Isoflavone workshop
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Extraction methods of botanicals for qualitative and quantitative analysis

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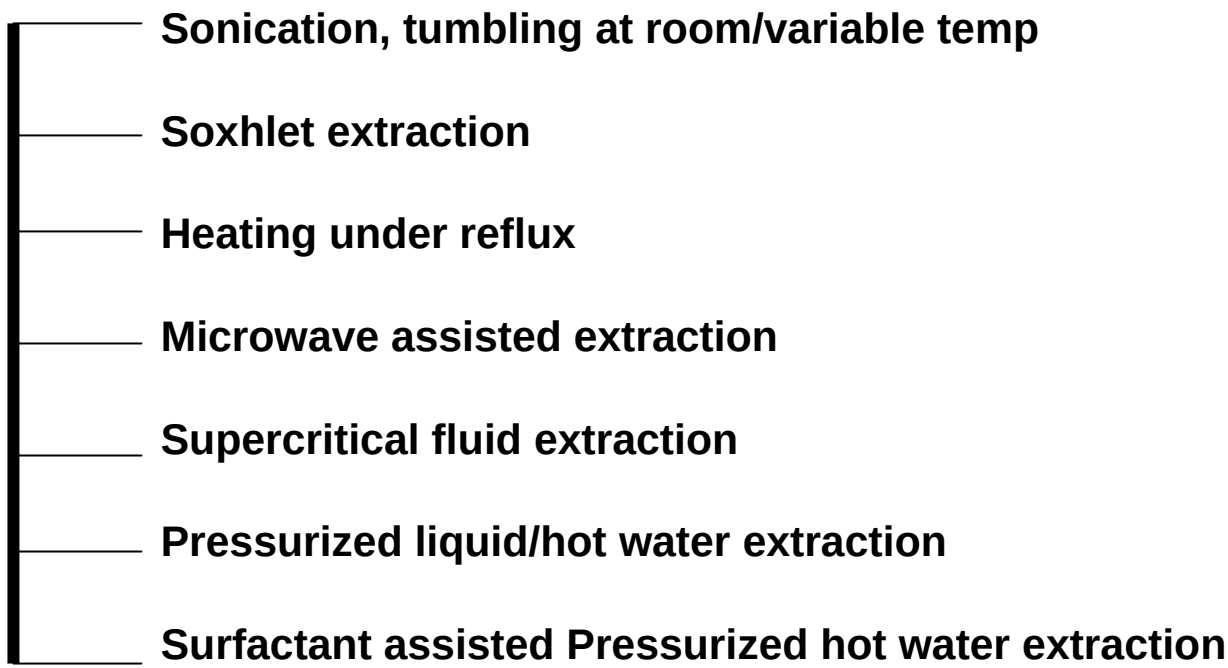
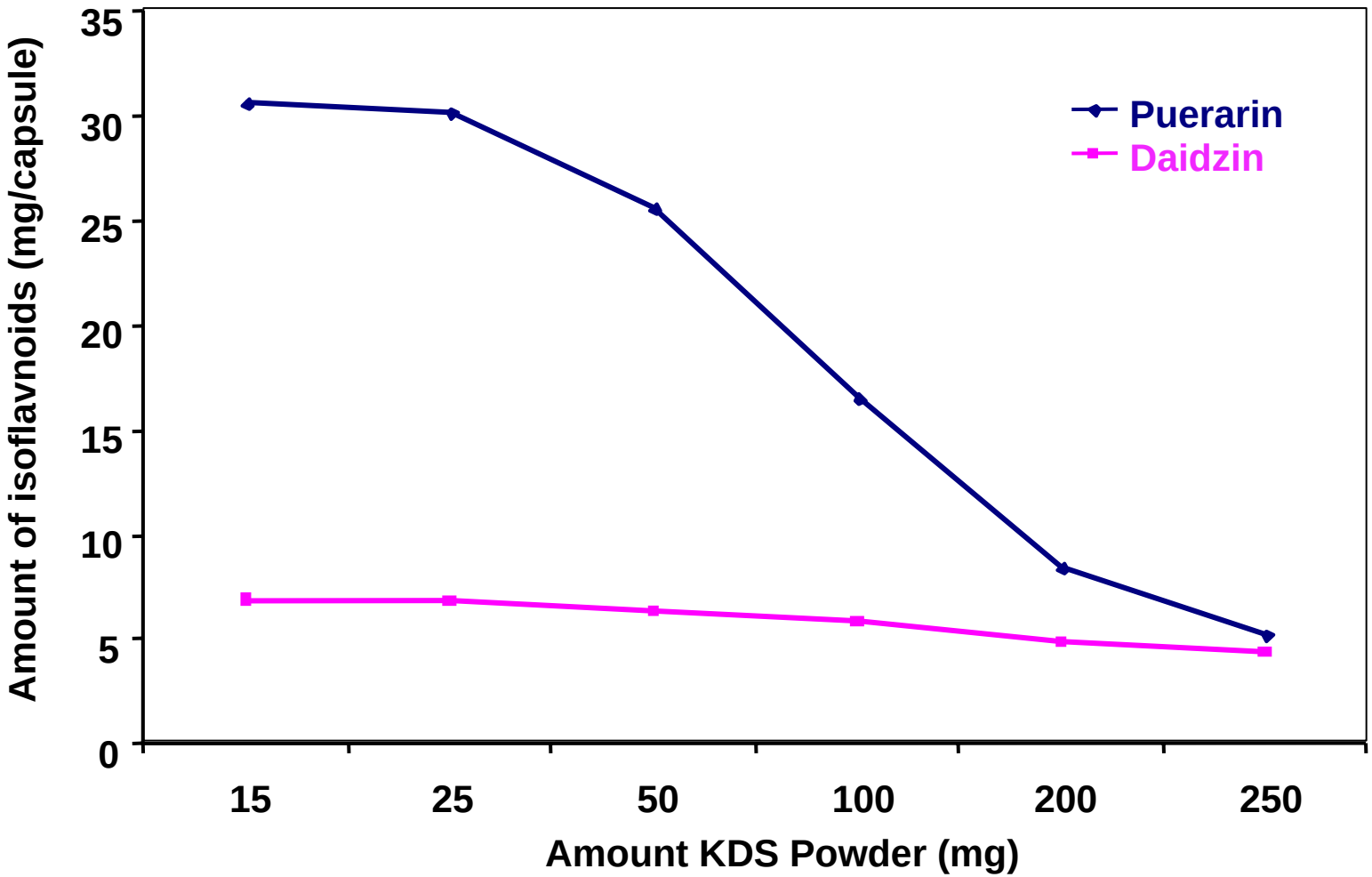
Commonly used extraction methods for botanicals



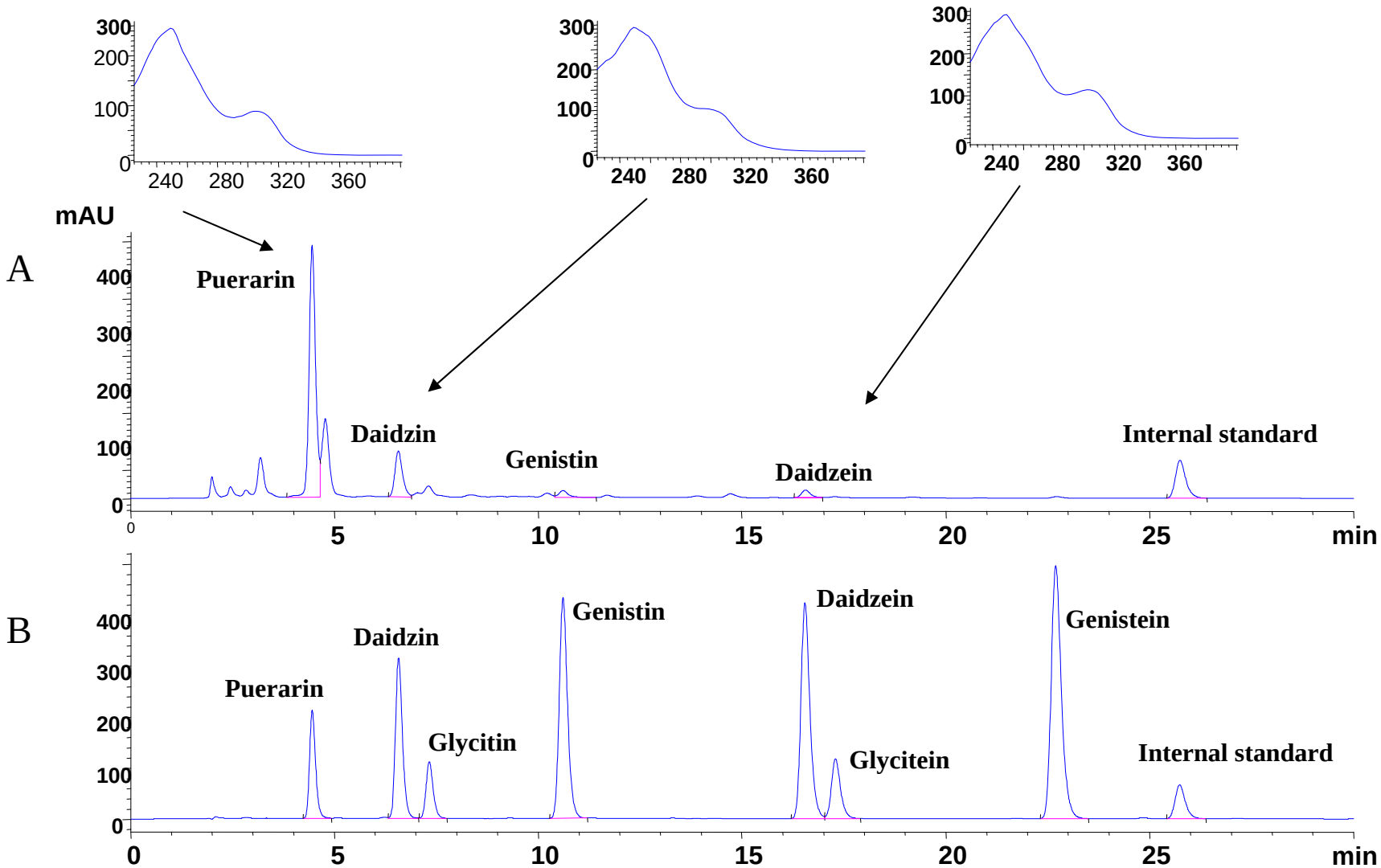
Among the modern sampling techniques, SFE seems to offer high selectivity, minimum degradation of thermally labile analytes, and elimination of the use of organic solvents (e.g. use of pure CO_2). On the other hand, such as difficulties in extracting polar compounds and high susceptibility to matrix effects, are problematic in the extraction of botanical materials.

Extractability of isoflavone glucoside conjugates puerarin and daidzin with various quantities of KDS powder in 5 mL of 80% aq. MeOH



The ratio of KDS powder/extracting solvent is critical for efficient extraction

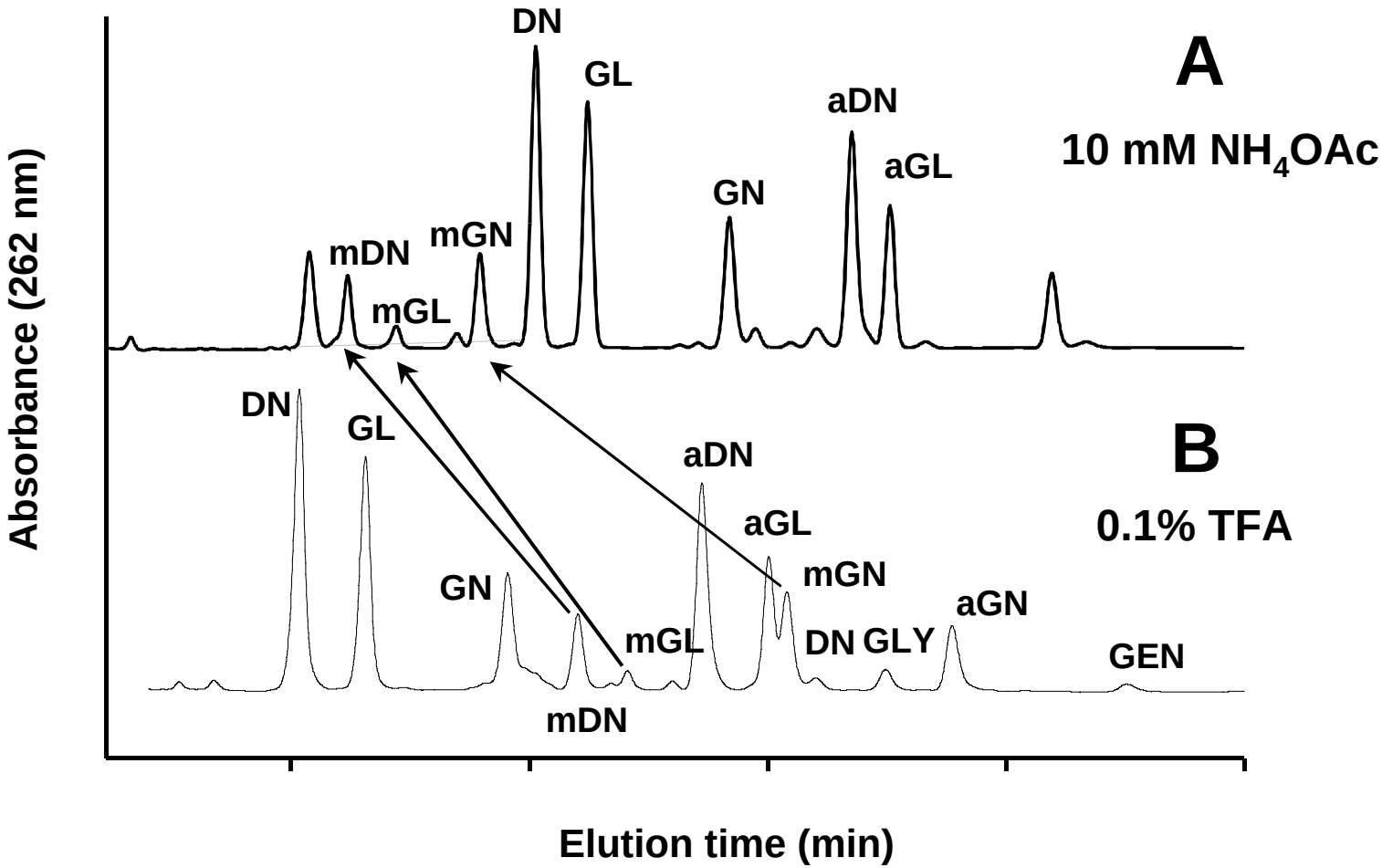
HPLC-chromatograms of [A] a kudzu dietary supplement; [B] isoflavone standards



Prasain et al., J Agric Food Chem 2003

RP-HPLC of isoflavone conjugates

value of changing the mobile phase pH



Extraction of malonyl/acetyl glycosides at room temperature or higher temperatures may lead to change in composition

TABLE I

Effect of extraction temperature on isoflavone concentrations in 80% aqueous methanol extracts of around soybeans[1]

Isoflavone and temperature	6*0*MalGlc	βGlc	6*0*AcGlc	Aglucone	Total
			μg/g		
Daidzein					
4°C	352.1 ± 5.0	55.6 ± 0.8	73.4 ± 0.6	ND	481.1
Room temperature	302.5 ± 1.3	71.0 ± 0.1	71.8 ± 0.7	ND	445.3
80°C	69.6 ± 7.3	393.7 ± 9.3	ND	ND	463.3
Genistein					
4°C	545.9 ± 8.8	57.2 ± 0.5	5.4 ± 0.1	ND	608.5
Room temperature	467.5 ± 3.7	87.9 ± 0.2	4.3 ± 0.1	3.8 ± 0.1	563.5
80°C	69.6 ± 7.3	393.7 ± 9.3	ND	ND	463.3
Glycitein					
4°C	139.5 ± 3.3	42.1 ± 0.4	ND	ND	181.6
Room temperature	125.7 ± 1.7	48.7 ± 1.4	ND	ND	174.4
80°C	ND	150.8 ± 2.3	ND	ND	150.8

[1] $\bar{x}$ ± SD. Concentrations are in aglucone units. 6*0*MalGlc, 6″-*0*-malonyl-β-glucoside; βGlc, β-glucoside; 6*0*AcGlc, 6″-0-acetyl-β-glucoside; ND, none detected.

Barnes et al., Am J Clin Nutr. 1998

Even prolonged storage at 4ºC causes conversion to the β-glucoside conjugates

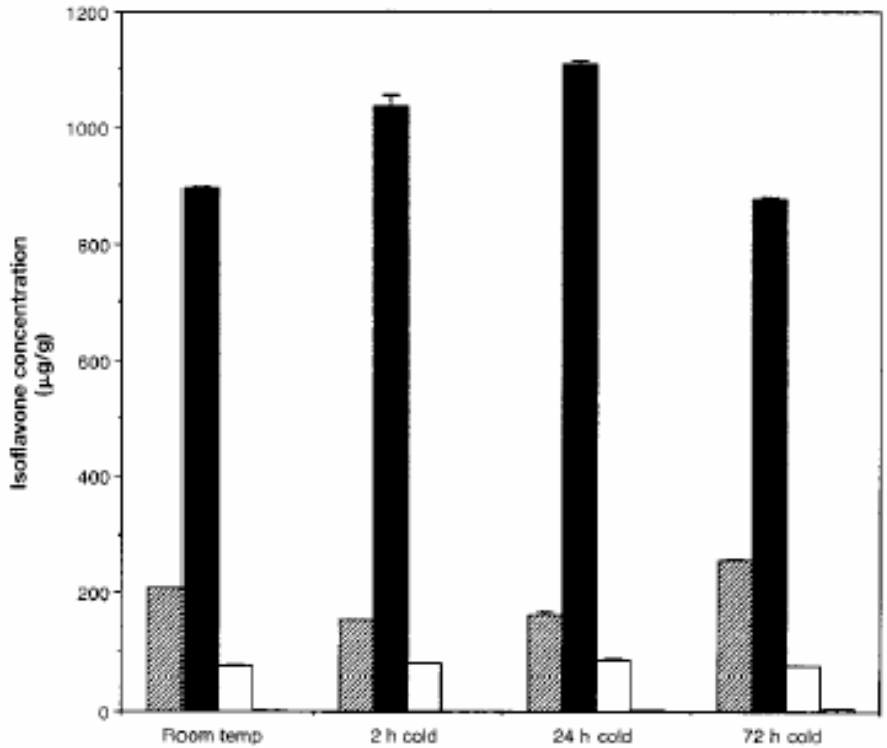


FIGURE 3. Mean (± SD) total concentrations of isoflavone glucoside conjugates from ground soybeans extracted with 80% aqueous methanol at room temperature (temp) for 2 h or at 4 °C (cold) for 2 and 24 h, or after storage at 4 °C for 24 or 72 h. For each set of bars, from left to right, the bars represent β-glucosides, 6″-*O*-malonyl-β-glucosides, 6″-*O*-acetyl-β-glucosides, and aglucones. $n = 2$.

An efficient isoflavone recovery could be achieved with extraction using 70% ethanol containing 0.1 % acetic acid for one hour with agitation

Table 1 - Average (± SD) concentration of total isoflavones (mg/100g) in soybean flour (Kinako), extracted with and without agitation (CV=6.8%)[1].

Extraction time (hours)	Constant agitation	Without agitation	Mean
1	205.4 aA (±13.3)	228.4 aA (±10.8)	216.9 a
4	218.2 aA (±6.2)	198.9 abA (±11.6)	208.9 ab
8	223.0 aA (±1.3)	164.6 bcB (±6.8)	193.8 ab
12	225.4 aA (±9.8)	135.6 cB (±18.9)	180.5 b
16	229.9 aA (±17.1)	183.3 abB (±37.6)	206.6 ab
20	229.6 aA (±5.5)	208.1 abA (±2.5)	218.8 a
24	189.0 aA (±4.9)	218.7 aA (±7.7)	203.6 ab

[1]Mean values followed by the same capital letter in the lines and the same small letter in the columns are not significantly different (Tukey P≤0.05).

Mercedes et al. Brazilian Archives of Biology and Technology, 2002.

Conclusions

- **The ratio of botanicals/dietary supplements to extracting solvents needs to be optimized for efficient extraction.**
- **The isoflavone glucoside conjugates are easily altered during extraction, processing, and cooking. However, the total concentration of isoflavones does not change.**
- **The 6*O*MalGlc conjugates are unstable when exposed to heat or prolonged times after extraction.**
- **Room temperature extraction also led to a loss of the 6*O*MalGlc conjugates, but at much slower rate.**