

Changes in the physicochemical characteristics, bioactive compounds, flavor volatiles and antioxidant properties of noni juice (*Morinda citrifolia*) during natural fermentation

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Research Article

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Abstract

Noni (*Morinda citrifolia*) is a tropical plant native to South-East Asia, the fruit of which is usually fermented to extract the juice as a drink, and for medicinal purposes. Despite its widespread consumption and good natural medicinal properties, little is known about its postharvest handling and compositional changes during processing. Here, changes in the physiochemical characteristics (pH value, titratable acidity (TA) and total suspended solid (TSS)), bioactive compounds (total phenolic compounds, flavonoids, lignins), flavor volatiles, and antioxidant capacity were monitored in the broth of naturally fermented noni fruit using conventional analytical methods. The pH value of the fermented noni fruit showed an overall declining trend (from 4.35 to 4.04) while TA increased slowly (from 2.91 g/kg) in the first 24 weeks, reaching 9.25 g/kg at week 36. Fermentation had little impact on TSS, and values remained largely unchanged (1.34 mg/mL). The total phenolic compounds revealed an overall decreasing trend (from 1.52 mg/L to 0.87 mg/mL) reaching a maximum value (1.79 mg/mL) during the first week. Within this group, the total flavonoids decreased rapidly in the first week of fermentation (from 0.531 mg/mL to 0.295 mg/mL) while lignins decreased slowly in the first few weeks before increasing to 0.314 mg/mL by week 4. Both species increased from week 36 to reach 0.732 mg/mL and 0.366 mg/mL at the end of fermentation. The DPPH scavenging capacity of the extract reached its highest value (100%) at week 4. A total of 112 volatile compounds were identified in the noni juice at week 1, and their compositions changed during fermentation over 1-24 weeks. Methyl caproate and caprylic acid were the dominant compounds, with the latter contributing to the distinctive odor of the fermenting juice. During the first week, a high concentration of total esters and increasing TA was the dominant characteristics; total flavonoids reached highest level in week 2; alcohols were the major class of compound in week 4, reaching maximum levels at 8. The changes in physicochemical characteristics, active and odor compounds at different stages of the fermentation may provide a foundation to understand and control key relationships during the commercial production of noni fruit.

Highlights

Changes of physiochemical characteristics, phenolic compounds, flavonoid compounds, lignan compounds and volatile compounds during the Noni fruit fermenting process. Antioxidant activity changes during the Noni fruit fermenting process. Taken together, We proposed that changes in bioactive compounds may be related to antioxidant activity, while knowledge of the behavior of volatile components could confirm the origin of the characteristic fermentation odor and its control. Our results also provide a reference for understand and control key relationships during the commercial fermentation of noni fruit.

Introduction

Noni (*Morinda citrifolia*), is a small fruit bearing tree of the coffee family (*Rubiaceae*), native to Southeast Asia. All parts of the plant are sources of traditional medicines for native aboriginal, Polynesian and Southeast Asian communities while the fermented juice is consumed as a beverage. Noni fruit is rich in

fiber, contains many seeds and the ripening fruit has been called “vomit fruit” due to its pungent odor/strong taste. Consequently, the fruit is usually fermented to extract the juice for consumption as a beverage and traditional medicine. During fermentation, the microbial metabolic processes degrade the insoluble high molecular weight materials in the raw fruit into smaller soluble compounds that can be utilized by the human body, as well as generating various substances with antioxidant activity.

Noni fruit has a moisture content of ~ 90% and is enriched with many bioactive compounds such as fatty acids, anthraquinones, flavonoids, and polysaccharides, which account for pharmacological properties. The main soluble solids are polysaccharides and phenolic compounds including flavonoids, coumarins, lignans, anthraquinones, and phenolic acids. Noni fruit juice has antioxidant, antimicrobial, bacteriostasis and anticancer properties, and can also induce cardiovascular protection, increased immune activity, and preventative effects towards liver damage from excessive alcohol consumption [1-9], etc.

The aim of this study was to determine the key changes in physicochemical characteristics (pH value, titratable acidity (TA) and total suspended solid (TSS)), phenolic (total phenolic (TPC), flavonoid (TFC), and lignin (TLC)) and flavor compounds, and antioxidant activity at different stages in the natural fermentation of noni fruit. We proposed that changes in bioactive compounds may be related to antioxidant activity, while knowledge of the behavior of volatile components could confirm the origin of the characteristic fermentation odor and its control. An understanding of these changes will provide a foundation to understand and control key relationships during the commercial fermentation of noni fruit.

Materials And Methods

2.1 Chemicals and materials

All chemicals were of analytical reagent grade. Deionized water (18.2MΩ·cm) was prepared in-house.

Noni fruit (*Morinda citrifolia*; obtained by crossbreeding of wild varieties in the Xisha Islands) were obtained as a single batch from Sanya, (Hainan Province, China). Undamaged fruit were selected and rinsed with deionized water to remove debris, and air dried before individually sealing in sterile containers prior to natural fermentation in the dark at 25-30°C. Individual samples were taken at the onset of the experiment (week zero) and at the end of weeks, 1, 2, 4, 8, 12, 16, 24, 36 and 48. Test samples were homogenized, centrifuged at for 10 min, and the supernatant was stored in amber reagent bottles in the dark at -80°C prior to analysis.

2.2 Physiochemical characteristics (pH, TA, and TSS)

The pH values were measured using a pH meter. TA was determined by acid-base titration according to Chinese National Standard (CNS) GB/T 12456-2008 [22], and the results were expressed as acetic acid. The TSS content of the fermentation broth was obtained at a given temperature using a Abbe refractometer based on CNS GB/T 12143-2008 [23].

2.3 Total phenolic content (TPC)

TPC was measured by the Folin-Ciocalteu assay [24]. Briefly, the absorbance of deionized water, gallic acid standard and sample solutions (1 mL each in 10 mL cuvettes) was measured. Folin-Ciocalteu's reagent (5 mL of 10%) was added, the solution was allowed to stand for 3-8 min, Na₂CO₃ solution (4 mL of 7.5%) was added, and shaken well. After incubation at room temperature for 60 min, the absorbance was measured at 740 nm and the results (determined by external standard quantification) were reported.

2.4 Total flavonoid content (TFC)

The TFC was measured by the colorimetric aluminum nitrate method [25]. The absorbance of deionized water, rutin standard and sample solutions (1 mL each in 10 mL cuvettes) were recorded on the UV-Vis. Spectrophotometer. Sodium nitrite solution (0.3 mL of 5%; stand for 6 min) was added, followed by sodium hydroxide solution (4.0 mL of 1 mol/L) and the volume was made to 10 mL with deionized water and shaken to mix. After standing for 15 min, the absorbance recorded 511 nm and the results (obtained by external standard quantification) were expressed as rutin equivalents.

2.5 Total lignin content (TLC)

The lignin content was measured by UV spectroscopy [26]. Sample solutions (0.5 mL in 10 mL cuvettes) were diluted to volume with methanol and shaken to mix. An aliquot of the solution (3 mL) was transferred to second 10 mL cuvette, diluted to volume with methanol, mixed well and centrifuged. The absorbance of the supernatant was measured at 285 nm and the system.

2.6 Antioxidant activity

Antioxidant activities of the fermentation broth samples were determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay using serial dilution. DPPH (2 mL of 3.94 mg/L in ethanol, used immediately after preparation) was added to each sample (2 mL) and maintained in the dark for 30 min; a control and blank control were prepared using ethanol (2mL) and water (2mL) respectively; and the absorbances were recorded 517 nm. The DPPH scavenging rate in each sample was calculated from Eqn. (1) [27]:

$$\text{DPPH scavenging rate (\%)} = [1 - (\text{AS}-\text{AC})/\text{A0}] \times 100 \tag{1}$$

Where AS, AC and A0 are the absorbances of the sample, control and blank control, respectively.

2.7 Analysis of volatile compounds

Volatile components in the fermentation broth were sampled between weeks 1 and 24 using solid phase microextraction (SMPE) for analysis on a 6890 GC coupled to a HP-5973 MS (Hewlett-Packard, USA) according to the conditions given in Table 1.

Table 1: SPME-GC-MS conditions used for the analysis of fermentation volatiles

SPME	Samples were mixed with saturated NaCl solution (1 mL) in 20 mL headspace vials and equilibrated at 70°C for 30 min. The headspace was sampled with a 50/30 µm divinylbenzene polydimethylsiloxane on a StableFlex fiber (Merck, USA) for 30 min, followed by desorption in a split injector for 5 min.	
GC-MS conditions	GC column:	HP-5MS (30 m × 0.25 mm × 0.25 µm; Agilent, USA)
	Split ratio:	None
	Carrier gas:	He at 1.0 mL/min
	Injection port temperature:	250°C
	GC column temperature program:	45°C, hold for 4 min; 6°C/min to 130°C hold for 6 min; 10°C/min to 250°C hold for 8 min.
	Scanning mode:	Full Scan
	Ion source temperature:	230°C
	Quadrupole temperature:	180°C

2.8 Statistical analysis

The experimental results were expressed as the mean ± SD of three parallel measurements, and data analysis was performed using IBM SPSS Statistics for Windows, Version 23 (IBM Corp., Armonk, NY, USA).

Results And Discussion

3.1 Physiochemical characteristics (pH, TA, TSS)

The changes in pH and TA are given in Tables 2 and 3. The initial pH showed no significant change during the first week, decreasing uniformly thereafter during fermentation (from 4.35 to 4.04; $p < 0.05$). This suggested that week 1 was a key stage in the fermentation process resulting in pH change. A corresponding increase in TA (from 2.91 g/kg to 10.39 g/kg) was recorded over the same period. The value at week 36 was significantly different from those at weeks 4, 8, 12, 16 and 24 ($p < 0.05$), indicating a key change in TA at this stage.

Table 2 pH of *Morinda citrifolia* fruit during fermentation $\bar{X} \pm \text{SD}$ $n=3$

Fermentation time week	pH	Fermentation time week	pH
0	4.35±0.08 a	12	3.99±0.04 c
1	4.30±0.10 a,b	16	4.05±0.05 c
2	4.07±0.07 b,c	24	4.04±0.09 c
4	4.09±0.06 b,c	36	3.98±0.11 c
8	3.99±0.04 c	48	4.12±0.34 b,c
P	P 0.05	P	P 0.05

Note: P < 0.05 refers to the group compared to the group, and if the group is represented by different letters, then there is a significant difference between the two groups, with the highest content being a, followed by b, and c.

Table 3 Total acid content of *Morinda citrifolia* fruit during fermentation $\bar{X} \pm SD$ n=3

Fermentation time week	Total acid content g/kg	Fermentation time week	Total acid content g/kg
0	2.91±0.16 e	12	5.04±0.74 b,c,d
1	3.20±0.64 d,e	16	5.44±0.99 b,c
2	3.98±0.40 c,d,e	24	6.20±1.47 b
4	4.64±1.04 b,c,d,e	36	9.25±1.62 a
8	5.35±0.79 b,c	48	10.39±1.98 a
P	P 0.05	P	P 0.05

Note: P < 0.05 refers to the group compared to the group, and if the group is represented by different letters, then there is a significant difference between the two groups, with the highest content being a, followed by b, c, d, and e.

Fermentation appeared to have little impact on TSS results (Table 4) which changed very little over the 48 weeks. The small initial increase and subsequent decrease between weeks 1 and 8 (from 1.3433 to 1.3408) indicated that first week may be a key stage for changes in TSS.

Table4 Soluble solids content of *Morinda citrifolia* fruit during fermentation n=3

Fermentation time week	Soluble solids content	Fermentation time week	Soluble solids content
0	1.3426 a,b	12	1.3412 b,c
1	1.3433 a	16	1.3412 b,c
2	1.3419 b,c	24	1.3412 b,c
4	1.3412 b,c	36	1.3415 b,c
8	1.3408 c	48	1.3408 c
P	P 0.05	P	P 0.05

Note: $P < 0.05$ refers to the group compared to the group, and if the group is represented by different letters, then there is a significant difference between the two groups, with the highest content being a, followed by b, and c.

3.2 Total phenolic content (TPC)

The TPC of the fermented fruit juice samples (Table 5) showed an overall decreasing trend (from 1.52 mg/L to 0.87 mg/mL), increasing to 1.79 mg/L initially, with some small variations between.

Table5 Total polyphenol content of *Morinda citrifolia* fruit during fermentation $X \pm SD$ $n=3$

Fermentation time week	Polyphenol content mg/mL	Fermentation time week	Polyphenol content mg/mL
0	1.52 $\pm$ 0.02 a,b,c	12	1.13 $\pm$ 0.19 d,e,f
1	1.79 $\pm$ 0.08 a	16	1.19 $\pm$ 0.10 b,c,d,e
2	1.54 $\pm$ 0.48 a,b	24	1.16 $\pm$ 0.07 c,d,e,f
4	1.66 $\pm$ 0.25 a	36	0.81 $\pm$ 0.06 f
8	1.28 $\pm$ 0.15 b,c,d	48	0.87 $\pm$ 0.08 e,f
P	P 0.05	P	P 0.05

Note: $P < 0.05$ refers to the group compared to the group, and if the group is represented by different letters, then there is a significant difference between the two groups, with the highest content being a, followed by b, c, d, e, and f.

3.3 Total flavonoid content (TFC)

The TFC decreased significantly in the first week (Table 6) ($p < 0.05$) from 0.531 mg/mL to 0.295 mg/mL, fluctuating thereafter before increasing again in week 36th ($p < 0.05$) to reach a maximum value at the end of fermentation. Hence, the periods between weeks 1 and 36 were considered important for the

changes in the flavonoid content, and these events may represent critical control points for the future optimization of the nutritional value and functionality of noni juice.

Table6 Total flavonoid content of *Morinda citrifolia* fruit during fermentation $X \pm SD$ $n=3$

Fermentation time week	Flavonoid content mg/mL	Fermentation time week	Flavonoid content mg/mL
0	0.531 $\pm$ 0.10 b	12	0.197 $\pm$ 0.03 d
1	0.295 $\pm$ 0.02 c,d	16	0.203 $\pm$ 0.02 c,d
2	0.314 $\pm$ 0.07 c	24	0.215 $\pm$ 0.02 c,d
4	0.203 $\pm$ 0.01 c,d	36	0.614 $\pm$ 0.08 b
8	0.229 $\pm$ 0.03 c,d	48	0.732 $\pm$ 0.12 a
P	P 0.05	P	P 0.05

Note: $P < 0.05$ refers to the group compared to the group, if the group is represented by different letters, then there is a significant difference between the two groups with the highest content of a, followed by b, c and d.

3.4 Total lignan content (TLC)

The changes in TLC of the noni juice are listed in Table 7. The overall trend of initial decrease and subsequent increase was significant at weeks 4 and 36 ($p < 0.05$), suggesting that these were key stages during fermentation.

Table7 Total lignin content during the fermentation of *Morinda citrifolia* fruit $X \pm SD$ $n=3$

Fermentation time week	Lignin content mg/mL	Fermentation time week	Lignin content mg/mL
0	0.273 $\pm$ 0.04 b,c	12	0.283 $\pm$ 0.01 b,c
1	0.290 $\pm$ 0.01 b,c	16	0.278 $\pm$ 0.01 b,c
2	0.250 $\pm$ 0.03 c	24	0.274 $\pm$ 0.01 b,c
4	0.244 $\pm$ 0.03 c	36	0.321 $\pm$ 0.01 a,b
8	0.314 $\pm$ 0.03 b	48	0.366 $\pm$ 0.05 a
P	P 0.05	P	P 0.05

Note: $P < 0.05$ refers to the group compared to the group, if the group is represented by different letters, then there is a significant difference between the two groups, with the highest content being a, followed by b, and c.

3.5 Antioxidant activity

The antioxidant capacity of the noni fruit during fermentation was determined by examining its ability to eliminate free radicals. The results obtained using the free radical scavenger DPHH showed significant changes in the antioxidant ability of the fermented noni fruit ($p < 0.05$) in weeks 8, 12, 16 and 24, implying that these were key transitional periods (Figure 1).

3.6 Volatile compounds

The composition of volatile compounds in the noni fruit were analyzed between weeks 1 and 24. A total of 112 volatile compounds could be divided into seven categories. During the first week, the concentration of the groups followed the order, esters (63.0%) > acids (25.1%) > ketones > alcohols > alkanes > aldehydes > other alkanes > heterocyclic hydrocarbons > phenols. The esters and acids accounted for the largest share of volatile compounds at weeks 1, 2 and 4, with the alcohols dominating thereafter. The changes in the different categories of volatile compounds over weeks 1 to 24 are presented in Figure 2. The results demonstrated that most of the volatile components were formed during the fermentation process, and their composition and quantity in the broth varied with time.

While the esters were the predominant group in the first week, their content declined significantly in week 2 ($p > 0.05$) and remained unchanged for the remainder of the sampling period. Methyl caproate was the dominant ester during week 1, followed by methyl caprylate and ethyl hexanoate, and their concentrations all decreased during fermentation. Farine et al., [10] and Pino et al., [11] obtained similar results in ripe noni fruits. The observed increase in fruity esters and decreases in acids could be attributed to esterification in the ripening process [11] while hydrolysis occurred in the fermentation process (Figure 3).

The organic acids ranked the second highest group during the first week, while TA remained at a high level in weeks 2 and 4 before declining for the remainder of the sampling period ($p > 0.05$). Caprylic acid was the main acid, and together with caproic (C6) and capric (C10) acids, they have been identified as the chemicals responsible for the unpleasant odor in the fermentation broth of noni fruit. Caprylic acid also possesses insecticidal properties [10-12]. The aliphatic organic acids, generated by the biosynthesis or degradation of fatty acids and the branched chain acids like 2-octenoic acid and 2-hexenoic acid, are associated with the synthesis of aliphatic amino acids [13]. Acetic acid was the second most abundant acid, and it appeared in the first four weeks of fermentation. Its generation could be ascribed to an increase in *Lactobacillus* species within this period [14, 15].

After week 4, alcohols were the dominant compounds, reaching maximum levels by week 8 ($p < 0.05$). Among these, 1-hexanol had the highest concentration, followed by ethanol. The subsequent conversion of alcohols to acids and esters, and vice versa, was consistent with the observed changes in the amounts of esters, acids and alcohols in the fermentation process.

In addition, terpenes and phenolic compounds, such as myrcene and eugenol, respectively, were found in the fermentation broth. These are important and potent odor compounds in culinary spices (e.g., *Syzygium aromaticum*) and the perfume industry, and may contribute to the unique odor of noni.

Overall, the concentrations of esters reduced significantly while the acids and ketones decreased slowly during fermentation. The alcohols, phenols and aldehydes initially increased to remain relatively stable beyond week 8.

3.7 Significance of the measured changes

3.7.1 Physiochemical changes

Values of pH and TA are important indices reflecting the degree of fermentation and hence yield of products. The overall decrease in pH, and corresponding increase in TA, indicated that this was due to the increased generation of organic acids by microorganism carbohydrate metabolism [16]. The small but statistically significant overall change in TSS could result from the initial processing of the samples. Squeezing of the fruit to extract the juice, followed by centrifugation without natural precipitation, increased the initial soluble fiber content which then decreased by microbial action.

3.7.2 Phenolic compounds

Phenolic compounds are important markers of visual appearance, flavor, nutritional value, and bioactivity in foods. Polyphenolic compounds bound to, or adsorbed, by celluloses, proteins, etc., were probably released by microbial action in the first week (rapid fermentation stage) [17]. Following dissolution, their overall decrease in the fermentation broth could occur via various reactions such as oxidation, combination with organic acids to generate phenolic acids ^[18], and combination with alkaloids or polysaccharides to form macromolecular substances ^[19-20].

The initial decreases in TFC and TPC may be due to their oxidation or transformation into other substances. On the other hand, the substantial increase in flavonoids from week 36 may result from increased transfer into the broth as the cellular material of the noni fruit degrades in late fermentation [21].

Conclusion

Changes in the physiochemical characteristics, bioactive compounds and flavor compounds and antioxidants were measured during the natural fermentation of noni fruit over 48 weeks. Overall decreasing and increasing trends in pH and TA respectively reflected increasing microbial activity; TA increased markedly at week 36. The overall declining trend in TPC agreed with the removal of phenolic compounds by oxidation or reaction with other components formed during fermentation. However, TFC and TLC both increased from week 36, were possibly due to increased breakdown of the fruit cellular material and release of the phenolics into the fermentation broth. A total of 112 volatile compounds were identified during the initial stage of the natural fermentation of noni fruit. Methyl caproate and caprylic acid were the dominant compounds, and the C8 acid contributed to the unique odor during fermentation. Antioxidant activity reached a maximum at week 4. While most compounds reduced during fermentation, those exerting antioxidant effects have yet to be identified. Key stages during the fermentation process

were as follows: During the first week, a high concentration of total esters and increasing TA were the dominant characteristics; TFC reached highest levels in week 2; and alcohols were the major class of compounds in week 4, reaching maximum levels at week 8. These key stages they may provide a foundation to understand and control key relationships during the natural fermentation of noni juice.

Declarations

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contribution Statement

Hanqiao Liang designed, supervised the study and revised the manuscript. Da Li, Jing Tian and Yufei Liang performed the experiments. Da Li and Jing Tian analyzed the data and drafted the manuscript. Jing Tian and Yikai Fan collected the samples for the study.

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Figures

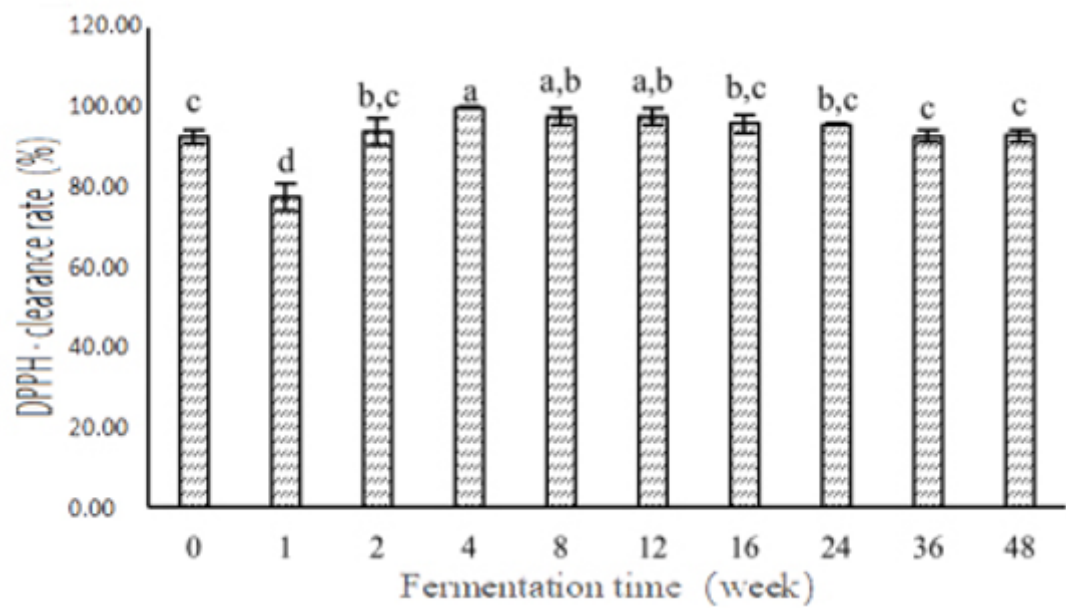


Figure 1

Changes results of free radical scavenger DPPH

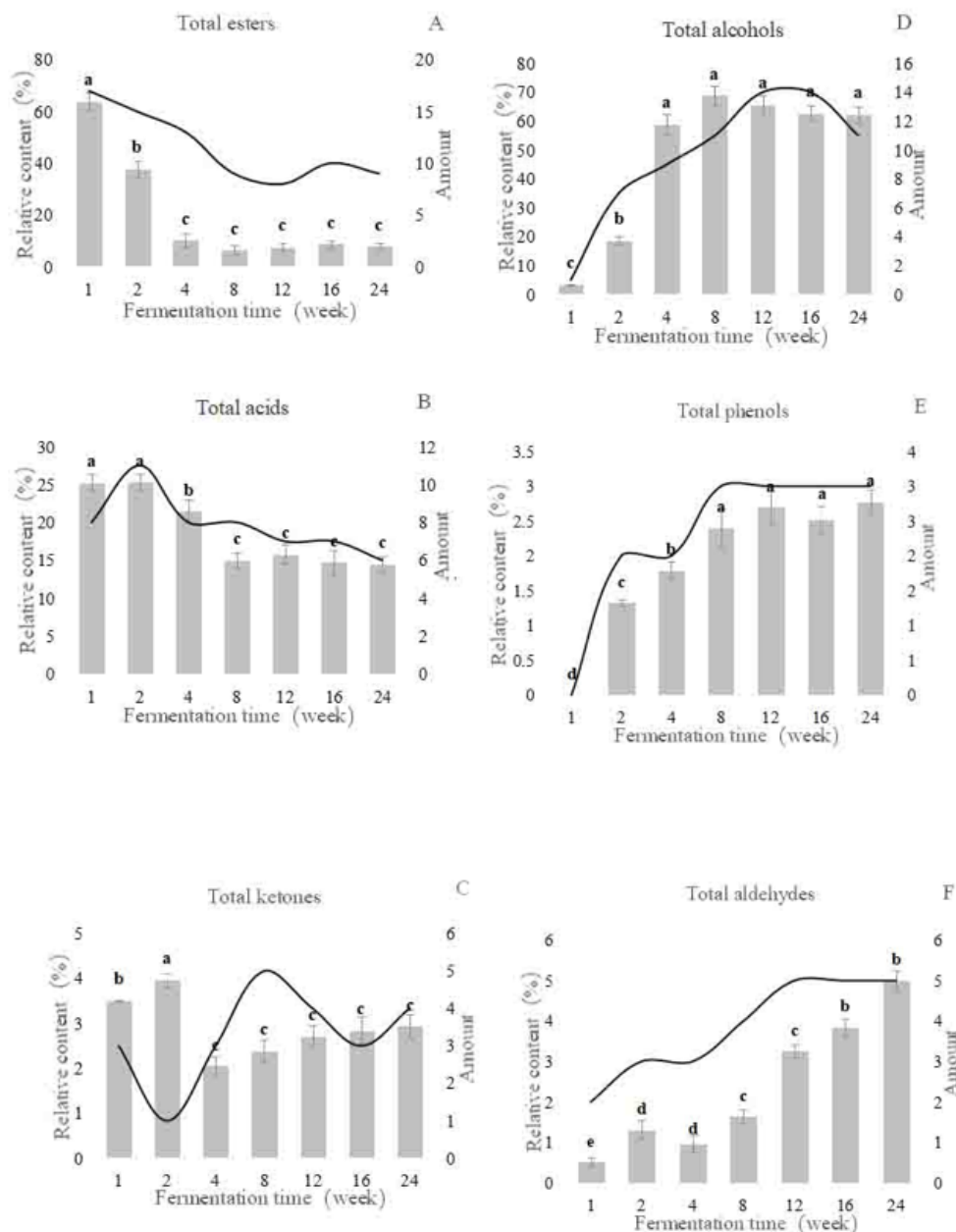


Figure 2

Changes in total esters (A), total acids (B), total ketones (C), total alcohols (D), total phenols (E) and total aldehydes (F) in green and fermented *Morinda citrifolia* fruits. The marked error lines indicate the standard error of the results for each treatment (n=3). Different letters indicate significant differences between samples ($P < 0.05$).

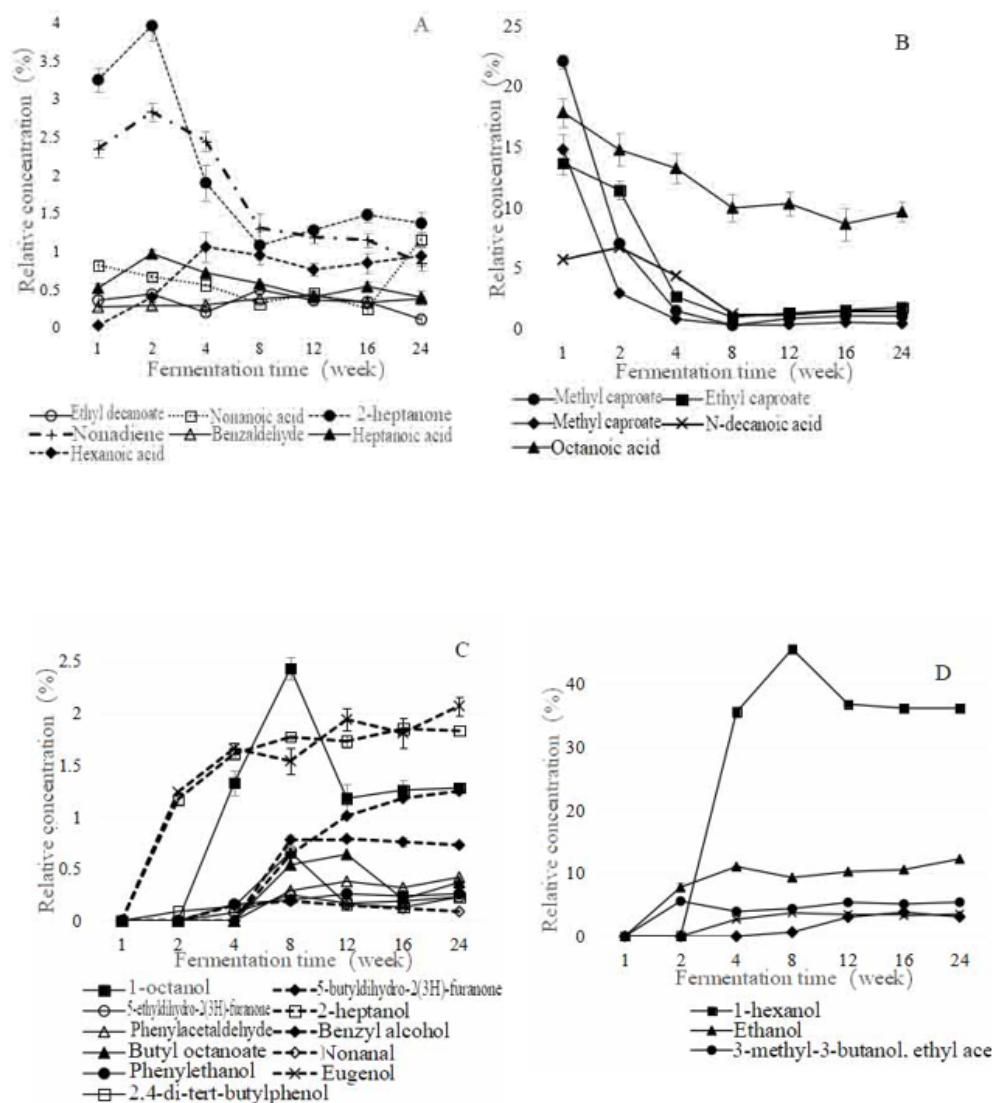


Figure 3

Changes in major volatile compounds during fermentation: (A) Changes in the contents of ethyl decanoate, nonanoic acid, 2-heptanone, nonadiene, benzaldehyde, heptanoic acid, and hexanoic acid, which were always present during fermentation. The contents of 2-heptanone and laurylenes decreased rapidly before the 8th week of fermentation, and the rest were not significantly different. (B) Changes in methyl caproate, ethyl caproate, methyl caproate, n-decanoic acid and octanoic acid, which were always

present during the fermentation process. The content of all three esters and both acids decreased significantly until the 8th week of fermentation. (C) and (D) Ten alcohols, two aldehydes, and two esters (1-octanol, 5-butylidihydro-2(3H)-furanone, 5-ethylidihydro-2(3H)-furanone, 2-heptanol, phenylacetaldehyde, benzyl alcohol, butyl octanoate, nonanal, phenylethanol, eugenol, 2,4-di-tert-butylphenol, 1-hexanol, ethanol, 3-methyl-3-butanol, ethyl acetate, and 1-butanol) began to appear after week 2 of fermentation and then continued to increase until week 8. 1-octanol and 1-hexanol decreased significantly over 8-12 weeks and then stabilized.