Variability of Lutein Content in Peas (*Pisum sativum* L.) in Relation to the Variety, Season and Chlorophyll Content

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Abstract: Lutein and β-carotene contents in 32 samples of field peas with green (*ii*), yellow (I.) and orange cotyledons (*OrcOrc*) were determined. The highest lutein concentration ranging between 0.768 and 1.394 mg/100 g was found in varieties with green cotyledons (median 0.978 mg/100 g). Yellow cotyledons contained lower amount of lutein, median = 0.692 mg/100 g. Big variation in lutein content was found in tested breeding lines with orange cotyledons, only 4 lines showed higher lutein content in comparison with the lowest value found in green cotyledons. Content of β-carotene in field peas with green cotyledons ranged between 0.1–0.2 mg/100 g. Field peas with yellow and orange cotyledons contain β-carotene in 10 times lower concentration. 27 samples of garden peas were tested. Lutein content fluctuated between 0.471 and 1.524 mg/100 g with median = 0.791 mg/100 g. Year-to-year differences in 54% of all repeatedly tested samples shoved coefficient of variation lower than 10%. Coefficient of variation under 20% was found in 96% of repeatedly analysed samples. A strong correlation ($r = 0.77$, $P < 0.01$) between lutein and chlorophyll contents was found.

Keywords: lutein; β-carotene; chlorophyll; peas

INTRODUCTION

Lutein is one of the most widely distributed carotenoids. The main sources of lutein are vegetables and fruit. Calvo (2005) collected data of lutein concentration in 74 species of fruit and vegetables reported by 44 authors since 1990. He observed that lutein concentration in green vegetables (lettuce, broccoli, water cress, parsley, pea, spinach) was higher than in yellowish-white vegetables. Red-orange vegetables are recognised as the weakest lutein source.

Main source of lutein in the diet depend on nutritional habits prevailing in a given country. Generally, green vegetables are reported the main source of lutein in the diet. In Poland legumes, mainly peas and beans represent below 1% of the dietary intake (Homulka *et al*. 2005). However lutein from peas contributed by more than 19% to its intake in Republic of Ireland and the UK. The results are based on food frequency questionnaire and carotenoid database comparing the carotenoid intakes in five European countries: UK, Republic of Ireland, Spain, France and the Netherlands (O'Neill *et al*. 2001).

Lutein has no provitamin activity, but it displays biological activity in relation to human health. Evidence suggests that its intake is inversely related to the occurrence of eye diseases such as age-related macular degeneration and cataracts. Lutein may also serve to protect skin from UV-induced damage and may reduce the risk of cardiovascular disease (Alves-Rodrigues & Shao 2004).

The aim of the study was the evaluation of field and garden peas as a source of lutein in relation to the variety, cotyledon type and season.

MATERIALS AND METHODS

Plant material. 15 recognised varieties and 17 breeding lines of field peas (round seeds *RR*)

and 13 recognised varieties and 24 breeding lines of garden peas (wrinkled seeds *rr*) were cultivated in field condition. Group of field peas samples consisted of 4 samples, 11 samples and 17 samples with green (*ii*) yellow (*I.*) and orange (*OrcOrc*) cotyledons, resp. (Swiecicki 1989; Chloupek 2000). 11 samples were analysed both in years 2007 and 2008. Samples of garden peas (13 recognised varieties and 24 breeding lines) originated from harvests in 2006, 2007 and 2008, 13 of them were grown in 2 or 3 following years. A field trials (2006–2008) were carried out for the evaluation of the yield potential of varieties and new breeding lines, TSW and healthy state. The trials were arranged as a randomised complete block design with 3 replications. Harvest of peas was performed by harvester. Seeds were dried in oven by cold air to 14% moisture and cleaned.

Determination of carotenoids content. For lutein and β-carotene determinations the HPLC method following saponification of the sample was used. After milling (IKA A11 basic), saponificaton with 10% KOH in ethanol (30 min at 95°C) and repeated extraction into diethylether was performed. Ascorbic acid was used as an antioxidant. Diethylether was evaporated, the last portion was removed by stream of nitrogen. The residue was dissolved in hexane and used for HPLC determination. Agilent HPLC system HP 1100 equipped with UV-VIS detector operating at 445 nm and Lichrosorb Si 60 (5 um, 250 × 4 mm, Merck) column was applied. Mobile phase consisted of n-hexane and 2-propanol (95:5). Standards Xanthophyll from alfaalfa (Fluka) and β-carotene (Sigma) were used for calibration. UV/VIS spectrophotometer Hélios α (Thermo Electron Corp.) and extinction coefficients $E^{1\%}_{1cm}$ = 2592 for β-carotene and $E^{1\%}_{1cm}$ = 2589 for lutein were applied in construction of calibration curves. All operations were done under dim laboratory light.

Determination of chlorophyll content. Dry seeds of pea with moisture content less than 10% were grinded by Tecator Cyclotec mill. 200 mg of the test samples were put into tubes and 5 ml of extraction solvent N,N'-dimethylformamide (DMFA) were added. Extraction was carried out for 24 hours at 4°C. Absorbance of filtered extract was measured at 647 nm, 664 nm and 710 nm by UV-VIS spectrophotometer (Wellburn 1994). The chlorophyll a and chlorophyll b concentrations (g/m^3) in extract were then calculated by the formulas:

$$c_{Chla} = 11.65 \times (A_{664} - A_{710}) - 2.69 \times (A_{647} - A_{710})$$
$$c_{Chlb} = 20.81 \times (A_{647} - A_{710}) - 4.53 \times (A_{664} - A_{710})$$

RESULTS AND DISCUSSION

Influence of variety

Field peas. 4 recognised varieties of field peas with green cotyledons (*ii*) and 11 recognised va-

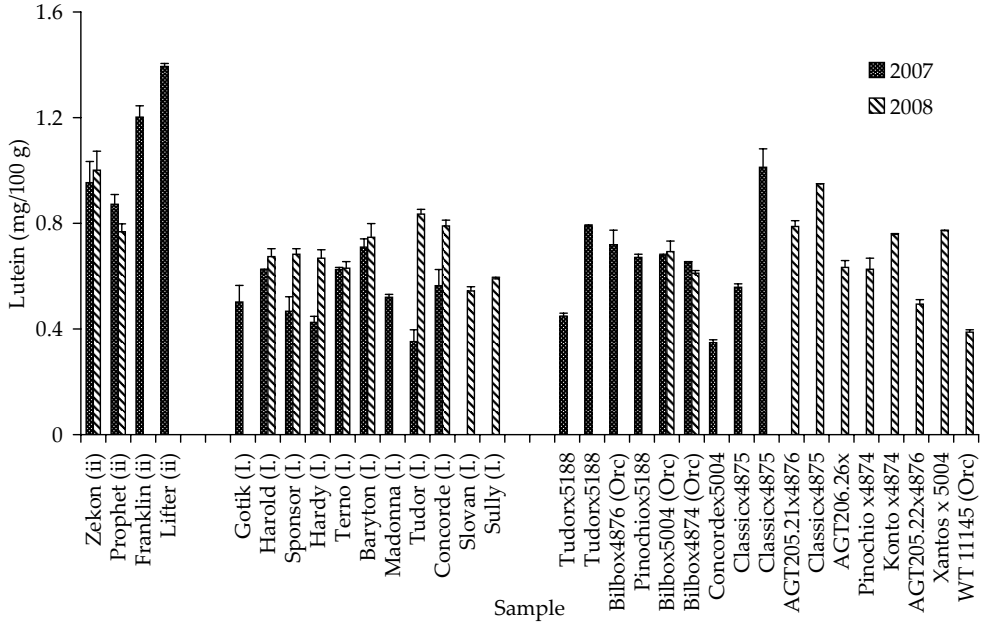

Figure 1. Lutein content in field peas (harvest 2007 and 2008). Data are expressed as average (n = 2) ± SD

rieties with yellow cotyledons (*I.*) were tested for lutein and β-carotene contents. 17 promising breeding lines with orange cotyledons (*OrcOrc*) were part of tested group of field peas as well. The results are presented in Figure 1. The highest lutein concentration ranging between 0.768 and 1.394 mg/100 g was found in varieties with green cotyledons (median 0.978 mg/100 g). Yellow cotyledons contained lower amount of lutein, median = 0.692 mg/100 g. Big variation in lutein content was found in tested breeding lines, only 4 lines showed higher lutein content in comparison with the lowest value found in green cotyledons. The hypothesis, that orange cotyledons might be better source of lutein was not confirmed.

Content of β-carotene in field peas with green cotyledons between 0.1–0.2 mg/100 g was found. Peas with yellow and orange cotyledons contained β-carotene in 10 times lower concentrations.

Garden peas. 27 samples of garden peas were tested, 13 of then represented recognised varieties and 24 of them the promising breeding lines. The results are demonstrated in Figure 2. Lutein content fluctuated between 0.471 and 1.524 mg/100 g with median = 0.791 mg/100 g. However, among samples of garden peas, 53% of them contain more than 0.8 mg lutein/100 g. For field peas only 19% of samples exceeded this level. Breeding lines SM 255/00 showed the highest lutein content. β-carotene content in garden peas is comparable with that in field peas with green cotyledons.

Literature data for lutein content differs, probably due to influence of variety, season, weather conditions, maturity or variabilities in the quantification process. Homulka *et al.* (2005) found lutein content in yellow peas between 0.81 mg to 1.13 mg/100 g. In thermally treated peas the increase of lutein concentration was reported, probably due to the improved extractability from the matrix (Edelenbos *et al.* 2001). On average of two years, lutein concentration varied from 1.2 to 1.9 mg/100 g in 6 cultivars of processed green peas (Edelenbos *et al.* 2001). Lower value for canned peas (0.662 mg/100 g) found Humphries & Khachik (2003).

Year-on-year variation

11 samples of field peas from both harvest 2007 and 2008 were obtained and analysed. 13 samples of garden peas were analysed in two or three fol-

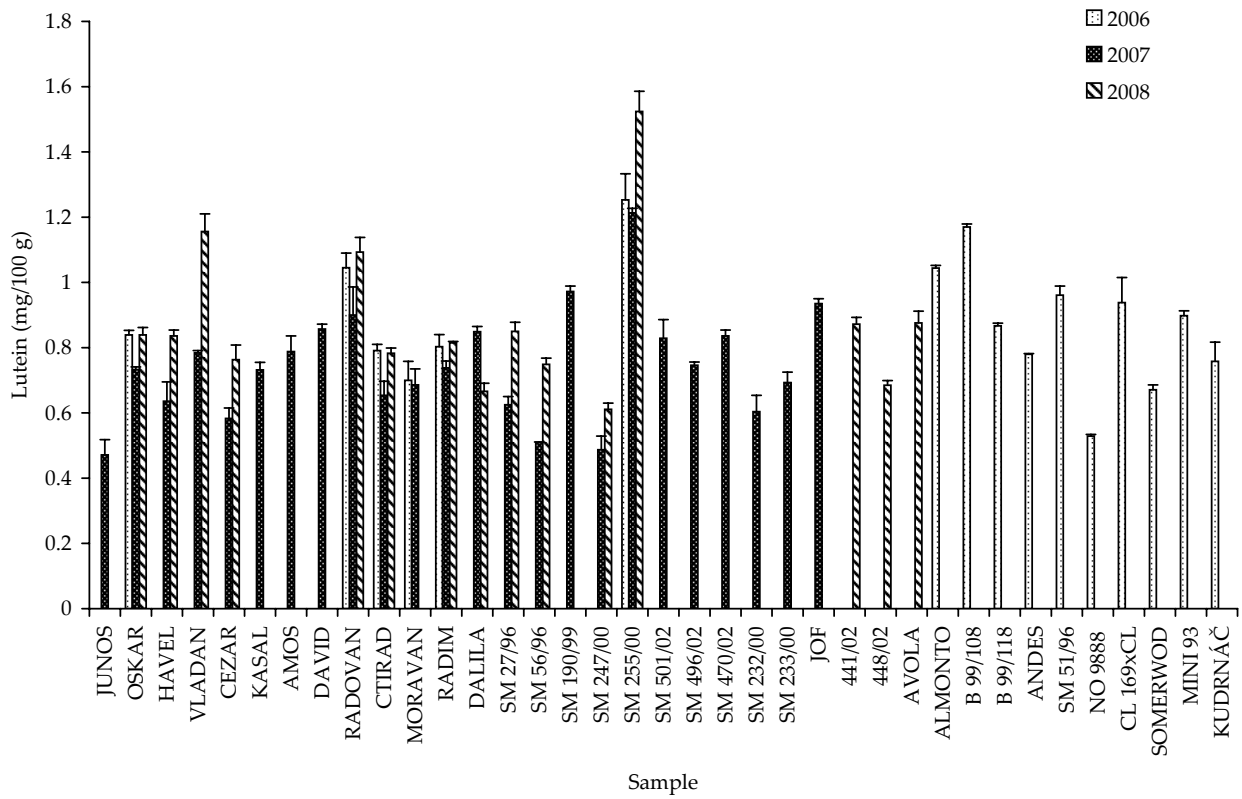

Figure 2. Lutein content in garden peas (harvest 2006, 2007 and 2008). Data are expressed as average (n = 2) ± SD

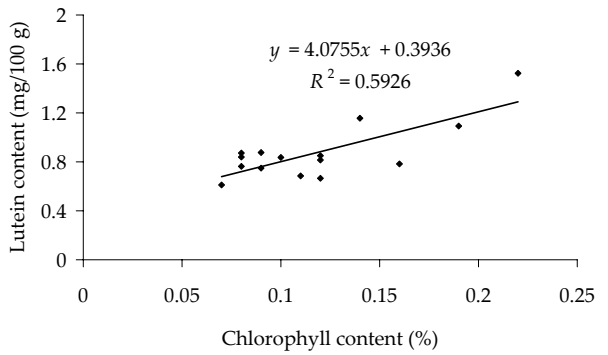

Figure 3. Correlation between lutein and chlorophyll contents in garden peas

lowing years (2006–2008). 54% of all repeatedly tested samples showed lutein content with coefficient of variation lower than 10%. Coefficient of variation under 20% was found in 96% of analysed samples. Season influence seems less pronounced in field peas, one of explanations might be the better uniformity in ripeness during the harvest.

Relationship of lutein and chlorophyll contents in garden peas

In 15 samples of garden peas both chlorophyll and lutein contents were determined. A strong correlation ($r = 0.77$, $P < 0.01$) between lutein and chlorophyll contents was found (Figure 3).

CONCLUSIONS

Tested varieties and breeding lines of field and garden peas differ in lutein content. Lutein content in field peas ranged between 0.348 and 1.394 mg/100 g. Field peas with green cotyledon (*ii*) is better source of lutein than field peas with yellow (*I.*) and orange (*OrcOrc*) cotyledons. The hypothesis, that orange cotyledons might contain more lutein was not confirmed. In garden peas lutein content between 0.471 and 1.524 mg/100 g was found. Lutein content in garden peas seems to be higher in comparison with field peas. Whereas 53% of results in garden peas were over 0.8 mg/100 g, for field peas only 19% of samples exceeded this level. Year-to-year differences in 54% of all repeatedly tested samples showed coefficient of variation lower than 10%. Coefficient of variation under 20% was found in 96% of repeatedly analysed samples. A strong correlation ($r = 0.77$, $P < 0.01$) between lutein and chlorophyll contents was proved.

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