Study of Resistant Starch (RS) Content in Peas during Maturation

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Abstract: Total starch (TS), amylose and resistant starch (RS) were determined in the sets of smooth pea and wrinkled pea varieties in the years 2006–2008. Starch content of smooth peas varied in the range 53.61–57.23%. Average amylose content was 27.8%. Resistant starch content varied from 2.07% to 6.31%. Content of starch at wrinkled pea varied from 26.57% to 32.55%. Average amylose content was 76.82% of total starch. Content of total starch increases continually during seed development. The dependence of total starch on determined dry mass in harvested sample can be defined by equation $y = 1.2427 \times -6.5611$, by determination coefficient $R^2 = 0.8936$ and highly significant correlation coefficient $r = 0.945$. Total starch content in dry seed reached final average value 29.56%. In garden pea, the level of maturity (by tenderometric measurement) and dry matter were determined. Resistant starch content of 11 garden pea cultivars was studied in three different terms of technological harvest.

Keywords: peas; total and resistant starch; amylose; dry matter; optimal term of harvest

INTRODUCTION

Peas, especially garden peas (with wrinkled seeds – *rr*), is very good source of resistant starch (Mikulíková *et al*. 2005). It is well established that dietary fibre plays an important role in maintaining healthy bowel function and hence may play an important role in preventing colon cancer. More recent research suggests that there are different types of dietary fibre with a greater diversity of health benefits. Starch is made up of amylose and amylopectin. Amylose has a linear molecular structure whereas amylopectin has a branched structure. Most plants contain about 20–25% amylose. But some, like garden pea starch have 70–80% amylose. Content of resistant starch is related to content of amylose, which is lower in field pea than in wrinkled pea. Such amylose content is classified as high – very high, according to classificatory scale for wrinkled peas (Hýbl *et al*. 2001). Resistant starch includes physically inaccessible starch /type RS_1/, native granular starch/RS_2/, retrograded starch /RS_3/ and chemically modified starch/RS_4/(Mikulíková *et al*. 2008). The aim of this work was to find out content of RS during maturation of garden peas, and relations to total starch and dry matter.

MATERIAL AND METHODS

Samples. Accessions of smooth (18) and wrinkle (16) seeded peas were analysed for total starch, resistant starch and amylose content in 2006–2008. RS content was compared on select set of wrinkled peas (11) in three different terms of harvest 2008. Registered cultivars and breeding lines of field peas (smooth seeded) and garden peas (wrinkled seeded) were represented.

Treatment. Dry seeds of field and garden pea (dry matter 84–86%) were cleaned and ground into the flour for chemical analyses. 11 samples of garden peas were harvested in three different terms (June 23–July 10, 2008). Green peas were husked and sorted on sieves. Part of the fresh seeds was dehydrated (t = 100°C) for determination dry matter (%), and the rest was used for determination T-value, (Tenderometer TU/(TM)-2). The

tenderometr test was used to estimate the degree of maturation (Ekval *et al.* 2006).

Chemical determinations. Changes in resistant starch (RS) content were monitored at wrinkled pea in different terms of technological harvest. The content of total starch and amylose in pea seeds was determined by Near Infra Red Spectroscopy (NIRS) method (Perten Instruments, Inframatic 8100). Results were re-counted to g/100 g dry weight. NIRS apparatus was calibrated by set of calibration standards with known starch and amylose content, that were determined using commercial kits Megazyme (Megazyme total starch assay procedure and Megazyme amylose assay procedure). Determination of resistant starch (starch, that is not hydrolysed by enzymes) was also carried out using commercial kit Megazyme (Resistant starch assay procedure). Starch at all these procedures is always hydrolysed by specific enzymes and glucose is then measured with glucose oxidase/peroxidase reagent.

The results were submitted to analysis of Simple Linear Correlation (Pearson, Spearson) and of variance ANOVA and LSD test, using the STATISTICA (StatSoft), considering $P < 0.01$ and $P < 0.05$ as minimum acceptable probability for difference between the means.

RESULTS AND DISCUSSION

In the period 2006–2008, contents of resistant starch at field and wrinkled peas were determined. Significant differences of resistant starch content in seed samples were found among different cultivars. Resistant starch content of field peas varied from 2.07% (cultivar Slovan) to 6.31% (cultivar Madonna, measured values are plotted in Figure 1. Starch content varied in the range 53.61–57.23%. Average amylose content was 27.8% (Figure 3). Such content is classified as high–very high to classification scale for smooth seeded pea ((Hýbl *et al.* 2001).

Resistant starch was analysed at 16 cultivars of wrinkled pea, that were harvested in the stage of biological ripeness (dry seeds). Content of RS at wrinkled pea varied from 12.17% (line CL 169 × CL) to 17.7% (line Mini 93), values are presented Figure 2. Content of starch at wrinkled pea varied from 26.57% to 32.55%. Such content is classified as low–medium according to classification scale for garden pea. Average amylose content was 76.82% of total starch. Such content is classified as high–very high according to classification scale for garden pea (Hýbl *et al.* 2001).

In the year 2008, contents of resistant starch (RS) in seed samples of 15 cultivars were significantly lower, then in previous years. Among cultivars, significant differences of resistant starch content in seeds were found. RS content in seeds varied between 4.45% (cultivar Radovan) and 5.4% (cultivar Dalila). Values are plotted in Figure 4.

In the period 2006–2007, correlations between amylose and resistant starch contents were studied at field and wrinkled pea. Using correlation analysis, highly significant correlation was found at both types of pea with highly conclusive correlation coefficient $r = 0.74^{**}$, that corresponds to many published data (Vasanthan *et al.* 1998; Sajilata *et al.* 2006) (Figure 5). Likewise, corre-

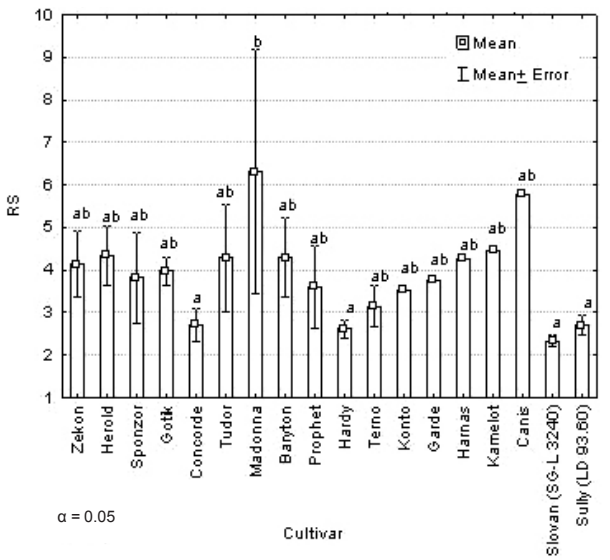

Figure 1. Resistant starch content % of field pea

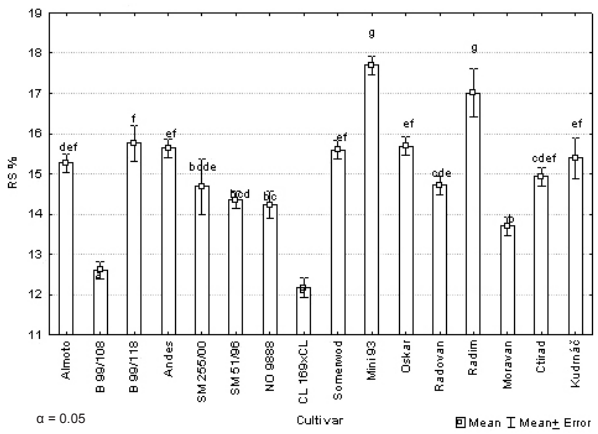

Figure 2. Resistant starch content % of garden pea

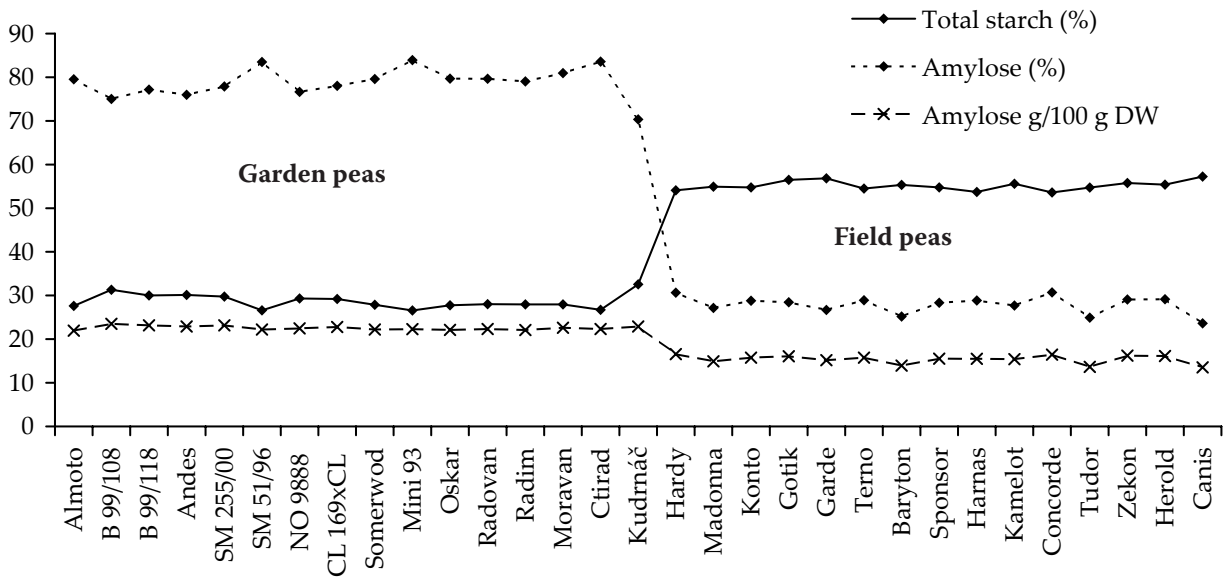

Figure 3. Total starch (%), amylose content (%) and amylose (g/100 g DW) on garden and field peas

lation betweeen total starch and resistant starch contents was found, with correlation coefficient $r = -0.94^{**}$ (Figure 6).

Total starch content (TS) constantly increases during seed development. Dependence of TS content on dry mattter of sample (Figure 7) can be formally expressed by the relation $y = 1.2427 \times -6.5611$, with determinative coefficient $R^2 = 0.8936$ and highly significant correlation coefficient $r = 0.945^{**}$. At dry seeds of wrinkled pea, content of total starch achieved final average value 29.76%.

Dependence of physical tenderometric values (TD values) on dry weight of samples (SV, %) is plotted on Figure 8. Cultivars of wrinkled peas were harvested just after optimal technological ripeness suitable for canning industry. Samples demonstrated mostly higher value then 100–115 (TD) and/or 21–22% (SV). Majority of harvested samples demonstrates gradual over-maturation of pea, that is characterised by accumulation of starch, proteins and fat. Seed maturity duration lowers the quality of pea forcanning purposes.

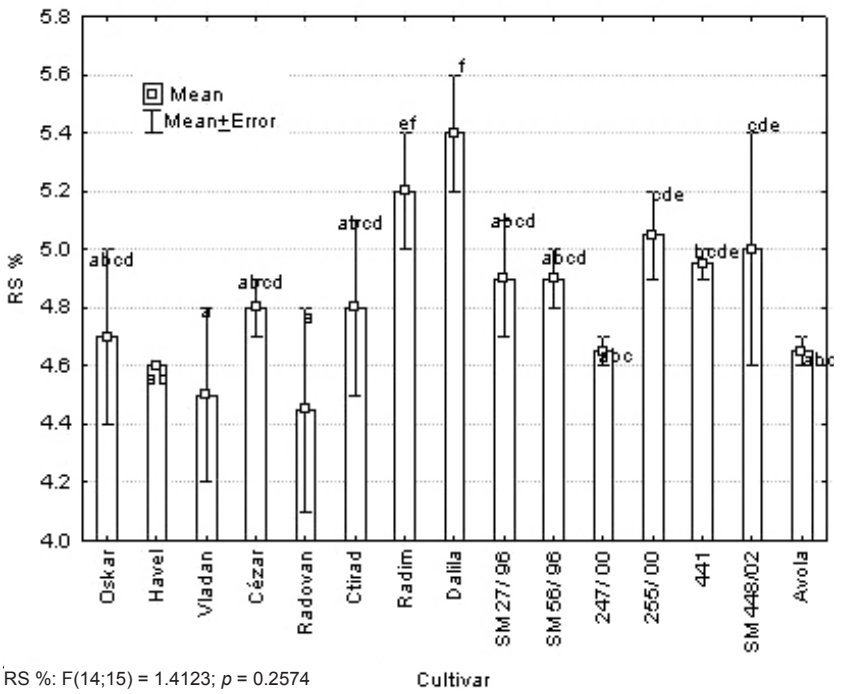

Figure 4. Resistant starch content of garden pea (dry seeds)

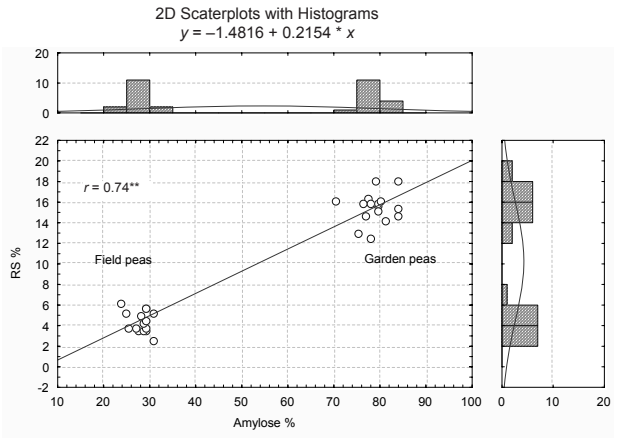

Figure 5. Amylose and RS content on peas

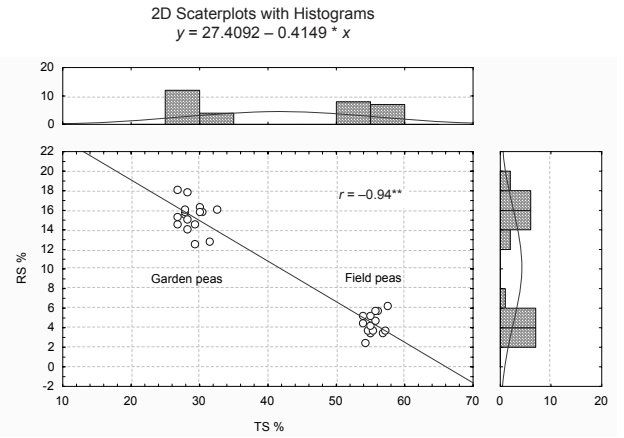

Figure 6. Total starch and RS content on peas

Hihgly significant correlation coefficient $r = 0.98^{**}$ confirms, that physical determination of rigidity of seeds is very good method, that is suitable for the practical evaluation of pea maturity in field and determination of optimal term of harvest.

Resistant starch content of 11 garden pea cultivars was studied in three different term of harvest. After calculation of minimal significant difference (Dt = 0.53) and calculation of differences of average values of individual cultivars, we can conclude, that:

1. Pea variety Oskar (RS = 7.91%) contained significantly higher level of resistant starch, than cultivars Havel, Dragon, Cesar, line SM441/02 (Tessa × Horymír × B99/118), Cetris, Radovan, Radim and Ctirad.
2. Pea variety Vladan (RS = 7.72%) contained significantly higher level of resistant starch, than cultivars Havel, Dragon, Cesar, line SM441/02 (Tessa × Horymír × B99/118), Cetris, Radovan and Radim.
3. Pea variety Dalila (RS = 7.44%) contained significantly higher level of RS than cultivars Havel and Dragon.
4. Pea variety Ctirad (RS = 7.23%) contained significantly higher level or resistant starch than variety Havel. Content of resistant starch in over-ripening samples of garden pea increases to maximal value (8.4% in variety Oskar), stagnate and decreases to an average content 4.79.

Correlations between resistant starch (RS %) and total starch (TS %) contents during the maturation (3 terms of harvest): The 1st term of harvest is characterised by linear dependency of RS on TS, that can be expressed by equation $y = 0.2041 \times +2.5745$ where $R^2 = 0.8158$, with statistically significant correlation coefficient $r = 0.903^{**}$. At this phase of over-maturation, content of total starch increases from 15 to 25%. At 2nd and 3rd term of harvest, resistant starch content stagnates and gradually decreases. In the year 2008, measured values of RS varied from 5.8% (1st term of harvest) up to

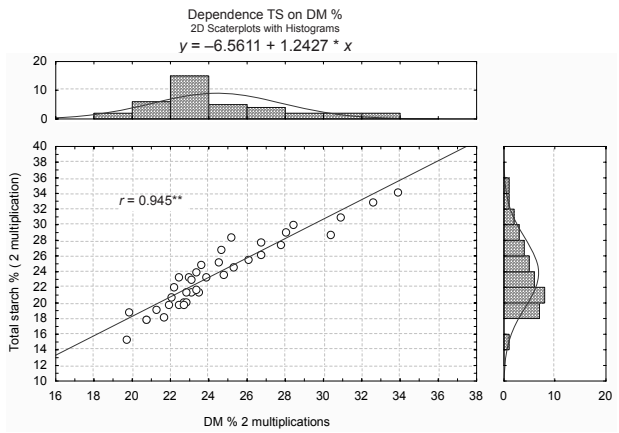

Figure 7. Total starch and dry matter increase during peas maturity

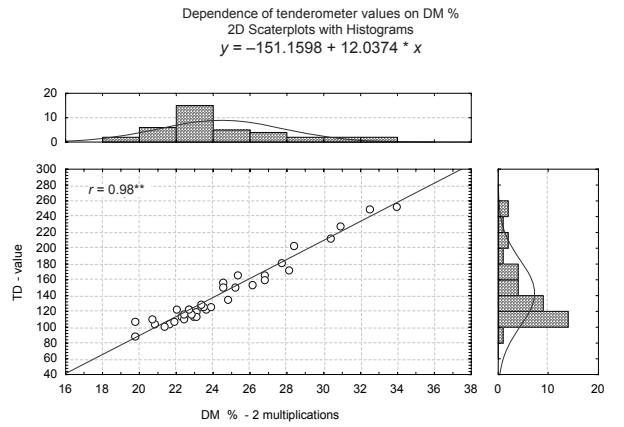

Figure 8. Tenderometer values and TS content during maturation

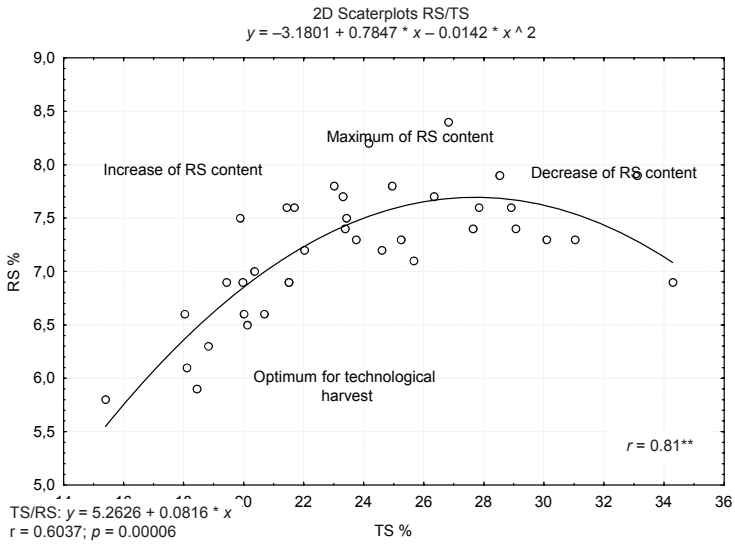

Figure 9. Total starch and dry matter increase during maturity

maximal value 8.2–8.4% (cultivar Oscar, 2nd and 3rd term of harvest). After the 3rd term of harvest, content of RS decreased up to 4.84%. Progress of RS changes can be evaluated by polynomic equation $y = -0.0142\,x^2 + 0.7847 \times -3.1801$ with correlation coefficient of determination $r = 0.81^{**}$.

In optimal technological ripeness, the quality of pea can be characterised both by tenderometric values (TD = 100–115), dry matter of samples (DM = 20–21%) and total starch (TS) content, that varied between 21–26% of dry matter. Average values of RS alter according to the term of harvest (with increasing solid of samples in %). In dry seed, content of RS reached the final average value 4.84%.

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