Carotenoids and vitamin E in seed, press cake and oil of rapeseed, sunflower, flax and safflower – Comparison of HPLC and photometric determination of carotenoids

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ABSTRACT

Carotenoids (carotenes and xanthophylls) and vitamin E (tocopherols and tocotrienols) in oils from the market - 6 rapeseed and 6 sunflower oils, half of each cold pressed and refined - and in the oil of rape, sunflower, flax and safflower as well as the respective seeds and press cakes from a local oil mill were quantified by HPLC. Furthermore, a photometric determination of carotenoid content was tested. In the cold pressed oils minor amounts of xanthophylls *(all-E)*-lutein and *(all-E)*-zeaxanthin were determined, carotenes as vitamin A active compounds did not occur. Carotenoids were not detectable in the refined oils. Cold pressed rapeseed oils contained total carotenoids in the range of 0.5 to 1.5 mg per 100 g which was manifold the content of sunflower, flax and safflower oils. Vitamin E was found in all vegetable oils at plant-typic tocopherol patterns. The photometric determination of carotenoids in oils resulted in significantly higher carotenoid concentrations compared to the HPLC analysis which can be explained by the nature of the carotenoids in these plant oils.

Key words: carotenoids, vitamin E, oilseeds, rapeseed, HPLC, photometry

INTRODUCTION

In the oils of home-grown oilseeds (rapeseed - *Brassica napus,* sunflower - *Helianthus annuus*, thistel - *Carthamus tinctorius* and linseed - *Linum usitatissimum*) and the respective press cakes, the tocopherols are valuable constituents representing vitamin E and antioxdant activity, resp. (BALZ et al., 1993, SCHÖNE et al. 1998). There are hints on remarkable concentrations of carotinoids not only as colouring substances but also with vitamin A-activity (Guizhen et al. 2007). In the former investigation only the „total carotene“ content was measured by a photometrical method. In recent years, concentrations of vitamin E and carotenoids of several grain products and vegetables were characterized by determination via HPLC (BÖHM 2001, PANFILI et al. 2004, BIEHLER et al. 2010) and this method should be used to differentiate between the carotenoids. The present investigation focused on carotenoids and tocochromanols, i.e. tocopherols and tocotrienols, in refined and cold pressed vegetable oils from the market and – as secondary series – in oils, but also in seeds and press cakes produced in a smaller local oil mill. In addition, known carotenoid rich materials - maize flour and carrot - were analysed. The photometric method was also tested to compare it with the HPCL method and to evaluate its use as a simple, fast and cheap oil quality control in the practice with limited laboratory and labour capacity.

MATERIALS AND METHODS

The samples from the market consisted of 12 different rapeseed and sunflower oils bought at the end of 2007 and at the beginning of 2008 in a supermarket of Jena. Each oil plant species represented three samples refined and three ones cold pressed oil. The samples of the 2nd series – seed, oil and press cake, each from rape, sunflower, flax and safflower – originated from a Monforts oil press with an capacity of 5-8 kg seed/hour. The material for comparison with well known high carotenoid content represented freshly mashed carrot and a maize flour bought 2008.Tocopherols and tocotrienols were analysed by HPLC according to the modified method reported by BALZ et al. (1993). Carotenoids were analysed by normal phase HPLC according to the method reported by PANFILI et al. (2004). The normal phase HPLC used is not well suited for determination of α- and β-carotene and so in first investigations, the carotenoids in the oil samples were determined by using a gradient HPLC method modified by BÖHM (2001) using a TRENTEC C_{30}-Column. This analysis was also performed for the carotenoid rich materials - carrot and maize flour. For details regarding samples, sample preparation, mainly extraction procedures, analyses (chromatography conditions and used standards) and statistical evaluation see FRANKE et al. 2010.

RESULTS AND DISCUSSION

Tocotrienols were detected neither in the oils from the market nor in the samples from the oil mill. There were high amounts of α- and γ-tocopherol, whereas only traces of β- and δ-tocopherol were found. The total tocopherol content of the rapeseed oils (for differentiation into tocopherol species see FRANKE et al., 2010) amounted to 63.8 - 68.0 mg/100 g – without a difference between the cold pressed and refined oils. In sunflower oils total tocopherol contents of 48.2 to 75.5 mg/100 g were determined. There was a slightly lower content of 1/10 to 1/5 of the refined versus cold pressed oils and this difference showed significance ($P<0.05$). The total tocopherol content of the thistel oil amounted to amounted to 68.4 mg/100 g, that of the linseed oil to 42.5 mg/100 g. For mean and standard deviation of the content of tocopherol species in all investigated oils, seeds and press cakes and for the discussion see FRANKE et al. 2010.

Among the oils that were studied, carotenoids were only detected in cold pressed oils (tab.1). As main carotenoid *(all-E)*-lutein was quantified. In one cold pressed rapeseed as well as in one sunflower oil also *(all-E)*-zeaxanthin in minor amounts was determined. The total carotenoid content ranged from 0.05 to 1.52 mg/100 g oil (traces of *(all-E)*-β-carotene detected in two cold pressed rapeseed). The cold pressed rapeseed oil had a higher carotenoid concentration than the cold pressed sunflower oil. The carotenoid contents determined in the cold pressed rapeseed and sunflower oils from the small oil press agreed by far with those of the respective oils from the market. The safflower oil contained only traces of carotenoids (comparable to sunflower oil) in the flax oil the content of *(all-E)*-lutein and carotenoids, resp., was in a range of 0.4 mg/100 g.
In seed and press cake of flax and safflower a lower or non-detectable carotenoid quantity points to an unequal distribution with dominance in the oil. In contrast, in rapeseed similar amounts of *(all-E)*-lutein and carotenoids in oil, seed and press cake stand for a relative homogenous distribution in oily and non-fat parts in these matrices.
Regarding the absence of carotenoids in refined oils, previous technological studies have shown that carotenoids, mainly lutein and zeaxanthin, are removed from the crude oil by various refining steps particularly through bleaching and deodorization (CMOLIK et al. 2000).

The photometric method detected carotenoids in all samples (FRANKE et al. 2010) in contrast to the previously shown non-detectabilty of the carotenoids by HPLC in seeds and press cakes of sunflower and safflower. In the samples with carotenoid analysis by both methods the photometry resulted in significantly higher concentrations (up to factor 3) compared to the HPLC. The highest carotenoid content of 4.6 mg/100 g, photometrically detected in the rapeseed, was in the range of 0.8 to 5.2 mg/100 g rapeseed given by GUIZHEN et al. (2007). Also rape press cake and rapeseed oil showed quite high carotenoid contents whereas the photometrically analysed carotenoid content of sunflower, flax and safflower was significantly lower. A decisive disadvantage of carotenoid content calculation based on photometrical detection at 445 nm is the use of one wavelength for the whole group of carotenoids with different absorbance maxima. To explain a possible effect of the carotenoid pattern, carrot and maize flour were compared regarding the results of photometry versus HPLC. Fig. 1 shows a photometrically determined total carotenoid content of 5.7 mg/100 g carrot fresh matter (FM), representing about half the concentration determined by HPLC (11.5 mg/100 g FM). Vice versa in the maize flour 1.9 mg total carotenoids/100 g FM via photometry contrasted with 1.1 mg/100 g FM by HPLC. Both these matrices differ substantially in the carotenoid pattern: the carrot represents almost only *(all-E)*-α- and *(all-E)*-β-carotene; in the maize flour lutein and zeaxanthin are dominating. This comparison strengthens the thesis of an overestimation of total carotenoid content with photometry at 445 nm for the xanthophylls-dominated samples maize or rapeseed oil and an underestimation by this method for the carotene-dominated carrot. Therefore, the photometric method is only of limited value for determination of a valid total carotenoid content in several foods and feeds. Knowing the percentage of carotenes and xanthophylls of a given matrix this simple and fast estimation of carotenoid content could be improved.

Table 1: Content of carotenoids in the samples from the market and from the oilmill (mean ± SD, n=3) determined by normal phase HPLC [1)]

Sample	Carotenoids, mean ± SD [mg/100 g FM]			
	***(all-E)*-lutein**		***(all-E)*-zeaxanthin**	**total**
market				
R/cp-1	**0.57** ± 0.03^{c}	*	n. d.	**0.57** ± 0.03^{c}
R/cp-2	**1.49** ± 0.02^{a}	*	**0.04** ± 0.00	**1.52** ± 0.02^{a}
R/cp-3	**1.16** ± 0.07^{b}	*	n. d.	**1.16** ± 0.07^{b}
R/rf-1	n. d.		n. d.	n. d.
R/rf-2	n. d.		n. d.	n. d.
R/rf-3	n. d.		n. d.	n. d.
Su/cp-1	n. qu.		n. qu.	n. qu.
Su/cp-2	**0.05** ± 0.00^{e}	*	n. qu.	**0.05** ± 0.00^{e}
Su/cp-3	**0.07** ± 0.00^{d}	*	**0.09** ± 0.00	**0.16** ± 0.00^{d}
Su/rf-1	n. d.		n. d.	n. d.
Su/rf-2	n. d.		n. d.	n. d.
Su/rf-3	n. d.		n. d.	n. d.
oil mill				
R	**1.47** ± 0.02^{a}	*	n. qu.	**1.47** ± 0.02^{a}
RS	**1.33** ± 0.06^{b}	*	n. d.	**1.33** ± 0.06^{b}
RP	**1.45** ± 0.11^{a}	*	n. d.	**1.45** ± 0.11^{a}
Su	**0.03** ± 0.00^{c}	*	**0.04** ± 0.00	**0.07** ± 0.01^{e}
SuS	n. d.		n. d.	n. d.
SuP	n. d.		n. d.	n. d.
F	**0.37** ± 0.01^{d}	*	n. qu.	**0.37** ± 0.01^{c}
FS	**0.25** ± 0.01^{e}	*	n. d.	**0.25** ± 0.01^{d}
FP	**0.09** ± 0.01^{c}	*	n. d.	**0.09** ± 0.01^{e}
Sa	**0.08** ± 0.01^{c}	*	**0.15** ± 0.01	**0.22** ± 0.00^{d}
SaS	n. d.		n. d.	n. d.
SaP	n. d.		n. d.	n. d.

abbr: R-rapeseed oil, Su-sunflower oil, F- flaxseed oil, Sa- safflower oil, cp- cold pressed, rf-refined, RS-rapeseed, RP-rape press cake, SuS-sunflower seed, SuP- sunflower press cake, FS-flaxseed, FP-flax press cake, SaS-safflower seed, SaP- safflower press cake, n. d. - not detectable, for limit of detection see material and method`s section! n. qu. - not quantifiable, for limit of quantification see material and method`s section!

^{abcde} means with different letters indicate significance (P<0.05)

* significant difference between concentrations of (*all-E*)-lutein and (*all-E*)-zeaxanthin (P<0.05)

1) In a screening run of the oils using a gradient HPLC method [11] traces of *(all-E)*-β-carotene were below the limit of quantification (see material and method`s section) with exception of cold pressed rapeseed oil which contained <11-195 µg *(all-E)*-β-carotene /100 g.

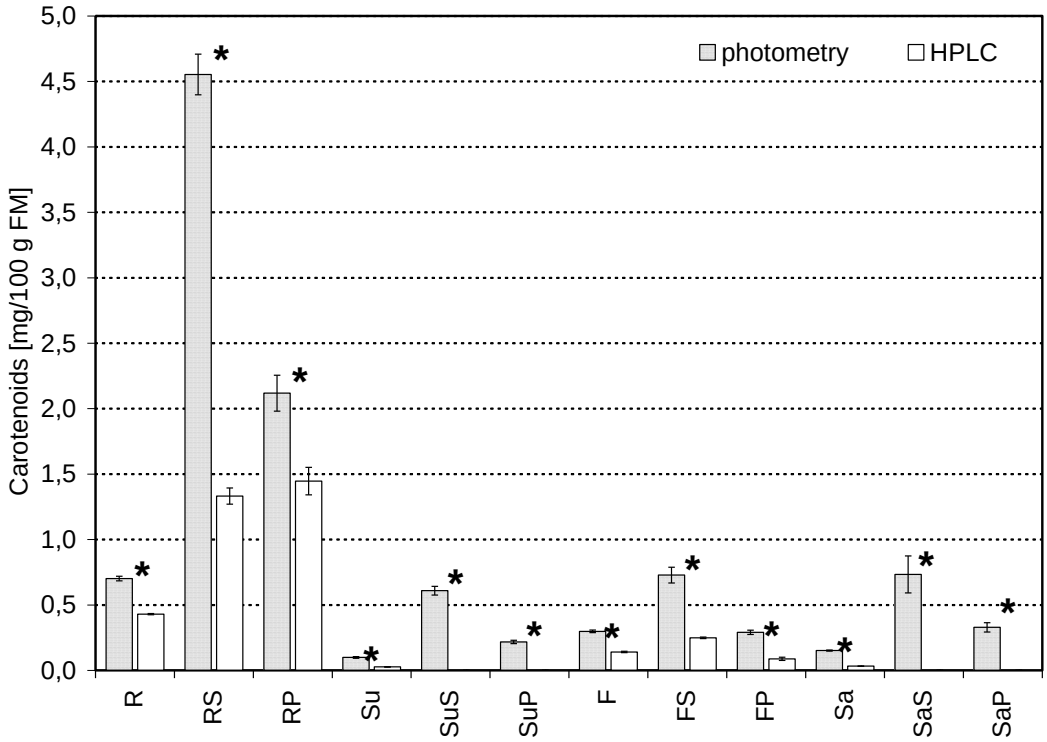

Figure 1: Comparison of the photometric and the chromatographic determination of the carotenoid content in seed, press cake and oil (mean ± SD, n=3), in cases of invisible bars for HPLC determination the values were below LOD (*(all-E)*-lutein 0.004 mg/100 g fresh matter (FM), *(all-E)*-zeaxanthin 0.011 mg/100 g FM).
* significant difference (P<0.05)
Abbr.: R - rapeseed oil, RS - rape seed, RP - rape press cake, Su - sunflower oil, SuS - sunflower seed, SuP - sunflower press cake, F - flaxseed oil, FS - flax seed, FP - flax press cake, Sa - safflower oil, SaS - safflower seed, SaP - safflower press cake

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