**Title** Chlorophyll fluorescence as a tool for non-destructive estimation of anthocyanins and total flavonoids in apples

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**Citation** Postharvest Biology and Technology, Volume 41, Issue 2 , August 2006, Pages 156-163

**Keywords** Epidermal transmission; Flavonols; *Malus domestica*; Polyphenols; UV radiation; UV screening

**Abstract**

Flavonoids are secondary plant metabolites assumed to have beneficial effects on human health. In this study, chlorophyll fluorescence (ChlF) was tested as a tool for non-destructive estimation of the flavonoid content in the skin of apples (*Malus domestica* Borkh., cv. Aroma). The method is based on the comparison of ChlF signals obtained with excitation beams absorbed and not absorbed by the various flavonoids. The use of three excitation beams allowed us to measure the total flavonoid content and the content of anthocyanins separately. The ChlF measurements were performed directly on the apple skin with portable fluorometers inducing ChlF with UV-A radiation at 375 nm ($F$(UV)), blue light at 470 nm ($F$(B)) and red light at 655 nm ($F$(R)). The relative absorbance of UV-A radiation was calculated as log[$F$(R)/$F$(UV)], whereas the relative absorbance of blue light was calculated as log[$F$(R)/$F$(B)]. For reference, the same area of the apple as subjected to the ChlF measurements was used for analysis of phenolic compounds by HPLC and reflectance spectra recordings. To determine the localisation of flavonoids in the apple skin, cross sections of the outer cell layers were examined under epifluorescence microscopy. The flavonoids were mostly concentrated in the epidermis above the chlorophyll. This distribution is in agreement with the prerequisites of the ChlF method. The relative UV-A absorbance calculated from ChlF measurements was well correlated with the total flavonoid content detected with HPLC ($r^2 = 0.899$, $P < 0.001$). The relative blue light absorbance calculated from ChlF measurements was linearly correlated to the content of anthocyanins quantified with both HPLC ($r^2 = 0.889$, $P < 0.001$) and reflectance measurements ($r^2 = 0.917$, $P < 0.001$). In conclusion, ChlF measurements can indeed be utilised for non-destructive estimation of both anthocyanins and total flavonoids in apples and can in this context function as a valuable tool for the commercial fruit industry as well as in plant and food research.