

**Title** The effect of freezing on solvent extraction efficiencies in the total polyphenol content of whole 'Royal Gala' apple fruit

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### Abstract

The Folin-Ciocalteu procedure is commonly used to measure the total polyphenol content (TPP) of fruit. There is no overall agreement on the most effective solvent for this assay, however, the most widely reported extractants are acetone or methanol based. There is limited knowledge on the affect of freezing the fruit on the extraction efficiency of the solvent used for the assay. The aim of this work was to examine the effects of freezing on the extraction efficiencies of a variety of solvents by measuring the TPP values for "Royal Gala" apples stored at -20°C for six months. The polyphenol composition was determined concurrently on each of the extracts by ultra high pressure liquid chromatography-photodiode array mass spectrometry (UHPLC-PDA-MS). Four different extractants were evaluated: acetone-water 50-50% (A50); acetone 70%, water 29.5% and acetic acid 0.5% (A WA); methanol-water 90-10% (M90); and methanol-water 50-50% (pH 2.0 by HCl) followed by acetone-water 70-30% (M50A 70). The TPP values were determined for fruit frozen for 1, 15, 30, 90 and 180 days and were compared with values for the fresh apples. A50 provided the highest TPP values (120 mgGAE/100gFW) in the fresh apples, followed by A WA (110 mgGAE/100gFW), M90 (84 mgGAE/100gFW) and M50A 70 (81 mgGAE/100gFW). There was a significant interaction between extractant type and freezing time. TPP values decreased over time for A50 (10-20%), remained constant with A WA and M90 and increased with M50A 70 (10-25%). A WA provided the most consistent TPP values throughout the experiment. A WA gave the highest values while M90 the lowest when the polyphenol levels were determined by UHPLC-PDA-MS. This experiment suggests that A WA is the preferred extractant for measuring the TPP values of frozen fruit after storage for up to 6 months. The extract is also suitable for identifying and quantifying selected polyphenols in fruit by UHPLC-PDA-MS.