

Characterization of Phenolic Content in Almond Industry “Waste” by Negative Ion ESI Capillary LC/MS

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Abstract:

Extraction of bioactive compounds from agricultural waste products is an important means of producing value-added products from inexpensive starting materials. Almond industry waste (e.g. the skins) is a potential source of natural bioactive compounds, attributed to their phenolic content. Phenolic compounds are antioxidants, antimicrobials, and anticarcinogenic.

The overall research objective was to develop a suitable capillary liquid chromatography/ mass spectrometry (LC/MS) method to qualitatively determine the effect of γ -irradiation on the phenolic content of almond industry waste. Microscale LC separations provided increased separation efficiencies, reduced sample consumption and reduced cost (solvent and waste generation). In addition, the capillary LC/MS method developed required 29-90% less time to achieve the same separation as conventional HPLC (10 vs. 35-101 min.)^{1,4}.

γ -irradiation is expected to increase the antioxidant capacity of the phenolics by cleaving attached sugars. Changes in phenolics before and after different irradiation doses were determined with positive and negative ESI to elucidate maximum phenolic content in the γ -irradiated samples.

Experimental:

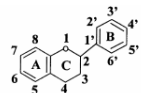
Sample Treatment/Preparation: Almond skins were obtained from Campos California Shelled Almonds (Caruthers, CA). Dried, powdered almond skins were defatted with hexane (1:3 w/v) and subjected to γ -irradiation at IBA Steragenics (Tustin, CA) at 0, 3.15, 4.25, 8.2 and 12.4 kGy. After irradiation, the phenolics were extracted from almond skins with HCl: water: MeOH (1:19:80). The extraction filtrate was then used in subsequent analyses after 1:1 dilution with water.

Phenolic compounds were separated and identified with an Eksigent ExpressLC™-100 capillary LC coupled to a ThermoFinnigan LCQ Advantage MAX MS. A mixture of 7 phenolic compounds (see Table 1) at 20 ppm each (prepared in 40% MeOH: 60% water) was used to optimize the chromatographic and mass spectrometric conditions.

Optimized chromatographic conditions:

- Eksigent ChromXP™ 3C18-EP-120 embedded polar column (150 mm x 0.3 mm i.d., 3 μ m)
- Mobile Phase: Eluent A (90% Water:10% MeOH w/ 0.1% formic acid) and Eluent B (ACN w/ 0.1% formic acid)
- Gradient: 30% B (2 min.), 30-95% B (5 min.), 95% B (2.5 min.), 95-30% B (0.25 min.), 30% B (1.25 min.) at a flow rate of 7 μ L/min. The total run time was 10.0 min.
- Samples injection volume ranged from 0.5 to 1.0 μ L.

Figure 1. Structure of Phenolics:



Results:

Table 1. Phenolic Standards

Phenolic Compound Standards	[M+H] ⁺	[M-H] ⁻	R. T. (min.) ^a
1 Chlorogenic acid	355.3	353.3	1.84
2 Catechin	291.3	289.3	1.96
3 Rutin	611.5	609.5	2.48
4 Quercetin-3- β -D-glucoside	465.4	463.4	3.23
5 Quercetin	303.2	301.2	6.77
6 Isohammetin	317.3	315.3	6.92
7 Kaempferol	287.2	285.2	7.60

^aR.T. = Retention Time in minutes with an average %RSD of 0.59% (n = 10)

Figure 2. Positive Ion ESI LC/MS.

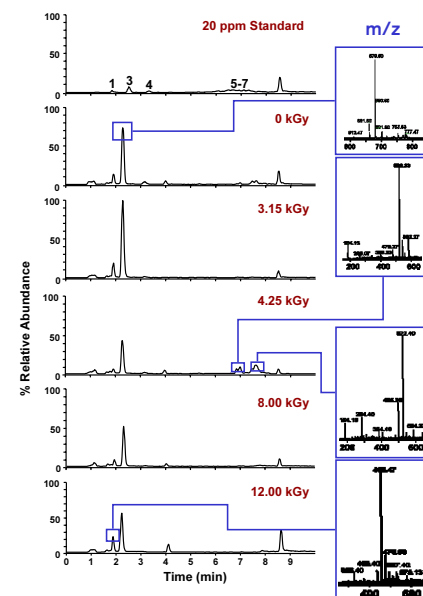


Figure 2. Comparison of positive ion chromatograms for select phenolic standards and almond skin extracts with different γ -irradiation doses. None of the expected phenolic compounds are present in the γ -irradiated samples. A steady decrease in the quantity of these compounds (R.T. < 3 min.) is observed with increasing γ -irradiation. 4.25 kGy produces additional compounds (R.T. 6.5-8 mins.) of interest, which are not observed at the other γ -irradiation doses and indicate an increased level of phenolics at this dose.

Figure 3. UV of Phenolic Stds.

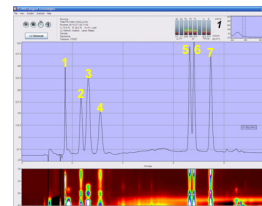


Figure 3. UV Spectrum of Phenolic Standards. As shown above, the UV flow cell in the Eksigent ExpressLC-100 system collects UV data in three-dimension (e.g. retention time, full UV spectrum, and intensity). Refer to Table 1 for peak identification.

Figure 4. Negative Ion ESI LC/MS

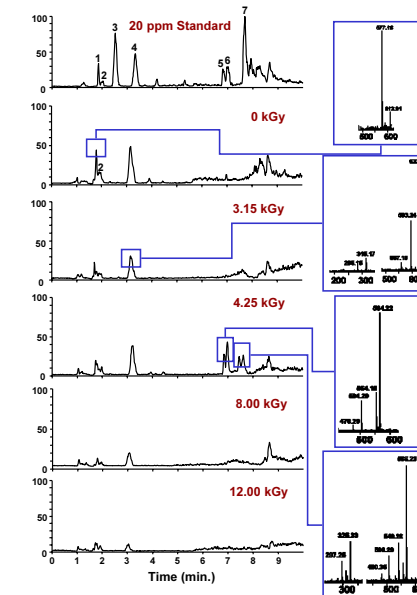


Figure 4. Comparison of negative ion chromatograms for select phenolic standards and almond skin extracts with different γ -irradiation doses. 4.25 kGy produces additional compounds (R.T. 6.5-8 mins.) not observed at the other γ -irradiation doses or positive ion mode. Negative mode provides greater selectivity and higher ionization efficiency for phenolics than positive mode.

Table 2. Tentative Assignments for Phenolics in Irradiated Samples

Tentative Assignments	[M+H] ⁺	[M-H] ⁻	R. T. (min.)
Naringenin 7-O- β -D-glucopyranoside ²		433	2.02
3'-O-Methylquercetin		477	3.95
3-O- β -D-glucopyranoside ¹			1.74
Procyanidin B1 ¹		577.1	
Kaempferol hexose-deoxyhexoside 2 ¹		593.2	3.25
3'-O-Methylquercetin 3-O-L-rhamnosyrosyl(1- β)- β -D-glucopyranoside (3) ¹		623.2	3.16
p-coumaric acid hexose derivative ¹	520.3		6.96

Conclusions:

The standards initially chosen, based on available literature for almonds², were not present in the γ -irradiated almond skins and subsequent quantitation of the antioxidant content within the γ -irradiated samples was not possible. However, a suitable capillary LC/MS method was developed for the analysis of the γ -irradiated almond skins. Initial analysis of the γ -irradiated almond skins and review of the literature has provided a clear direction for future work. The 4.25 γ -irradiated samples are of particular interest because of the increased chromatographic and mass spectral complexity associated with increased antioxidant content. It is believed that the disappearance of these compounds at higher γ -irradiation doses results in the conversion of these phenolic antioxidants to pro-oxidants.

Further identification of all suspected phenolic compounds in the irradiated samples by MS/MS and retention time correlation to a new set of standards is necessary. Upon identification, the quantitation of phenolics in the γ -irradiated almonds skins will be possible, thus allowing the determination of the optimal γ -irradiation dose for increased antioxidant content. The switch to capillary LC/MS proved beneficial due to reduced sample consumption (and the associated cost of γ -irradiation). Additional benefits of the capillary LC/MS system include reduced solvent usage and significantly shorter inject-to-inject times, which provide faster method development and increased sample throughput.

References:

1. Maatta, Kaisu R., et al. J. Agric. Food Chem. 2003, 51, 6736-6744.
2. Sang, Shengmin, et al. J. Agric. Food Chem. 2002, 50, 2459-2463.
3. Kammerer, Dietmar, et al. J. Agric. Food Chem. 2004, 52, 4360-4367.
4. Cai, Yi-Zhong, et al. J. Agric. Food Chem. 2005, 53, 9940-9948.