Histolocalization of the pigments in the pumpkin seed which are the source of dichromatic colour in edible oil

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Introduction: The roasted pumpkin (Cucurbita pepo L.) seeds are used to press edible oil. The characteristic colour of the pumpkin seed oil has been previously described as dark green to red ochre [1]. A survey on students in Graz showed that most participants perceived pumpkin seed oil both reddish and greenish [2]. Recently, the physicochemical properties of the variable colour of the pumpkin seed oil were elucidated. Due to the specific absorption spectrum, the pumpkin seed oil appears green in thin layers and red in thick layers [3]. Such perceptual shift in colour appearance was termed dichromatism. The protochlorophyll pigment is the main source of colour in pumpkin seed oil. The pigment is consensually assigned to the green layer (chlorenchyma) of the seed testa (Figure 1); however, the selective histolocalization was not yet reported.

In the present study, microscopic fluorimetry was employed to localize protochlorophyll pigment to chlorenchyma. The *in situ* emission spectrum was compared to the two-dimensional fluorescent spectrum of the pumpkin oil.

Materials and Methods: For microscopical examination perpendicular sections of the pumpkin seeds were cut with a scalpel knife. The fluorescent images were taken by Zeiss LSM 510 Meta confocal microscope with the spectral detector and analysis. For autofluorescence images, the 458 nm line of the Argon laser was used. The emission spectra in the range from 478 nm to 799 nm were taken for every image pixel, and then average spectra were calculated for each of the region-of-interest, depicted in Figure 2.

Results and Discussion: The localisation of the pigment in the pumpkin seed was investigated by microscopical study of botanical morphology-anatomy. The red autofluorescent pigment is located to the thin layer (4 to 7 μm) in the lowest part of testa (Figure 1). The emission spectra of this layer measured by microscopical fluorimetry has a maximum in the red (emission peak at 700 nm). The peak at 700 nm in the fluorescent spectrum of chlorenchyma is however not at the same position as in the emission spectrum measured in pumpkin oil as it can be seen from the two-dimensional fluorescent spectrum (Figure 2), where pumpkin oil has maximal emission at 655 nm regardless of the excitation wavelength. It was suggested that the protochlorophyll in pumpkin seed is in a form of molecular aggregate [4]. This is supported by our measurements, since a secondary peak of emission fluorescence in fluorescent images (Figure 1) at around 650 nm was observed. Similarly, in measurements of oil fluorescence a secondary peak of fluorescence at 700 nm was also observed (Figure 2) [5].

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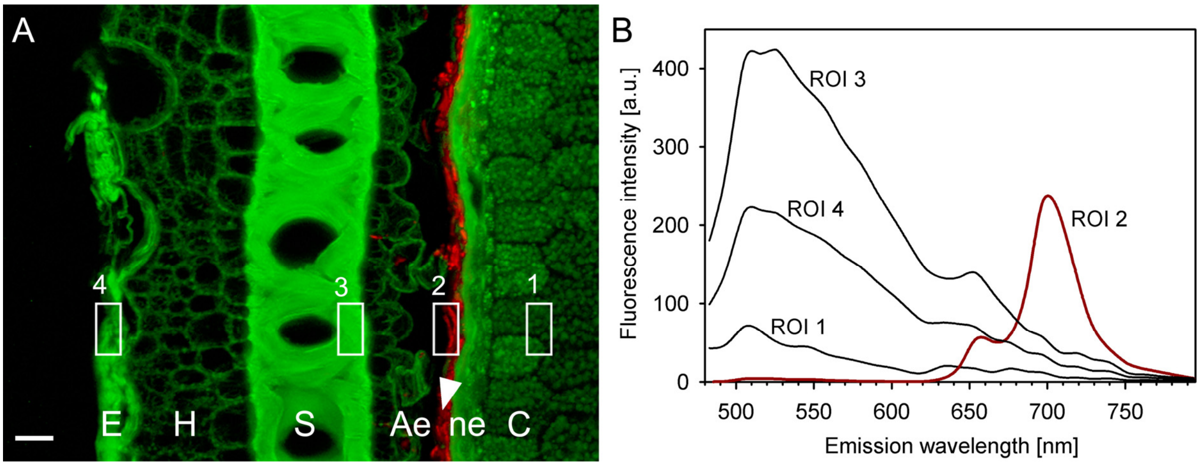

Figure 1. A. The autofluorescent image of the perpendicular section of the pumpkin seed. Layers of the testa epidermis (E), hypodermis (H), sclerenchyma (S), aerenchyma (Ae) and chlorenchyma (arrowhead) are shown. Thin layers of the nucellus (n) and the endosperm (e) are also visible on the right side of the cotyledon parenchyma with oil droplets (C). Scale bar: 20 μm. B. Spectral properties of fluorescence acquired at the regions of interest (ROI 1-4) marked in A. The excitation was at 458 nm. The layer enclosed in the ROI 2 displays red florescence, whereas all other regions display green fluorescence.

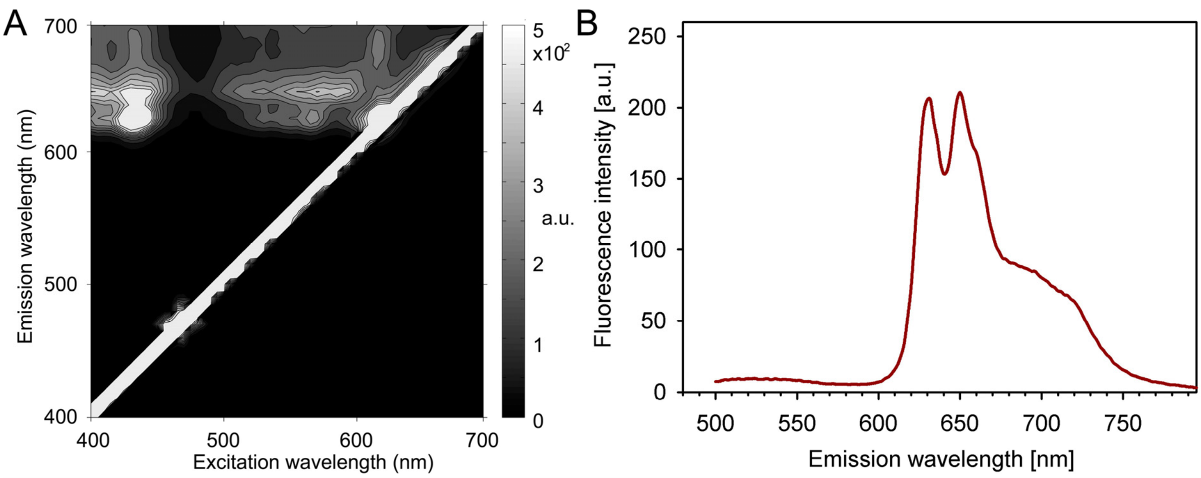

Figure 2. A: Two-dimensional fluorescent spectrum of the pumpkin oil. B: The fluorescent spectrum of the pumpkin oil at excitation: 458 nm. The pumpkin oil has maximal emission at 655 nm regardless of the excitation wavelength, but the second peak at 700 nm is also observed as in chlorenchyma fluorescence.

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