Effects of Juice Processing on Anti-inflammatory Flavonoids in Plant Foods

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ABSTRACT

Apigenin glycosides are abundant in parsley, celery, and chamomile tea and possess unique anti-inflammatory properties *in vitro*. Apigenin O-glycosides have been evaluated in foods by hydrolysis and comparison to aglycone standards, but intact O-glycosides of apigenin cannot be directly identified by these methods. As the stability and bioavailability of flavonoids depends on their structure, it is important to identify and quantify intact apigenoids (apigenin O- and C-glycosides) in fresh and processed foods.

Apigenoids in fresh parsley and celery were compared with those in juices. Fresh samples were lyophilized and extracted with 70% (v/v) aqueous methanol (3 ml solvent:50 mg sample), and juices extracted with 50% (v/v) methanol. Extracts were analyzed by reversed-phase HPLC with photodiode array detection to identify and quantify flavonoids versus standards. Peak identities were determined by electrospray mass spectrometry using in-source fragmentation to liberate aglycones.

In fresh celery and parsley, the predominant apigenoid was apigenin O-malonylapiosylglucoside (malonylapiin), with lesser amounts of apigenin O-apiosylglucoside (apiin); in celery and parsley juices, apiin was the predominant form. Total apigenoid concentrations were 5-25 mg/100 g (wet basis) in celery and juice, and 130-160 mg/100 g in parsley and juice. We found that anti-inflammatory flavonoids were slightly modified but largely retained during juice processing. Our method could be used to rapidly screen for other flavonoids, including C-glycosides, and similar derivatives in foods.

INTRODUCTION

Apigenin, like many flavonoids present in plants, is conjugated to sugars, simple acids (acetyl and malonyl) and cinnamic acids (Fig. 1). The acid functionalities are easily hydrolyzed in the gut when plant foods are consumed while the action of epithelial brush border beta-glucosidase can cleave the O-glycosidic bond, leaving the aglycone to be absorbed and metabolized (1). In vitro studies have shown that apigenin aglycone inhibits cancer cell proliferation (2) and reduces monocyte adhesion to LDL, a cause of heart disease (3). In addition, animal studies with apigenin demonstrate the potential to inhibit lung cancer (4) and reduce the inflammatory response (5). These studies underscore the need for methods to identify and quantify apigenin O-glycosides in foods.

Although flavonoid glycosides can be hydrolyzed prior to analysis to measure total aglycones in a sample, determining the native glycosides is potentially useful when developing functional foods. For example, flavonoid glycosides are differentially hydrolyzed in saliva based on their sugar conjugates (6), affecting their bioavailability.

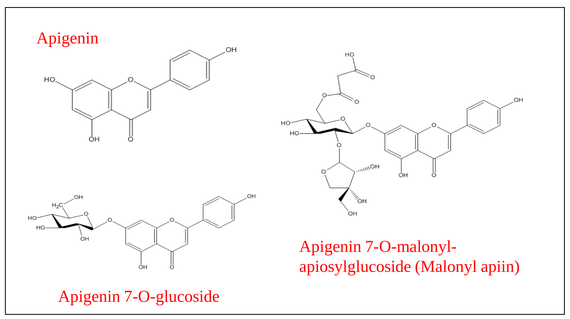

Figure 1. Apigenin aglycone and two common glycosides

MATERIALS AND METHODS

Juices were prepared from fresh parsley and celery with an Omega 8005 juice extractor, acidified to pH 4.2 with $NaHSO_4$, and heated to 93ºC for 2 min or 20 min to simulate commercial juice processing (Fig. 2). Aliquots of juice were taken each step of the process and frozen at -20ºC for later analysis. Subsamples of fresh parsley and celery were lyophilized and extracted with 70% (v/v) aqueous methanol (15 ml solvent:200 mg sample). Juices were extracted with methanol as a 50% (v/v) extract. All samples were extracted in triplicate and analyzed directly by reversed-phase HPLC with photodiode array (PDA) detection. UV chromatographic peaks were identified first according to parent and daughter ion m/z in electrospray mass spectrometry, and PDA data was used to quantify apigenin glycosides. Apigenin 7-O-apiosylglucoside (apiin) and luteolin 4'-methyl ether (diosmetin) standards were used to identify flavonoids in parsley, while apiin, luteolin, and luteolin 3'-methyl ether (chrysoeriol) were used for celery. Apiin was used to quantify apigenin glycosides.

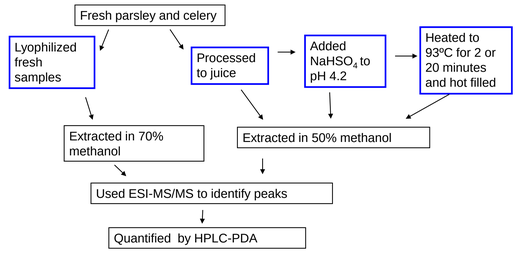

Figure 2. Methodology for processing of parsley and celery juices. Blue boxes indicate samples analyzed for apigenoids.

HPLC eluate was interfaced with an electrospray (ESI) probe into a hybrid quadrupole-time-of-flight mass spectrometer (QTof Premier, Micromass, UK). ESI was used in both positive and negative ion modes to detect parent ions and subsequently fragment them by collision-induced dissociation (CID) for daughter ion generation. To identify aglycone substituents in-source fragmentation was applied to liberate aglycones and then fragment the aglycone by CID to determine aglycone identity with aglycone standards. This approach was validated for apigenoids with apigenin 7-O-glucoside and apiin and demonstrated to be different from genistein fragmented from genistein glycosides, a common isoflavone that is isobaric with apigenin. The isobaric flavones diosmetin and chrysoeriol could also be differentiated using MS-MS as shown previously (7) (Fig. 3).

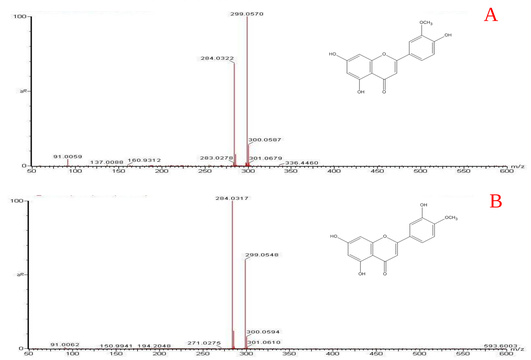

Figure 3. Mass spectra showing CID of the 299 aglycone parents chrysoeriol in celery extract (A) and diosmetin in parsley extract (B). The ratio of parent to daughter ions allows differentiation between the isobaric flavonoids.

RESULTS AND DISCUSSION

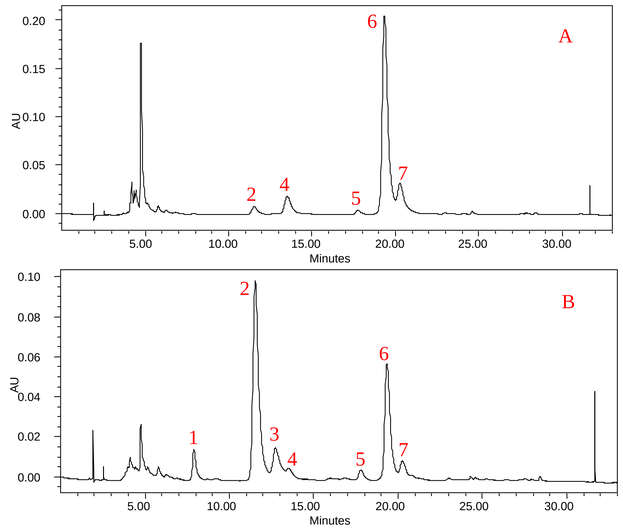

Figure 4. Chromatograms of fresh celery (A) and acidified celery juice heated 20 minutes (B) by PDA at 340 nm. Peak assignments by ESI-MS:

1. Luteolin apiosylglucoside
2. Apigenin apiosylglucoside (apiin)
3. Chrysoeriol apiosylglucoside
4. Luteolin malonylapiosylglucoside
5. Acetylapiin
6. Malonylapiin
7. Chrysoeriol malonylapiosylglucoside

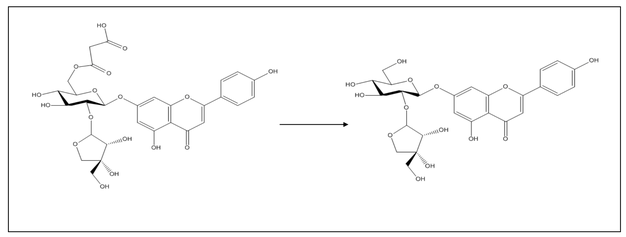

Figure 5. Hydrolysis of malonylapiin to apiin.

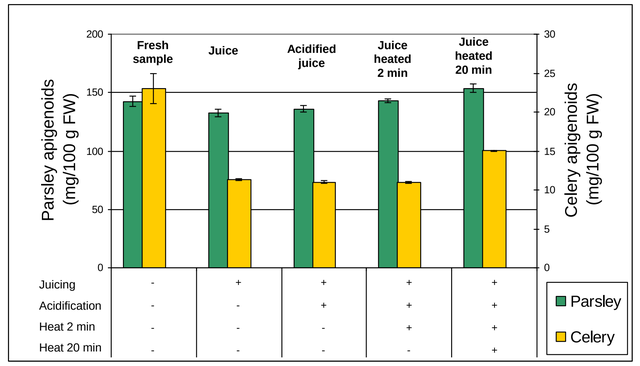

Figure 6. Concentration of apigenin glycosides in parsley and celery samples at each stage of juice processing, measured as apigenin aglycone equivalents.

Processing celery and parsley into juice resulted in the conversion of malonylapiin to apiin (Figs. 4 and 5). This occurred rapidly and was likely the result of enzymatic activity (8). Untreated juice contained almost no malonylapiin, suggesting that acidification preserved the malonylated form of the compound. Demalonylation of apiin in parsley after oven drying has also been observed, and the ratio of malonylapiin to apiin can be used to determine if it is freeze dried or oven dried (9). Loss of malonyl can also occur after the extraction process, as has been noted with other flavonoid glycosides (10).

While apigenin glycosides were slightly modified by processing into juice, the apigenin aglycone was very stable to acidification and heat treatment in this study. In parsley juice, there was little effect of processing on the total apigenoid concentration in fresh parsley or parsley juice (Fig. 6). Apigenin glycoside concentrations in celery juice were substantially lower than in fresh celery, and this was likely due to the large concentration of flavnoids remaining in the pulp after juicing.

Our results were similar to those of studies on other juices. Retention of apigenoids and other flavonoids in pomace has also been observed after processing artichoke heads into juice (11). Gil-Izquierdo (12) found that heating orange juice to 95ºC had little effect on flavonoid concentrations.

CONCLUSIONS

❖The predominant apigenin glycosides in parsley and celery include apigenin 7-O-malonylapiosylglucoside (apiin), acetylapiin, and malonylapiin.

❖Malonylapiin is converted to apiin in parsley and celery juices, and this conversion is slowed by acidification of the juice.

❖Acidification and heat treatment had little effect on total apigenoid concentrations in parsley and celery juices.

❖HPLC MS-MS was successfully used to identify unknown flavonoids in parsley and celery.

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