Thesis of PhD Dissertation

PHENOLOGICAL, MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATIONS OF FLOWER BUD- AND FRUIT DEVELOPMENT OF SOME IMPORTANT APRICOT CULTIVARS

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INTRODUCTION

The chemical parameters of apricot (*Prunus armeniaca* L.), especially its distinctive flavour and aroma, make it one of the most important stone fruits. On a global scale the annual yield is 3.8 million tonnes, EU member states grow 800 - 900 thousand tonnes apricots, almost 30 - 40 000 t of which has been grown in Hungary in recent years. However apricot is not a native fruit species in Hungary as well as our country is located on the northern border of apricot production therefore its growing is more difficult because of the cold winters and fluctuant spring temperature. The low temperature can cause problems during reproductive development so the crop will be less.

Increase of frost tolerance is come into prominence in apricot breeding. The flower buds are the most frost sensitive overwintering organs. Changing of frost tolerance correlates with developmental time and their position on plant, therefore it is also important to analyse phenological processes and morphological characterizations during dormancy. The plants responses to abiotic stresses depend on cultivars, species and ecological conditions. Therefore it is important to examine the phenological, enzymatic, accumulation and morphological changes in the apricot buds during dormant period. Most fruits grew on short shoots in extensive plantations, on the other hand the fruit production grew on long shoots in the intensive plantations. Our aim was to examine the developmental time and frost tolerance of flower buds on different shoots.

Improvement of fruit quality is the other big part of apricot breeding. The utilization of apricots are various. The fruit quality and use depend on the cultivar and ripening stage. Therefore the process of ripening is very important research topic. Apricot has valuable compounds, the beta-carotene and polyphenol contents have great importance in aspect of human nutrition. The knowledge of processes in fruit during ripening is essential for determination of optimal picking time.

AIM OF THE STUDY

Our main objectives were the followings:

1. to determine the process of flower bud development, description of phenological processes from beginning of paradormancy to end of endodormancy;
2. to examine the changes of antioxidant enzymes in flower buds during dormancy in aspect of frost resistance;
3. to determine the changes of carbohydrate compounds in flower buds of different frost tolerant cultivars during dormancy;
4. to monitor the changes of physical and chemical parameters of fruit during development and ripening;
5. to determine the essential nutritional contents of apricot during ripening, which are important in aspect of human nutrition.

MATERIALS AND METHODS

Introduction of the test orchards

The samples were taken from the experimental orchard of the Horticultural Faculty of Corvinus University, Budapest, in Soroksár, Hungary. Grafts on myrobalan rootstocks were planted at a spacing of 5 × 3 m in 2004 and were trained to give a compact, vase-shaped canopy form, there were six trees by varieties.

Most of the investigation was made in laboratories of the Department of Pomology, We also used other laboratories for further investigations. Which will be mentioned later.

Cultivars

In our experiment we choose Hungarian and foreign cultivars, which are used in Hungary (table 1.).

Table 1: Cultivars involved

Cultivar	Paradormancy	Microsporo-genesis	Pollen morphology	Enzyme activity, carbohydrate content in flower buds	Fruit development and ripening
Bergeron					
Ceglédi bíborkajszi					
Ceglédi óriás					
Ceglédi Piroska					
Gönci magyar kajszi					
Harcot					
Ligeti óriás					
Mandulakajszi					
Orange Red					
Pannónia					
Rózsakajszi C. 1406					

Flower bud development phenology

The paradormancy was observed by preparing longitudinal section. We have noticed flower bud development between 1st August and 30th September in three years (2007, 2008, 2009). We picked the samples from 1,5-2 m height of canopy two times a week. We examined two different fruiting branches, the spur (20 cm length) and long shoot (50 - 80 cm length). The sections were made from flower buds of central part of long and short shoots with freezing microtome and were examined under transparent light microscope.

The microspogenesis was observed by squash (colour: carminic acid), together with semithin (1 μm, colour: toluidin blue) and thin (70 nm, colour: uranyl acetate and lead citrate solution) sections. The observations were made by light microscope (Olympus BH2 DIC), and Hitachi 7100 TEM microscope (accelerating voltage 5 kV). We investigated the process of microsporogenesis in two different fruiting branches (short and long shoots).

The pollen grains were coated with gold by vacuum, then those were examined by SEM (Hitachi 2360N, accelerating voltage 25 kV) in laboratory of Department of Plant Anatomy, Eötvös Lóránd University.

Examination of stress tolerance of flower buds

Enzyme activities were measured in the Department of Applied Chemistry of the Corvinus University of Budapest in 2007/2008 and in the Department of Pomology in the next year (2008/2009). We examined flower buds from long shoots.

Peroxidase activity was determined by spectrophotometer (λ = 460 nm) with H_2O_2 substrate and ortho-dianizidine choromegenic reagent (ε = 11.3) (Shannon et al., 1966). **Polyphenol oxidase activity** was measured also by spectrophotometer (λ = 420 nm) using catechol (Bassuk et al., 1981).

The **sucrose, glucose, fructose and sorbitol** contents of flower buds were determined by HPLC.

Fruit development and ripening

We analyzed physical and chemical parameters during fruit development. The fruits were classified in ten maturity categories on the basis of ground colour and flesh firmness (table 2.).

Table 2: Picking time of fruit development (2009)

Cultivar	Picking time									
	1	2	3	4	5	6	7	8	9	10
Harcot	15 Apr	28 Apr	13 May	27 May	14 June	20 June	28 June	03 July	05 July	07 July
Gönci magyar kajszi	15 Apr	28 Apr	13 May	27 May	16 June	22 June	30 June	08 July	10 July	13 July
Bergeron	15 Apr	28 Apr	13 May	27 May	16 June	22 June	02 July	10 July	16 July	18 July

Changes in the carbohydrate (sucrose, glucose, fructose), sugar alcohol (sorbitol), soluble solid content, acid (malic, citric, succinic, titratable acidity) content and physical (fruit height,

ventral width, lateral width, weight) parameters of the fruits samples were measured. We made regression analysis.

The **acid, sugar and sorbitol contents** were determined by HPLC.

The **β-carotene content** was determined using the method of De Ritter and Purcell (1981), as modified by the Canning Research and Development Co. Ltd. (KPKI, 1990). After thawing, the β-carotene was extracted from 1 g fruit samples using methanol and acetone, followed by separation with diethyl ether. The absorbance of the ethereal solution was measured with a Hitachi U-2800A spectrophotometer at 450 nm.

The **total polyphenol content** was measured by spectrophotometer (λ = 765 nm) using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). Each measurement was repeated three times.

RESULTS AND DISCUSSION

Flower bud development

The flower organs were appeared in acropetal order during **paradormancy** of flower bud development. Paradormancy of flower buds could be divided into five stages: flattering of shoot apical meristem (numbers of cells are increased below central zone the shoot apical meristem), sepal primordia formation, petal primordia formation, stamen development, and carpel initial formation. There were no important morphological differences in tissue structure of flower organs among the examined varieties during the paradormant period. After this period the development slowed down and almost stopped.

There were important differences which were based on varieties, types of fruiting branches, and year effect in speed of flower bud development during the paradormant period. The formation started early on the spurs; there were 5-10 days different, which depends on the varieties. The temperature affected the speed of flower bud development. We determined, that the development of different varieties was similar during paradormancy (figure 1.).

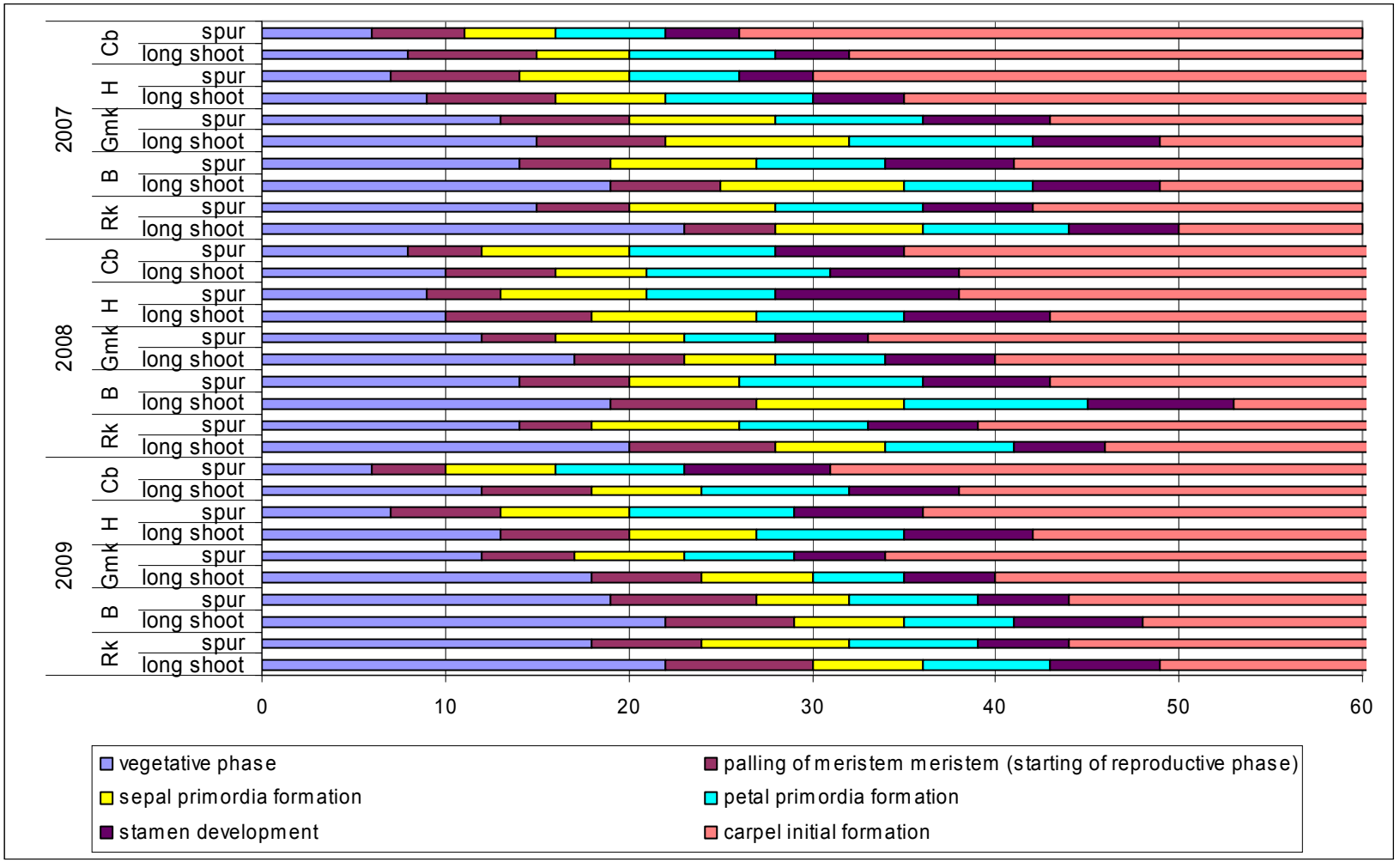


Figure 1. Phenological process of flower bud development during paradormancy (Soroksár, 2007-2009)

Cb: 'Ceglédi bíborkajszi'; H: 'Harcot'; Gmk: 'Gönci magyar kajszi', B: 'Bergeron'; Rk: 'Rózsakajszi C. 1406'

End of endodormancy was marked by inside tissue differentiation of anthers (first station of this formation is string stage). After this period the flowers entered into ecodormancy. Floral development can be traced by examining of **microsporogenesis** which means the pollen development can be checked on the short and long branches. There are six different stages in the microsopogenesis: archesporium, string stage, pollen mother cell stage, tetrad stage, microspore stage, pollen. Speed of microsporogenesis was different which varied by location of the flower buds. The pollen development started couple of days earlier on the spurs and it also finished earlier compared to the long branches. There were different in varieties and year effects during our study. We can consider based on our results which were completed during the last three years that the stages could be changed gradually and this transitional period took long time inside of the flower bud. It took some weeks for some genotypes to change from archesporium to string stage. Finally, the development speeded up and the transitional periods were ever shorter (figure 2.).

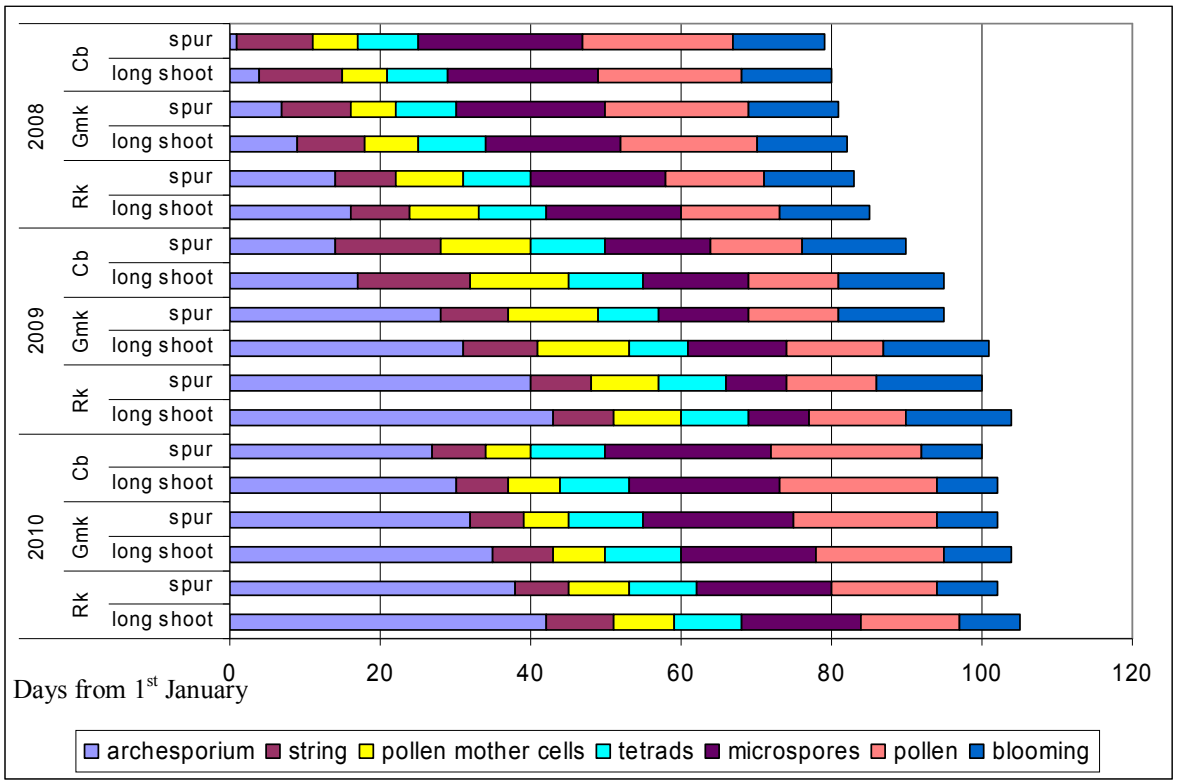
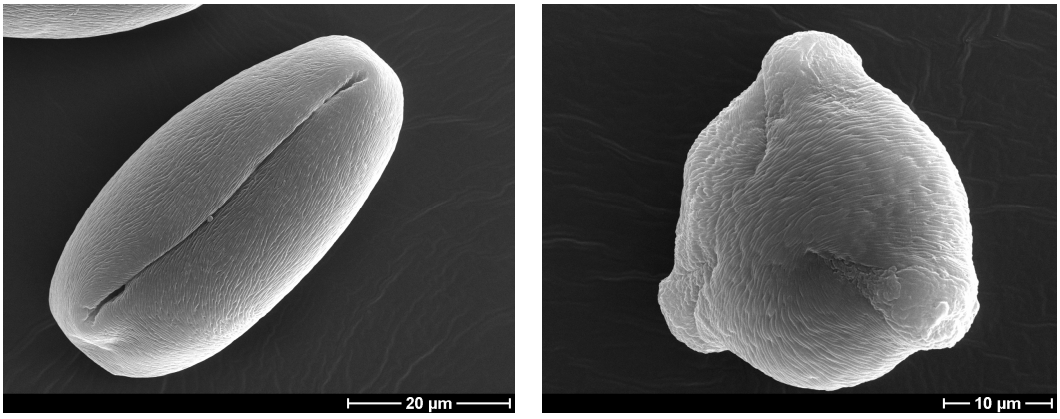


Figure 2. Phenological stages of microsporogenesis of apricot cultivars on different fruiting branches (Soroksár, 2007-2009)

Cb: 'Ceglédi bíborkajszi'; Gmk: 'Gönci magyar kajszi', Rk: 'Rózsakajszi C. 1406'

During change from achesporic cells to pollen cells light microscopy and detailed electron observations were made by ‘Gönci magyar kajszi’. The development can be traced well on pictures which were taken during the histological and cytological observations. Cytoplasm which was before the proliferation was firm, and contained a lot of cell organelles. The tapetal cells which were less vacuolated and were around the mother pollen cells in closed ordination broke up and destroyed. Four haploid microspores which are close to each other and surrounded by a callose wall started up from the pollen mother cells. This is the tetrad status. The microspore can be stayed inside of the callose wall until the late tetrad stadium. After imbibitions of the callose wall the microspore split up and development of pollen starts. The developing pollen is unicellular at the beginning. Lately, vegetative and generative cells are created by mitotic proliferation and special cell wall expansion of endothecium’s cells appears.

During our research **pollen morphological examination** of ten apricot varieties was finished. Two different pollen types were determined: the prolate elliptic shape and sub-oblate triangular. Aperture shape of both types was tricolpate. For which is characteristic the striate pollen surface markings as well as rugulate types was observed by two samples (figure 3.).



’Pannónia’ ’Ceglédi Piroska’

Figure 3. Two types of exine and shape of pollen

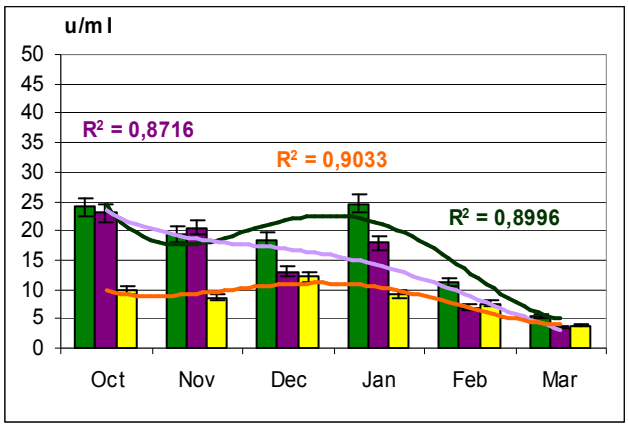
Stress tolerance of flower buds

Peroxidase and polyphenol oxidase activities were measured in 2007/2008 and 2008/2009 dormant period. Three groups of varieties with different frost tolerant (good frost tolerant, medium frost tolerant, frost sensitive) were involved in the trial. The activities of enzymes were significantly different in analyzed varieties. Changes of enzyme activity depend on the temperature and hardiness.

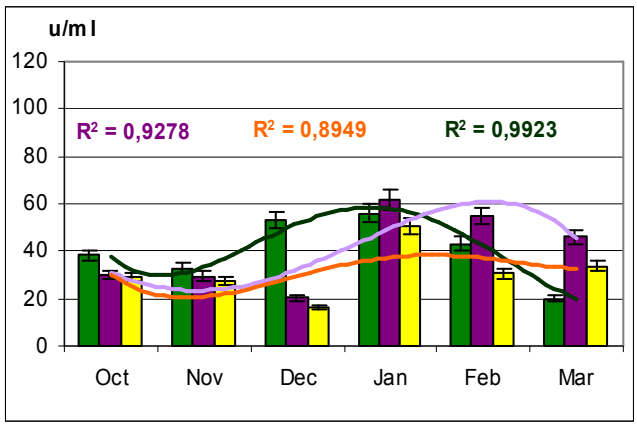
We measured the highest **peroxidase** activity in flower buds of frost sensitive 'Ceglédi bíborkajszi' in both years. The enzyme activity was less in flower buds of good frost tolerant 'Rózsakajszi C. 1406'. The enzyme activity of 'Gönci magyar kajszi' was between other cultivars. When the temperature decreased the enzyme activity also changed because of the increscent hardiness of flower buds. Based on our results we can consider that activity of peroxidase decreased in the flower buds of frost sensitive variety when the temperature fell in December and January. By this time the good frost tolerant and medium frost tolerant varieties acquired hardiness. Therefore the activity of peroxidase increased in their flower buds. The hardiness of flower buds started to decrease parallel with increase of temperature.

The activity of **polyphenol oxidase** showed opposite trend in case of good and medium frost tolerant cultivars. First the activity of polyphenol oxidase decreased in line with temperature. The good frost tolerant cultivar responded to the growth of enzyme activity to the fall in temperature between December-January, while we did not observe change in flower buds of the frost sensitive cultivar.

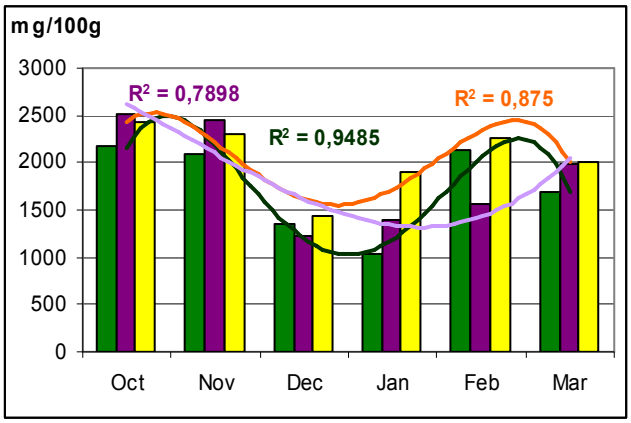
Quantity of **carbohydrate compounds** which were measured in the flower buds changed dynamically during chilling, its concentration increased. The carbohydrate compounds which were measured in the generative buds of good frost tolerant cultivar and activity of perioxidase enzyme showed an opposite trend. Change of sucrose was the most dynamic of other carbohydrates. The changes of glucose, fructose and sorbitol were similar in winter. We observed the accumulation of glucose, fructose and sorbitol in flower buds during chilling. In this time the quantity of them was the highest in generative buds (figure 4.).



Peroxidase enzyme



Polyphenol oxidase enzyme



Sucrose

Figure 4. Changes of peroxidase-, polyphenol oxidase activities and sucrose in flower buds of ’Ceglédi bíborkajszi’ (Cb), ’Gönci magyar kajszi’ (Gmk) and ’Rózsakajszi C. 1406.’ (Rk) (2008/2009)

Fruit development and ripening

We analyzed the **changes of the size parameters and gain in weight** from ovary growing to mature fruit. Four parameters can represent the fruit size: lateral width, ventral width, height, and weight of the fruit. Changing of parameters was described with two connected logarithmic graphs. The equation of first section is $y = \frac{p_1}{1+\exp(-p_2(x-p_3))}$ and the second is $y = p_2 + \frac{p_4 - p_2}{1+\exp(-p_5(x-p_6))}$. The p_1 parameter is the saturation value of first section, p_2 is the slope, and p_3 is the inflection point. The p_4 parameter is the saturation value of the second section, p_5 is the slope, and p_6 is the inflection point. The second section starts by the saturation value of first section.

We analyzed three different ripening times of cultivars (’Harcot’, ’Gönci magyar kajszi’, ’Bergeron’), we could to describe the different. The first logistic curve shows the first part of fruit development, the second curve shows the second part, and the stone solidification is between two curves.

During our research determination of organic acid compounds (malic-, citric- and succinic acids) and titratable acidity were measured by three varieties during the fruit development. Among the organic acids detected, **malic acid** was present in the greatest quantities in the early section of ripening. First the malic acid content increased, and then it decreased rapidly in fruits of ’Harcot’ and ’Gönci magyar kajszi’ since 4th picking time. Changing in malic acid reciprocal curve was fitted ($y = \frac{b}{\text{érésidő}}$) since 3rd picking time because of the dissolving of malic acid was continuous. The explained variances are 93.3%, 96% and 97.7% for varieties ‘Harcot’, ‘Gönci magyar kajszi’ and ‘Bergeron’, respectively. Significant difference was between effects (time, cultivar, interaction) by repeated measure anova. The malic acid content depended on picking time; however the malic acid content did not change considerably since 5th picking time.

Change of **succinic acid** was fitted reciprocal curve. The explained variances are 87.8%, 95.1% and 94.1% for varieties ‘Harcot’, ‘Gönci magyar kajszi’ and ‘Bergeron’, respectively. The quantity of succininc acid decreased suddenly after first picking time at all three varieties. After 2nd picking time the amount increased slightly, the amount of succinic acid decreased again from 4th picking time. The most succinic acid was in fruits of ‘Bergeron’ at first. We detected less (35% and 28%) succinic acid amount at varieties ’Harcot’ and ‘Gönci magyar kajszi’. The quantity was similar in fruits of all varieties at last picking time. Significant

difference was between effects (time, cultivar, interaction) by repeated measure anova; all effects affected the quantity of succinic acid in fruit. 'Harcot' varied significantly in amount of succinic acid in fruit.

Citric acid decreased in the first three measuring time, after it started to increase. This increase was small in fruits of 'Gönci magyar kajszi' and 'Bergeron', while increase was bigger in samples of 'Harcot'. The second part of ripening citric acid remained stabile. Significant difference was between effects by repeated measure anova. The varieties differed from each other by Post Hoc analysis.

The organic acids increased between 3rd and 4th ripening stages, then their amount started to decrease. This period is equaled the stone solidification.

Changing of **titratable acidity** was characterized by decreased logistic curve ($y = p_0 + \frac{p_1 - p_0}{1 + \exp(-p_2(x - p_3))}$) at varieties 'Gönci magyar kajszi' and 'Bergeron', and by decreased saturation curve ($y = p_1 + p_2 * (1 - \exp(-p_3 * (10 - X)))$) at 'Harcot' variety. The titratable acidity was decreased in fruits of all varieties during fruit development; 'Harcot' and 'Bergeron' were similar. The explained variances are 90.1%, 99% and 97.9% for varieties 'Harcot', 'Gönci magyar kajszi' and 'Bergeron', respectively.

Significant difference was between cultivars by sugar content. **Sucrose** was the main sugar component of apricot. Sucrose content of all cultivars increased during fruit development, however we could not detect traceable amount in the first two picking times. Logistic curve was fitted on the aggregation of sucrose ($y = \frac{p_1}{1 + \exp(-p_2(x - p_3))}$). The explained variances are 94.7%, 99% and 94.1% for varieties 'Harcot', 'Gönci magyar kajszi' and 'Bergeron', respectively. End of stone solidification change of sucrose stopped at varieties 'Harcot' and 'Bergeron', whilst we described balanced increase in samples of 'Gönci magyar kajszi'. We detected the highest sucrose content in samples of 'Harcot', the sucrose content was lowest by 38% and 19% in samples of 'Gönci magyar kajszi' and 'Bergeron'. The time, cultivar and both interaction were significant. The varieties 'Harcot' and 'Bergeron' were similar, 'Gönci magyar kajszi' was in other group by Post Hoc test.

Large quantities of **glucose** were also detected. We considered that the changing of glucose was very special. Changing of glucose content could be divided in two stages: curve of the second degree ($y = b_1 t + b_2 t^2$; t is time) was fitted during the first stage of the ripening period and it was constant during the second stage. During first period the glucose increased till 3rd picking, after it decreased. The explained variances are 91.3%, 97.5% and 96.9% for varieties 'Harcot', 'Gönci magyar kajszi' and 'Bergeron', respectively. The effect of cultivar is

not significant in all picking times, at the same time the other effects (time, interaction) are significant. If we disassociate the first five stages and second five stages, the effect of cultivar is significant in both periods. The varieties were in different group by Post Hoc test.

The **fructose** content was very similar in samples of cultivars. The fructose content in fruits of 'Harcot' increased at 6th ripening stage, after a mildly decreasing remained stabile. In this time fructose content increased in samples of 'Bergeron' and 'Gönci magyar kajszi' too. The all three effects acted on quantity of fructose by repeated measure anova. The 'Gönci magyar kajszi' and 'Bergeron' were similar, 'Harcot' was different by Post hoc test.

Sorbitol was one polyalcohol, which we measured by HPLC. Sorbitol content increased significantly at 6th ripening stage in all varieties, after a fast decreasing remained stabile. Increasing was 17% in 'Harcot', 05% in 'Gönci magyar kajszi' and 61% in 'Bergeron'. Decreasing was appreciable also, it was 78% in 'Harcot', 82% in 'Gönci magyar kajszi' and 47% in 'Bergeron'. In this period the temperature decreased and it may be hypothesized this gave rise to change of metabolism. Change of oxidation and reduction was the response to stress. The effect of time, cultivar and interaction was significant to change of sorbitol. The 'Harcot' and 'Bergeron' were similar, 'Gönci magyar kajszi' was different by Post Hoc.

The **soluble solid content** increased during ripening. Increase was described by logistic curve ($y = p_0 + \frac{p_1 - p_0}{1 + \exp(-p_2(x - p_3))}$). The explained variances are 96.5%, 99.7% and 98.4% for varieties 'Harcot', 'Gönci magyar kajszi' and 'Bergeron', respectively. 'Bergeron' and 'Gönci magyar kajszi' were significant different, 'Harcot' was similar the other two varieties by Post Hoc test.

Changes in the **β-carotene content** of the fruit during ripening are illustrated for the different varieties in Figure 5. Significant differences in β-carotene content were observed between the various stages of maturity and cultivars. ‘Bergeron’ had much higher carotene contents than ’Harcot’ and ’Gönci magyar kajszi’. The level of β-carotene content in ‘Bergeron’ was already very high at 60% maturity; this notable difference remained during ripening. ‘Bergeron’ had almost double β-carotene content in 100% maturity than ’Harcot’, and it had 29% higher β-carotene content than ’Gönci magyar kajszi’. We measured similar beta-carotene content in fruits of ’Harcot’ and ’Gönci magyar kajszi’ in 60-80% ripening stages, and then the β-carotene content increased suddenly in Hungarian variety.

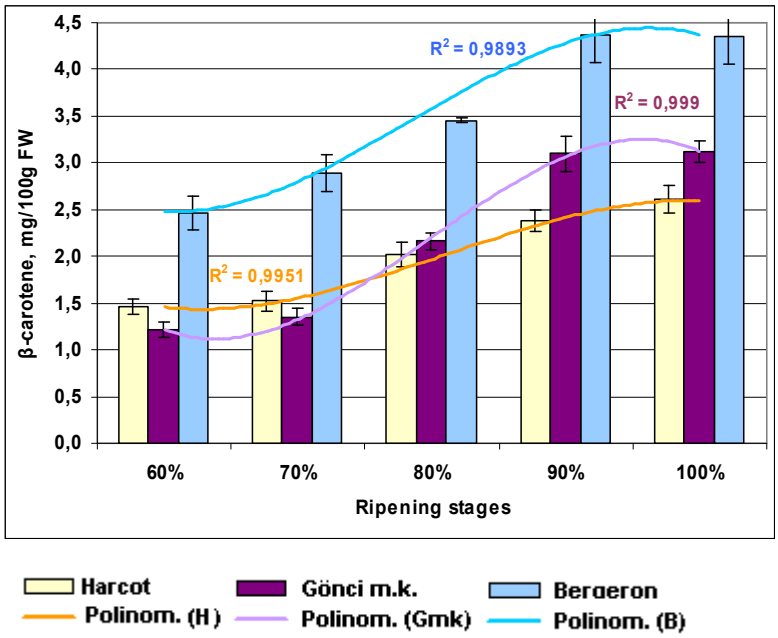


Figure 5. Changes in the β-carotene content during ripening in apricot cultivars, 2010.

The β-carotene content increased steadily in all examined cultivars, and the increase culminated in end of ripening process. Significant difference was not observed in 90 and 100% mature fruits, so the most varieties had characteristic quantity of β-carotene in commercial maturity. However significant difference was between 80% and 90% mature fruits. Fruits in 80% ripening stage are good for industrial use. The 80% ripe fruits were lower by 16-20% than 90% ripe fruits.

Changes in the **total polyphenol content** of the fruit during ripening are illustrated for the different varieties in Figure 6. We observed significant difference among varieties. 'Gönci magyar kajszi' had higher polyphenol content in 60-70% ripening stages, after the polyphenol content of 'Harcot' in 80% ripening stage was higher than other cultivars. The polyphenol content of Gönci magyar kajszi' was grown slightly, changes of other cultivars were more intensive. The polyphenol content of 'Bergeron' was the least in 60% mature fruits, and then the polyphenol content grew and it was similar to the Hungarian variety in 90% ripening stage. We observed significant difference between 80 and 90% ripening stages only in case of 'Bergeron', the others had same polyphenol contents in 80 and 90% ripening stages.

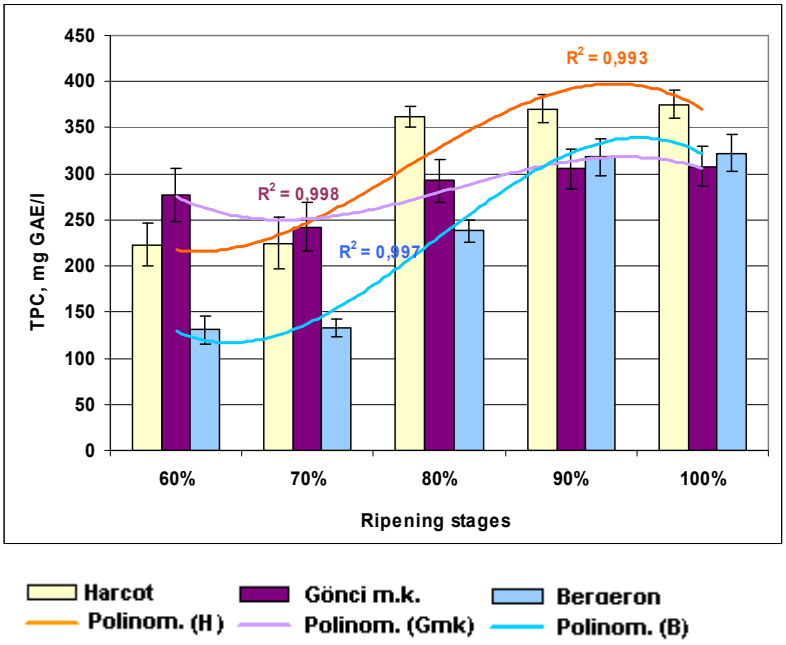


Figure 6. Changes of polyphenol content during ripening in flesh of apricot cultivars, 2010

On the whole the total polyphenol content grew during ripening, this increase was low at 'Gönci magyar kajszi'.

New scientific results

1. Description of phenological processes of flower bud development of five apricot cultivars in three years from beginning of paradormancy to end of endodormancy.
2. Histological analysis of flower bud development of an important apricot cultivar.
3. Grouping of ten apricot cultivars by shape of pollen, ornamentation of exine and surface of pollen, as unknown features of cultivars.
4. Determination of changes of antioxidant enzymes (peroxidase, polyphenol oxidase) together with the changes of carbohydrate compounds in flower buds of different frost tolerant cultivars during dormancy.
5. Description of changes of physical and chemical parameters (carbohydrates, organic acids) of fruit during development and ripening by mathematical models in three different apricot cultivars.
6. Determination of changes of two essential nutritional contents, the β-carotene and total polyphenol contents of apricot during ripening

Publications of the author in the topic of the dissertation

Scientific journals

Journals with IF

Németh, Sz., Szalay, L., Ficzek, G., Stéger-Máté, M., Sándor, G., Végvári, Gy. Tóth, M. (2011): Analysis of chemical parameters determining the fruit quality of apricot varieties during ripening. Acta Alimentaria. 40: 109-119. IF: 0.379 (2010)

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