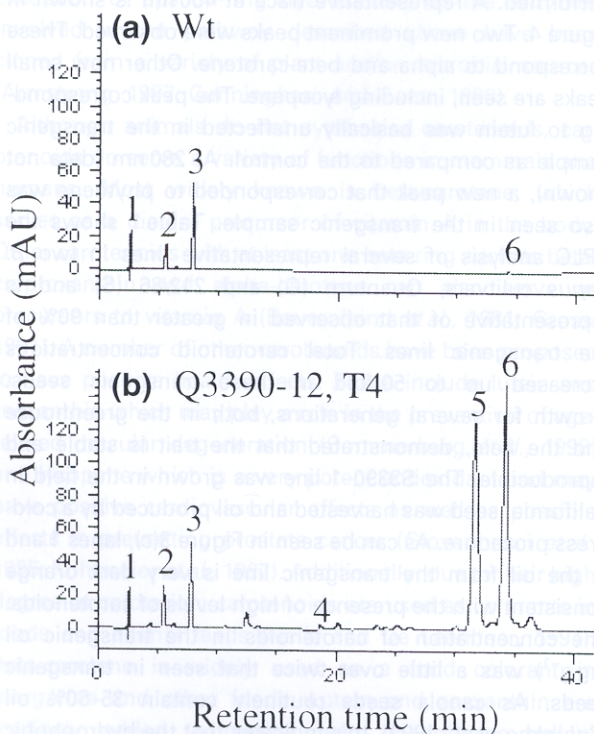


**Figure 3.** Napin-*crtB* embryos, plants and oil. (a) Twenty-eight d.p.a. silique from S3390-1 (T2 seeds). (b) Thirty-five d.p.a. embryos from S3390-1 (T3 homozygote) and non-transgenic control. (c) Lane 1: cold-pressed oil from non-transgenic S control; lane 2: cold-pressed oil from S3390-1 from Brawley field trial (homozygous seed); lane 3: complete extraction of 12 control S seeds in 2 ml hexane; lane 4: complete extraction of 12 S3390-1 seeds (T4 homozygous seed) in 2 ml hexane.



**Figure 4.** HPLC analysis of mature control and pCGN3390 seed. (a) S control. (b) S3390-1 – Homozygous seed. Peaks are 1, solvent front; 2, lutein; 3, internal standard; 4, lycopene; 5, alpha-carotene; and 6, beta-carotene. Extracts were from similar tissue weights.

promoter. Similarly, a Western blot for *crtB* protein (Figure 5b) demonstrated that *crtB* protein was absent at 15 d.p.a., and accumulated as the seed aged. An immunoreactive

polypeptide of the expected size for the processed protein, 33 kd, was present in addition to a smaller band that may come from either incorrect processing or degradation. Samples beyond 24 d.p.a. were also analyzed by Western blot, however, as the highly abundant seed storage protein cruciferin (Crouch and Sussex, 1981; Sjö Dahl *et al.*, 1991) migrates at the same molecular weight as *crtB* and interferes with the signal from *crtB*, these data are not shown. Carotenoid analysis on this same time course indicated that carotenoid production in the napin-*crtB* transgenic line began to rise above the control between 18 and 21 d.p.a. and continued to rise throughout development (Figure 5c). The beta-carotene level continued to rise until the seed was mature, while the alpha-carotene concentration leveled off around 35–40 d.p.a. The increase in carotenoid level correlates both with the production of *crtB* mRNA as well as the presence of *crtB* protein. However, the fact that the carotenoid levels continue to rise while the mRNA levels drop, indicates that the *crtB* protein is probably quite stable in the transgenic seed or that little protein is needed to maintain the increase in carotenoid production.

#### *Analysis of other isoprenoids and fatty acids reveals other metabolic changes*

The substrate for phytoene synthase, GGPP, is also a precursor for tocopherols and chlorophylls in the plastids. To ascertain the effect of the overexpression of *crtB* on the levels of these compounds, HPLC analysis was performed on the developing seed of several pCGN3390 lines (Table 2). In normal canola seeds, chlorophyll levels are high during development and decline somewhat with age.