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Full Length Research Paper

Chemical composition changes of post-harvest coconut inflorescence sap during natural fermentation

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Coconut inflorescence sap (CIS) is sweet, oyster-white and translucent and was reported to be highly nutritive and a good digestive agent. The chemical composition changes including total sugar, reducing sugar, ethanol, total acidity, volatile acid, amino acid, vitamin C and total phenolic contents of post-harvest coconut inflorescence sap (PCIS) were investigated during a 12-day natural fermentation, and the variety and content of phenolic compounds of fresh coconut inflorescence sap (FCIS) and natural fermented coconut inflorescence sap (NCIS) were also studied by an high performance liquid chromatography (HPLC) system. Total acid, volatile acid and total phenolic contents increased during natural fermentation, while total sugar contents decreased during natural fermentation. The amino acid content declined steadily after harvesting until 3 days, but then remained almost constant. Vitamin C content decreased on day 1, slowly rose to 20.7 mg/L on day 3, and then decreased obviously. Five kinds of phenolic compounds were detected by HPLC. These compounds all increased in NCIS compared with FCIS; both NCIS and FCIS also contained other kinds of phenolic compounds.

Key words: Coconut inflorescence sap (CIS), post-harvest, natural fermentation, chemical composition.

INTROTUCTION

Coconut palm (*Cocos nucifera* L.) is an important member of the monocotyledons. This tree mainly grows in tropical coastal areas. Every part of the tree is useful in one way or another (Borse et al., 2007). Therefore, coconut palm is an important economic crop of the local places. In China, more than 90% coconut palms grow in Hainan Province and have long been the symbol and the characteristic tourism product of the region. Fresh coconut inflorescence sap (FCIS), obtained by tapping the unopened spadix of coconut palm, is sweet, oyster-white and translucent (Gupta et al., 1980) and was reported to be highly nutritive and also function as a good digestive agent (Devdas et al., 1969). FCIS is usually consumed as a juice by local people in Southeast Asia and is also used as raw material for the production of sugar, alcoholic beverages, vinegar and acetic acid (Purnomo, 1992).

FCIS is rich in sugar (10 ~ 15%) and is at a nearly neutral pH. It contains 16 kinds of amino acids and various vitamins such as vitamin C, vitamin B complex, especially nicotinic acid (Aalbersberg et al., 1997). The rich nutritious components make coconut inflorescence sap (CIS) highly susceptible to spontaneous fermentation even during the process of harvesting, especially in sunlight. Many researchers have studied the chemical or microbiological compounds of FCIS and naturally fermented coconut inflorescence sap (NCIS). Borse et al. (2007) studied the chemical composition of volatiles from FCIS and NCIS which were fermented for 24 h at 30 ± 2°C; 21 compounds making up more than 98% of the volatiles in FCIS, and 12 compounds representing more than 95% of the volatiles in NCIS, were characterized. Atputharajah et al. (1986) studied the distribution of microorganisms and changes of physical and chemical

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contents during natural fermentation of CIS and 166 isolates of yeasts and 39 isolates of bacteria were identified, while 17 species of yeasts belonging to eight genera were recorded. Tomomatsu et al. (1996) also studied for a whole day the changes of sugar and acidity of post-harvest coconut inflorescence sap (PCIS). However, there have been few reports about the changes of functional components such as polyphenol compounds and vitamin C contents of CIS during natural fermentation.

In this study, the chemical composition changes including total sugar, reducing sugar, ethanol, total acidity, volatile acid, amino acid, vitamin C and total phenolic content of PCIS were analyzed during a 12-day natural fermentation, and the variety and content of phenolic compounds of FCIS and NCIS were also studied by an HPLC system.

MATERIALS AND METHODS

Ascorbic acid, 2,2`-dipyridyl, Folin-Ciocalteu reagent, trichloroacetic acid, gallic acid, protocatechuic acid, caffeic acid, p-coumaric acid and galangin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other reagents were of analytical or HPLC grade.

Collection of FCIS

FCIS was collected by tapping the unopened spadix of the palm from three trees of the *C. nucifera* L. (Hainan local tall cultivar) respectively in the experimental farm in Wenchang, Hainan, China, and were stored in a container which had been thoroughly washed with boiled water, and then desiccated completely as a precautionary measure to avoid microbial contamination. The FCIS was collected overnight and kept at 4°C with an ice bath. The containers were transported to the laboratory and stored at 4°C until processing.

A portion of each sap was quickly filtered (Whatman No.1), put into a conical flask covered with cheese cloth, and then fermented for about 12 days at 25 ± 2°C, respectively in triplicate. An aliquot of the sap was removed from each conical flask and the physicochemical compositions were investigated periodically.

Determination of total acidity and volatile acid

Total acidity was determined by titrating 10 ml of each sample with 0.1 mol/L sodium hydroxide using phenolphthalein as indicator and expressed as gram lactic acid per 100 ml of the sample (Jackson et al., 2004). Volatile acid was determined by distilling the sample and then titrating the remaining nonvolatile acid using 0.1 mol/L sodium hydroxide and using phenolphthalein as indicator. The content of volatile acid was then calculated by subtracting the content of the remained nonvolatile acid from the content of total acidity.

Determination of amino acid content

Amino acid content was measured using ninhydrin colorimetric analysis according to Rosen (1957). 0.5 ml cyanide-acetate buffer and 0.5 ml 3% ninhydrin solution in methyl cellosolve were added to a 1 ml sample. The resulting solution was heated for 15 min in a 100°C water bath, then it was immediately removed from the water bath, 5 ml isopropyl alcohol-water diluents was added, and shaken vigorously to homogenate. The mixture was cooled to room temperature and its absorbance was determined in a colorimeter at 570 nm.

Determination of total sugar and reducing sugar

Total sugar was transformed into invert sugar. To obtain invert sugar, 50 ml of clarified sap was heated and was boiled for 2 min, and then 15 ml of 5% hydrochloric acid was added. The resulting solution was then neutralized with sodium bicarbonate. Reducing sugar was calculated as invert sugar too (Jackson et al., 2004).

Determination of ethanol content

Ethanol in the sap was estimated using an ebulliometer. 50 ml distilled water was added to 50 ml sap. The fluid was distilled until the distillate was up to 50 ml. Then, ebulliometer was used to determine the ethanol content (Samarajeewa and Tissera, 1975).

Determination of total phenolic content

The total phenolic contents of samples were quantified by the Folin-Ciocalteu's reagent and were expressed as gallic acid equivalents. Aliquots of test samples (100 µl) were mixed with 2.0 ml of 2% Na_2CO_3 and incubated at room temperature for 2 min. After the addition of 100 µl of 50% Folin-Ciocalteu's phenol reagent, the reaction tube was further incubated for 30 min at room temperature, and finally absorbance was determined at 720 nm (Fernandez and Carandang, 1990).

Determination of vitamin C content

Vitamin C content was determined spectrophotometrically. 0.2 ml of sample was mixed with 0.8 ml of 2 mol/L phosphate buffer, 0.8 ml of 43% phosphoric acid, 0.8 ml of 4% 2, 2`-dipyridyl, 1.0 ml of 10% trichloroacetic acid, and 0.4 ml of 3% ferric trichloride. The mixture was then incubated at 42°C in a water bath for 40 min and the absorbance was read at 525 nm (Samarajeewa et al., 1985).

Identification of sugars

Sugars were identified by thin-layer chromatography (TLC). The saps were centrifugated at 11,180 *g* for 30 min (Universal 32R, Hettich Zentrifugen, Germany). The supernatant was removed to a Teflon pestle (plastic bottle) and diluted to five times the original volume by 95% ethanol so as to be used in TLC analysis. The optimum conditions for TLC were: 5 µl of each diluted sample was applied to silica gel G plates, developed by acetic acid/ chloroform/95% ethanol (25:23:15 v/v/v) and then detected by spraying with aniline/diphenylamine/85% phosphoric acid (2:1:10, v/w/v) in acetone, and was heated at 120°C for 10 min. Sugars from the sap were identified by comparing their R_f values with those of authentic samples of sugars.

Identification and determination of phenolic compounds

The test materials were FCIS and NCIS. The NCIS had been fermented at 25 ± 2°C for 24 h. Sap samples of 5.0 g each were dissolved with ultra pure water and made constant to a 10 ml measuring flask. They were put into ultrasonic cleaners (WF-

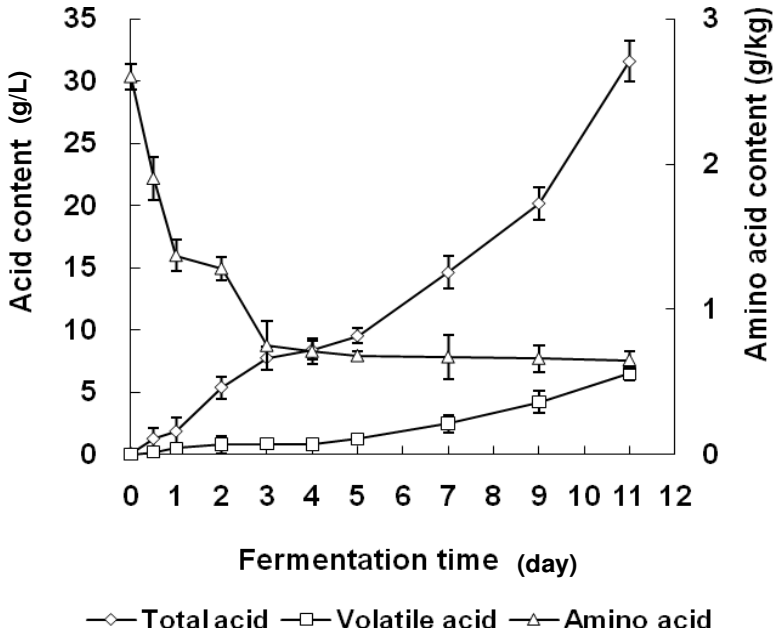

Figure 1. Changes of total, volatile and amino acid contents of PCIS during natural fermentation. The results are representative of three independent experiments and values are expressed as mean ± SD from three experiments.

180EH, WUFANG Ultrasonic Equipment CO., LTD, Ningbo, China) and dissolved for 5 min within. An aliquot sample (5 µl) was analyzed by an HPLC system (Agilent 1100 Series, Santa Clara, CA) equipped with a diode array detector (DAD)(G1315A) covering a spectral range of 190 to 800 nm and by a Zorbax SB-C18 column (150 × 4.6 mm, 5.0 µM; Agilent, Santa Clara, CA). The eluent was a mixture of methanol and 2% acetic acid by a linear programming and the flow rate of the eluent was 1.0 ml/min. The elution gradients were methanol/2% acetic acid (2:98 v/v) for 0 to 6 min, methanol/2% acetic acid (8:92 v/v) for 6 to 10 min, methanol/2% acetic acid (15:85 v/v) for 10 to 18 min, methanol/2% acetic acid (35:65 v/v) for 18 to 23 min, methanol/2% acetic acid (55:45 v/v) for 23 to 28 min and methanol/2% acetic acid (70:30 v/v) for 28 to 33 min. The column temperature was 30°C. The wavelength of DAD was set at 280 nm. The data were stored and processed by HP Chemstation.

Statistical analysis

The data were statistically analyzed using SPSS 13.0 software package (SPSS, Shanghai, China) and reported as mean ± SD. The differences among the experimental groups were identified by one-way analysis of variance (ANOVA) using Duncan's multiple range test. The statistical significance was considered at $P < 0.05$. All experiments were repeated at least three times.

RESULTS AND DISCUSSION

Changes of total acid, volatile acid and amino acid contents of PCIS during natural fermentation

Natural fermentation of CIS consists of three stages: an initial lactic acid fermentation, a middle alcoholic fermentation and a final acetic fermentation. At each stage, the microbial activity helps the activity of the micro-organisms in the next stage (Atputharajah et al., 1986). The acids present in CIS include lactic, acetic, tartaric, malic and citric acid, but the volatile acid mainly consists of acetic acid (Samarajeewa et al., 1985). Figure 1 shows the changes of total acid and volatile acid of PCIS during natural fermentation. The total acid increased constantly from day 1 to 3 and we believed it to be in the lactic acid fermentation. This increased slowly from day 4 to 5 until PCIS began its ethanol fermentation. This condition might therefore enhance the growth and invertase activity of the yeasts (Atputharajah et al., 1986). The total acid rose sharply after day 5, which could be the acetic acid fermentation phase. Moreover, the volatile acid content increased slightly from day 1 to 5 and it increased sharply thereafter, which also showed that it was in acetic acid fermentation phase after day 5. These changes of the total acid do not however completely agree with the results of Atputharajah et al. (1986) where the total acid content increased from day 1 to 5, but slowly decreased thereafter. The difference might be due to the different microorganisms in CIS. Atputharajah et al. (1986) did not report the changes of volatile acid.

The amino acid content was 2.6 g/kg of FCIS. Amino acid content decreased dramatically on day 1 with the reproduction of microorganisms. However, this decrease was not obvious on day 2, which might be due to the degradation of protein in the sap. The amino acid

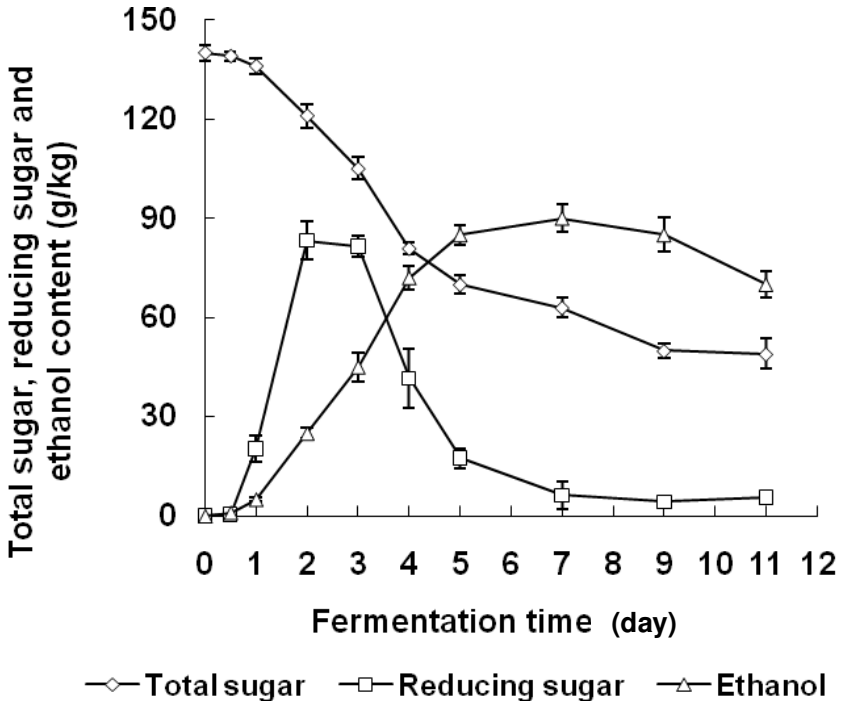

Figure 2. Changes of total sugar, reducing sugar and ethanol contents of PCIS during natural fermentation. The results are representative of three independent experiments and values are expressed as mean ± SD from three experiments.

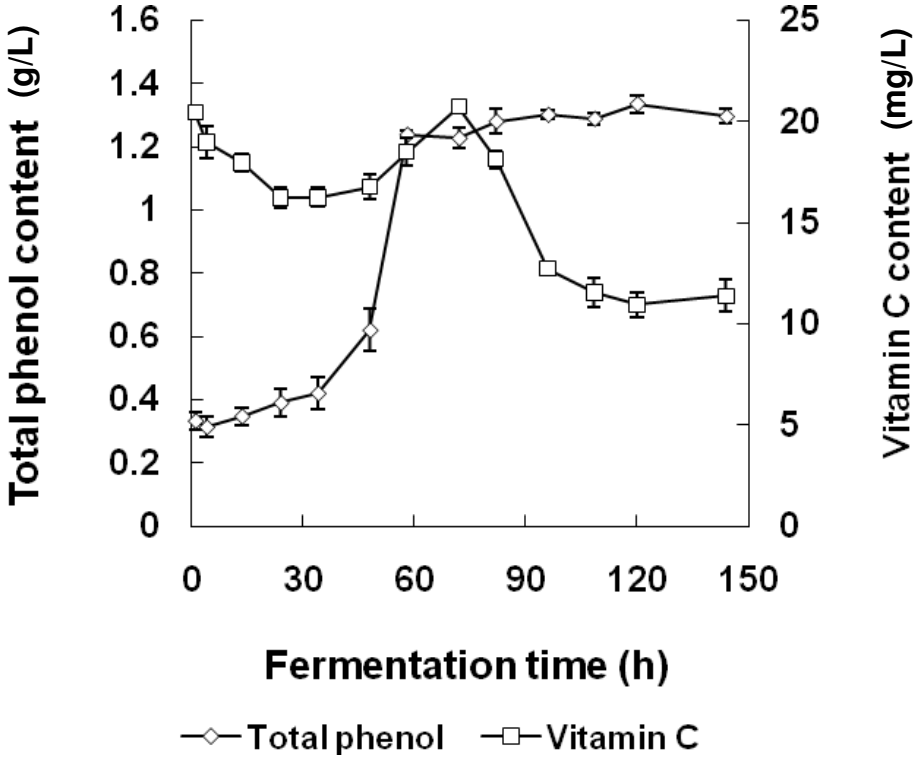

Figure 3. Changes of total phenolic and vitamin C contents of PCIS during natural fermentation. The results are representative of three independent experiments and values are expressed as mean ± SD from three experiments.

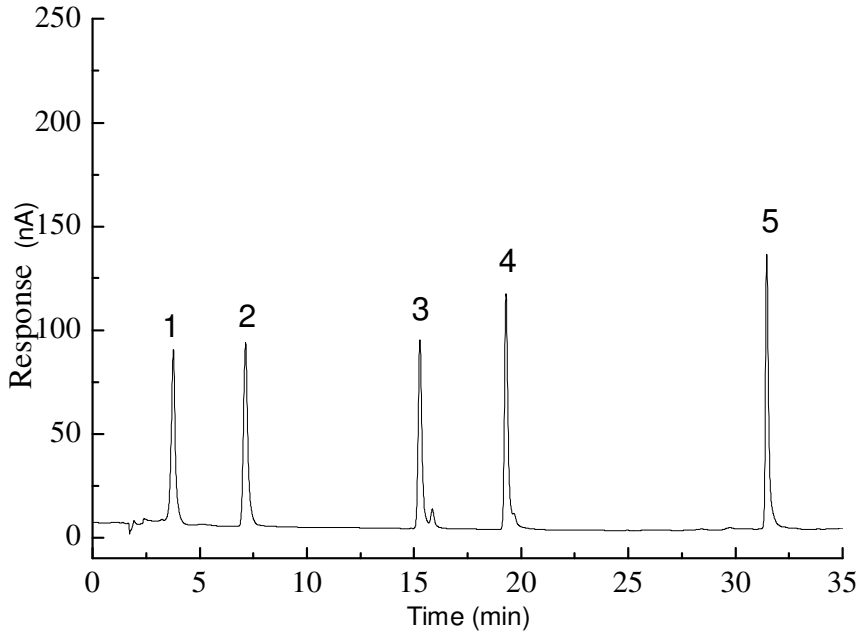

Figure 4. High performance liquid chromatograms of phenolic compounds standards. 1, Gallic acid; 2, Protocatechuic acid; 3, Caffeic acid; 4, p-Coumaric acid; 5, Galangin.

decreased significantly again on day 3, but resumed its insignificant decrease later. The latter situation might be due to the small amount of amino acid produced by the death or autolysis of part of the yeast (Penn et al., 1983) (Figure 1).

Changes of total sugar, reducing sugar and ethanol content of PCIS during natural fermentation

TLC analysis showed that only sucrose (R_f = 0.32) was present in FCIS. Fructose (R_f = 0.51) and glucose (R_f = 0.47) were detected at 7 h during natural fermentation. The spot color of fructose and glucose became dark but that of sucrose became light, which meant that the content of fructose and glucose increased but sucrose decreased. Almost no sucrose was detected after 58 h of natural fermentation which also implies that almost all the sucrose had been degraded into fructose and glucose.

Changes of total sugar, reducing sugar and ethanol content are shown in Figure 2. Inversion of sugars occurred during natural fermentation, total sugar dropped steadily, while reducing sugar increased constantly until day 3 but quickly decreased thereafter. This is due to sucrose being converted into fructose and glucose during initial fermentation, and the reducing sugar was consumed by microorganisms at the later stage. Ethanol content increased significantly from day 1 to 5 of fermentation and achieved its maximum of 90 g/kg at day 7 but it decreased later. These results agree with those reported by Atputharajah et al. (1986).

Changes of vitamin C and total phenolic contents of PCIS during natural fermentation

Vitamin C is an important indicator of juice quality, but it is easily oxidized and degraded. Phenolic compounds are a class of chemical compounds consisting of phenolic hydroxyl group. Vitamin C and phenolic compounds are important antioxidant agents that protect biomacromolecule from the damage induced by free radical. They also possess anti-aging, anti-tumor and antimutagen functions (Chimi et al., 1991; Nardini et al., 1995; Milić et al., 1998). Figure 3 shows the changes of vitamin C and total phenolic contents.

The vitamin C content of FCIS was 20.4 mg/L. It however reduced slowly on day 1 of fermentation, but rose on day 2, then reached its maximum of 20.7 mg/L on day 3. This increase might be due to the activity of yeast which synthesized vitamin C in fermentation (Bremus et al., 2006). After day 3, the vitamin C fell sharply, which might be due to the decreased activity of the yeast. However, after day 5, the vitamin C almost stopped decreasing, which might be the protection of vitamin C by antioxidant phenolic compounds.

The total phenolic content of FCIS was 0.33 g/L. It increased slowly from day 1 to 2 of fermentation and rapidly thereafter reached a peak value of 1.24 g/L at 58 h. Later, it showed no obvious change. The increase of phenolic content might be caused by plant polyphenols binding with sugar, protein, cellulose and starch and forming glucosidic bonds. Glucoside bonds are degraded by the acids produced during natural fermentation and

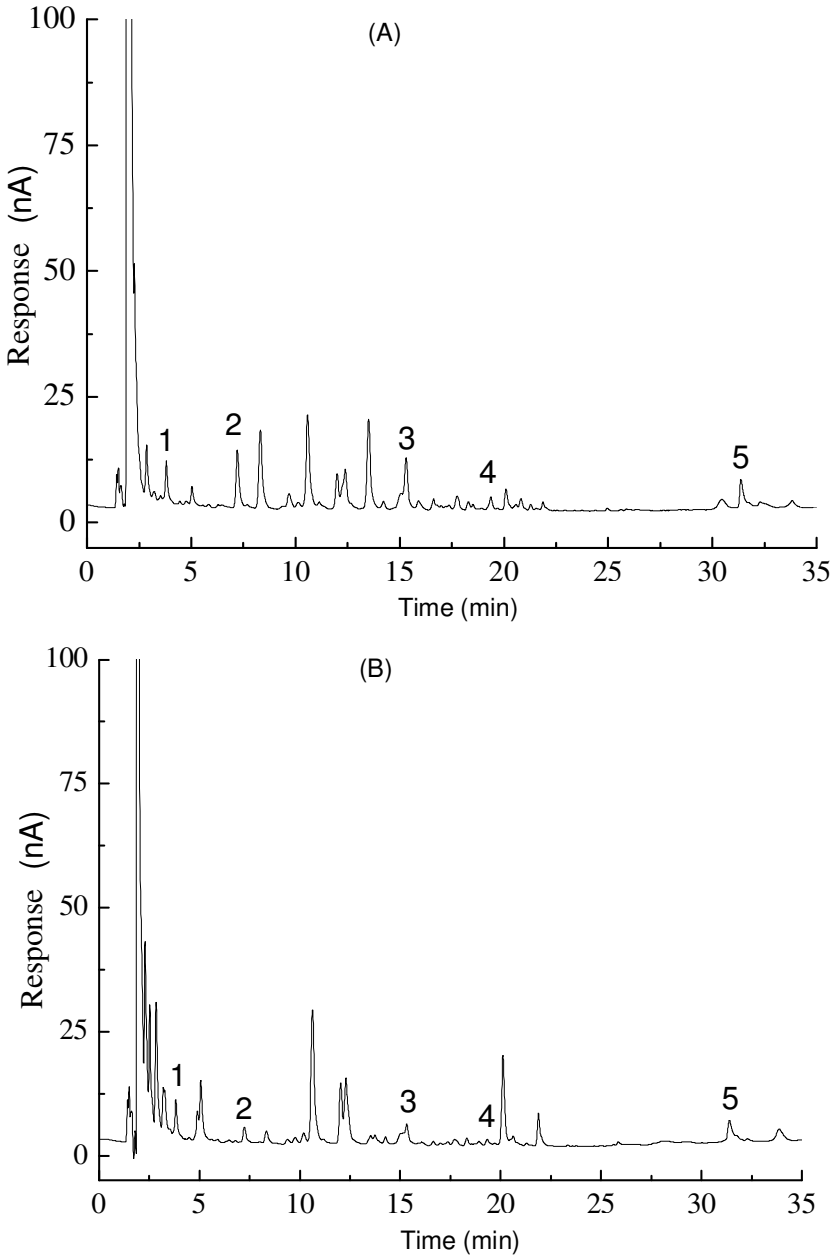

Figure 5. High performance liquid chromatograms of phenolic compounds in NCIS (A) and FCIS (B). 1, Gallic acid; 2, Protocatechuic acid; 3, Caffeic acid; 4, p-Coumaric acid; 5, Galangin.

phenolic compounds are yielded (Landbo and Meyer, 2004). The metabolism of some microorganisms might also contribute to the production of phenolic compounds.

Variety and content of phenolic compounds in FCIS and NCIS

Five varieties of standard phenolic compounds were sufficiently separated (Figure 4). The retention time obtained for gallic acid, protocatechuic acid, caffeic acid, p-coumaric acid and galangin were 3.774, 7.127, 15.262, 19.292 and 31.468 min, respectively. Figure 5A and Figure 5B indicate that NCIS and FCIS contained the five and several other phenolic compounds. In addition, Table 1 shows that the contents of the five phenolic compounds were higher in NCIS than in FCIS. In NCIS, caffeic acid content was highest at 730 µg/L compared with the other four compounds, while in FCIS, gallic acid content was the highest at 350 µg/L.

Table 1. Concentrations of phenolic compounds in FCIS and NCIS (μg/L).

Sample	Gallic acid	Protocatechuic acid	Caffeic acid	p-Coumaric acid	Galangin
FCIS	350 ± 1.36	86 ± 0.37	56 ± 0.21	27 ± 0.13	100 ± 0.65
NCIS	500 ± 1.51	210 ± 1.02	730 ± 1.29	150 ± 0.98	120 ± 1.07

The results are representative of three independent experiments and expressed as the mean ± SD from three experiments.

Conclusion

CIS contain many nutritious ingredients and the content of each component has great changes during natural fermentation. We researched the chemical components of PCIS produced in Hainan, China for the first time. Due to the different microorganisms in Hainan, China with the other countries, however, we obtained some different and new results. The changes of the total acid were different from Atputharajah et al. (1986) report. The changes of volatile acid, amino acid and vitamin C contents, the variety and content of polyphenol of PCIS before now were rarely reported. More also, five kinds of phenolic compounds were detected by HPLC and these compounds all increased in NCIS compared with FCIS, thus showing that NCIS can also be used as good functional food.

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