

Quantification of amygdalin in nonbitter, semibitter, and bitter almonds (*Prunus dulcis*) by UHPLC-(ESI)QqQ MS/MS

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Highlights

- Developed highly sensitive method to analyze for amygdalin levels in almonds
 - Can be used to reliably quantify amygdalin at trace levels: ≥ 0.013 mg/100 g almond
- Compared the trace amygdalin contents of raw kernels of 10 commercial nonbitter (sweet) varieties of California almonds obtained from four major growing regions (2010 harvest)
 - Mean amygdalin levels ranged from ~ 0.2 to 16 mg/100 g in sweet varieties
 - Aldrich and Fritz almonds had statistically higher amygdalin contents
 - Growing region had a significant effect on amygdalin contents
- Measured the amygdalin content of 4 semibitter (slightly bitter) and 6 bitter varieties of almonds provided by the UC Davis almond breeding program
 - Mean amygdalin levels ranged from ~ 52 to 180 mg/100 g in semibitter varieties
 - Mean amygdalin levels ranged from $\sim 3,300$ to 5,400 mg/100 g in bitter varieties

Summary

Almonds can be characterized into three flavor phenotypes: sweet (nonbitter), slightly bitter (or semibitter) and bitter. In general, a cyanogenic diglucoside, *amygdalin*, is responsible for the bitterness of almonds. Amygdalin is found naturally in almond kernels and in the seeds of stone fruits (e.g., apricots).

Bitter almonds contain high levels of amygdalin (3–5%), whereas only trace levels are found in almonds from sweet varieties ($< 0.05\%$ amygdalin) and from slightly bitter varieties ($< 0.2\%$ amygdalin). *Accurate quantification of amygdalin may be useful for distinguishing between sweet and slightly bitter almonds and to categorize flavor.* Also, there is little information available on varietal differences in trace amygdalin levels among the commercial almond varieties. In some previous studies on amygdalin in almonds, the extraction solvents and methods used may have resulted in lower recoveries of amygdalin, and many analytical methods lack the sensitivity to measure amygdalin at the trace levels.

Effect of variety: The following table (adapted from Lee et al., Tables 2 and 3) compares amygdalin levels in the sweet, slightly bitter and bitter almonds analyzed. Mean amygdalin levels ranged from ~ 0.2 to 16 mg/100 g in the sweet varieties. Among the 10 commercial California almond varieties analyzed, Fritz and Aldrich almonds had statistically higher amygdalin contents. Amygdalin contents also were significantly different among the slightly bitter and bitter almond varieties within each group. Compared with sweet almonds, the slightly bitter almonds had a ~ 16 -fold greater overall mean amygdalin level.

Effect of growing region: Growing region of the commercial almond varieties significantly affected the trace amygdalin content (not shown in table below). However, almond samples were obtained from only one harvest year (2010); multi-harvest sampling would be useful to confirm the growing region effect. Growing region was not studied for the slightly bitter or bitter almond varieties.

Methodology

In this study, methodology was developed that was highly sensitive and selective for analyzing even trace amounts of amygdalin in almonds. The new methodology employed solid-phase extraction of finely ground almond kernels followed by analysis of the extract with an ultrahigh-pressure liquid chromatography system (UHPLC) interfaced to a triple-quadrupole mass spectrometer (QqQ MS/MS) with electrospray ionization (ESI). The limit of detection (LOD) for amygdalin with this UHPLC-(ESI)QqQ MS/MS analytical method is 0.04 mg/kg almond, and the limit of quantification (LOQ) is 0.13 mg/kg almond (or 0.013 mg/100 g). The LOD is about 400 times lower than the lowest mean level of amygdalin observed in the almond samples.

Amygdalin levels in almonds¹

Flavor	Variety	Amygdalin concentration (mg/100 g) ²	
		Varietal mean	Overall mean
Sweet (nonbitter)	Butte	0.22 ± 0.12	
	Padre	0.43 ± 0.24	
	Sonora	0.78 ± 0.60	
	Nonpareil	1.2 ± 0.4	
	Monterey	6.2 ± 2.7	
	Wood Colony	7.5 ± 0.8	
	Carmel	7.7 ± 1.5	
	Mission	9.0 ± 3.2	
	Fritz	14.5 ± 3.6	
	Aldrich	15.7 ± 5.4	
	All 10 varieties		6.3 ± 5.8
Slightly bitter (semibitter) ³	(4 varieties)		99 ± 51
Bitter ³	(6 varieties)		4000 ± 790

¹ Table adapted from Lee et al. (Tables 2 and 3).

² Mean values ± standard deviation.

³ Slightly bitter and bitter almond varieties were obtained from UC Davis breeding program.