

Thermal Degradation of Flavonol Glycosides in Noni Leaves During Roasting

Shixin Deng, Brett J. West and C. Jarakae Jensen

Research and Development Department, Tahitian Noni International,
737 East, 1180 South, American Fork, Utah, 84003 USA

Abstract: Noni leaves have been used for a variety of health benefits for thousands of years. Noni leaf tea, a commercial product made by a roasting process, is attracting more attention due to its potential health benefits. Flavonoids appear to be some of the predominant constituents in noni leaves. As flavonoids exist mostly in the forms of glycosides or polymers, degradation to corresponding metabolites is usually needed for bio-absorption. This study investigates the effects of thermal treatment (non-aqueous roasting) on flavonoids in noni leaves. Rutin and kaempferol glycoside contents decreased dramatically as roasting time and/or temperature increased, while quercetin and kaempferol aglycones were produced. A quantitative comparison demonstrated that quercetin and kaempferol concentrations were 3.74 and 6.28 times greater in noni leaf tea than in raw noni leaves, respectively. These findings indicate that the roasting process for the noni leaf tea could induce the degradation of flavonol glycosides, and produce their aglycone metabolites, which in turn, may lead to more beneficial bioactivities and bioavailability.

Key words: Flavonoids, leaf, *Morinda citrifolia* L., noni, roasting process, thermal stability

INTRODUCTION

Noni (*Morinda citrifolia* L., family Rubiaceae) is a tropical plant prevalent in the islands of French Polynesia, such as Tahiti, as well as Hawaii and South Asia, etc. Like the fruit of this tree, the leaves have a long history of traditional use for a variety of conditions, including arthritis, bruises, colds, diabetes, headaches, inflammation, pain, etc. (McClatchey, 2002; Wang *et al.*, 2002). The leaves were also consumed as a traditional food and are reported to have very good nutritional value (South Pacific Commission, 1995). Noni leaf has been used as an ingredient in many health products. Among those, noni leaf tea, made by roasting raw noni leaves, increasingly attracts more attention due to its recent approval within the European union as a safe novel food (European Commission, 2008) and its potential health benefits (West *et al.*, 2007). Noni leaf contains many biologically active phytochemicals, with flavonoids appearing to be predominant constituents (Deng *et al.*, 2008).

Flavonoids are well-known antioxidants, and widely present in natural resources, such as vegetables, fruits, and herbs. They are beneficial in the prevention and improvement of many diseases, including cancer, inflammation, and cardiovascular diseases, among others (Harborne and Williams, 2000). Flavonoids commonly exist as glycosides or polymers in foods and need to be degraded to corresponding metabolites for bio-absorption (Kelly *et al.*, 2002). Thermal treatments in food production may produce many by-products. It was shown

that boiling, frying, and cooking may result in the loss of flavonoids in foods (Crozier *et al.*, 1997). Our study aims to investigate the effects of non-aqueous roasting process on flavonoids in noni leaves at selected experimental conditions (roasting time: 10-60 min; temperature: 100-250°C).

MATERIALS AND METHODS

The experiments were conducted in 2009 at the Research lab of Tahitian Noni International, USA.

Chemicals and reagents: Methanol (MeOH) and water (H₂O), HPLC grade, were obtained from Fisher Scientific Co. (Fair Lawn, NJ, USA). HPLC grade acetonitrile (MeCN), ethanol (EtOH) and analytical grade trifluoroacetic acid (TFA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Quercetin-3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (rutin, 1), kaempferol-3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (kaempferol-3-*O*-rutinoside, 2), quercetin (3), and kaempferol (4) were isolated in our lab from noni fruit and leaves from Tahiti. The purity was above 99% as determined by HPLC, MS, and NMR (Deng *et al.*, 2007). Their structures are given in Fig. 3. The standards 1-4 were accurately weighed and then dissolved in an appropriate volume of MeOH to produce corresponding stock standard solutions. Working standard solutions for calibration curves were prepared by diluting the stock solutions with MeOH at different

concentrations. All stock and working solutions were maintained at 0°C in a refrigerator.

Plant materials and sample preparation: Raw noni leaves were collected from Tahiti, French Polynesia, and air-dried. The noni leaf samples were identified by our Research and Development Department, and a voucher specimen was deposited in our laboratory. Noni leaves were divided into two groups: the first group had six noni leaf samples (5 g/each). These samples were roasted at 175°C in an Isotemp oven (Fisher Scientific, USA) for 10, 20, 30, 40, 50, and 60 min, respectively. The second testing group consists of 5 samples (5 g/each), which were roasted for 20 min at 100, 150, 175, 200, and 250°C, respectively. After cooling to room temperature, 2 g of each sample in the two groups was extracted with 100 mL of EtOH in a sonicator for 30 min. The extractants were filtered and evaporated to dryness by using a rotary evaporator under vacuum at 45°C. The resulting noni leaf EtOH extracts was re-dissolved with MeOH for HPLC analysis. Noni leaf tea is the sample made from raw noni leaves through a roasting process at 175°C for 20 min during the experiment. To determine the influence of the roasting process on flavonol aglycones, we compared the quercetin (3) and kaempferol (4) contents in raw noni leaf and noni leaf tea. All sample solutions prepared were passed through a 0.45µm micropore membrane filter before HPLC injection.

Instrumentation and chromatographic conditions:

Chromatographic separation was performed with a Waters Alliance™ 2690 separations module coupled to a Waters 2996 photodiode array (PDA) detector, utilizing a Waters Atlantis analytical C₁₈ column (5 µm, 4.6 × 250 mm, Wexford, Ireland) at a flow rate of 1 mL/min. A triplet solvent system [A; MeCN, B; MeOH, and C; 0.1 TFA% in H₂O (v/v)] was used as the mobile phase. The mobile phase was programmed consecutively in linear gradients as follows: 0 min, 10% A, 10% B, and 80% C; 15 min, 20% A, 20% B, and 60% C; 26 min, 40% A, 40% B, and 20% C; 28-39 min, 50% A, 50% B, and 0% C; and 40-45 min, 10% A, 10% B, and 80% C. The injection volume was 10 µL. The column temperature was maintained at 25°C. The UV spectrum was monitored in the range of 210-400 nm, and HPLC chromatograms were integrated and quantified at 365 nm. Data collection and integration were performed using Waters Millennium software version 32. All experiments were run in triplicate, unless elsewhere specified. Results were averaged and standard deviations calculated.

Identification and quantification: Identification of flavonoids was conducted by comparison of their spectroscopic and chromatographic characteristics, including UV absorptions and HPLC retention, with those of standard compounds 1-4. Quantification was performed

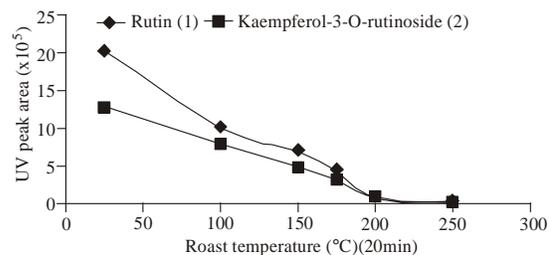


Fig. 1: The effects of non-aqueous roasting on flavonoid glycoside contents of noni leaves at varying times and temperatures

on the basis of linear calibration plots of the UV absorption peak area against concentration. At the selected wavelength (365 nm), no interference from undesired components was observed. In the experiments, the amounts of 1-2 in all samples, including raw and roasted leaves, as well as the contents of 3-4 in the raw leaf and the leaf tea were determined by using the method described above.

RESULTS AND DISCUSSION

Thermal processing techniques used in food and dietary supplement industries, such as cooking, boiling, frying, roasting, etc, may have an impact on flavonoid structures, and therefore, influence their bioavailability and bioactivities (Rohn *et al.*, 2007). Flavonol, flavone, and isoflavone glycosides usually require hydrolysis to their respective aglycons to become bioactive (Formica and Regelson, 1995; Pietta, 2000). Rutin needs to be hydrolyzed by cecal microflora, with the resulting quercetin being absorbed from the small intestine. Therefore, quercetin is more easily absorbed than rutin (Manach *et al.*, 1997). In animals, quercetin, not its glycoside rutin, was observed to have anticarcinogenic activity in azoxymethane-treated rats, suggesting that the lower protective effect of rutin may be attributed to its low bioavailability (Dihal *et al.*, 2006). Another study showed that rutin was rapidly degraded into quercetin during roasting. A roasting process of 60 min at 180°C led to loss of more than 40% of the original rutin content (Rohn *et al.*, 2007). During roasting under experimental conditions, flavonol glycosides are converted into their aglycones, and thus, more potent bioactivities may be expected. In addition to well-known antioxidant activities, quercetin and kaempferol were also found to exhibit anti-inflammatory activities by interfering with many stages of eicosanoid metabolism (Deng *et al.*, 2007; O’Leary *et al.*, 2004; Takano-Ishikawa *et al.*, 2006).

In our experiments, we examine the effects of non-aqueous roasting at various times and temperatures, on flavonoid glycosides in noni leaves with an HPLC/UV

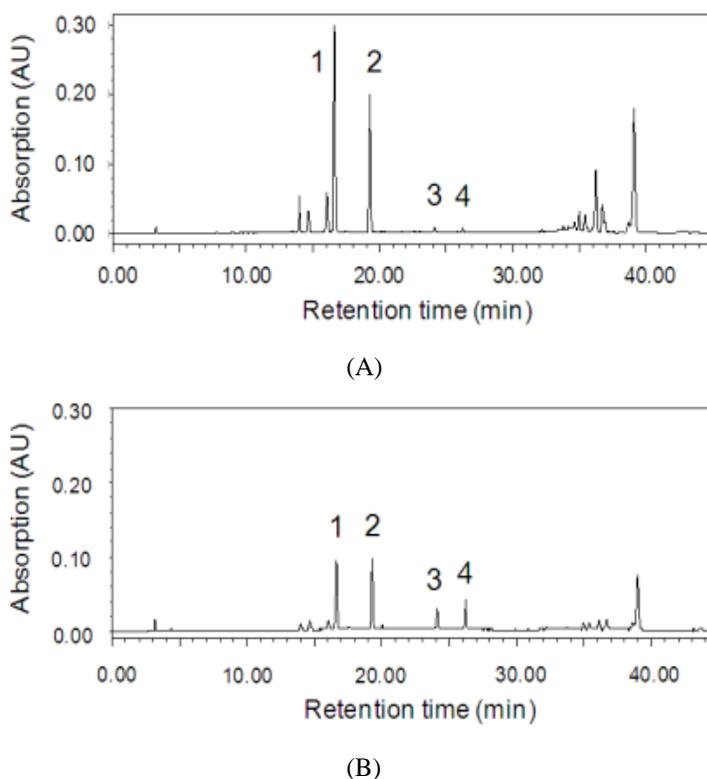


Fig. 2: Comparison of phytochemical profiles of raw noni leaf (A) and noni leaf tea (B). (1-rutin; 2-kaempferol-3-O-rutinoside; 3-quercetin; 4-kaempferol)

technique. Analytical results (Fig. 1) indicate that the amounts of 1 and 2 decreased dramatically as the roasting time and/or temperature increased. At 175°C, a roasting time of 10 min resulted in a 65% and 50% loss of 1 and 2, respectively. A 75-80% loss of 1 and 2 were detected after being roasted for 20 min. As the roasting process continued beyond 20 min, the amounts of 1 and 2 continued to decrease, at a relatively slow rate. 60 min of roasting resulted in approximately 95% loss of 1 and 2. In contrast, increasing roasting temperatures caused a linear decrease in the contents of 1 and 2. Temperature ranging from 100 to 200°C led to 50-95% and 40-95% drop of contents of 1 and 2, respectively. However, the noni leaf roasted at 200°C exhibited burned smell and color. The experimental results indicate that thermohydrolysis of flavonol glycosides in noni leaf depends largely on roasting temperature and time.

Flavonol aglycones quercetin (3) and kaempferol (4) in raw noni leaf and noni leaf tea were also analyzed in the experiments. Figure 2 depicts the phytochemical profiles of raw noni leaf and noni-leaf tea. The results revealed that raw leaf and noni leaf tea exhibit similar HPLC fingerprint profiles, but with different peak intensity. Further quantitative analysis revealed that the

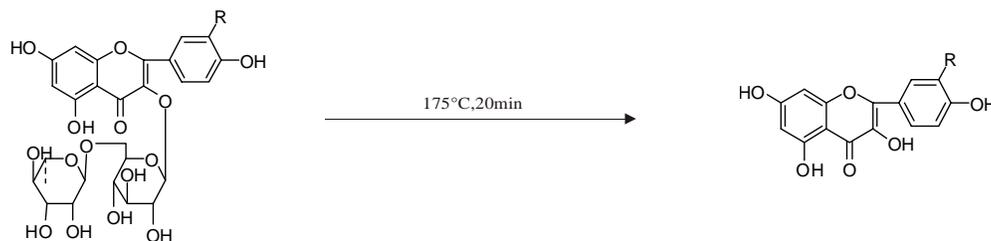
Table 1: Determination of flavonoids 1-4 in raw noni leaf and noni leaf tea

Samples	Contents of analytes* (mg/100 g)			
	1	2	3	4
Raw noni leaf	355.13±3.70	260.33±4.45	3.01±0.22	1.97±0.13
Noni leaf tea	116.03±3.98	126.13±7.84	11.27±0.25	12.37±0.15

*: Data represent mean ± S.D of triplicates

concentrations of 3 and 4 were significantly higher in roasted leaf tea compared to raw leaf, as shown in Table 1. The contents of 3 and 4 in raw noni leaf were 3.01 and 1.97 mg/100 g and 11.27 and 12.37 mg/100 g in the leaf tea. Quercetin and kaempferol in the leaf tea were found to be, respectively, 3.74 and 6.28 times more than those in raw leaves, suggesting that the roasting conditions induced the degradation of flavonol glycosides 1 and 2, and produced additional aglycones 3 and 4. A degradation scheme for flavonoid glycosides during the roasting process of noni leaf tea is proposed in Fig. 3.

The thermal stability of quercetin and kaempferol during the manufacturing process of noni leaf tea was investigated by comparing HPLC chromatograms before and after roasting. The HPLC analysis indicated that no degradation of 3-4 occurred during thermal treatment. It is apparent that quercetin and kaempferol are stable under the experimental conditions.



quercetin-3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (1): R=OH quercetin (3): R=OH
 kaempferol-3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (2): R=H kaempferol (4): R=H

Fig. 3: The degradation mechanism of flavonol glycosides to corresponding aglycones during the roasting process of noni leaf tea. Roasting conditions resulted in a thermal-induced degradation of quercetin and kaempferol glycosides

The roasting used to process noni leaf produces flavonol aglycones from glycosides and could lead to more beneficial bioactivity (Kim *et al.*, 1991; Rohn *et al.*, 2007). It also likely that more other biometabolites could be generated during this process, which may also contribute to bioavailability.

In conclusion, the findings in this experiment indicate that the roasting processes could induce the degradation of noni leaf flavonol glycosides, and produce their aglycone metabolites, which in turn, may lead to increased bioavailability. This information may help to further understand the mechanism of medicinal uses of noni leaf.

ACKNOWLEDGMENT

The authors thank Tahitian Noni international (Utah, USA) for the financial support of this project.

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