, however without significant difference (P > 0.05). Furthermore, the electrical conductivities of fresh juices were lower than those of the cider samples (P < 0.05).

The sugar, acid, and alcohol contents in ripe and overripe wax apple juices and their ciders are shown in Table 1. The fermentable sugars sucrose, D-glucose, and D-fructose were significantly higher in the overripe fruit juice than in the ripe juice. On the other hand, the cider samples had no detectable sugars after fermentation, suggesting that all sugar was converted to alcohol by yeast. In accordance with the sugar results, the total soluble solid (TSS) level was strongly decreased to below 3.2 °Bx in the cider samples, while before fermentation, it was between 10.5 and 10.8 °Bx in the fresh juices. Generally, titratable acidity (TA) relates to the buffering capacity that maintains pH during the fermentation, which could add and/or remove acid or alkali in the cider. TA was slightly decreased in the cider samples compared to fresh juice, possibly consumed to maintain the pH. pH of the fresh samples (4.29–4.39) was a bit lower than of the cider samples (4.62–4.63). Volatile acidity (VA) was plentiful in the cider samples relative to fresh wax apple juices. Overripe cider and juice samples had slightly elevated VA. Among alcohols, mainly ethanol and methanol were identified in the cider samples: ethanol was predominant while methanol contents were small (0.08–0.12%). A minute level of fermentation had occurred in the unfermented fruit juices, as evidenced by the presence of ethanol (see Table 1). This could be due to fermentation by natural yeasts on the fruit surfaces. The proximate compositions of ripe and overripe wax apple juice and their cider are shown in Table 1. Moisture was the dominant component in all proximate compositions. Ripe and overripe wax apple juices did not differ in moisture content (P > 0.05), but the ciders had slightly increased moisture. Normally, anaerobic fermentation does not influence the moisture content of a beverage. Ananias et al.10 reported increased moisture levels in the fermented beverages, which might be from the conversion of alcohol into acetic acid and water in the presence of oxygen during handling. The carbohydrates were higher in the fruit juices than in the ciders. Ash content was stable and did not differ among the samples (P > 0.05). In addition, the results showed less protein in the ciders than in the juices. The decreased protein in cider could be due to many reasons, it could be metabolized by yeast to form amino acids or fatty acids11 and/or changed pH during fermentation could degrade the protein.12 On the other hand, the fat content was elevated in the cider samples. Fat could be accumulated from dead yeast cell membranes. Phospholipids are the key factor strengthening yeast cells and increasing the ethanol level during fermentation, adversely changing the proportion of phospholipids and ergosterol in the cell membrane and thereby making an unsuitable environment for yeast to survive.13,14 The fresh fruit juices had higher caloric contents than the cider samples. Ascorbic acid was slightly decreased in the cider samples relative to the unfermented juices. Ascorbic acid is highly susceptible to chemical and enzymatic oxidation during processing. Escudero-López et al.14 observed a slight decrease in ascorbic acid in fermented orange juice. Among the different minerals, calcium, magnesium, potassium, copper, and zinc were predominant. The overripe fruit ciders had higher levels of calcium, magnesium, iron, potassium, copper, and zinc than the other samples. Overripe juice and cider
The polyphenolic compounds and antioxidant efficiencies of ripe and overripe wax apple juices and their ciders are presented in Table 3. There were a total of 9 phenolic compounds identified in the samples, namely, gallic acid, chlorogenic acid, caffic acid, p-coumaric acid, hydroxycinnamic acid, ferulic acid, vanillic acid, cyanidin-3-glucoside, and cyanidin-3-galactoside. Among these, chlorogenic acid, caffic acid, ferulic acid, and gallic acid were predominant in all the samples. The majority of phenolics was observed at higher levels in the cider than in the unfermented juices, except for the anthocyanins cyanidin-3-glucoside. In another study, Gomez et al.22 and Zhao and Dixon23 reported that cyanidin-3-glucoside could move into the yeast vesicles and another study, Gomez et al.22 and Zhao and Dixon23 reported that cyanidin-3-glucoside could move into the yeast vesicles and another study, Gomez et al.22 and Zhao and Dixon23 reported that cyanidin-3-glucoside could move into the yeast vesicles and another study, Gomez et al.22 and Zhao and Dixon23 reported that cyanidin-3-glucoside could move into the yeast vesicles and another study, Gomez et al.22 and Zhao and Dixon23 reported that cyanidin-3-glucoside could move into the yeast vesicles and another study, Gomez et al.22 and Zhao and Dixon23 reported that cyanidin-3-glucoside could move into the yeast vesicles and another study, Gomez et al.22 and Zhao and Dixon23 reported that cyanidin-3-glucoside could move into the yeast vesicles and another study, Gomez et al.22 and Zhao and Dixon23 reported that cyanidin-3-glucoside could move into the yeast vesicles and another study, Gomez et al.22 and Zhao and Dixon23 reported that cyanidin-3-glucoside could move into the yeast vesicles and another study, Gomez et al.22 and Zhao and Dixon23 reported that cyanidin-3-glucoside could move into the yeast vesicles and another study, Gomez et al.22 and Zhao and Dixon23 reported that cyanidin-3-glucoside could move into the yeast vesicles and another study, Gomez et al.22 and Zhao and Dixon23 reported that cyanidin-3-glucoside could move into the yeast vesicles and another study, Gomez et al.22 and Zhao and Dixon23 reported that cyanidin-3-glucosid
Table 4. Volatile Compounds in Ripe and Overripe Wax Apple Juices and Ciders*  

| volatile compound (relative concentration %) | wax apple juice | | wax apple cider | |
|---------------------------------------------|-----------------|---|----------------|---|
| butan-1-ol, 3-methyl-                        | 0.20 ± 0.01     | 0.35 ± 0.05 |                   | 0.10 ± 0.01 |
| furan, 2-ethyl-                              | 0.06 ± 0.00     | 0.06 ± 0.00 |                   | -              |
| propane, 1-methoxy-                          | -               | -            |                   | -              |
| pentanal                                     | 0.2 ± 0.05      | 0.8 ± 0.08  |                   | 0.24 ± 0.10    |
| ethene, fluoro-                              | 5.98 ± 0.80     | 7.60 ± 0.50 |                   | 6.20 ± 0.10    |
| 2-propanol, 1-chloro-                        | 2.44 ± 0.10     | 4.20 ± 0.10 |                   | -              |
| 1(R)-,2,6,6-trimethylbicyclo[3.1.1]hept-2-ene | 1.42 ± 0.30     | -            |                   | -              |
| bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methyl-ethyl)- | 0.84 ± 0.10   | 1.93 ± 0.10 |                   | -              |
| butanoic acid, ethyl ester                  | -               | -            | 0.12 ± 0.00       | 2.78 ± 0.10    |
| 1-propanol                                  | -               | -            | 0.43 ± 0.05       | 1.27 ± 0.20    |
| ethanol                                     | -               | -            | 0.67 ± 0.10       | 1.87 ± 0.10    |
| hexanol                                     | 38.36 ± 2.00    | 47.56 ± 1.50 |                   | -              |
| methanamine                                 | -               | -            | 3.89 ± 0.10       | 4.97 ± 0.30    |
| isocyanic acid                              | -               | -            | 0.54 ± 0.01       | 0.77 ± 0.01    |
| 1-butanol, 3-methyl- acetate                 | 0.63 ± 0.01     | 0.96 ± 0.01  | 9.27 ± 0.01       | 12.94 ± 0.01   |
| 7-hydroxyheptene-1                          | 2.00 ± 0.10     | 3.61 ± 0.20  |                   | -              |
| 2-hexanal, (E)-                             | 0.98 ± 0.01     | 1.02 ± 0.01  |                   | -              |
| hexanoic acid, ethyl ester                  | -               | -            | 0.45 ± 0.10       | 0.89 ± 0.10    |
| 3-methyl butanol                            | 0.23 ± 0.01     | 0.58 ± 0.01  |                   | -              |
| α-cymene                                    | 3.91 ± 0.30     | 7.41 ± 0.10  |                   | -              |
| 1-butanol, 3-methyl-                         | -               | -            | 21.07 ± 0.50      | 29.11 ± 0.80   |
| hexan-1-ol                                  | 0.29 ± 0.01     | 0.37 ± 0.05  |                   | -              |
| acetic acid, 3-methylpentyl ester            | -               | -            | 2.25 ± 0.04       | 3.54 ± 0.01    |
| 1-pentanol                                  | -               | -            | 0.12 ± 0.01       | 0.61 ± 0.05    |
| 4-hexen-1-ol, acetate                       | -               | -            | 0.23 ± 0.01       | 2.19 ± 0.10    |
| 3-hexen-1-ol, acetate, (Z)-                  | 0.14 ± 0.10     | 0.28 ± 0.20  | 0.31 ± 0.10       | 1.79 ± 0.20    |
| 1-pentanol, 2-methyl-, acetate               | -               | -            | 1.95 ± 0.01       | 2.24 ± 0.30    |
| cyclohexyl acetate                          | -               | -            | 0.11 ± 0.05       | 0.18 ± 0.10    |
| 2,2-dimethyl-3-isopropenylcyclobutane-1-ethyl acetate | -           | -            | 0.14 ± 0.10       | 0.14 ± 0.20    |
| formamide, N,N-bis(2-cyanoethyl)-            | 12.3 ± 0.60     | 23.61 ± 0.40 |                   | -              |
| 3-hexen-1-ol, (E)-                           | 0.87 ± 0.10     | 1.97 ± 0.10  | 0.46 ± 0.01       | 1.05 ± 0.01    |
| 3-hexen-1-ol, (Z)                            | 5.77 ± 0.50     | 10.13 ± 0.20 |                   | -              |
| 4-hexen-1-ol, (E)                            | -               | -            | 3.77 ± 0.20       | 7.51 ± 0.60    |
| 2,4-hexadienial                             | 0.91 ± 0.10     | 1.31 ± 0.10  |                   | -              |
| 2-hexen-1-ol, (E)                            | 0.29 ± 0.05     | 0.79 ± 0.06  |                   | -              |
| ethyl octonate                              | -               | -            | 3.09 ± 0.20       | 5.08 ± 0.50    |
| α-cymene                                    | 1.21 ± 0.10     | 2.47 ± 0.10  |                   | -              |
| 1-hexanol, 2-ethyl-                          | 0.19 ± 0.01     | 2.59 ± 0.01  | 0.07 ± 0.01       | 0.21 ± 0.01    |
| benzaldehyde                                | 0.28 ± 0.30     | 0.35 ± 0.20  |                   | -              |
| 2,3-butanediol, [R-(R*,R*)]-                 | -               | -            | 1.13 ± 0.10       | 4.17 ± 0.50    |
| 1-octanol                                   | -               | -            | 0.22 ± 0.01       | 0.26 ± 0.05    |
| propanoic acid, 2-methyl-                    | -               | -            | 0.16 ± 0.05       | 0.31 ± 0.01    |
| caryophyllene                               | 4.22 ± 0.60     | 5.56 ± 0.50  |                   | -              |
| 1-isopropyl-4-methyl-3-cyclohexen-1-ol       | 0.47 ± 0.01     | 0.92 ± 0.10  |                   | -              |
| ethyl decanoate                             | -               | -            | 0.42 ± 0.01       | 0.77 ± 0.01    |
| α-humulene                                  | 0.38 ± 0.05     | -            |                   | -              |
| butanoic acid, 2-methyl-                     | -               | -            | 0.49 ± 0.01       | 0.92 ± 0.04    |
| 1-propanol, 3-(methylthio)-                  | -               | -            | 0.40 ± 0.01       | 1.10 ± 0.01    |
| 1,3-propanediol, diacetate                  | -               | -            | 0.15 ± 0.03       | 0.37 ± 0.01    |
| Δ-cadinene                                  | 0.59 ± 0.10     | 1.17 ± 0.10  |                   | -              |
| Oxime-, methoxy-phenyl- acetide             | 0.83 ± 0.22     | 1.62 ± 0.30  | 1.45 ± 0.06       | 3.34 ± 0.50    |
| β-phenylethyl butyrate                       | -               | -            | 0.78 ± 0.20       | 1.57 ± 0.10    |
| propanoic acid, 2-methyl, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester | 0.24 ± 0.01 | 0.58 ± 0.10 |                   | -              |
| benzamidine, 3-methoxy-                      | -               | -            | 5.98 ± 0.70       | 6.71 ± 0.90    |
| octanoic acid                               | 0.75 ± 0.10     | -            | 0.14 ± 0.10       | 0.24 ± 0.20    |
| decanoic acid                               | -               | -            | 0.72 ± 0.10       | 1.79 ± 0.10    |

*(-) indicates no detection of an amino acid. Data presented are mean and SEM from six replications.
Budak et al.\textsuperscript{25} also reported high antioxidant activities in apple ciders compared to unfermented apple juice. The results showed that wax apple juice and its cider were more efficient in scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and hydroxyl radicals than ABTS\textsuperscript{+} radicals. Khandaker et al.\textsuperscript{24} have found that red wax apple fruit exhibits high DPPH scavenging activity. Fakruddin et al.\textsuperscript{26} observed similar antioxidant efficiencies, particularly in DPPH radical scavenging, reducing power potentiality, and hydroxyl radical scavenging, in yeast that extracted from fruit as compared to standard ascorbic acid. This could also be one of the reasons for slightly higher antioxidant activities in wax apple ciders.

The flavor profiles of ripe and overripe wax apple juices and their ciders are presented in Table 4. A total of 57 flavor compounds in 10 categories, namely, alcohols (18), acids (12), esters (8), terpenes (6), aldehydes (3), amines (2), and other compounds (8), were observed in the juices and ciders. The overripe fruit cider (37 compounds) showed more volatile compounds than the other samples: ripe fruit cider (35 compounds), ripe fruit juice (29 compounds), and overripe fruit juice (26 compounds). The results revealed that fermentation helps develop more flavor compounds in the wax apple juice. The unfermented ripe fruit juice exhibited more flavor compounds compared to overripe fruit juice. On the other hand, the overripe fruit cider showed more volatile compounds than the ripe juice cider. Overall, the different ciders and juices varied significantly. Yeast plays a vital role in developing various flavor compounds through its metabolic activity. Among the various flavor compounds alcohols, esters and their acetates, acids, and fatty acids are important flavor contributors in alcoholic beverages.\textsuperscript{27} Normally, alcohol is a byproduct of yeast performing anaerobic fermentation of sugar in a fruit juice. It has an intense and pungent odor that contributes significantly to the various flavor compounds. The alcohol flavor in cider is normally categorized as “higher alcohol,” and it is also a precursor for synthesizing esters in the cider. The present study showed that the wax apple ciders had abundant levels of alcoholic flavor compounds. Among the various alcohols, hexanal and 3-hexen-1-ol, (Z)- were predominant in the juices, while 1-butanol, 3-methyl- and 4-hexen-1-ol, (E)- were predominant in the ciders. Overall, 7 alcoholic compounds were identified in the juice samples, and 10 compounds were observed in the cider samples. Bicyclo[3.1.0] hex-2-ene, 2-methyl-5-(1-methyl ethyl)- and 1-hexanol, 2-ethyl- were identified in the juice and cider samples.

Many studies have reported ester compounds as the major contributors of sweet and fruity odor in cider, and these are mainly formed through esterification of alcohols with fatty acids during the fermentation and post-fermentation stages (Peng, Li, Cui, & Guo, 2015). Some esters, particularly 3-hexen-1-ol, acetate, (Z)-, acetic acid, 2-phenylethyl ester, 7-hydroxy heptane-1 and 1-butanol, 3-methyl-, acetate were observed in both juices and ciders. Qiao et al.\textsuperscript{28} found various natural fruity flavor ester compounds in unfermented orange juice. Qin et al.\textsuperscript{29} reported that fresh apple juice had esters before it was fermented. The overripe fruit cider contained a higher level of ester compounds than the ripe fruit cider. This could be due to the higher concentration of alcohol in the overripe samples (see Table 1). Carboxylic acids are abundant volatile compounds in ciders, and furthermore, fatty acids are responsible for the cider’s fruity, cheesy, and rancid notes.\textsuperscript{30} The present results show that the more acidic flavor compounds were present in the ciders than in the juices.

Qin et al.\textsuperscript{29} reported that acids normally show a high odor detection threshold, but they did not affect much cider flavor. There are four fatty acids, namely, ethyl octanoate, ethyl decanoate, octanoic acid, and decanoic acid observed in the cider samples, and octanoic acid was also found in the ripe juice samples. On the other hand, acetic acid was observed at a lower concentration in both juice and cider samples, and the overripe juice cider had a higher concentration of it (1.57%). Acetic acid is responsible for vinegar flavor in the cider samples, and it is synthesized by the oxidation of acetaldehyde by alcoholic or malolactic fermentation.

Terpenes are pungent oils contributing fruity odor, and in the present study, they were only found in the ripe and overripe juice samples, not in the ciders. Zhang et al.\textsuperscript{31} reported that terpenes were generated in red wine through esterification. P-Cymene, caryophyllene, α-humulene, 1-isopropyl-4-methyl-3-cyclohexen-1-ol, and Δ-cadinene were identified in wax apple ripe and overripe samples. Caryophyllene was predominant among the others, and the overripe juice had a higher level of terpenes than the ripe juice. Aldehydes such as pentanal, 2,4-hexadienal, and benzaldehyde were identified only in the fruit juice samples, and the overripe fruit juice had slightly more aldehydes. Ye et al.\textsuperscript{30} reported that a lower concentration of aldehydes could provide a pleasant fruity flavor. On the other hand, amines (methanamine and 3-methoxy benzamine) were only observed in the cider samples. The overripe fruit cider had more amines than the ripe fruit cider. Garai et al.\textsuperscript{32} stated that biogenic amines are closely related to ripening and fermentation and are normally generated into fermented beverages by microbial decarboxylation of amino acids. The other compounds observed were two ethers (propane, 1-methoxy- and 2-propanol, 1-chloro), two cycloalkanes \{[(1R)-2,6,6-trimethylbicyclo [3.1.1] hept-2-ene and bicyclo [3.1.0] hex-2-ene, 2-methyl-5-(1-methyl ethyl)-]}, two fluorocarbons (furan, 2-ethyl- and ethene, fluoro), and two phenols (oxime-, methoxy-phenyl-). Among the various other compounds, 2-propanol, 1-chloro- was dominant and was found in the overripe fruit cider. Overall, the cider made of overripe wax apple fruit juice had more numerous volatile compounds than the other samples.

**CONCLUSIONS**

The present study had provided information regarding the impacts of the ripening stage on the chemical profiles of juices and ciders made of the wax apple fruit. The results revealed that over ripeness of the fruit influenced the physicochemical properties, phytochemicals, and flavor compounds widely. Fruit juice and its cider did not differ much in terms of synthesis and/or degradation of bioactive chemical compounds. A slight decrease in amino acids was observed in the cider samples from their level in juice, but the changes were smallish. As compared to ripe fruits, overripe fruits are possibly suitable for producing fermented beverages, as they have abundant vitamins and antioxidants. The cider produced from overripe wax apple fruit juice contains more than 6% of ethanol, and it is considered to be a hard cider. The fruit at the overripe stage normally has a low economic value because of its short shelf life; therefore, producing value-added products such as cider could improve the competitiveness of this unique seasonal fruit.
EXPERIMENTAL SECTION

Raw Material Preparation. The ripe and overripe wax apples (*Syzygium aqueum* Alston cv. Taapirimjan) were hand-picked from a commercial orchard in Surat Thani province of southern Thailand. The collected samples were screened for uniform size [height (7–9 cm) and width (6–8 cm in diameter)], color (dark red), total soluble solids (10–14 °Bx), and for being free of defects (apparent damage or disease). The procured fruit was taken to the laboratory within 8 h and then sorted into two groups (ripe and overripe). Wax apples were cleaned of dust with tap water and then washed again with distilled water. After that, the fruit was thoroughly juiced using a food processor (Philips centrifugal juicer) in a cold environment (4–7 °C), then roughly strained with cheese close, and a part of the juice extracts was stored at −18 °C for further analysis while the remaining juice was used for cider preparation.

Cider Preparation. The cider fermentation process was carried out based on the method of Alberti et al. with some modifications. The wax apple juice extract (6 L) from each group was fermented in a fermenter. Prior to fermentation, the wax apple juice was thawed to the ambient temperature before yeast inoculation. The cider yeast strain, *Saccharomyces bayanus* (specific for cider and wine fermentation and obtained from FERMENTIS, France), was inoculated (10⁶ cells/mL) according to manufacturer’s instructions. Alcoholic fermentation took place in an anaerobic environment at 25 °C for 10 days (the duration was set based on a preliminary study). After the fermentation, the cider was racked, bottled, and frozen (−18 °C) until further analyses.

Physicochemical Analysis. The color of the juice and cider was measured using a Hunter Lab Ultra Scan colorimeter (Hunter Associates Laboratory, Inc. Reston, VA, USA). The color coordinates L, a, and b were recorded and further converted to chroma (C*) and hue (H°) angle. The kinetic viscosity was obtained using a Brookﬁeld viscometer (Model LVDV-II+, Brookfield Engineering Laboratory, Inc., Middleboro, USA) and is reported as apparent viscosity (cP). The density of samples was measured using a hydrometer based on the buoyant force acting on a tared body floating in the liquid. The results are expressed in g/cm³. The electrical conductivity of the sample was monitored using a conductivity meter, giving the result in mS/cm² (YSI Incorporated, Yellow Springs, OH, USA). For chromatographic analysis of sugar and ethanol, the samples were diluted to 1:10 (mL/mL) with ultrapure water and then filtered through a membrane prior to analysis. The injection volume was set to 10 μL, and the flow rate was 0.5 mL/min in isocratic conditions with ultrapure water (Milli-Q). The column used was a Waters Sugar Pak 1 (300 × 6.5 mm), and identification of components was done by comparing to the retention times of reference standards. For quantification, calibration curves were made for sucrose, d-glucose, d-fructose, ethanol, and methanol. For TSSs, a sample of a few drops was analyzed using a digital handheld refractometer (Atago, Tokyo, Japan), and the results are expressed in °Bx. For determination of acids and VA, the TA was determined by volumetric neutralization with sodium hydroxide, using phenolphthalein as the indicator. The TA is expressed in equivalent tartaric acid (g/L). VA was measured based on the method proposed by Kourkoutas et al. The results are expressed as equivalent acetic acid (g/L). The pH of wax apple juice and cider samples was measured using a digital pH meter (pH30 Tester, CLEAN, Shanghai, China).

Proximate Analysis. Wax apple juice and cider were subjected to proximate analysis. The analysis included protein, carbohydrate, fat, ash, moisture, vitamins, and minerals. The Soxhlet method was used for fat content, and the moisture content was measured using a moisture analyzer. The Kjeldahl method according to AOAC was used for protein determination. For ash content, the sample was first dried in an oven at 100 °C before being transferred to a muffle furnace at 550 °C until a white or light gray ash remained. Ascorbic acid content in the juice and cider samples was quantified using a modified high-pressure liquid chromatography (HPLC) assay suggested by Lee and Coates. The minerals iron, copper, zinc, calcium, sodium, potassium, and magnesium were measured using atomic absorption spectrophotometry with a flame emission spectrophotometer (PerkinElmer 460).

Amino Acid Profile. The identification of amino acids in juice and cider samples was done by the chromatographic method developed by Alberti et al. The identification and quantification were accomplished by HPLC according to the AccQ-Tag reagent kit methodology with a Pico-Tag column (4 mm, 3.9 × 150 mm). Twenty-three amino acids were quantified in the sample and are reported in mg/100 mL.

Polyphenolic Compounds and Antioxidants. The extraction and identification of phenolics (by HPLC) in the samples were conducted using the method described by Alberti et al. The retention times and spectra of the selected polyphenolic standards were used to identify the polyphenolic compounds in the samples by using calibration curves of standards. The polyphenolic compounds, mainly gallic acid, chlorogenic acid, caffeic acid, hydroxyl cinnamic acid, ferulic acid, vanillic acid, cyanidin-3-o-glucoside, and cyanidin-3-o-rutinoside; and the flavonoids such as catechin, quercetin, kaempferol, and rutin, were identified and quantified, and the results are expressed in μg/mL. The total polyphenol and total flavonoid contents were also calculated based on the method of Alberti et al. The DPPH scavenging activity was determined as described by Alberti et al. The results are expressed as percentages. The ABTS⁺ radical cation scavenging activity of the samples was determined using the method suggested by Campodonico et al. The results are expressed as percentages. The method suggested by Alberti et al. was applied for identifying the ferric reducing ability of the samples. The results are expressed as percentages. The hydroxyl radical (OH⁺) scavenging ability of the samples was measured by the deoxyribose method described by Halliwell et al. The results are expressed as percentages.

Chromatographic Analysis of Volatile Compounds. The analysis was performed by mixing 6 mL of a sample with 50 μL of the internal standard (heptanoic acid) in a 20 mL glass vial. Prior to analysis, the samples were incubated for 10 min at 60 °C under agitation in the oven of the automatic injector. After that, the volatile compounds were identified using the static headspace method proposed by Pietrowski et al.

Statistical Analysis. The fermentation of wax apple juice extracts was performed in six replications. The data are presented as mean and SEM (n = 6). Pearson correlations (r) of the observed variables were assessed. Two-way ANOVA was performed to study the interactions between the evaluated variables of the juice and cider. All the statistical analyses were run in SPSS v6 for Windows (IBM, NY, USA).
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