# HORTICULTURAL SCIENCE

## Zahradnictví

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# The influence of oxygen concentration in the atmosphere on anaerobic metabolites produced by several apple cultivars

J. GOLIÁŠ, A. NĚMCOVÁ, L. ŠUDERLOVÁ, J. BALÍK, J. VEVERKA, M. KYSELÁKOVÁ

*Mendel University of Agriculture and Forestry, Brno, Faculty of Horticulture, Lednice na Moravě, Czech Republic*

**ABSTRACT**: Storage of Golden Delicious, Jonagold, Melrose, Idared apples (*Malus domestica* Borkh.) in ultra-low oxygen (0.9% $O_2$), anaerobiosis (0.2% $O_2$) and regular atmosphere (21% $O_2$), plus 0.3% $CO_2$ and 0.03% $CO_2$ respectively, indicated that an exposure to ULO suppressed the production of metabolic compounds such as acetaldehyde, ethanol and ethyl acetate. Fruit tolerance to specific atmospheres yielding anaerobic products was determined. The tissue ethanol content in ULO and RA atmosphere ranged from 40 to 112 mg/l and from 4 to 7 mg/l, respectively. The concentration of ethanol in anaerobiosis (< 0.2% $O_2$) reached 790–830 mg/l in 30 days. The oxygen stress also caused a high concentration of acetaldehyde (350 mg/l) after 30 days in anaerobiosis, the concentrations of this metabolite in ULO and RA being statistically significant ($P < 0.05$) but fluctuating only in a narrow range of mg/l units. The presence of acetaldehyde in the fruit pulp indicates a degree of injury by low oxygen which nonetheless figures at the concentration of 0.9% $O_2$. The gaseous metabolites released across the skin were measured in µg/kg per h being captured in the sorbent and then gas-chromatographically analysed for acetaldehyde, ethanol and ethyl acetate. As their accumulation in the liquid phase leads to their release into the environment, the variant RA could be dramatically distinguished from ULO and AN. There were only minimal differences between ULO and RA in nonvolatile compounds such as sucrose, glucose, fructose and malic acid. Tukey's test could not distinguish between the means of individual cultivars and treatments. An increase in the consumption of malic acid was identified between the ULO and the AN storage. Pulp firmness was depressed only in RA.

**Keywords**: *Malus domestica*; ethanol; acetaldehyde; ethyl acetate; low oxygen; firmness; emission of volatiles

Many apple cultivars are stored commercially for extended periods under refrigerated controlled atmosphere conditions. Typically oxygen is lowered to 1–3% and $CO_2$ allowed to accumulate to 1–3% depending on what cultivars are being stored. CA storage, in particular ULO, diminishes firmness, acidity, chlorophyll and sugar in the fruit and reduces the incidence of some physiological disorders in comparison with cold storage of apples (BOHLING, HANSEN 1985; LITTLE, PEGGIE 1987; CHEN et al. 1989).

The knowledge of the lower oxygen limit is critical for optimising the gaseous storage of fruits. The optimum storage atmosphere occurs just above the lowest achievable level of aerobic respiration without development of anaerobic metabolism. There seems to be an agreement that reducing $O_2$ to levels that are as low as possible without inducing fermentative metabolism would be desirable (GRAN, BEAUDRY 1993).

It is generally accepted that the optimal storage atmosphere for a given temperature is at an oxygen concentration above the point of the accumulation of products of anaerobic respiration resulting in off-flavours. Low oxygen limits of crops are typically reported in relation to the external atmosphere often with ethylene, total volatile evolution and fruit characteristic (LIDSTER et al. 1987; RIZZOLO et al. 1997; HANSEN et al. 1992).

Ethanol is produced in ripening apples stored in the air but the metabolic activity favouring the production of ethanol increases during low-oxygen-induced anaerobic respiration. The fruit ethanol concentration can increase several hundredfold depending on the duration of anaerobic respiration and the variety of apples (FIDLER, NORTH 1971; PATTERSON, NICHOLS 1988). CAMERON et al. (1995) proposed the detection of ethanol would provide a sensitive technique for low oxygen injury identification since there is a positive correlation between quality deterioration due to low oxygen injury and the headspace ethanol partial pressure. SMYTH et al. (1999) demonstrated a biosensor for the detection of gas phase ethanol. Hence low oxygen injury could be detected indirectly in lightly processed cut vegetables in modified atmosphere.

The purpose of this investigation was to determine the effect of storage conditions, especially ULO, on the

anaerobic volatiles production in the headspace evaluated through the skin of intact fruit and the same volatiles accumulated in the fruit pulp. In addition, the effect of the ULO-storage duration on the quality retention (sugar, acidity, firmness of fruit) was determined.

## MATERIALS AND METHODS

### Fruit supply, initial measurement, treatment and storage

Apple fruits of the cultivars Jonagold and Golden Delicious were harvested at a climacteric stage monitored by starch content. Immediately after picking, the fruits were selected for uniformity and stored at 3°C for one week (cv. Jonagold and Golden Delicious) and five weeks (cv. Melrose and Idared) before the ULO atmosphere and anaerobic conditions were applied. Fruits of cv. Golden Delicious were sealed in the same storage conditions in mid-storage.

### Storage treatment

For the ULO storage, 40 kg of apples of each cultivar were placed in a 1,050 l container. The AN storage was carried out in a 500 l container with 25 kg of the cultivar. There were three levels of oxygen (0.2% $O_2$ anaerobiosis – AN), (0.9% $O_2$ ultra-low oxygen – ULO), (21% $O_2$ regular atmosphere – RA), each maintained to the nearest ± 0.1% and combined with one level $CO_2$ (0.3% to the nearest 0.1%), yielding 3 environments in a factorial arrangement with 4 apple cultivars. To achieve the end concentration of $O_2$, flushing with $N_2$ was carried out. The storage temperature was 3°C.

Concentrations of $CO_2$ and $O_2$ were continuously monitored by gas analysis (Arelco, ARC, France) connected to a process computer. Gas concentrations were verified every hour. After 30 days in gas storage, each chamber was opened and a 40-kg lot (subsequently divided into six replicates from each chamber) was transferred into air atmospheres. Golden Delicious apples in the mid-storage period were put into differential oxygen regimes for 30 days. Then they were stored in the air at 3°C. During the following two months, they were analysed for the content of metabolic compounds.

## VOLATILE COMPOUNDS ANALYSIS

The experimental arrangement for trapping compounds in a column with sorbent (Tenax GC) in a closed circuit with the chamber represents a two-phase system. By recycling the gaseous phase in one direction, volatiles from apples can be transported by percolating gas in the trapping column.

Headspace volatiles were measured by placing one fruit in a spherical jar with about 500 ml volume with 6 replicates, passing storage atmosphere (ULO, AN) through the jar with a velocity of 30 ml/min continually. For the sufficient trapping of compounds, 60 ml of percolating gas was passed into the trapping column

with 40 mg (± 1 mg) of Tenax GC. Trapped volatiles were thermally desorbed (130°C) in a chromatographic packed column with Carbowax 2OM on Carbopack AW. Quantification was achieved using flame-ionisation detection.

The mass of analyte in percolating gas is given by

$$W_i = (A_i/A_s) c_s v_s \qquad (\mu g) \qquad (1)$$

where: $A_i, A_s$ – areas of the peak of the given compound and the standard,
$c_s$ – concentration of standard ($\mu$l/l),
$v_s$ – volume of standard ($\mu$l).

Quantification was done by the external standard method. Defined amounts of the analysed material and the standard were separately injected. Subsequently, corresponding chromatographic peaks were obtained by comparing the sizes.

The concentration of analyte in the percolating gas can be written

$$c_i = W_i/V \qquad (\mu g/l) \qquad (2)$$

where: $V$ – the volume of percolating gas via the trapping column.

Production $G_i$ of the given compound was expressed as the velocity of percolating gas ($F$ l/h) and mass of fruits m (kg) placed in the spherical jar

$$G_i = c_i F/m \qquad (\mu g/kg \text{ per h}) \qquad (3)$$

### Quality evaluation of fruit

Six apples were removed from each chamber. Fruits were individually analysed for skin and flesh firmness (8 mm plunger) with two opposite measurements for each fruit. In the fresh fruit, the following properties were measured: titratable acidity (TA), soluble solids (SS), and active acidity (pH). From homogenised frozen pulp, sugars (sucrose, glucose, fructose), and organic acids (malic, citric, succinic and acetic acids) were analysed by HPLC of the aqueous extract from the pulp. Of all acids, only malic and citric acids have been calculated.

### Anaerobic compounds in the pulp

Acetaldehyde, ethanol and ethyl acetate in the frozen pulp were analysed by GC (packed column with Porapak P). 1 $\mu$l aqueous samples were injected in a sample block fitted with Teflon, an inert material. Eight peaks from the chromatogram were evaluated, three of them were quantified with the external standard of these compounds (acetaldehyde, ethanol, ethyl acetate) and expressed in mg/l for each compound.

### Statistical analysis

Six replicates were provided for each measurement above, means and standard errors being reported in tables. The experiment was analysed as a factorial one (treatment × variety × 14 parameters) using the analysis of means and the analysis of multiple comparison. The means were compared by Tukey's test to separate treatment means and variety means.

Table 1. Anaerobic metabolic emissions (µg/kg per h) from 4 apple varieties after 30 days of storage in different oxygen regimes*

| | Treatment | Jonagold | Golden Delicious | Melrose | Idared | Tukey's HSO significance |
|---|---|---|---|---|---|---|
| Acetaldehyde (µg/kg per h) | ULO | 0.30 ± 0.05 | 0.81 ± 0.14 | 1.15 ± 0.17 | 0.53 ± 0.06 | a |
| | AN | 0.51 ± 0.15 | 1.40 ± 0.23 | 1.53 ± 0.19 | 0.98 ± 0.10 | b |
| | RA | 0.26 ± 0.04 | 0.23 ± 0.02 | 0.44 ± 0.05 | 0.30 ± 0.04 | c |
| Tukey's HSO significance | | | a | ab | c | ab |
| Ethanol (µg/kg per h) | ULO | 13.1 ± 2.8 | 16.3 ± 2.9 | 21.7 ± 2.8 | 11.3 ± 2.5 | b |
| | AN | 12.6 ± 2.9 | 16.2 ± 2.4 | 22.7 ± 1.6 | 13.0 ± 2.0 | b |
| | RA | 3.7 ± 0.5 | 4.5 ± 0.4 | 4.7 ± 0.3 | 3.0 ± 0.4 | a |
| Tukey's HSO significance | | | a | ab | b | a |
| Ethyl acetate (µg/kg per h) | ULO | 0.5 ± 0.11 | 0.62 ± 0.12 | 2.5 ± 0.22 | 0.80 ± 0.08 | a |
| | AN | 0.49 ± 0.09 | 0.79 ± 0.13 | 1.04 ± 0.19 | 2.04 ± 0.44 | b |
| | RA | 0.28 ± 0.05 | 0.28 ± 0.05 | 1.44 ± 0.23 | 0.78 ± 0.12 | a |
| Tukey's HSO significance | | | a | a | b | b |

*All fruits were stored at 3°C, $CO_2$ was in ULO and AN 0.2% $O_2$, RA, regular atmosphere 21% $O_2$
Values are means and standard errors of six replicate samples, each containing one apple (0.15 kg)

## RESULTS AND DISCUSSION

### ANAEROBIC METABOLITES RELEASED THROUGH THE SKIN OF AN INTACT FRUIT INTO THE ENVIRONMENT

The present method makes it possible to capture the metabolites from intact fruits in two steps: first, the concentration in the percolating gas of the compound being analysed is measured (equation 2), then these experimentally obtained values: m - mass of the fruit in kg, and F – the volume rate in l/h serve to ascertain the production of the compound being analysed as it is captured in the trapping column (µg/kg per h). The volume of the percolating gas V passed through the concentration tube in ml (equation 2) had to be tested in advance. The production values of the respective metabolites in Table 1 are means from 6 fruits with the standard deviation of the mean, each measurement concerning only one fruit. Given the mass of the adsorber (Tenax GC) in the trapping column as 40 mg and the volume rate (F) 1.8 l/h, the volume of the percolating gas (V) of 120 ml, or only 60 ml is completely sufficient to capture the analyte.

The concentration of ethanol in the percolating gas from the variant RA was obtained by conversion of Table 1 figures into the values corresponding to equation 2. It oscillates around the level 4.1–5.2 µg/l (1.6–2.1 µl/l). The ethanol biosensors (SMYTH et al. 1999) which were designed for the detection of the oxygen stress of minimally processed fruits and vegetables are capable of discovering 10 µl/l in the gaseous phase at the temperature of the sample 5°C.

The conservation version of the complete capture of the compounds into the trapping column is a very efficient means of relatively quick suction into the concentration tube. The condition to be kept is, however, adhering to the given volume rate (F) and the time of suc-

tion through the tube. Should the compound be captured up to the equilibration, then tens of litres of the ambient atmosphere would have to be put through which would make the verification rather lengthy. Apart from this drawback, other aroma volatiles would be included and their flushing into the analytic column would prolong the analytic procedure.

If the values of the emission of the anaerobic metabolites from intact fruits are processed by Tukey's test, the cultivars cannot be separated. On the other hand, the metabolite productions from RA and ULO are clearly distinguished from the storage in anaerobic conditions (AN). Although our results were obtained at the close of the storage period, the present method is capable of measuring the emissions of the anaerobic respiration at any time of the storage.

### THE ACCUMULATION OF THE ANAEROBIC PRODUCTS IN THE FRUIT FLESH

The increased concentrations of acetaldehyde in the anaerobic conditions (Table 2) are several times above both the normal oxygen-containing atmosphere (RA) and that of oxygen at 0.9% (ULO). Tukey's test confirms that the technological variant AN is dramatically different from ULO and RA. Comparing the cultivars, Golden Delicious has a generally higher acetaldehyde concentrations.

PATTERSON and NICHOLS (1988) give the concentration of acetaldehyde from 4 to 9 µl/l after 14 days of exposition in 0.5% $O_2$. On the other hand, the incidence of this metabolite at 1% $O_2$ in the ambient atmosphere is totally negligible. The concentration of ethanol in the studied cultivars is easily comparable with that of acetaldehyde and ranges between 700 and 830 mg/l. The conditions are very similar (Table 2). Notice that technologically the two atmospheres ULO and RA are strongly different from AN.

Table 2. Anaerobic metabolites in the flesh (mg/l) of apple varieties at the beginning of storage and in different oxygen regimes after 30 days of storage*

| | Treatment | Jonagold | Golden Delicious | Melrose | Idared | Tukey's HSO significance |
|---|---|---|---|---|---|---|
| Acetaldehyde | IN | 0.6 ± 0.1 | 1.1 ± 0.5 | 0.8 ± 0.2 | 0.9 ± 0.1 | a |
| (mg/l) | ULO | 0.2 ± 0.1 | 0.6 ± 0.3 | 1.1 ± 0.1 | 0.8 ± 0.2 | a |
| | AN | 3.4 ± 0.4 | 6.2 ± 0.6 | 4.3 ± 0.6 | 2.9 ± 0.3 | b |
| | RA | 0.1 ± 0.0 | 0.6 ± 0.2 | 0.7 ± 0.1 | 0.6 ± 0.1 | a |
| Tukey's HSO significance | | a | b | a b | a | |
| Ethanol | IN | 4 ± 2 | 26 ± 20 | 8 ± 3 | 6 ± 1 | a |
| (mg/l) | ULO | 41 ± 16 | 112 ± 58 | 8 ± 2 | 13 ± 2 | a |
| | AN | 835 ± 30 | 831 ± 35 | 693 ± 17 | 794 ± 29 | b |
| | RA | 4 ± 1 | 7 ± 3 | 2 ± 1 | 3 ± 0 | a |
| Tukey's HSO significance | | a | a | a | a | |
| Ethyl acetate | IN | 2.2 ± 0.7 | 2.6 ± 0.8 | 2.8 ± 0.8 | 0.3 ± 0.1 | a |
| (mg/l) | ULO | 1.4 ± 0.4 | 2.4 ± 0.7 | 6.2 ± 0.6 | 0.5 ± 0.1 | a |
| | AN | 13.6 ± 1.7 | 14.5 ± 1.9 | 8.6 ± 1.3 | 1.8 ± 0.5 | b |
| | RA | 2.2 ± 0.5 | 1.0 ± 0.2 | 2.6 ± 0.5 | 0.9 ± 0.3 | a |
| Tukey's HSO significance | | b | b | b | a | |

*Storage conditions and statistical analysis were the same as indicated in Table 1
IN – initial storage. Values are means of six replicate samples, Tukey's HSD test $P \leq 0.05$

The ethanol point is defined as the production or concentration of ethanol (acetaldehyde) related to the lowest oxygen content which does not yet lead to an increased formation of both metabolites. The traces of ethanol inside the fruit pulp are normally identified at the beginning of storage. They are at the level of a few units of mg/l. The only exception is Golden Delicious, which has 26 mg/l.

However, in Spartan apples fruit ethanol content was in excess of 20 mg/100 g and may have contributed to the incidence of low-$O_2$ injury (LIDSTER et al. 1987). The resultant low-$O_2$ atmosphere injures the stored apples when acute levels of 120 mg/100 g (FIDLER, NORTH 1971) are reached.

## The ripening of apple varieties in different oxygen regimes

The main sugars were obtained using the method HLPC in one measurement together with organic acids from 6 replicated samples. The ratio of sucrose, glucose

Table 3. Sugar content (mg/l) at the beginning of storage and after 30 days in different oxygen regimes*

| | Treatment | Jonagold | Golden Delicious | Melrose | Idared | Tukey's HSO significance |
|---|---|---|---|---|---|---|
| Sucrose | IN | 46,343 ± 3,046 | 32,884 ± 1,652 | 19,036 ± 1,110 | 41,020 ± 1,118 | a |
| (mg/l) | ULO | 50,177 ± 2,891 | 32,998 ± 1,186 | 17,951 ± 483 | 37,635 ± 1,304 | a |
| | AN | 42,328 ± 775 | 31,835 ± 1,107 | 13,304 ± 1,834 | 31,813 ± 725 | a |
| | RA | 46,761 ± 2,237 | 34,060 ± 2,064 | 16,509 ± 531 | 39,439 ± 1,449 | a |
| Tukey's HSO significance | | d | b | a | c | |
| Glucose | IN | 11,561 ± 1,036 | 9,766 ± 502 | 20,621 ± 1,176 | 9,023 ± 351 | a |
| (mg/l) | ULO | 16,692 ± 1,264 | 11,139 ± 641 | 21,543 ± 840 | 10,072 ± 474 | a |
| | AN | 14,041 ± 1,088 | 11,864 ± 553 | 20,893 ± 507 | 9,980 ± 390 | a |
| | RA | 15,229 ± 1,601 | 9,481 ± 284 | 17,311 ± 684 | 9,625 ± 529 | a |
| Tukey's HSO significance | | b | a | c | a | |
| Fructose | IN | 69,048 ± 4,662 | 64,054 ± 2,780 | 70,420 ± 1,218 | 63,319 ± 585 | ab |
| (mg/l) | ULO | 78,500 ± 1,766 | 65,862 ± 1,781 | 68,304 ± 1,396 | 62,979 ± 1,161 | b |
| | AN | 72,116 ± 2,165 | 67,629 ± 2,251 | 63,479 ± 3,673 | 60,752 ± 1,397 | ab |
| | RA | 73,215 ± 3,626 | 59,016 ± 1,518 | 59,906 ± 1,604 | 60,704 ± 1,891 | a |
| Tukey's HSO significance | | b | a | a | a | |

*Values are means and standard errors calculated from six apples subject to treatment, Tukey's test for variety and treatment, $P \leq 0.05$

Table 4. Effect on pH and quality attributes – (titratable acidity as malic acid/l) and soluble solids content at the beginning of storage and after 30 days of storage*

| | Treatment | Jonagold | Golden Delicious | Melrose | Idared | Tukey's HSO significance |
|---|---|---|---|---|---|---|
| pH | IN | 3.03 ± 0.03 | 3.27 ± 0.02 | 3.07 ± 0.02 | 3.04 ± 0.01 | ab |
| | ULO | 2.96 ± 0.01 | 3.31 ± 0.04 | 3.19 ± 0.01 | 3.16 ± 0.01 | b |
| | AN | 2.98 ± 0.03 | 3.34 ± 0.02 | 3.21 ± 0.02 | 3.18 ± 0.02 | b |
| | RA | 2.98 ± 0.02 | 2.97 ± 0.02 | 3.11 ± 0.01 | 3.14 ± 0.01 | a |
| Tukey's HSO significance | | a | c | b | b | |
| Titrable | IN | 0.60 ± 0.04 | 0.42 ± 0.01 | 0.41 ± 0.03 | 0.59 ± 0.03 | a |
| acid | ULO | 0.53 ± 0.03 | 0.42 ± 0.02 | 0.41 ± 0.02 | 0.51 ± 0.02 | a |
| (g/100 g) | AN | 0.53 ± 0.04 | 0.39 ± 0.01 | 0.38 ± 0.02 | 0.50 ± 0.02 | a |
| | RA | 0.49 ± 0.02 | 0.49 ± 0.02 | 0.43 ± 0.02 | 0.55 ± 0.02 | a |
| Tukey's HSO significance | | b | a | a | b | |
| Soluble | IN | 14.2 ± 0.4 | 12.0 ± 0.3 | 12.1 ± 0.2 | 12.3 ± 0.3 | a |
| solids | ULO | 14.9 ± 0.2 | 12.4 ± 0.2 | 11.9 ± 0.1 | 12.3 ± 0.2 | a |
| (°Rf) | AN | 14.5 ± 0.2 | 12.6 ± 0.3 | 11.4 ± 0.4 | 11.8 ± 0.2 | a |
| | RA | 14.6 ± 0.4 | 14.6 ± 0.4 | 11.1 ± 0.1 | 11.7 ± 0.2 | a |
| Tukey's HSO significance | | c | b | a | a | |

*Storage conditions, sampling and statistical analysis were the same as indicated in Table 2 legend

and fructose was constant in the studied cultivars, fructose being prevalent (Table 3). During the whole experiment, the reserve of sucrose was not consumed and so it did not turn through enzymatic hydrolysis into simple sugars. Only cv. Jonagold and Idared showed a higher consumption of sugars in anaerobic conditions and ultralow oxygen, which corresponds to the Pasteur effect (CHERVIN et al. 1996).

No significance of the differences between cultivars could be established by Tukey's test ($P \le 0.05$). Between the technological variants, a significant change was the loss of fructose in AN storage and of glucose in the normal atmosphere (RA). BRACKMANN et al. (1994) reports that the ULO storage in combination with higher $CO_2$ was the most efficient

of all tested storage conditions in retaining soluble solids, acidity, flesh firmness and green skin colour.

Soluble solids – SS (Table 4) were obtained in fresh mass. According to this parameter a slight drop occurred in anaerobic conditions but, on the whole, the differences between technological variants were not statistically separated.

**The contents of organic acids in apple varieties**

The prevailing acid occurring in fruits is malic acid, there is also a minimal presence of citric acid (Table 5). The results obtained through the method HLPC were checked by establishing titratable acidity in values g/100 g as given in Table 5. The metabolic consumption during the experiment was high only in Golden Delicious

Table 5. Content of malic and citric acids in the flesh of apple fruits at the beginning of storage and after storage in different oxygen regimes*

| | Treatment | Jonagold | Golden Delicious | Melrose | Idared | Tukey's HSO significance |
|---|---|---|---|---|---|---|
| Malic acid (mg/l) | IN | 6,939 ± 479 | 5,525 ± 133 | 5,065 ± 264 | 7,265 ± 231 | b |
| | ULO | 6,084 ± 375 | 5,303 ± 256 | 4,952 ± 166 | 6,332 ± 181 | ab |
| | AN | 5,446 ± 221 | 5,066 ± 112 | 4,443 ± 231 | 5,680 ± 170 | a |
| | RA | 5,689 ± 165 | 4,227 ± 250 | 4,740 ± 241 | 6,565 ± 245 | a |
| Tukey's HSO significance | | b | a | a | b | |
| Citric acid (mg/l) | IN | 67 ± 6 | 52 ± 3 | 30 ± 3 | 33 ± 4 | b |
| | ULO | 32 ± 6 | 32 ± 4 | 24 ± 2 | 40 ± 5 | a |
| | AN | 25 ± 4 | 30 ± 5 | 31 ± 5 | 27 ± 5 | a |
| | RA | 40 ± 7 | 39 ± 8 | 33 ± 4 | 41 ± 5 | ab |
| Tukey's HSO significance | | b | ab | a | ab | |

*Storage conditions, sampling and statistical analysis were the same as indicated in Table 2 legend

Table 6. Firmness of subepidermis, skin and flesh at the beginning of storage and after 30 days of storage in different oxygen regime*

| | Treatment | Jonagold | Golden Delicious | Melrose | Idared | Tukey's HSO significance |
|---|---|---|---|---|---|---|
| Subepidermis (MPa) | IN | 0.31 ± 0.04 | 0.30 ± 0.02 | 0.31 ± 0.06 | 0.55 ± 0.07 | a |
| | ULO | 0.27 ± 0.04 | 0.29 ± 0.05 | 0.30 ± 0.04 | 0.63 ± 0.05 | a |
| | AN | 0.38 ± 0.04 | 0.26 ± 0.05 | 0.23 ± 0.03 | 0.51 ± 0.05 | a |
| | RA | 0.32 ± 0.03 | 0.34 ± 0.05 | 0.25 ± 0.03 | 0.48 ± 2.04 | a |
| Tukey's HSO significance | | a | a | a | b | |
| Skin (MPa) | IN | 0.94 ± 0.05 | 1.04 ± 0.03 | 1.17 ± 0.02 | 1.01 ± 0.02 | a |
| | ULO | 1.05 ± 0.04 | 0.92 ± 0.04 | 1.05 ± 0.04 | 0.86 ± 0.15 | a |
| | AN | 1.11 ± 0.05 | 1.00 ± 0.05 | 0.97 ± 0.03 | 0.93 ± 0.03 | a |
| | RA | 1.08 ± 0.05 | 1.01 ± 0.05 | 1.00 ± 0.04 | 0.80 ± 0.03 | a |
| Tukey's HSO significance | | b | Ab | b | a | |
| Flesh (MPa) | IN | 0.75 ± 0.04 | 0.79 ± 0.02 | 0.84 ± 0.04 | 0.79 ± 0.02 | b |
| | ULO | 0.82 ± 0.04 | 0.78 ± 0.03 | 0.74 ± 0.02 | 0.74 ± 0.02 | ab |
| | AN | 0.82 ± 0.03 | 0.81 ± 0.04 | 0.82 ± 0.02 | 0.73 ± 0.03 | b |
| | RA | 0.75 ± 0.04 | 0.78 ± 0.03 | 0.70 ± 0.03 | 0.61 ± 0.02 | a |
| Tukey's HSO significance | | b | b | ab | a | |

*Values are means and standard errors from 12 measurements (two replicates for each fruit), storage conditions are the same as indicated in Table 2

in the variant RA. A statistical significance was attributed by Tukey's test only to the different values of malic acid between Idared and Jonagold on the one hand, and Golden Delicious and Melrose on the other. The consumption of acid is statistically significant for all varieties but there is a difference between AN and RA.

The actual contents of citric acid are between 30 and 70 mg/l. They do not respond to metabolic processes. pH-values oscillate in a narrow range.

### Firmness of apple fruits in oxygen regimes

Three parameters could have been identified on the basis of the deformation curve obtained by stressing an intact fruit with slow deformation indentation by means of a plunger 8 mm in diameter. Integrating strain and penetrating length, one obtains the subcutaneous (subepidermal) injury which is measured before the skin is pierced. Subepidermal injury is statistically non-significant (Table 6). The firmness of the skin is not affected by oxygen contents and it is comparable for AN and ULO storage. Statistically significant losses of firmness were observed in the pulp of the fruits stored in the regular atmosphere (RA).

### Changes of anaerobic metabolites after storage in low oxygen

During storage not only ethanol, but also acetaldehyde as ethyl acetate levels can increase. Low oxygen or high $CO_2$ concentrations in parenchymal tissues are well known to produce such accumulations, hence increases in acetaldehyde and ethanol are often taken as an indication of fermentation in the tissues. In anaerobic condi-
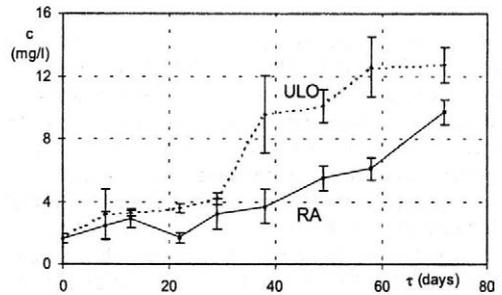


Fig. 1. Content of acetaldehyde of cv. Golden Delicious apples under ULO (0.9% $O_2$) and RA (regular atmosphere 21% $O_2$) regimes at 3°C for 76 days. Each point is the mean of 6 replicates. Vertical bars indicate standard error
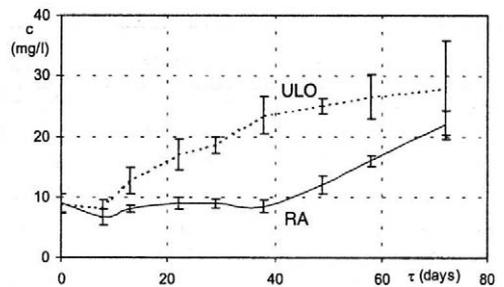


Fig. 2. Effect of $O_2$ levels on ethanol content in the pulp of cv. Golden Delicious apples at 3°C for 76 days. Ambient atmosphere is the same as Fig. 1. Each point is the mean of 6 replicates. Vertical bars indicate standard error
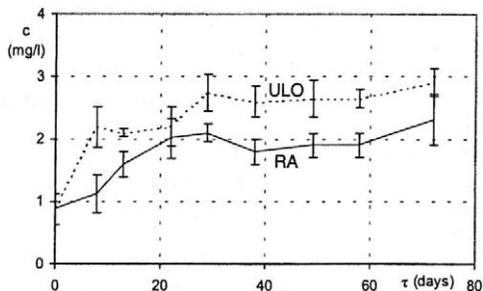
Fig. 3. Effect of $O_2$ levels on ethyl acetate content in the pulp of cv. Golden Delicious apples at 3°C for 76 days. Ambient atmosphere is the same as Fig. 1. Each point is the mean of 6 replicates. Vertical bars indicate standard error



Fig. 5. Amount of ethanol cv. Golden Delicious apples under anaerobic environments (0.2% $O_2$) at 3°C for 20 days, 29 days respectively, followed by 70 days and 76 days, respectively in air. Each point is the means of 6 replicates. Vertical bars indicate standard error. Arrow indicates the date of transfer to air. Apple in term onset immediately stored, mid-storage about 3.5 month in air



Fig. 4. Effect of exposure to the indicated level of $O_2$ at 3°C for 30 days, followed by 76 days on acetaldehyde content of cv. Golden Delicious apples. Each point is the means of 6 replicates. Vertical bars indicate standard error. Arrow indicates the date of transfer to air



Fig. 6. Ethyl acetate concentration in pulp of cv. Golden Delicious apples exposed to anaerobic environment (0.2% $O_2$). Vertical bars indicate standard error. Arrow indicates the days of transfer to air

tions, the production od acetaldehyde is high: more than 300 ml/l were produced in 30 days. The subsequent aeration of the fruits caused this high concentration to drop to 60–70 mg/l (Fig. 4). In spite of this, the eventual concentration was still higher than the measured in the same variety kept in ULO and RA (Fig. 1).

The concentration of acetaldehyde in the parenchyma of apple fruits can be used as a diagnostic for finding out the time point of the transition from basic metabolism to pyruvate metabolism and a glycolytic end-product. In the senescence which is concurrent with low but still enough oxygen not to trigger the production of th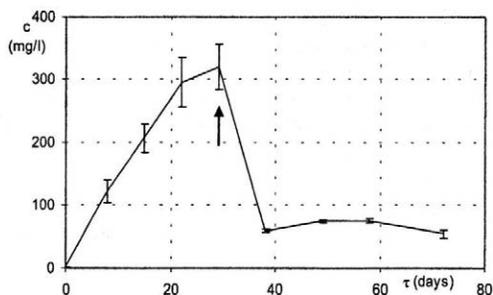e mentioned metabolites, the values of acetaldehyde in the variant ULO are always statistically higher, confirmed at $P < 0.05$, compared with the fruits constantly stored in RA (Fig. 1).

Acetaldehyde is more toxic for the parenchymal tissues than ethanol (CHERVIN et al. 1996). Acetaldehyde is also associated with browning and softening disorders in the fruit. The evidence of acetaldehyde suggests that its accumulation is a result, rather than a cause, of tissue disorganisation (SMAGULA, BRAMLAGE 1977).

The concentration of ethanol in the fruits which were constantly kept in anaerobic conditions has a linearly increasing course in time with slight differences only in the velocity (Fig. 5). Consequently, the oxidation of ethanol in the fruits exposed to a regular oxygen atmosphere is either rather slow (Fig. 5 – onset) as in the case of fruits treated with ultra-low oxygen immediately after the harvest, or the ethanol quantity hardly changes at all (Fig. 5 – mid-storage) as in the case of fruits that have previously been kept only in cold storage for 3.5 months. PATTERSON and NICHOLS (1988) suggested that ethanol levels decrease within a week in the air at 20°C, following 14 weeks in experimental atmospheres.

The technological variants ULO and RA can easily be identified according to the increased concentration of acetaldehyde and ethyl acetate (Figs. 1 and 3). It is possible to conclude that the concentration of oxygen in the ambient atmosphere at a level of 0.9% does not physiologically harm the tissues of apples by producing increased quantities of acetaldehyde and ethyl acetate.

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# Účinek koncentrací kyslíku na tvorbu anaerobních metabolitů při skladování jablek

**ABSTRAKT:** Skladování odrůd jablek Golden Delicious, Jonagold, Melrose, Idared (*Malus domestica* Borkh.) v ultranízké koncentraci kyslíku (ULO: 0,9 % $O_2$), v anaerobních podmínkách (An: 0,2 % $O_2$) a v normální atmosféře (RA: 21 % $O_2$) s přidáním 0,3 % $CO_2$ nebo 0,03 % $CO_2$ ukázalo, že vystavení plodů atmosféře ULO potlačuje vytváření metabolitů jako etanol, acetaldehyd a etylacetát. Byla zjišťována tolerance plodů na jednotlivé atmosféry vedoucí k tvorbě anaerobních zplodin. Koncentrace etanolu v anaerobních podmínkách uložení (< 0,2 % $O_2$) dosáhla za 30 dnů 790–830 mg/l. Kyslíkový stres za 30 dnů v anaerobních podmínkách rovněž vyvolal souběžně vysokou koncentraci acetaldehydu (350 mg/l). Přitom byly koncentrace tohoto metabolitu v ULO a RA statisticky rozlišeny (*P* < 0,05), ale pohybovaly se v úzkém rozmezí jednotek mg/l. Obsah acetaldehydu v ovocné pulpě indikuje stupeň poškození nízkým obsahem kyslíku, který se však stále nachází v koncentracích 0,9 % $O_2$. Přes slupku uvolňované plynné metabolity, hodnocené v µg/kg za h, byly zachyceny do sorbentu a následně plynově chromatograficky stanoveny na obsah acetaldehydu, etanolu a etylacetátu. V důsledku toho, že nahromadění těchto metabolitů v kapalné fázi vede k jejich částečnému uvolňování do okolního prostředí, se výrazně odlišuje varianta RA od ULO a An. Tukeyovým testem nejsou rozlišeny průměry odrůd a ošetření. Vyšší spotřeba kyseliny jablečné byla rozpoznána mezi uložením metodou ULO a AN. Ke snížení pevnosti stanovené měřením dužniny došlo pouze v normální atmosféře.

**Klíčová slova:** *Malus domestica*; etanol; acetaldehyd; etylacetát; nízký obsah kyslíku; pevnost plodu; emise těkavých látek

*Corresponding author:*

Prof. Ing. JAN GOLIÁŠ, DrSc., Mendelova zemědělská a lesnická univerzita, Brno, Zahradnická fakulta, Ústav posklizňové technologie zahradnických produktů, Valtická 337, 691 44 Lednice na Moravě, Česká republika
tel.: + 420 627 34 01 05–7, fax: + 420 627 34 01 59, e-mail: golias@mendelu.cz

# Evaluation and prognosis of stored vegetable losses

K. Kopec[1], M. Valšíková[2], J. Bartoš[3]

[1]Mendel University of Agriculture and Forestry, Brno, Czech Republic
[2]Vegetable Research Institute, Nové Zámky, Slovak Republic
[3]Vegetable Producer's Union, Olomouc, Czech Republic

**ABSTRACT**: Reduction of losses during vegetable storage can advance the economics of production and utilization of these important foodstuffs. The results of many years of research into the evaluation of losses in basic species of vegetables (cabbage, kohlrabi, carrots, parsnips, celeriac, onions, garlic), numerically and graphically elaborated, lead to the conclusion that the course of these losses during storage can be generalized by a polynomial quadratic third grade equation. This equation is valid regardless of preharvest factors (variety, conditions, method and date of harvest, growth soundness, etc.) or postharvest factors and storing conditions (pit, cold-storage room or ventilated warehouse). Graphs present time curves of the loss process under optimal and even substandard conditions. From this equation or from graphical presentation we can determine the prognosis of further losses and in combination with prices it is also possible to determine an economically acceptable date for unloading. Designed methodology of determination and evaluation of losses are applicable to total quality management according to ISO series 9000, alternatively to determine the critical point of exposure for the technology of vegetable storage.

**Keywords**: vegetable; equation of losses; storage prognosis; cabbage; kohlrabi; carrots; parsnips; celeriac; onions; garlic

The transformation of national economy and transition to market economy make it necessary to consider all the possibilities of cutting production costs and increasing economic effectiveness and at the same time improving the quality of products (KOPEC 1997).

One of the effective ways to increase general production at low-cost is the utilization of reserves in grower technology and reduction of losses during harvest and postharvest operations and vegetable storage. According to qualified evaluation these reserves and losses are always high and represent on average one quarter to one half of the currently achieved crop. In some cases they can be even higher (SALUNKHE, KADER 1998).

## DEFINITION OF LOSSES

Losses usually occur in the final phases of production and are defined as negative deviations in the quantity and quality of final products. In a wider sense (besides losses in the final product) there is also damage to growth during vegetation, possibly damage to useful waste. Damage also leads to losses of quantity and quality, but mostly it is not possible to anticipate them (hailstorms, vernal slight frost, high occurrence of pests, etc.).

Underestimation of losses is mostly due to lack of exact data. Analysis of the extent of losses is lacking, there is no stable method of assessing them. Losses during storage are determined only by a qualified estimate. Detailed complex analysis of their cause is also missing.

For evaluation of losses a sufficient number of informatory sources is essential.

We have worked out a method for judging the probable development of losses during storage of some sorts of vegetables and also the possible causes of these losses. We assumed that storage was frequently a necessary operation, which makes it possible to market short-term harvest crops over a longer period of time and especially to overcome the time discrepancy between supply and demand (VALŠÍKOVÁ, KOPEC 1999).

## MATERIAL AND METHODS

We assessed the following vegetables: onion from seeds and bulbils, garlic from spring and autumn planting, parsley (root), carrots, celeriac, kohlrabi Gigant, white cabbage.

Before storage we used a three-point scale to evaluate preharvest factors: variety applicability, harvesting conditions, method and date of harvesting and disease occurrence. Further we evaluated storage conditions (pit, air-cooled warehouse, cold-storage room).

During storage we checked weighed samples, inserted in piles or pallets, namely in relation to of losses of weight and quality. Representative average samples in reticular bags were first weighed, provided with large labels on a long piece of string placed visibly outside the pallets or piles of vegetables. The label included the name of the sample, storing (and control) date, quantity and further

Table 1. Relevancy of individual storage loss causes

| Cause of losses | Onion | Garlic | Carrots | Parsnips | Celeriac | Cabbage |
|---|---|---|---|---|---|---|
| Bad state of health in growth | +++ | + | ++++ | +++ | ++ | + |
| Wrong date and mode of harvest | +++ | ++ | ++ | ++ | + | ++++ |
| Excessive damage during harvest | ++ | + | ++++ | ++ | +++ | ++++ |
| Failure to discard damaged products | +++ | + | + | ++ | ++ | ++++ |
| Incorrect postharvest treatment | ++++ | ++++ | ++++ | ++ | ++ | + |
| Incorrect storage conditions | ++ | ++++ | ++++ | ++++ | ++++ | ++++ |
| High level of unsaleable goods | +++ | + | ++++ | + | + | ++++ |
| Total low level of quality care | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ |

details. In the course of storage we took out samples one by one, evaluated losses in quality and weight and processed the results statistically and graphically. For equation calculation we used a computing programme. The size and number of samples were established according to the sort, size of stored batches and the presumed duration of storage, namely:

For determination of losses in stored onion we inserted into each batch of onion in piles or on pallets an adequate number of samples (usually 10) in reticular bags containing 10 kg, and every month qualitative and material changes were checked three times.

For garlic we inserted into the pallets of each section (1 t) 2 kg samples in reticular bags and evaluated in quadruplet repetition once a month.

Carrots and parsley (roots): 15 samples of 3 kg each were put into each storage batch in reticular bags and their qualitative and quantitative changes were evaluated two or three times in the course of storage. Celeriac was evaluated likewise with samples of 30 roots.

Control samples of cabbage were inserted in six reticular bags containing 10 heads each, individually numbered on the stalks. Every 6 weeks we took out 2–3 bags,

evaluated the quality and weight of every head and put the bags back.

## RESULTS

Hundreds of dates on the dynamics of losses during storage, obtained by assessing the crops, can be generalized by regression behaviour, which is characterized by a common polynomial third order quadratic equation:

$$y = ax^3 + bx^2 + cx + d$$

The equation is valid regardless of preharvest factors and storage conditions even for different vegetable sorts. Particular causes of loss were established according to growing practice and the results of extensive research in previous years (BARTOŠ et al. 1988; KOPEC et al. 1997; VALŠÍKOVÁ et al. 1996–1998, and others).

Some results are plotted in diagrams. Curve A (the lowest) represents development of standard (unavoidable) losses under optimal growing and storage conditions. Curve B represents losses in better storage conditions (air-cooled storage-rooms, pits), curve B1 to B4 and C represent running losses affected by inferior growing conditions.



$y = 0.4621x^3 - 5.2327x^2 + 23.424x - 18.214$ (C)

$y = 0.3066x^3 - 3.4565x^2 + 14.113x - 11.007$ (B)

$y = 0.0821x^3 - 0.98x^2 + 5.11654x - 4.1429$ (A)

- ◆- ·A          - ▲- ·B          - ▲- ·B1
- ▲- ·B2          - ▲- ·B3          - ■- ·B4
- ■- ·C          —— Polynomial (C)          —— Polynomial (A)
—— Polynomial (B)
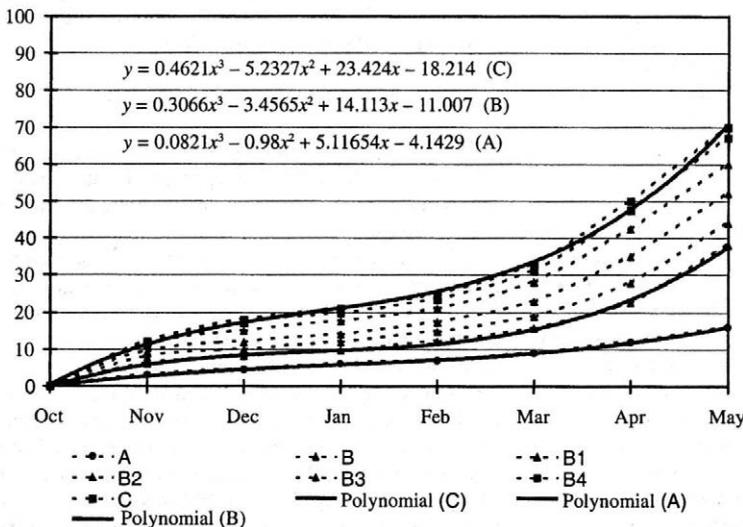
Fig. 1. Losses in stored onion (grown from seeds)

$y = 0.7778x^3 - 7.619x^2 - 30.032x - 22.429$ (C)

$y = 0.4167x^3 - 3.9405x^2 + 15.286x - 11.571$ (B)

$y = 0.3333x^3 - 3.5x^2 + 13.167x - 10$ (A)

■ A  ▲ B  ■ C   — Polynomial (C)   — Polynomial (B)   — Polynomial (C
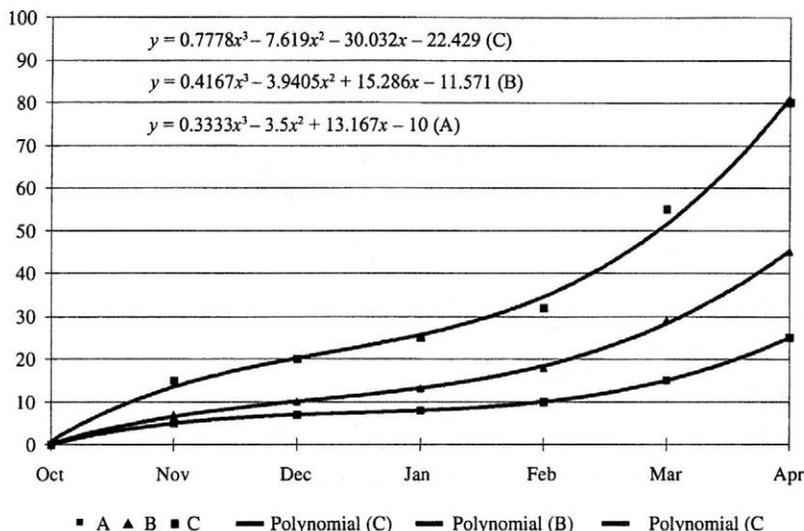
Fig. 2. Losses in stored onion (grown from bulbils)

## DISCUSSION

From the analysis of results we defined the principal preharvest factors which decide on the level of storage losses (see Table 1):
- suitable composition of type and sorts (for gradual and single harvest),
- selection of special varieties with long-term shelf life, suitable for the given locality and growing method,
- appropriate cultivation (growth density, irrigation),
- reliable preservation of healthy state before harvesting,

- determination and adherence to exact harvesting dates and directive harvest operations,
- organization of harvest with complex continual material flow,
- optimal utilization of harvesting machines and their tuning so as to minimize damage to crops,
- harvesting in optimal soil and weather conditions,
- careful preparation of crops for storage (for example predrying of onions),
- choice of suitable storage methods (cold-storage),
- rapid introduction and constant adherence to storage regime for the given species or variety,



$y = 0.0547x^3 - 0.7504x^2 + 7.5044x - 7.1825$ (C)

$y = 0.0387x^3 - 0.6587x^2 + 6.3264x - 6.4841$ (B)

$y = 0.0417x^3 - 0.6456x^2 + 3.539x - 2.9048$ (A)

♦ A  ■ B  ▲ C  -- Polynomial (C)   — — Polynomial (B)   — Polynomial (A)
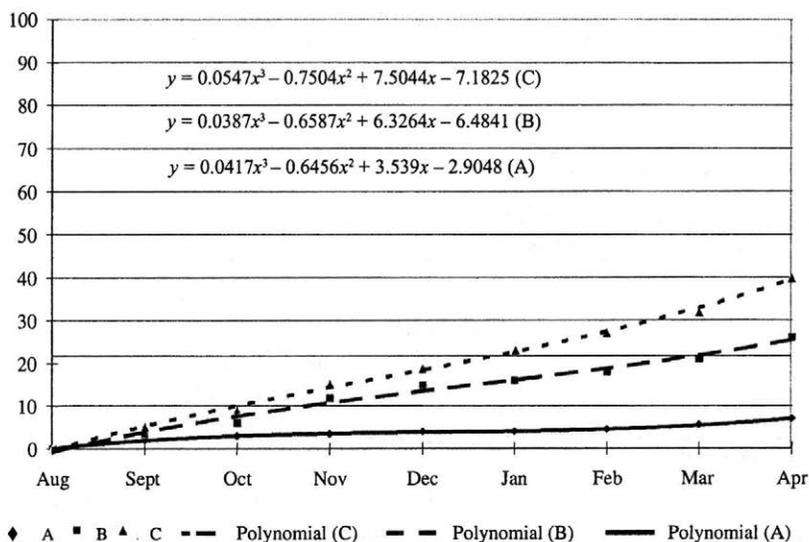
Fig. 3. Losses in stored garlic from bulbils planted in spring

$$y = 0.101x^3 - 1.7327x^2 + 19.714x - 19.143 \text{ (C)}$$
$$y = 0.351x^3 - 3.697x^2 + 15.595x - 11.643 \text{ (B)}$$
$$y = 0.0096x^3 - 0.0333x^2 + 1.459x \text{ (A)}$$
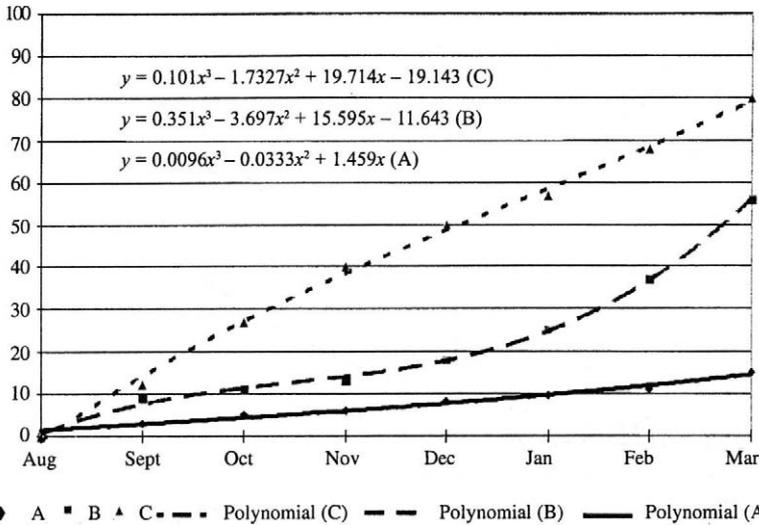
Fig. 4. Losses in stored garlic from bulbils planted in autumn

♦ A ■ B ▲ C ▪—▪—▪ Polynomial (C) — — Polynomial (B) —— Polynomial (A)

– current evaluation of losses in each particular post-harvest operation,
– timely unloading.

During storage the losses in weight and quality must be expressed in the price, which can change in the course of storage. However, there need not be incurred economic losses. Losses can be divided into unavoidable (standard) and superfluous. Unavoidable losses are caused by physiological processes which proceed in live crops. Superfluous losses arise through non-performance of technological discipline, but also as the result of unfavourable climatic conditions. Total losses can be suffered in disastrous situations when some factors exceed the critical point (high temperatures resulting in overheating, wilt, total mechanical damage, failure of service, etc). It is not always possible to anticipate them. All losses can further be divided into losses through negligence and accidental ones, and the measure of negligence must be judged individually. These losses have a wide range of causes. Microbial losses increase exponentially during storage.

Suitable storage conditions must be ensured by:
– full and timely sanitation of all storage areas and equipment,
– constructional and technological adaption of warehouses, especially improved heat insulation,
– individual elements (or whole systems) of automatic regulation and recording of storage conditions (temperature, humidity, air composition and airing),
– rapid cooling of crops before storage,
– technological discipline and adherence to operating rules, prescribed for the given storage subject,
– organizational steps destined to improve work achievement and observance of operational discipline (training, perks, etc).

The actual losses should not be higher than those given in the cited equations (norms) of weight losses during each month of storage. The equation parameters must



$$y = 0.3889x^3 - 3.4286x^2 + 13.611x - 10.571 \text{ (C)}$$
$$y = 0.1667x^3 - 1.7619x^2 + 8.4286x - 6.9286 \text{ (B)}$$
$$y = 0.0972x^3 - 1.0655x^2 + 5.4802x - 4.5 \text{ (A)}$$
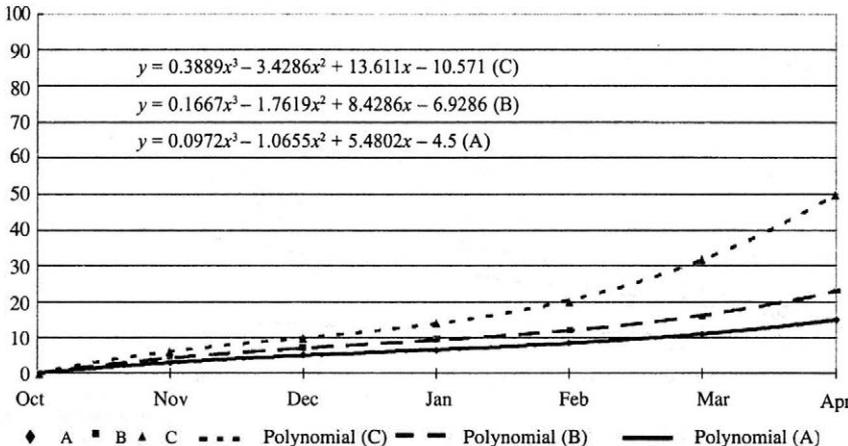
Fig. 5. Losses in stored carrots

♦ A ■ B ▲ C ▪▪▪ Polynomial (C) — — Polynomial (B) —— Polynomial (A)
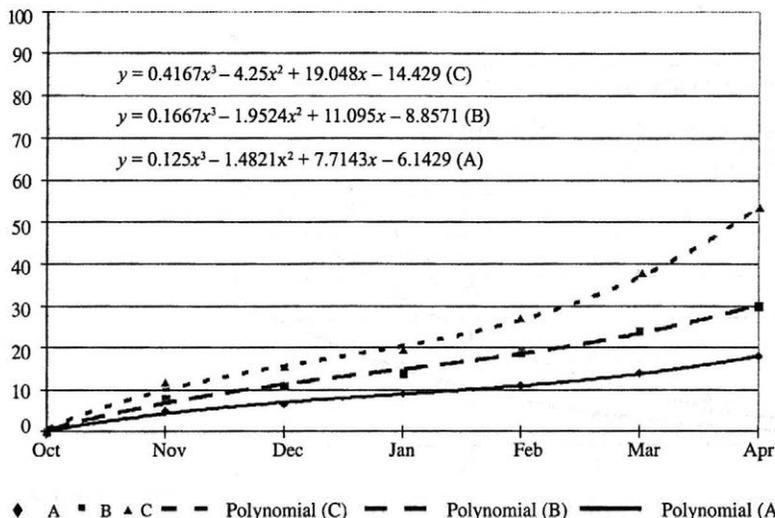
Fig. 6. Losses in stored parsley (roots)

Legend: ♦ A ■ B ▲ C — - Polynomial (C) — — Polynomial (B) —— Polynomial (A)

Equations shown in figure:
$$y = 0.4167x^3 - 4.25x^2 + 19.048x - 14.429 \ (C)$$
$$y = 0.1667x^3 - 1.9524x^2 + 11.095x - 8.8571 \ (B)$$
$$y = 0.125x^3 - 1.4821x^2 + 7.7143x - 6.1429 \ (A)$$

be experimentally set by the above method for each particular warehouse and factor, results of several year' observation analyzed and then used as limits. Thus the actual cause of losses can be determined and cut down to achieve the required economic effect.

## CONCLUSIONS

This method enables us to assess whether the losses were accidental or due to negligence. Further it is possible to determine outlines for reducing losses during storage in the given locality. From the equation and from graphic illustrations of standard (unavoidable) and maximum losses it is possible to make the prognosis of dynamic loss increase and with respect to prices also economically acceptable terms of unloading. The suggested method and mathematical model of loss evaluation are perspectively applicable to a system of quality management according to ČSN ISO series 9000, to determine the critical point of endangered quality during the technological process of storage.
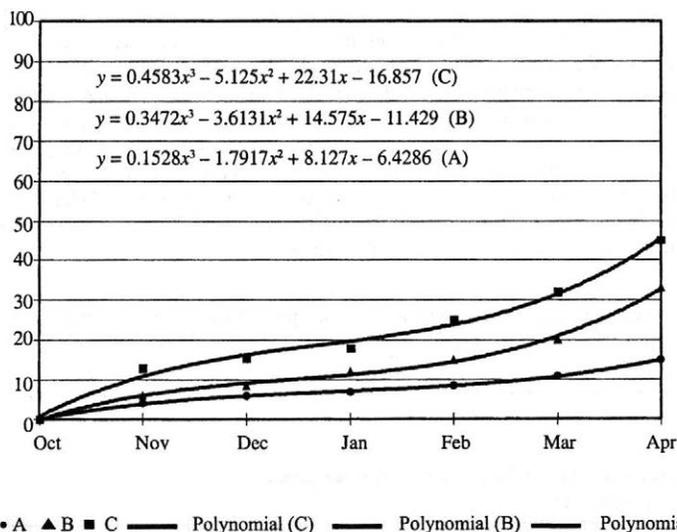


Fig. 7. Losses in stored celeriac

Legend: • A ▲ B ■ C —— Polynomial (C) —— Polynomial (B) —— Polynomial (A)

Equations shown in figure:
$$y = 0.4583x^3 - 5.125x^2 + 22.31x - 16.857 \ (C)$$
$$y = 0.3472x^3 - 3.6131x^2 + 14.575x - 11.429 \ (B)$$
$$y = 0.1528x^3 - 1.7917x^2 + 8.127x - 6.4286 \ (A)$$

$$y = 0.6111x^3 - 4.2976x^2 + 16.091x - 12.714 \text{ (C)}$$

$$y = 0.2222x^3 - 0.7738x^2 + 4.3611x - 3.4286 \text{ (B)}$$

$$y = 0.1667x^3 - 0.8571x^2 + 3.2619x - 2.1429 \text{ (A)}$$

Fig. 8. Losses in stored white cabbage

◆ A ■ B ▲ C  - - - Polynomial (C)   — — Polynomial (B)   —— Polynomial (A)

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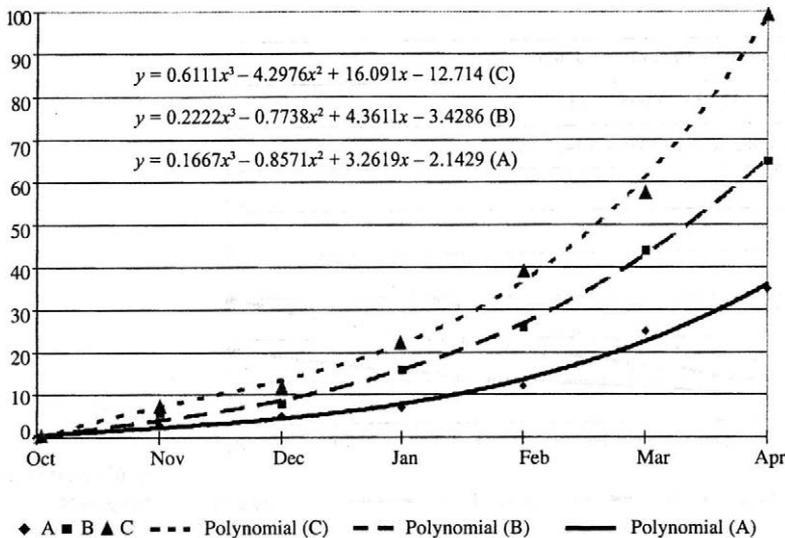KOPEC K. et al., 1997. Stavby a zařízení pro posklizňovou úpravu a skladování ovoce a zeleniny. In: Požadavky na stavby a zařízení pro rostlinnou výrobu. Praha, MZe ČR: 80–119.

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## Metodika hodnocení a prognózování ztrát skladované zeleniny

**ABSTRAKT**: Omezení ztrát během skladování zeleniny může zefektivnit ekonomiku produkce i zpracování těchto významných potravin. Víceleté výsledky hodnocení ztrát základních druhů zelenin (cibule, česnek, mrkev, petržel, celer, zelí, kedluben), zpracované číselně a graficky, vedou k závěru, že průběh těchto ztrát během skladování lze zevšeobecnit polynomickou rovnicí třetího stupně. Tato rovnice platí bez ohledu na předsklizňové faktory (vhodnost odrůdy, podmínky, způsob a termín sklizně, zdravost porostu) anebo na skladovací podmínky (chladírenský či větraný sklad, krecht). Křivky znázorňují časový průběh ztrát za normálních i nestandardních podmínek. Z těchto rovnic nebo z jejich grafického vyjádření lze prognózovat další vývoj ztrát. Také je možné s přihlédnutím k cenovým změnám určit ekonomicky přijatelný termín vyskladnění. Navržená metodika stanovení a vyhodnocení ztrát může být uplatněna pro komplexní řízení jakosti v souladu s normami ČSN ISO řady 9000, event. ke stanovení kritických bodů v technologii skladování zeleniny.

**Klíčová slova**: zelenina; rovnice ztrát; prognóza skladování; zelí; kedlubny; mrkev; petržel; celer; cibule; česnek

*Corresponding author:*

Prof. Ing. KAREL KOPEC, DrSc., Nádražní 20, 691 44 Lednice na Moravě, Česká republika
tel.: + 420 627 34 02 61, e-mail: Kopcovi@centrum.cz

# Using image processing and analysis techniques for counting apple fruits in the orchard

D. Stajnko, M. Lakota

*University of Maribor, Faculty of Agriculture, Maribor, Slovenija*

ABSTRACT: Counting objects by using blob analysis technique was found to be a very practical method for determining the number of apple fruits in specified digital pictures captured under natural lighting conditions by CCD camera. The RGB and IHS palettes were applied to each picture before being analysed by edge detection and blob analysis procedures processed by our own programs developed in G-language. Fruits of all colours were successfully detected in the pictures captured on the bright sunny side of the apple tree. However, pictures including red fruits had to be converted to hue images by implementing IHS palette before being further analysed. Counting the red fruits on the hue images converted from the pictures captured on the sunny side of the apple trees indicated a very close correlation to the manually counted fruits ($R^2 = 0.89$), but counting little yellow and green-yellow fruits has been less successful because the pre-processing could not sufficiently remove the noise. The edge detection method did not produce better results but required more processing time in comparison with blob analysis; therefore it can be accepted for precise measurements of objects only.

Keywords: RGB, IHS palettes; apple fruit counting; edge detection; blob analysis

The question of future yield and its quality has always been a crucial problem for apple producers and the basis for prediction of prices and storage capacities for apples in the ULO refrigerating chambers. Since 1989, the yield has been estimated using the Winter method which calculates on the basis of the counting and measuring of fruit samples that are additionally corrected for the plant age, health and rootstock type. However, the differences between the calculated and measured yield have varied up to 4 tons per hectare depending on the year, apple variety and orchard age, so that a significant loss of profit is induced when the production was overestimated.

The main objective of this paper is to research and evaluate a new approach to yield prediction which is based on the machine vision and image processing that establishes the capturing procedure from the pictures in the apple orchard taken under natural light conditions, and to investigate the best analysis techniques for counting immature apple fruits that would provide better yield estimation.

## REVIEW OF LITERATURE

Practically no research has been reported concerning the use of image analysis techniques for counting fruits on apple trees in the orchard under natural lighting conditions. The reason for sparse research in this field of image analysis can be deduced from the following citations.

According to PETERSON et al. (1999) lighting is a critical part of image processing and is particularly difficult when operating outdoors. Therefore a fibre-reinforced drapery was installed on the apple harvesting robot to block the influence of natural light conditions, but, in turn, created a need for artificial lighting.

When harvesting the cucumbers, a colour difference between leaves and ripe fruits was so close that the only imaging technique capable of detecting cucumbers was the use of the near infrared range (NIR) frequencies (KOLLENBURG-CRISSAN et al. 1998). It was determined that the reflectance of fruits in the near infrared range was different from that of their leaves.

KONDO et al. (1998) also mentioned that the testing of three different strawberry harvesting robots showed the need for an artificial light source although the ripe fruits were red in colour.

For counting the particles or cells in blood samples the blob analysis procedure was reported by HANKS (1997) to be very successful but it depends on the quality of image pre-processing techniques and the objects investigated.

## METHODS

Colour images were captured with a charge coupled device (CCD) digital camera, KODAK DC290 with 1,792 × 1,200 pixel resolution in the Faculty's five-year-old apple orchard planted with the varieties Golden Delicious and Jonathan on the M9 rootstock.

One series of 50 pictures was captured on the sunny side of the trees and another on the shadow side both from the distance of 3.0 m at an angle of 90 degrees to the planting row. Concurrently, the fruits were manually counted on the photographed trees and the results were compared with the machine analysis.

For image digitalisation and processing Labview IMAQ Vision Software has been used on a Pentium II 350 MHz Intel processor with 64 Mb RAM. Images were directly transferred from the CCD camera to the computer through the USB port.

## IMAGE PRE-PROCESSING

Since it was impossible to block the sunlight on all trees in the orchard, every digital image should be pre-processed to suppress undesired distortions or to enhance features important for further analysis. In the first step, the RGB and IHS palettes were applied for researching the effect of the basic colour areas of red, blue and green on the image histogram and the inspection of objects. The RGB palette yielded three images in the red (R), green (G) and blue (B) colours which were also the basis for calculating the IHS palette that produced images for I (intensity), H (hue) and S (saturation) according to the following equations from SONKA et al. (1998):

$$I = \frac{R+G+B}{3}$$

$$H = \cos^{-1}\left( \frac{\frac{1}{2}[(R-G)+(R-B)]}{\left[ (R-G)^2 + (R-B)(G-B)^{\frac{1}{2}} \right]} \right)$$

$$S = 1 - \frac{3}{R+G+B}\min(R;G;B)$$

In the second step, from every new image, a histogram was processed and analysed according to the frequency of pixels for each value level. Only images consisting of outstanding picks in the histogram were selected for the thresholding procedure, which fragmented the image into object region and background region. After this process, all pixels that fell in the interval background were given the value zero and became black in the binary image. Otherwise, the threshold interval was given the value one and became white.

Two different methods, i.e. the edge detection and the blob analysis procedures, were used for counting fruitlets in the processed binary images.

The edge detection pre-processing procedure is based on the different gradient operators that define region boundaries in the image. There were three parameters – contrast, width, and steepness of pixels that affected the quality of calculations. The blob analysis procedure is based on a blob which was defined as a group of connected pixels that have the same intensity and represent an object displayed in the image, HANKS (1997). Before getting blobs, the binary image was filtered using a specified number of 3 × 3 erosions to remove noise and the connectivity – 4 function for determining whether an adjacent pixel was the same or different particle. The particles kept after the procedure were exactly the same as those detected in the original image.

## SEARCHING AND COUNTING PROCEDURE

Once the particles were detected in the binary image, a template of a fruit object was selected from the image by using a pencil tool to explain to the computer what to search for and count. The matching process labelled the image to give each object in the image a unique ID before performing the operation. The searching analysis processed with the implementation of the outside contours of the object known as the mask. Due to the heterogeneous shape of particles, the mask tolerance must be determined for increasing the efficiency of the procedure.

For all image analyses our own programs called *APPLESEARCH*, *APPLECOUNT*, and *APPLEFILTER*, were designed and written in the G-language developed for National Instruments® program packages Labview 5.0.1 and IMAQ Vision 4.1.1.

Statistical analysis for the regression equations between all machine counting results and manually counted immature apple fruits was developed by applying an SPSS Package Program.

## RESULTS

### THE EFFECT OF IHS SEGMENTATION ON THE IMAGE ANALYSIS

The efficiency of the analysis procedure for detecting objects in the images depends very much on the brightness conditions of the sunlight while capturing pictures in the orchard.

According to the pre-processing procedures required, three types of pictures were classified on the basis of the grey image histogram. The pictures with Golden Delicious fruits captured on the sunny side of the trees were already acceptable for further analysis and they were just converted into grey images before final analysis. The second group of pictures including Jonathan apple fruits captured on the sunny or shadow side of the trees required transformation to the hue images by implementing the IHS palette which proved the most appropriate for further analysis (Fig. 1).

However, the use of RGB and IHS palettes in pictures including Golden Delicious fruits captured on the shadow side of the trees neither affected the quality of the pre-processing nor increased the correlation to the manually counted fruits. For improving the image quality, an artificial light source needs to be used in future research so that the correlation between manual and machine counting would be closer.

### COMPARISON BETWEEN EDGE DETECTION AND BLOB ANALYSIS

As seen from Figs. 2 and 3, the difference between edge detection and blob analysis results depended very much on the remaining information in the binary image after
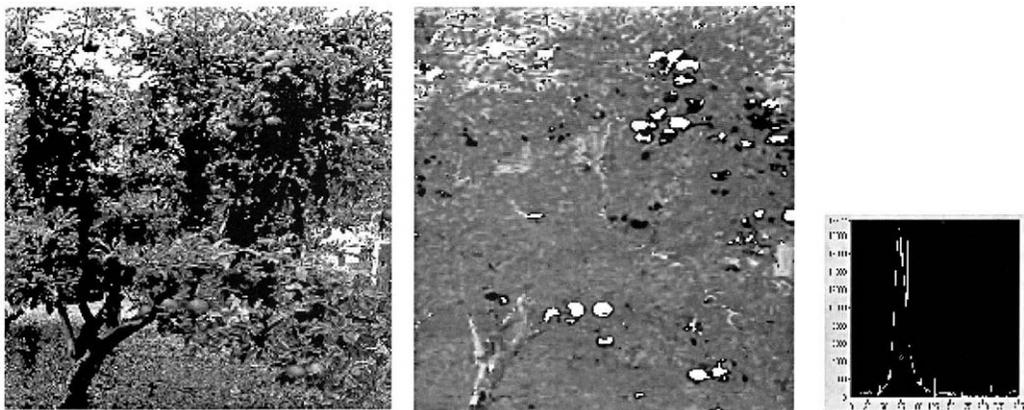
Fig. 1. Original colour picture (left), hue image (middle) and hue's histogram (right)
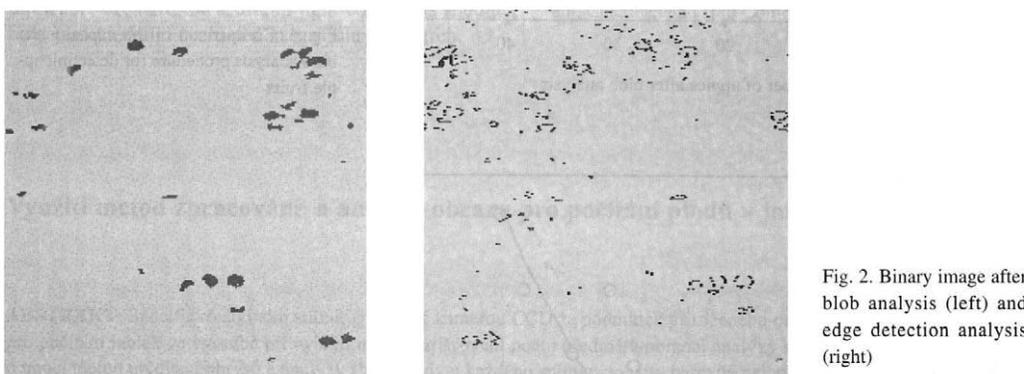


Fig. 2. Binary image after blob analysis (left) and edge detection analysis (right)

the thresholding process succeeded in converting the grey image to the binary image.

The edge detection method was discovered to be very sensitive to all small light intensity differences between pixels. Each determined contrast between pixels was detected as part of a new object and the procedure produced five times more objects in the same image than the blob analysis. Therefore, the new image included a lot of unusable edges that decreased further processing effective-

ness by consuming too much time. However, this method was selected as very useful for calculating the width or area of certain objects needed for calculation of fruit growth because it contains very clear edges.

On the other hand, the blob analysis process after the thresholding detected fewer objects than edge detection (Fig. 3) but they were more appropriate for counting because they contained pixels with the same intensity, thus being processed faster.
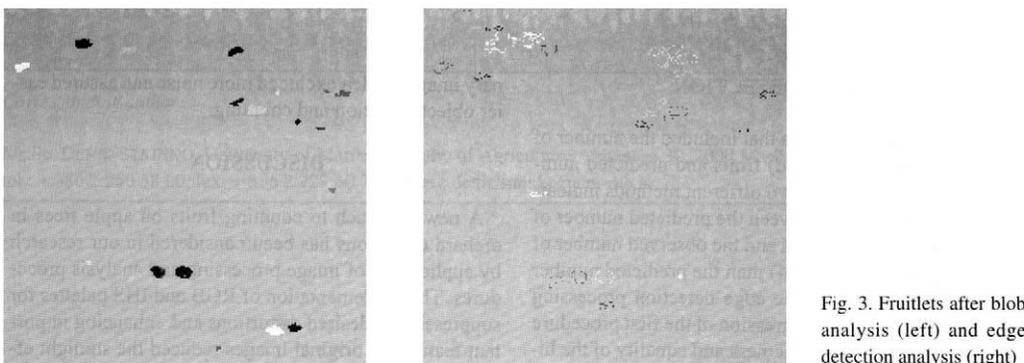


Fig. 3. Fruitlets after blob analysis (left) and edge detection analysis (right)
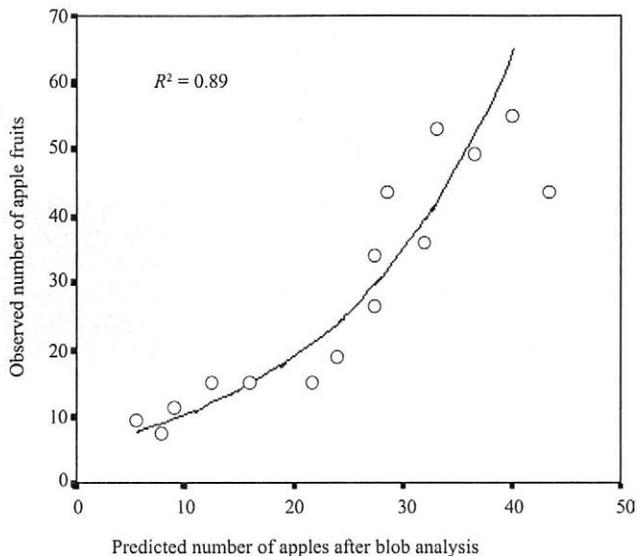
Fig. 4. Comparison of the capacity the blob analysis procedure for detecting apple fruits
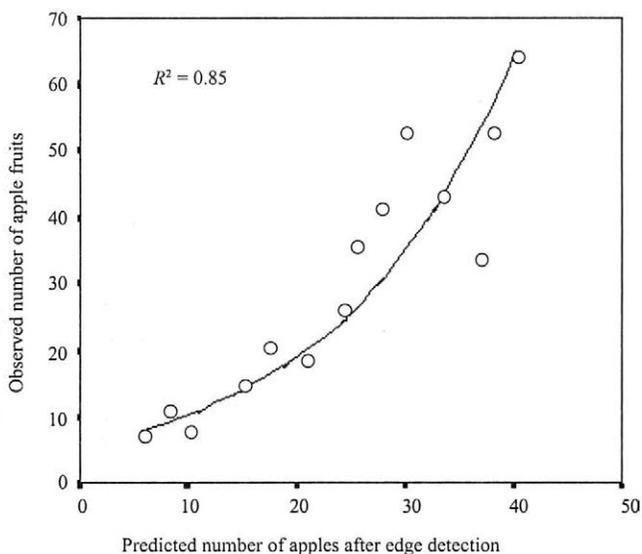


Fig. 5. Comparison on the capacity of the edge detection procedure for detecting apple fruits

## OBJECT DETECTION

Statistical analysis of data that included the number of manually counted (observed) fruits and predicted number of fruits processed by two different methods indicated a closer correlation between the predicted number of apple fruits by blob analysis and the observed number of apple fruits $R^2 = 0.89$ (Fig. 4) than the predicted number of apple fruits based on the edge detection processing $R^2 = 0.85$ (Fig. 5). Better regression of the first procedure was attributed to the compactness and equality of the bi-

nary images which excluded more noise and assured easier object detection and counting.

## DISCUSSION

A new approach to counting fruits on apple trees in orchard conditions has been considered in our research by application of image processing and analysis procedures. The implementation of RGB and IHS palettes for suppressing undesired distortions and enhancing important features in original images reduced the sunlight ef-

fect on the captured pictures and affected the speed and quality of the analysis. When counting the Jonathan fruits all images were converted to the 'hue' images by applying IHS palette prior being analysed independently of the light conditions. The detection of Golden Delicious fruits processed in the pictures captured on the bright side of the trees resulted in a very high correlation to the manually counted fruits. However, when pictures were captured on the shadow side of the trees the image procedure produced a very low correlation.

The comparison between the edge detection and blob analysis procedure resulted in no differences in the fruit detection, but the former method required more time for processing and requires creating a template because it produced three to four times more objects.

The developed vision system has proved a new procedure for counting immature apple fruits *in vivo* for some types of images of different apple varieties. To be used in the whole orchard, the artificial light conditions should be determined and evaluated in our future research.

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# Využití metod zpracování a analýzy obrazu pro počítání plodů v jabloňové výsadbě

**ABSTRAKT:** Specifické digitální snímky pořízené kamerou CCD za podmínek přirozeného osvětlení byly úspěšně použity pro počítání jablek ve výsadbě při využití metody zjišťování počtu předmětů pomocí analýzy shluků. Před vlastní analýzou pomocí metod určování obrysů a analýzy shluků byly u každého snímku použity barevné palety RGB a IHS a výsledky byly zpracovány pomocí vlastních programů vytvořených v jazyku G. Zjišťování počtu plodů všech vybarvení bylo úspěšné na snímcích pořízených na osluněné straně jabloně. Nicméně snímky, na kterých byly červené plody, musely být před další analýzou převedeny na obrazy odstínů pomocí palety IHS. Počítání červených plodů na obrazech odstínů vzniklých převodem ze snímků pořízených na osluněné straně jabloně vykazovalo velmi těsnou korelaci s počty plodů zjišťovanými při ruční sklizni ($R^2 = 0,89$), zatímco počítání drobných žlutých a žlutozelených plodů bylo méně úspěšné, protože se během přípravného zpracování nepodařilo v dostatečné míře odstranit šum. Lepší výsledky nepřinesla ani metoda určování obrysů, která si navíc ve srovnání s analýzou shluků vyžádala více času na zpracování. Tuto metodu lze akceptovat pouze pro přesná měření předmětů.

**Klíčová slova:** palety RGB a IHS; počítání jablek; určování obrysů; analýza shluků

*Corresponding author:*

Mr.Sc. DENIS STAJNKO, University of Maribor, Faculty of Agriculture, Vrbanska 30, 2000 Maribor, Slovenija
tel.: + 386 2 250 58 00, fax: + 386 2 229 60 71, e-mail: denis.stajnko@uni-mb.si

# Factors influencing growth of sweet cherries in a dense planting

J. BLAŽKOVÁ

*Research and Breeding Institute of Pomology, Holovousy, Czech Republic*

ABSTRACT: Selected characteristics and parameters were evaluated for 5 growing seasons in an experimental orchard that was established in the spring 1996. It included four rootstocks (Colt, P-HL-A, P-HL-B and P-TU-2), three cultivars (Kordia, Těchlovan and Vanda) and two different tree spacings (6 × 1 m and 6 × 1.5 m). Just after planting trees were cut back, since that time they have not been pruned or trained any more. On the basis of presented results it can be estimated that more than 50% variability of tree vigour was induced by rootstocks, up to 30% by cultivars and nearly 20% by different planting densities. Regarding rootstocks a small part of this variability could be explained by a natural growth potential that is typical of trees before fruiting, whereas the greater part of it was generated indirectly by differences in their productivity and cropping. Differences in yields between the cultivars were much less expressive. Differences in vigour of cultivars and in part also of the rootstocks were caused by differences in the length of shoots, in the rate of ramification, in the crotch angles and in the strength of apical dominance, which influences distribution and length of shoots within the canopy. Both the length of shoots and rate of ramification were depressed in direction from the top of the canopy to its basis. In middle and low parts of canopy they are also greatly influenced by shading. During the fruiting stage of trees, abundant blossoming on the bases of the last year shoots also significantly reduces the rate of ramification. Excessive growth of shoots on terminals, especially in the second year after planting on vigorous rootstocks requires attention during training of trees.

Keywords: sweet cherry; rootstocks; cultivars; tree growth; dense planting; tree spacing; ramification; shading; correlations

Present-day agricultural economics requires intensive commercial sweet cherry growing on small trees that come rapidly into production. It is necessary to get an early return on capital and allow easy, and thus relatively cheap, orchard management. Traditional sweet cherry trees are large and difficult to manage. Especially hand picking of fruits on them is very expensive and dangerous. Moreover plant protection against pests and diseases on small trees is more efficient than that with large trees. Finally, today in many areas sweet cherry orchards show a shorter economic lifespan that allows growers to profit from new cultivars.

Recent interest in growing sweet cherry trees which produce quickly following planting, bear abundantly and consistently thereafter and give rapid economic returns, has stimulated interest in higher intensity culture (more trees per hectare). For these reasons both rootstocks and cultivars inducing weak growth vigour have been recently searched (RIESEN, WAGNER 1998; SANSAVINI, LUGLI 1996; SCHIMMELPFENG 1996; STRADA et al. 1994).

Knowledge of the fundamentals of the sweet cherry tree's growing habit in the new growing system and its variability can provide a basis for the choice of the most suitable cultivars and rootstocks, their proper spacing and training. With these purposes the present research is carried out, following on the studies of growth and ramification with maiden sweet cherry trees in the nursery and on the studies of behaviour of sweet cherry trees in a very dense planting without any pruning (BLAŽKOVÁ 1997, 1998, 1999).

## MATERIAL AND METHODS

In the spring of 1996 an experimental orchard was established at Holovousy using two-year-old trees with side branches set at the height of 0.8–1.0 m. Three cultivars: Kordia, Těchlovan and Vanda on four rootstocks: Colt, P-HL-A, P-HL-B and P-TU-2 (Mazzard) were planted in two blocks with spacings 6 × 1 m and 6 × 1.5 m. From every cultivar, rootstock and spacing two replications were used with 4 or 5 trees in each. Climatic conditions of Holovousy are characterised by the average annual temperature of 8.1°C and the average annual rainfall of 650 mm. The soil was medium loam sandy with rather deep cultivated layer on gravelly substrate. The orchard was located at the altitude of 280 m a.s.l. and it was situated on a very gentle slope facing north. Just after planting these trees were cut back (a reduction of branch length was about two thirds), but since that time they have not been pruned or trained any more. No irrigation was applied in the orchard. Clean strips were kept under trees by contact herbicides whereas frequently cut sod was kept in alleys between tree rows. Fertilisers were applied according to soil analyses.

Table 1. Selected characteristics of shoot growth in sweet cherry cultivars according to their position in canopy*

| Cultivar | Position within canopy | Average length of shoots (cm) | Average maximum length of shoots (cm) | Share of last year's shoots without new growth (%) | Average maximum number of shoots per tip | Average number of new shoots on all last year's shoots | Average number of new shoots on growing last year's shoots |
|---|---|---|---|---|---|---|---|
| Kordia | terminal | 34.8 | 56.3 | 0 | 6.3 | 5.0 | 5.0 |
| | sub-terminal | 29.9 | 48.2 | 0 | 5.5 | 4.5 | 4.5 |
| | middle | 23.3 | 46.8 | 4.6 | 3.7 | 1.9 | 1.9 |
| | low | 18.2 | 36.5 | 38.9 | 1.2 | 0.6 | 1.0 |
| | total | 26.5 | 46.9 | 10.9 | 3.7 | 3.1 | 3.2 |
| Těchlovan | terminal | 40.4 | 67.3 | 0 | 6.8 | 5.5 | 5.5 |
| | sub-terminal | 38.4 | 56.0 | 0 | 6.5 | 4.8 | 4.8 |
| | middle | 29.1 | 48.5 | 13.6 | 3.2 | 1.9 | 2.2 |
| | low | 19.6 | 37.6 | 42.6 | 1.3 | 0.6 | 1.1 |
| | total | 31.9 | 52.3 | 14.1 | 4.0 | 3.3 | 3.5 |
| Vanda | terminal | 42.2 | 59.7 | 0 | 7.3 | 5.9 | 5.9 |
| | sub-terminal | 34.4 | 50.8 | 0 | 6.3 | 5.0 | 5.0 |
| | middle | 25.5 | 44.8 | 4.7 | 4.3 | 2.2 | 2.3 |
| | low | 16.5 | 34.7 | 27.5 | 1.7 | 0.8 | 1.1 |
| | total | 29.7 | 47.5 | 8.0 | 4.4 | 3.6 | 3.7 |
| Average | | 29.4 | 48.9 | 11.0 | 4.0 | 3.3 | 3.4 |
| I.S. ($P = 0.05$) | | 3.6 | 7.5 | 10.2 | 0.5 | 0.4 | 0.3 |

* Values from all rootstocks

Several measurements or countings were done on experimental trees every year after the growing season: number and length of all shoots according to their position within the canopy, characteristics necessary for calculations of canopy volume and trunk cross-section area, blossom set, fruit set and yield in kilograms. During 2000, the measuring of shoot length also angles between tips of branches and imaginary vertical axis were estimated. Furthermore in 2000 evaluation of "shading" conditions in relation to growth of shoots was made on several trees on Mazzard. In this case shoots were classified into 3 grades: 1. shoots in the upper fully lit parts of the canopy; 2. shoots in partially shaded areas between trees inside row; 3. shoots deeper inside canopy. All results were processed by ANOVA and regression analysis.

## RESULTS AND DISCUSSION

The rootstock has generally the greatest impact on tree vigour after five growing seasons since planting (Fig. 1). With all three cultivars trees on P-HL-A explicitly showed the weakest vigour. Nevertheless, trees on P-HL-B grew only slightly stronger. Unfortunately, only one cultivar (Těchlovan) was evaluated on this rootstock. Trees on the two remaining rootstocks (Colt and P-TU-2) grew vigorously and on average there was no significant difference between them. The differences between these two groups of rootstocks were larger in the total length of shoots and canopy volume and smaller in the case of trunk cross-section area.

The impact of cultivars on tree vigour was much less pronounced in comparison to rootstocks. The most vigorous from them was Těchlovan followed by Vanda, whereas Kordia produced relatively the smallest trees. Canopies of Těchlovan were the largest but those of Vanda were more compact and dense. These differences are confirmed by previous findings (BLAŽKOVÁ 1993). Considering particular combinations of rootstock and cultivar the most vigorous were trees of Těchlovan on P-TU-2 and the smallest ones those of Kordia on P-HL-A.

Results of evaluation of tree vigour in this study were to a great extent in agreement with results of evaluation
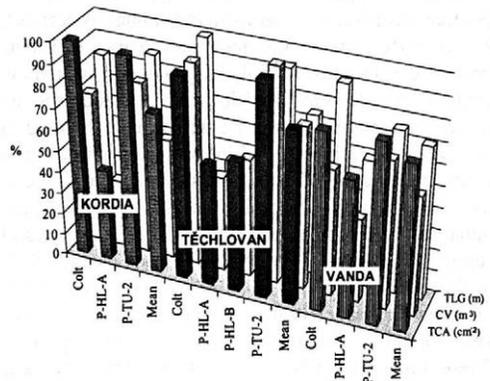


Fig. 1. Tree vigour of cultivars and rootstocks according to 3 criteria

Table 2. Selected characteristics of shoot growth according to their position in canopy in sweet cherries on different rootstocks*

| Rootstock | Position within canopy | Average length of shoots (cm) | Average maximum length of shoots (cm) | Share of last year's shoots without new growth (%) | Average maximum number of shoots per tip | Average number of new shoots on all last year's shoots | Average number of new shoots on growing last year's shoots |
|---|---|---|---|---|---|---|---|
| Colt | terminal | 39.7 | 70.2 | 0 | 7.1 | 5.6 | 5.6 |
| | sub-terminal | 38.0 | 56.5 | 0 | 6.0 | 4.7 | 4.7 |
| | middle | 26.2 | 44.4 | 7.0 | 4.4 | 1.8 | 1.9 |
| | low | 18.2 | 35.7 | 42.6 | 1.3 | 0.6 | 1.0 |
| | total | 30.6 | 51.7 | 12.4 | 4.2 | 3.3 | 3.4 |
| P-HL-A | terminal | 35.9 | 51.0 | 0 | 6.2 | 4.9 | 4.9 |
| | sub-terminal | 28.9 | 45.6 | 0 | 5.4 | 4.1 | 4.1 |
| | middle | 21.8 | 41.0 | 19.4 | 3.2 | 1.5 | 1.7 |
| | low | 12.9 | 29.4 | 62.7 | 1.5 | 0.4 | 1.1 |
| | total | 24.9 | 41.8 | 20.5 | 3.6 | 2.8 | 3.0 |
| P-HL-B | terminal | 35.2 | 54.6 | 0 | 6.0 | 4.8 | 4.8 |
| | sub-terminal | 30.7 | 51.8 | 0 | 5.5 | 4.5 | 4.5 |
| | middle | 26.4 | 48.3 | 5.9 | 3.0 | 1.9 | 2.0 |
| | low | 22.0 | 46.5 | 32.6 | 2.0 | 0.8 | 1.1 |
| | total | 28.6 | 50.3 | 9.6 | 3.6 | 3.0 | 3.1 |
| P-TU-2 | terminal | 44.0 | 73.1 | 0.0 | 7.1 | 5.9 | 5.9 |
| | sub-terminal | 34.8 | 56.2 | 0 | 6.7 | 5.5 | 5.5 |
| | middle | 30.4 | 46.2 | 1.9 | 4.7 | 2.7 | 2.8 |
| | low | 21.1 | 41.9 | 8.5 | 1.7 | 1.0 | 1.1 |
| | total | 32.6 | 54.4 | 2.6 | 4.5 | 3.8 | 3.9 |
| Average | | 29.3 | 48.8 | 10.9 | 3.9 | 3.2 | 3.3 |
| I.S. ($P = 0.05$) | | 3.9 | 11.2 | 12.9 | 0.7 | 0.5 | 0.4 |

* Values from all cultivars

of this material in the nursery (BLAŽKOVÁ 1997). The only distinct exception is however, the behaviour of Kordia on P-HL-A. In the nursery this combination was rather vigorous, whereas in the orchard it grew the most weakly. This difference agrees with the results of a previous work (BLAŽKOVÁ 1999).

The average shoot length with particular cultivars roughly corresponds to their total tree vigour, though differences in this parameter are relatively small (Table 1). For tree vigour the shoot growth on terminals is decisive. A difference between the maximum lengths of shoots (mostly the strongest central elongation shoots) and average length of shoots (which includes all side shoots) shows the strength of apical dominance. According to this criterion Těchlovan had the strongest apical dominance, followed by Kordia, whilst Vanda had the relatively weakest apical dominance. Stronger apical dominance of Těchlovan relates to wider crotch angles of setting side shoots or branches, which are characteristic of this cultivar (BLAŽKOVÁ 1997, 1998). The strength of apical dominance is also related to a share of the last year's shoots without any new (elongation) growth. These shoots are typical of the middle and lower parts of canopy.

Both the average and maximum lengths of shoots were depressed in direction from the top of the canopy to its basis. Still more emphatic, however, in this direction was a decrease in the number of shoots that were set on the growth axis. The rate of ramification was the highest with Vanda, intermediate with Těchlovan and the lowest with Kordia. Differences in length of shoots and rate of ramification within canopy were explicitly determined by angles between growth tips and the imaginary upright position (Figs. 2–5). The more horizontal position of the branch tips caused the production of shorter shoots in more restricted number. This relationship can explain weaker growth and limited ramification of Kordia. Shoots of the cultivar (even on terminals) do not often grow upright, but they bend more or less aside.

Differences in the length and number of shoots between rootstocks agree to their general vigour (Table 2). P-TU-2 (Mazzard) was the most vigorous but it did not differ significantly from Colt. A weaker growth was shown by P-HL-B and the weakest by P-HL-A. Colt had the largest difference between maximum length of shoots and average length of shoots on terminals that indicated stronger apical dominance. In spite of the vigour, the trees on Colt are easier to manage, because the branch angles were greater and the trees were more compact according to canopy volume. P-HL-A had the greatest branch angles and generated shortest shoots in the least numbers, particularly in the medium and low parts of the canopy.
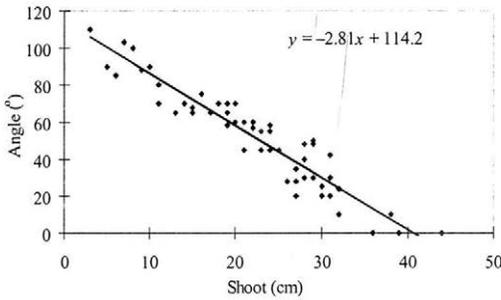
Fig. 2. Dependence of shoot length on angle between vertical axis and branch tip in Kordia cv.
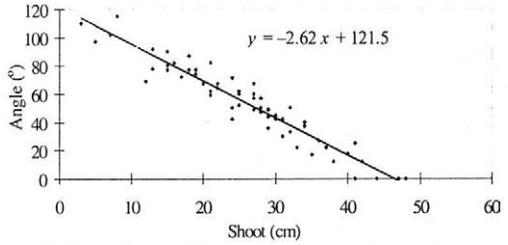


Fig. 3. Dependence of shoot length on angle between vertical axis and branch tip in Těchlovan cv.
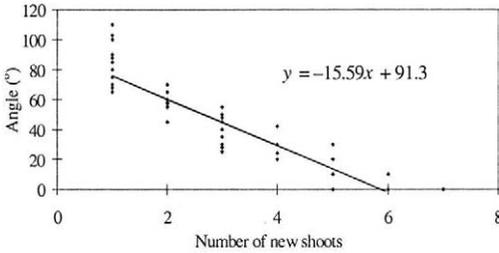


Fig. 4. Dependence of the number of new shoots on angle between vertical axis and branch tip in Kordia cv.
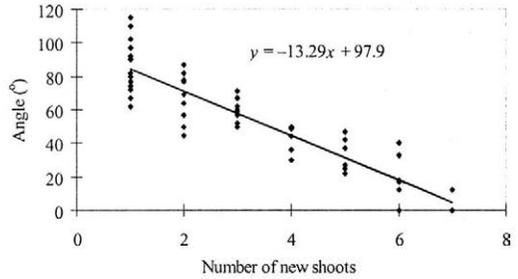


Fig. 5. Dependence of the number of new shoots on angle between vertical axis and branch tip in Těchlovan cv.

From all rootstock and cultivar combinations the weakest shoot vigour was observed in Kordia on P-HL-A (Table 3). From the point of view of shaping the tree into a small canopy excessive strong growth of terminal shoots in the second year after planting can be a problem. On both vigorous rootstocks there were shoots over 1 m in length produced (Table 3). As these very long shoots were not shortened by a cut, side laterals later developed too high on the axis. Moreover, blind wood frequently appeared on lower parts of the axis in such cases. For these cases, trees would have needed slight heading of shoots by pruning, as it was recommended before (SOUTHWICK et al. 1989).

Shading had a distinct influence on the length and number of shoots (Table 4). In conditions of slight shading shoots were longer (significantly only with Těchlovan), but after heavy shading shoots were markedly shorter with all cultivars. Both levels of shading had a marked influence on decrease in shoot number of all the cultivars. The heavier the shading was the stronger was this influence.

Table 3. Selected characteristics of shoot growth in terminals of sweet cherries according to cultivar and rootstock interaction

| Cultivar | Rootstock | Average length of shoots (cm) | Average maximum length of shoots (cm) | Maximum length of shoots in 2nd year (cm) | Average maximum number of shoots per tip | Average number of new shoots on all last year's shoots |
|---|---|---|---|---|---|---|
| Kordia | Colt | 37 | 59 | 105 | 7.5 | 5.8 |
| | P-HL-A | 27 | 42 | 66 | 6.0 | 4.2 |
| | P-TU-2 | 40 | 68 | 102 | 5.5 | 5.0 |
| Těchlovan | Colt | 47 | 73 | 117 | 7.1 | 6.3 |
| | P-HL-A | 35 | 57 | 59 | 5.4 | 4.5 |
| | P-HL-B | 40 | 68 | 57 | 7.2 | 4.5 |
| | P-TU-2 | 46 | 77 | 114 | 7.5 | 6.5 |
| Vanda | Colt | 45 | 63 | 110 | 7.4 | 6.1 |
| | P-HL-A | 36 | 53 | 56 | 6.6 | 5.5 |
| | P-TU-2 | 45 | 61 | 107 | 7.8 | 6.2 |
| I.S. ($P = 0.05$) | | 4.1 | 12.6 | – | 0.9 | 0.7 |

Table 4. Influence of shading on the length and number of shoots in the 5th year on P-TU-2

| Cultivar | Shading | Average length of shoots (cm) | Number of shoots |
|---|---|---|---|
| Kordia | none | 35.5 | 5.1 |
| | slight | 38.4 | 2.3 |
| | heavy | 20.2 | 1.4 |
| | I.S. ($P = 0.05$) | 7.7 | 0.6 |
| Těchlovan | none | 40.1 | 6.4 |
| | slight | 46.9 | 1.8 |
| | heavy | 19.9 | 1.5 |
| | I.S. ($P = 0.05$) | 5.0 | 1.1 |
| Vanda | none | 39.1 | 6.6 |
| | slight | 41.9 | 3.7 |
| | heavy | 18.4 | 2.0 |
| | I.S. ($P = 0.05$) | 8.4 | 0.8 |

Table 5. Yields per tree (kg)

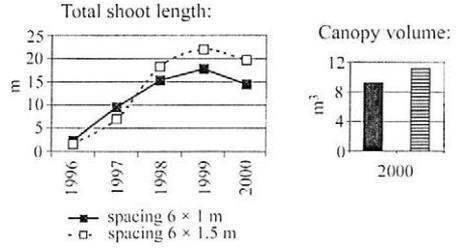| Rootstock | Cultivar | 1997 | 1998 | 1999 | 2000 |
|---|---|---|---|---|---|
| Colt | Kordia | 0.2 | 1.8 | 4.8 | 9.1 |
| | Těchlovan | 0.1 | 0.6 | 2.0 | 8.7 |
| | Vanda | 0.4 | 1.1 | 3.8 | 9.4 |
| | mean | 0.2 | 1.0 | 3.2 | 9.1 |
| P-HL-A | Kordia | 0.6 | 2.2 | 3.5 | 10.9 |
| | Těchlovan | 0.8 | 1.4 | 2.8 | 14.9 |
| | Vanda | 1.0 | 1.7 | 4.0 | 11.4 |
| | mean | 0.8 | 1.8 | 3.6 | 12.1 |
| P-HL-B | Těchlovan | 0.4 | 2 | 2.3 | 8.1 |
| P-TU-2 | Kordia | 0 | 0.2 | 2.5 | 7.6 |
| | Těchlovan | 0 | 0.3 | 0.8 | 4.8 |
| | Vanda | 0 | 0.42 | 4.2 | 8.4 |
| | mean | 0 | 0.3 | 2.5 | 6.9 |
| Total | | 0.4 | 1.2 | 3.0 | 9.7 |
| I.S. ($P = 0.05$) | | 0.9 | 0.6 | 0.7 | 0.7 |



Fig. 6. Influence of spacing on the total shoot length of Vanda cv. on P-HL-A

Favourable influence of shading on restriction of vegetative growth of cherry trees has only a very short-term effect. If the corrective pruning does not follow soon, such parts of the canopies suffer from progressive dieback as was shown in the previous work (BLAŽKOVÁ 1998).

Fruiting and yields were not primarily the main objective of this study, but since they influenced the growth of sweet cherry trees to a large extent, they are given in Table 5. P-HL-A was the most precocious rootstock with the highest yields per tree with all three trialled cultivars. Colt was more precocious than P-TU-2 despite nearly the same vigour. Among the cultivars Vanda was the most productive followed by Kordia, whereas productivity of Těchlovan was remarkable only on P-HL-A.

The vigour of sweet cherry trees seems to be the most influenced by fruit set and yield according to correlation coefficients that were calculated between selected characters (Table 6). Both the fruit set and yield are inversely related to vigour expressed by length of shoots and their number. Both these characteristics were also positively correlated with the share of shoots without new elongation growth. But of the other correlations, the most interesting is a negative relationship between flower set and rate of ramification.

Table 6. Correlation coefficients between selected characters of sweet cherry trees

| Character | TLS | MLS | SSWNG | MNSPT | ANSPT | FLS | FRS | YKG | FLSPY | FRSPY |
|---|---|---|---|---|---|---|---|---|---|---|
| TLS | 1 | | | | | | | | | |
| MLS | 0.66** | 1 | | | | | | | | |
| SSWNG | −0.71** | −0.74** | 1 | | | | | | | |
| MNSPT | 0.63** | 0.39* | −0.32* | 1 | | | | | | |
| ANSPT | 0.80** | 0.70** | −0.92** | 0.51** | 1 | | | | | |
| FLS | −0.37* | −0.42** | 0.50** | −0.45** | −0.52** | 1 | | | | |
| FRS | −0.66** | −0.51** | 0.70** | −0.27 | −0.60** | 0.54** | 1 | | | |
| YKG | −0.72** | −0.55** | 0.64** | −0.43** | −0.62** | 0.31* | 0.64** | 1 | | |
| FLSPY | −0.08 | −0.16 | 0.25 | −0.39* | −0.30* | 0.86** | 0.38* | 0.05 | 1 | |
| FRSPY | −0.25 | −0.35 | 0.24 | −0.14 | −0.28 | −0.08 | 0.04 | 0.23 | −0.12 | 1 |

Notes: TLS – total length of shoots, MLS – maximum length of shoots, SSWNG – share of shoots without new growth, MNSPT – maximum number of shoots per tip, ANSPT – average number of shoots per tip, FLS – flower set, FRS – fruit set, YKG – yield (kg) per tree, FLSPY – flower set of previous year, FRSPY – fruit set of previous year

The last factor being studied in this experiment was different tree spacings (Fig. 6). An increase of tree density per unit of area by 50% decreased the total vigour of trees by nearly 20%. This reduction of vigour is an effect of shading and competition for nutrients and water supply.

## CONCLUSIONS

Summarising the results, which were obtained in this study, it can be estimated that more than 50% variability of tree vigour was induced by rootstocks, up to 30% by cultivars and nearly 20% by different planting densities (Figs. 1 and 6). Regarding rootstocks a smaller part of this variability can explained by a natural growth potential that is typical of trees before fruiting, whereas the larger part of it is generated indirectly by differences in their productivity and cropping. Differences in yields between the cultivars were much less expressive. Differences in vigour of cultivars and in part also of rootstocks are caused by differences in length of shoots, rate of ramification, crotch angles and strength of apical dominance, which influences shoot distribution and growth within canopy. Both the length of shoots and rate of ramification are depressed in direction from top of the canopy to its basis.

Differences in the length of shoots and rate of ramification within the canopy are determined by angles of growth tips with imaginary upright position. In middle and low parts of canopy they are also greatly influenced by shading. During the fruiting stage of trees, abundant blossoming on the bases of the last year shoots also sig- nificantly reduces the rate of ramification. Excessive growth of shoots on terminals, especially in the second year on vigorous rootstocks requires attention during the training of trees.

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## Faktory ovlivňující růst třešní v husté výsadbě

**ABSTRAKT**: Vybrané charakteristiky a parametry byly po dobu pěti vegetačních období hodnoceny v pokusné výsadbě, která byla založena na jaře 1996. Tři odrůdy (Kordia, Těchlovan a Vanda) naštěpované na čtyřech podnožích (Colt, P-HL-A, P-HL-B a P-TU-2) byly v této výsadbě vysazeny ve dvou různých sponech (6 × 1 m a 6 × 1,5 m). Po výsadbě byly stromky zpětně seříznuty, avšak později již nebyly upravovány řezem ani jinak tvarovány. Na základě získaných výsledků lze odhadnout, že více než 50 % variability ve vzrůstnosti stromů bylo indukováno podnoží, do 30 % odrůdou a téměř 20 % rozdílným sponem. V případě podnoží menší podíl této variability lze vysvětlit rozdíly v růstovém potenciálu stromů před vstupem do plodnosti, zatímco větší podíl této variability byl vyvolán nepřímo rozdíly v jejich výnosech a plodnosti. Rozdíly ve výnosech mezi odrůdami byly mnohem méně výrazné. Rozdíly ve vzrůstnosti odrůd a částečně také podnoží byly způsobeny rozdíly v délce výhonů, v intenzitě rozvětvování, v úhlech odklonu výhonů a větví a v síle apikální dominance, která ovlivňuje rozmístění a délku výhonů v rámci koruny. Jak délka výhonků, tak i intenzita rozvětvování jsou potlačovány ve směru od vrcholu koruny k její bázi. Ve středních a spodních částech koruny jsou rovněž značně ovlivňovány zastíněním. Intenzitu rozvětvování po vstupu stromů do období plodnosti rovněž významně ovlivňuje intenzita kvetení na bázích jednoletých výhonů. Nadměrný růst výhonů na terminálech, zejména v druhém roce po výsadbě u stromů na bujně rostoucích podnožích, vyžaduje pozornost při tvarování stromů.

**Klíčová slova**: třešeň; podnože; odrůdy; růst stromů; hustá výsadba; spony; rozvětvování; zastínění; korelace

*Corresponding author:*

Ing. JITKA BLAŽKOVÁ, Výzkumný a šlechtitelský ústav ovocnářský Holovousy, s.r.o., Holovousy 1, 508 01 Hořice v Podkrkonoší, Česká republika

tel.: + 420 435 69 28 21, fax: + 420 435 69 28 33, e-mail: blazkova@vsuo.cz

# The effect of various methods of thinning on the quality of apricot fruits

**B. Krška, M. Močička**

*Mendel University of Agriculture and Forestry Brno, Faculty of Horticulture, Lednice na Moravě, Czech Republic*

**ABSTRACT**: The aim of this study was to analyse the effect of various methods of thinning on the quality of apricot fruits. Hand thinning, thinning by pruning, thinning by pruning followed by chemical treatment and various methods of chemical thinning were used on cultivars Hargrand and Lemira (1997) and Lejuna and LE-4725 (1998), respectively. Within this two-year period of testing hand thinning was the most suitable method. Its suitability was mostly demonstrated by the higher weight of fruits (+51% on average), improved attractiveness, increased percentage of extra large fruits, decreased percentage of 2nd quality categories and by acceleration of fruit ripening. Soluble solids increased by 28% on average. Thinning by pruning was completely unsuitable and no increase in fruit weight was observed. Its unsuitability was demonstrated also by the lower attractiveness of fruits, decreased share of extra class and increased share of fruits of 2nd category of quality. The harvest time was delayed due to a decrease in soluble solids (by −17% on average). In the case of cv. Hargrand, no effects on fruit attractiveness, quality, and maturity were observed. The method of thinning by pruning combined with the chemical treatment showed no positive effect either. Only in cv. Hargrand was a positive influence of the chemical thinning with the product Racine demonstrated on the fruit drop by +9% when applied 20 days after the blossom in 1997. Its efficiency was manifested as increased weight (by +47%) and attractiveness of fruits, and a higher share of extra class fruits. Chemical thinning did not influence the fruit drop. Spraying 30 days after the blossom showed a negative effect on fruit drop (+16% on average). This resulted in a decreased weight of fruits (by 24% on average), lower attractiveness, and a reduced proportion of extra class fruits. The share of fruits of the 2nd class of quality increased. Based on the obtained results it is possible to conclude that the hand thinning in apricots is the most efficient method of reducing fruit numbers.

**Keywords**: apricot; thinning; fruit quality; standards of quality

In apricots, a great variability in yields is caused by climatic conditions existing during the winter and early spring, by too high harvests and also by some other factors.

An optimum yield and corresponding quality of the crop depend on the numbers of fruits set on annual shoots within the whole tree. Numbers of fruits and their uniform distribution within the canopy should correspond with the numbers of leaves. VACHŮN and JINDROVÁ (1976) mentioned that altogether 10 leaves per one fruit are necessary for a normal course of differentiation of flower buds and for the development of each fruit in the cultivar Velkopavlovická.

A decrease in too high numbers of fruits shows positive effects on the growth of trees, fruit quality and formation of flower buds in the next year. Thinning is important especially in cultivars showing a small variability in yields because it increases the proportion of extra quality fruits (BLAŽEK et al. 1983). Thinning will be necessary especially in the case of new cultivars that show a higher resistance to frost on the one hand and a tendency to produce more but smaller fruits on the other (SVOBODA 1999).

Hand thinning could become a routine method of reduction of fruit numbers in apricot orchards because it improves the weight and quality of fruits and contributes to more uniform ripening (SANSAVINI, GIANNERINI 1991).

Chemical treatment with NAA acids (SURÁNYI 1982) or gibberellins (CLANET et al. 1983) has been described as a possible method of thinning, but it is not reliable and depends on air humidity and temperature too much.

SOUTHWICK et al. (1995) described another method of preventing too high yields using a reduction of flower buds by spraying the trees with 100 ppm of GA$_3$, 2 to 6 weeks after the harvest. This treatment eliminated chemical and/or hand thinning in cv. Patterson.

## MATERIAL AND METHODS

The suitability of five methods of thinning for a reduction of excessive numbers of fruits was tested in different apricot cultivars within the period of 1997–1998.

All cultivars were planted within the framework of pilot experiments in the demonstration area of the Institute of Fruit Growing and Viticulture, Faculty of Horticulture at Lednice.

*The experiment involved the following cultivars:*

Lejuna – an early cultivar showing a tendency to too high yields and small fruits (the average size of fruits ranges from 35 to 45 g). This cultivar is suitable either for direct consumption or for the freezing of whole fruits.

LE-4725 – a seedling originating from an open pollination of the cultivar Lejuna. A very fertile hybrid, very early, with a tendency to too high yields. Average weight of fruits 40 g, the maximum weight as much as 57 g.

Hargrand – a Canadian cultivar, semi-early, with large fruits of the weight 65–85 g.

Lemira – by 5 days earlier than Velkopavlovická, frost-resistant, fruit weight without thinning 50–55 g.

The experiment was established in 1993 on a flat plot with degraded Chernozem, loamy with sandy subsoil. Climatic conditions: average annual temperature (1901–1990) 9.1°C, annual rainfall sum 495 mm.

During the winter 1996/1997, a marked damage of flower buds was observed. In 1997/1998 season, the trees were frost damaged in the period of flowering and after the blossom. In 1998, the course of weather conditions during the period of dormancy was very bad; January was very warm nearly all month. A wave of frosts occurred at the end of this month and this resulted in great damage to flower buds.

All trees were trained to an open centre. Trees were planted in rows with spacing 6 × 4 m. Rootstock is M-LE-1 (an apricot seedling). Normal tillage were carried out in this orchard during the whole experimental period.

*Methods of thinning in 1997:*

1. hand thinning
2. thinning by pruning
3. thinning by pruning + chemical treatment
4. chemical treatment 20 days after blossom (chemical treatment A)
5. chemical treatment 30 days after blossom (chemical treatment B).

– Control – variant without any thinning of fruits.
– Hand thinning was carried out 20 days after the end of blossom. On long annual shoots, individual small fruits were removed to the distance of 7–10 cm, on fruiting spurs only 1 fruit was left.
– Thinning by pruning was carried out 20 days after the end of blossom. Semi-scaffold limb branches with small fruits, situated in morphologically identical parts of canopy were shortened to a half so that the number of fruits was also reduced to a half. Semi-scaffold limbs were cut to wood of the age 2–3 years.
– Thinning by pruning + chemical treatment was carried out 20 days after the end of blossom. Pruning was the same as in variant without chemical treatment. After pruning, experimental trees were treated with Racine (conc. 0.15%).

– There were two variants of chemical thinning. Racine (conc. 0.15%) was applied either 20 or 30 days after the end of blossom (chemical treatments A and B, resp.). Spraying was performed in morning hours.

In 1997, cultivars Hargrand and Lemira were used for thinning of flowers and small fruits.

Individual methods of thinning were applied not only on selected skeletal branches (that were used for the evaluation of general results) but also within the whole crown. For each cultivar altogether 18 trees were selected and each variant consisted of 3 trees (i.e. three replications).

*Methods of thinning in 1998:*

All methods of thinning were the same as in 1997 with the exception of variants with chemical treatments. In the variant pruning + chemical treatment, Racine was applied 7 days after the end of blossom. In the case of chemical treatment A, application of Racine was carried out already 7 days after the end of blossom and the variant B was not used. For each cultivar altogether 15 trees were selected and each variant involved also 3 trees.

In 1998, cultivars Lejuna and LE-4725 were selected for the evaluation due to smaller damage of flower buds and fruitlets by frost.

The product Racine, used in this experiment, has the same composition as Atonik and contains an addition of α–naphthylacetic acid (NAA).

Pomological characteristics – attractiveness and uniformity of fruits were evaluated using the method described by VACHŮN et al. (1991), in accordance with the Standard ČSN 46 3020 and also with the international standard of OECD. The weight of fruits was estimated as an average of 90 fruits from each variant (i.e. 30 fruits from each replication). Fruits were weighed, their uniformity was rated, and expressed as percentage of control. Using 90 fruits of each variant, the following parameters were evaluated: attractiveness of fruits, their uniformity, and correspondence with standards. Dry matter content of fruits was measured by refractometry.

The results were statistically processed using a single-factorial method of variance analysis. The significance of differences was tested by means of intervals of confidence at significance level of 95 and 99%.

## RESULTS AND DISCUSSION

As compared with the control, the highest increase in fruit weight was observed in the variant with hand thinning. For individual cultivars, statistical analysis indicat-

Table 1. Evaluation of average fruit weight in 1997

| Cultivar | Hargrand | | | Lemira | | |
|---|---|---|---|---|---|---|
| Variant | Fruit weight (g) | Test criterion $F$ | % of control | Fruit weight (g) | Test criterion $F$ | % of control |
| Control | 54.58 | | 100 | 54.12 | | 100 |
| Hand thinning | 90.04 | 490.3[++] | 165 | 58.77 | 18.8[+] | 109 |
| Pruning | 57.78 | 8.6 | 106 | 50.44 | 15.3 | 93 |
| Pruning + chemical | 80.46 | 263.9[++] | 147 | 50.12 | 4.1 | 93 |
| Chemical A | 59.77 | 29.2[+] | 109 | 52.20 | 3.7 | 96 |
| Chemical B | 43.89 | 48.2[++] | 80 | 39.31 | 204.6[++] | 73 |

Table 2. Evaluation of average fruit weight in 1998

| Cultivar | | LE-4725 | | | Lejuna | |
| Variant | Fruit weight (g) | Test criterion F | % of control | Fruit weight (g) | Test criterion F | % of control |
|---|---|---|---|---|---|---|
| Control | 26.06 | | 100 | 19.02 | | 100 |
| Hand thinning | 43.00 | 71.5 [++] | 165 | 31.08 | 591.7[++] | 163 |
| Pruning | 24.94 | 0.8 | 96 | 20.54 | 7.2 | 107 |
| Pruning + chemical | 24.60 | 1.2 | 94 | 19.58 | 1.4 | 103 |
| Chemical A | 24.42 | 1.5 | 94 | 20.30 | 2.7 | 107 |

ed an increase ranging from 63 to 65%. In the case of cv. Lemira this increase was only by 9%, but also this difference was significant. It is possible that in the control the number of fruits could be optimal for the nutrition of leaves (Svoboda, pers. commun. 1999). Thinning by pruning did not show any positive effect on an increase in fruit weight. However, no decrease in the weight of fruits was observed either, probably due to the unsuitable time of pruning. When applied 20 days after the end of blossom, pruning resulted in growth depression and all metabolic processes were retarded (Svoboda, pers. commun. 1999). In the variant thinning by pruning + chemical treatment, growth depression occurred similarly like in the case of pruning alone and the obtained results were similar like after thinning by pruning. In cv. Hargrand, application of the product Racine 20 days after the end of blossom resulted in a loss of 9% of fruits and a positive effect of this loss was reflected in an increased weight of fruits by 47%, as compared with control. There was a highly significant difference between the variants thinning by pruning and pruning + chemical treatment in the cv. Hargrand. A possible explanation of this fact is a larger assimilation area available for the remaining fruits. Application of Racine spray 7 days after the end of blossom did not affect the weight of fruits. Similarly like thinning by pruning, chemical thinning did not show any significant effect on the average weight of fruits either. A significant and positive effect on the weight of fruits (by 9.0%) was observed only in the case of cv. Hargrand. Application of Racine 20 days after the end of blossom reduced the number of fruits by 8% and increased their average weight (cv. Hargrand). On the other hand, chemical treatment showed to be quite unsuitable 30 days after the end of blossom. The loss of fruits was reduced by 15–17% and this resulted in a reduced weight of fruits by 20–27%. The NAA-product Racine influences the development of fruits in two ways. When the endosperm is liquid, it causes the loss of fruits

while when it is hard, it prevents such a loss. Thirty days after the end of blossom the endosperm was hard and for that reason there was no loss of small fruits after the application of Racine (Svoboda, pers. commun. 1999). The application 7 days after the end of blossom did not result in any loss, and, thus, an increase in the weight of fruits (Tables 1, 2).

The variant of hand thinning influenced the attractiveness and uniformity of fruits, especially as far as the weight of fruits and their coloration were concerned. Thinning by pruning, on the other hand, resulted in growth depression and thereafter in reduced attractiveness (pale coloration) and uniformity of fruits. No changes in these two parameters were recorded only in the case of cv. Hargrand. The variant of pruning + chemical treatment improved attractiveness and uniformity of fruits in cv. Hargrand due to their increased weight. In other cultivars, the weight of fruits was reduced. Chemical thinning A did not influence either attractiveness or uniformity of fruits. A negative effect was observed in chemical treatment B. Attractiveness of fruits was decreased due to their reduced loss and lower weight. As far as the uniformity of fruits was concerned, it was improved in cv. Hargrand while in cv. Lemira this trait was deteriorated. Reduced attractiveness was manifested in the reduced weight and size of fruits (Table 3).

The highest increase in soluble solids was observed in fruits after the hand thinning. Their contents were reduced by 19–34% (Tables 5, 6) and the harvest ripeness was accelerated. On the other hand, thinning by pruning reduced soluble solids by 12–26% and the harvest was delayed. This could be explained on the basis of a reduced leaf area after pruning. No effect was demonstrated in cv. Hargrand. Similarly like in the variant of pruning, also in the variant of pruning + chemical treatment a highly significant and significant effect on the reduction of soluble solids content was observed (by 12–29%). Chemical treatment A did not show any sig-

Table 3. Evaluation of attractiveness and uniformity of fruits in 1997

| Cultivar | Hargrand | | Lemira | |
| Variant | Uniformity (1–9) | Attractiveness (1–9) | Uniformity (1–9) | Attractiveness (1–9) |
|---|---|---|---|---|
| Control | 6 | 7 | 7 | 7 |
| Hand thinning | 9 | 9 | 8 | 8 |
| Pruning | 6 | 7 | 5 | 3 |
| Pruning + chemical | 8 | 8 | 5 | 3 |
| Chemical A | 6 | 7 | 7 | 7 |
| Chemical B | 7 | 5 | 6 | 5 |

Table 4. Evaluation of attractiveness and uniformity of fruits in 1998

| Cultivar | LE-4725 | | Lejuna | |
|---|---|---|---|---|
| Variant | Uniformity (1–9) | Attractiveness (1–9) | Uniformity (1–9) | Attractiveness (1–9) |
| Control | 6 | 6 | 6 | 6 |
| Hand thinning | 8 | 9 | 9 | 8 |
| Pruning | 4 | 3 | 4 | 4 |
| Pruning + chemical | 4 | 3 | 4 | 4 |
| Chemical A | 6 | 6 | 6 | 6 |

Table 5. Evaluation of soluble solids in 1997

| Cultivar | Hargrand | | | Lemira | | |
|---|---|---|---|---|---|---|
| Variant | Soluble solids (%) | Test criterion $F$ | % of control | Soluble solids (%) | Test criterion $F$ | % of control |
| Control | 10.80 | | 100 | 7.00 | | 100 |
| Hand thinning | 12.83 | 35[+] | 119 | 9.25 | 157[++] | 132 |
| Pruning | 9.50 | 14 | 88 | 5.16 | 120[++] | 74 |
| Pruning + chemical | 9.70 | 10.6 | 90 | 5.00 | 999[++] | 71 |
| Chemical A | 10.90 | 0.1 | 101 | 7.30 | 3.1 | 104 |
| Chemical B | 10.43 | 0.9 | 97 | 6.75 | 4.6 | 97 |

Table 6. Evaluation of soluble solids in 1998

| Cultivar | Lejuna | | | LE-4725 | | |
|---|---|---|---|---|---|---|
| Variant | Soluble solids (%) | Test criterion $F$ | % of control | Soluble solids (%) | Test criterion $F$ | % of control |
| Control | 10.60 | | 100 | 8.67 | | 100 |
| Hand thinning | 13.33 | 128[++] | 126 | 11.67 | 162[++] | 134 |
| Pruning | 9.33 | 32[+] | 88 | 7.33 | 32[+] | 84 |
| Pruning + chemical | 8.67 | 34[+] | 81 | 7.67 | 18[+] | 88 |
| Chemical A | 10.60 | 0 | 100 | 8.33 | 2 | 96 |

nificant effect on sugar content. In the case of the variant chemical treatment B no effect on soluble solids was observed either (Tables 5, 6).

When evaluating fruits according to commercial standards it could be observed that hand thinning increased the share of extra large fruits and reduced the percentages of those of the 1st and non-standard classes. This fact was more explicit in the case of evaluation in accordance with the Czech national standard (ČSN) because it is stricter than the international ones. In the case of cv. Lejuna, there were no marked differences when evaluating their fruits in accordance with the Czech standard because they are small, and although their weight was increased, it did not reach the required size.

On the other hand, thinning by pruning reduced the proportion of extra large fruits and increased the percentages of the 1st, the 2nd and non-standard classes of quality due to growth depression. In cv. Hargrand these parameters were the same as in the control. In the variant of thinning by pruning + chemical treatment, the quality of cv. Hargrand was improved due to an increased weight of fruits. In other cultivars the share of extra large fruits decreased and those of the 1st, 2nd and non-standard classes of quality were increased. Chemical treatment A did not influence the percentages of the fruit size classes as compared with the control. In the case of cv. Hargrand,

the percentage of extra large fruits increased due to their loss and increased weight, while that of the 1st class of quality decreased. In 1997, chemical treatment B reduced the proportion of extra large fruits due to a lower loss of fruits and their reduced weight on the one hand, and increased percentages of the 1st and, especially, the 2nd class of quality on the other (Tables 7, 8).

It can be concluded that hand thinning is the most efficient and positive but also highly laborious intervention. The use of this method in apricots is not justified due to its high costs (Svoboda, pers. commun. 1998). On the other hand, thinning by pruning was shown to be completely inefficient. SVOBODA (1997), however, mentioned that thinning by pruning proved to be the best method of all as far as the quality, weight of fruits, and the general condition of fruit-bearing wood were concerned. This observation concerned detailed spring pruning that was carried out prior to the beginning of blossom. In our experiment thinning by pruning was carried out 20 days after blossom. Thinning by pruning in combination with chemical treatment gave similar results like pruning itself while chemical thinning alone could be evaluated as inefficient. In apricots, the reduction of fruitlets by chemical treatment is not very elaborate, and for that reason it is mostly inefficient (Svoboda, pers. commun. 1999).

Table 7. Evaluation of fruit quality in LE-4725 cv. according to Czech (ČSN) and OECD standards in 1998

| Variant of thinning | Quality of fruits | ČSN (%) | OECD (%) | Differences from the control (%) ČSN | OECD |
|---|---|---|---|---|---|
| Hand thinning | Extra class | 6.67 | 53.33 | 100 | 33.32 |
| | Fruit quality class I | 83.33 | 46.67 | 100 | −22.22 |
| | Fruit quality class II | 10 | | −90 | |
| | Nonstandard | 0 | 0 | 0 | 0 |
| Pruning | Extra class | 0 | 23.34 | 0 | −41.65 |
| | Fruit quality class I | 0 | 63.33 | 0 | 5.55 |
| | Fruit quality class II | 86.67 | | −13.33 | |
| | Nonstandard | 13.33 | 13.33 | 100 | 100 |
| Pruning + chemical | Extra class | 0 | 18.89 | 0 | −52.77 |
| | Fruit quality class I | 0 | 67.78 | 0 | 12.97 |
| | Fruit quality class II | 86.67 | | −13.33 | |
| | Nonstandard | 13.33 | 13.33 | 100 | 100 |
| Chemical A | Extra class | 0 | 38 | 0 | −5 |
| | Fruit quality class I | 0 | 62 | 0 | 3.33 |
| | Fruit quality class II | 100 | | 0 | |
| | Nonstandard | 0 | 0 | 0 | 0 |
| Control | Extra class | 0 | 40 | | |
| | Fruit quality class I | 0 | 60 | | |
| | Fruit quality class II | 100 | | | |
| | Nonstandard | 0 | 0 | | |

Table 8. Evaluation of fruit quality in Hargrand cv. according to Czech (ČSN) and OECD standards in 1997

| Variant of thinning | Quality of fruits | ČSN (%) | OECD (%) | Differences with the control (%) ČSN | OECD |
|---|---|---|---|---|---|
| Hand thinning | Extra class | 70 | 73.33 | 90.89 | 11.87 |
| | Fruit quality class I | 28.88 | 26.67 | −43.49 | −22.56 |
| | Fruit quality class II | 1.11 | | −90.92 | |
| | Nonstandard | 0 | 0 | 0 | 0 |
| Pruning | Extra class | 37.78 | 64.44 | 3.03 | −1.69 |
| | Fruit quality class I | 51.11 | 35.55 | 0 | 3.22 |
| | Fruit quality class II | 11.11 | | −9.08 | |
| | Nonstandard | 0 | 0 | 0 | 0 |
| Pruning + chemical | Extra class | 66.67 | 68.89 | 81.81 | 5.09 |
| | Fruit quality class I | 32.22 | 31.11 | −36.96 | −9.67 |
| | Fruit quality class II | 1.11 | | −90.92 | |
| | Nonstandard | 0 | 0 | 0 | 0 |
| Chemical A | Extra class | 43.33 | 67.77 | 18.16 | 3.39 |
| | Fruit quality class I | 43.33 | 32.22 | −15.22 | −6.45 |
| | Fruit quality class II | 13.33 | | 9.08 | |
| | Nonstandard | 0 | 0 | 0 | 0 |
| Chemical B | Extra class | 20 | 58.89 | −45.46 | −10.16 |
| | Fruit quality class I | 51.1 | 41.11 | −0.02 | 19.37 |
| | Fruit quality class II | 28.89 | | 136.42 | |
| | Nonstandard | 0 | 0 | 0 | 0 |
| Control | Extra class | 36.67 | 65.55 | | |
| | Fruit quality class I | 51.11 | 34.44 | | |
| | Fruit quality class II | 12.22 | | | |
| | Nonstandard | 0 | 0 | | |

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## Vliv různých způsobů probírky na kvalitu plodů meruněk

**ABSTRAKT:** Cílem práce bylo zhodnotit vliv probírky plodů na kvalitu plodů u meruněk a vyhodnotit její účinnost z hlediska jednotlivých způsobů probírek. Pokus byl založen v poloprovozu meruněk na demonstračním pozemku Ústavu ovocnictví a vinohradnictví Zahradnické fakulty v Lednici na Moravě. V pokusu byly provedeny tyto varianty: ruční probírka, probírka řezem, probírka řezem v kombinaci s probírkou chemickou a chemická probírka a její varianty. Jednotlivé způsoby probírek se porovnávaly s kontrolními, neprobíranými stromy. V pokusu byly hodnoceny následující ukazatele – hmotnost plodů, atraktivnost a vyrovnanost plodů, jakost plodů a refraktometrická sušina. Za dvouleté období bylo zjištěno: nejvhodnějším způsobem probírky plodů u meruněk je *ruční probírka*. Vhodnost byla prokázána ve zvýšení hmotnosti plodů v průměru o +51 %. Její účinnost se projevila také ve zvýšení atraktivnosti plodů, zvýšení podílu výběru a snížení I., II. jakosti plodů především u normy ČSN a urychlení dozrávání plodů, zvýšení refraktometrické sušiny v průměru o +28 %. Pouze u odrůdy Lemira se zvýšila hmotnost plodů průkazně pouze o + 9 %. Zcela nevhodnou se projevila *probírka řezem*. Nebyl prokázán vliv na zvýšení hmotnosti plodů. Nevhodnost se projevila také ve snížení atraktivnosti plodů, snížení výběru a zvýšení I., II. jakosti plodů a nestandardu u norem ČSN a OECD a oddálení termínu sklizně, snížení refraktometrické sušiny v průměru o –17 %. U odrůdy Hargrand nebyl prokázán vliv na snížení atraktivnosti, jakosti plodů a oddálení termínu sklizně. *Probírka řezem v kombinaci s chemickou* neprokázala pozitivní vliv. Vlivem neprůkaznosti chemické probírky na propad plodů se závěry shodují s probírkou řezem. Pouze u odrůdy Hargrand byl prokázán pozitivní vliv chemické probírky přípravkem Racine 20 dní po odkvětu v roce 1997 na propad plodů o +9 %. Její účinnost se projevila ve zvýšení hmotnosti plodů o +47 %, zvýšení atraktivnosti plodů, podílu výběru a snížení podílu I. a II. jakosti plodů u normy ČSN. Postřik sedm dní po odkvětu v roce 1998 neprokázal vliv na propad plodů. Chemická probírka neprokázala dobrou účinnost na propad plodů. Účinnost byla opět prokázána u odrůdy Hargrand při postřiku 20 dní po opadu květů v roce 1997. Účinnost byla prokázána ve zvýšení propadu plodů o +8 % a zvýšení hmotnosti plodů v porovnání s kontrolou o +9 %. Zvýšil se podíl výběru a snížil se podíl I. jakosti plodů u normy ČSN. Naopak postřik 30 dní po odkvětu v roce 1997 prokázal negativní vliv chemické probírky na zabránění propadu plodů v průměru o +16 %. Tento vliv se projevil ve snížení hmotnosti plodů v průměru o –24 %, snížení atraktivnosti, snížení výběru a zvýšení II. jakosti plodů hlavně u normy ČSN. Postřik sedm dní po odkvětu v roce 1998 neměl průkazný vliv na propad plodů. Je možné konstatovat, že ruční probírka je nejúčinnějším způsobem probírky plodů u meruněk. Na základě její vysoké pracovní náročnosti, která je o +86 % vyšší ve srovnání s chemickou probírkou, ji lze doporučit pro malopěstitele na malých plochách. Regulace násady plodů u meruněk je nedořešený problém, kterému bude nutné i nadále věnovat patřičnou pozornost.

**Klíčová slova:** meruňka; probírka; kvalita plodů; norma jakosti

*Corresponding author:*

Dr. Ing. BORIS KRŠKA, Mendelova zemědělská a lesnická univerzita, Brno, Zahradnická fakulta, Ústav ovocnictví a vinohradnictví, Valtická 337, 691 44 Lednice na Moravě, Česká republika
tel.: + 420 627 34 01 05–7, fax: + 420 627 34 01 59, e-mail: krska@zf.mendelu.cz

# Use of HPLC for rapid determination of vitamin C in garlic

R. Pokluda, K. Petříková

*Mendel University of Agriculture and Forestry, Brno, Faculty of Horticulture, Lednice na Moravě, Czech Republic*

ABSTRACT: Vitamin C determination is possible by different methods and high performance liquid chromatography (HPLC) is one of the most important. An isocratic reverse-phase HPLC method of L-ascorbic acid determination in garlic was tested. The column SGX C18 (150 × 3 mm) with 7 μm sized sorbent particles was used. The mobile phase consisted of tetrabutylammonium hydroxide, oxalic acid and water. Six garlic varieties from 2 different localities were included in the experiment. Samples were mixed with a mobile phase, filtered and centrifuged before analysis. The detection limit of this method is 1 mg/kg of vitamin C. Mean retention time was 3.25 minutes, reproducibility was good with RSD (relative standard deviation) only 1.9%. The stability of vitamin C in samples mixed with mobile phase was given within 6 hours. Mean vitamin C content in garlic was 315 mg/kg in fresh matter (f.m.). The mean lowest level was shown by variety Vladan (273 mg/kg f.m.), while the mean highest one was found in Vekan (403 mg/kg f.m.). Using this method, it was possible to quantify vitamin C in less than 18 minutes with precision and accuracy.

Keywords: HPLC; vitamin C; analysis; garlic

Vitamins belong to the nutritionally important substances of vegetables and vitamin C plays a major role among them. Vitamin C content in garlic is very variable, from 92 mg/kg f.m. (KOPEC 1998), through 312 mg/kg f.m. (ANONYMOUS 1999) to 665 mg/kg f.m. (KONVIČKA 1998).

Common and traditional Tillman's titration method of vitamin C analysis (DAVÍDEK 1977) does not require any special laboratory equipment, but its sensitivity and reproducibility is mostly based on personal experiences during the process of colour changes. One of the sensitive methods is isocratic HPLC (VANDERSLICE, HIGGS 1993; CZERWIECKI, WILCZYNSKA 1999). ORUNA-CONCHA et al. (1998) used UV detection (254 nm) with C18 column. On the other hand, ROMERO et al. (1992) tested $NH_2$ column of the size 250 × 4.6 mm. Optimal sorbent size is in the range of 3–7 μm.

A mobile phase composed of 40 mM sodium acetate, 5 mM tetrabutylammonium hydrogen sulphate and 0.05% meta-phosphoric acid was used by BEHRENS and MADERE (1992). ROMERO et al. (1992) used deionised water with sulphuric acid (pH 2.2) and ALBRECHT and SCHAFER (1990) mentioned a mobile phase prepared from water solution of 5 mM $KH_2PO_4$ (pH 4.6) and acetonitrile at a ratio 30:70 (v/v).

A constant temperature in the column system increases reproducibility of measurements. PERÉZ et al. (1997) published the method with column temperature at 23°C. Sample preparation must be rapid due to the low stability of L-ascorbic acid. Its preservation with dithiothreitol was maintained. Such samples can be stored up to 8 hours

before analysis (MOSER et al. 1999). BENLLOCH et al. (1993) found the good stability of vitamin C if samples were extracted in acid conditions. Phosphoric acid prevents the decay of L-ascorbic acid within a few hours after sample preparation (GENNARO, BERTOLO 1990). ALBRECHT and SCHAFER (1990) extracted vitamin C from samples with 5% meta-phosphoric acid solution.

The length of analysis depends on the method and sample type. Total elution time of 22 minutes in strawberry analysis was reported by PERÉZ et al. (1997). Retention time of vitamin C was 6.37 minutes if Spherisorb ODS column was used (ROMERO et al. 1992). A commonly acceptable detection limit is 0.8–1 mg/kg of vitamin C (ALBRECHT, SCHAFER 1990; MOSER et al. 1999).

The aim of this study was to test the rapid HPLC method of vitamin C determination in garlic by evaluation of reproducibility, detection limit, vitamin C sample stability and comparison of vitamin C content in selected garlic varieties from two different localities.

## MATERIAL AND METHODS

### Plant material

The 6 following garlic (*Allium sativum* L.) varieties were used in the experiments: Ropal, Vekan, Alan, Vladan, Lukan and new breeding P10E (Ing. Kozák, Poběžovice). Garlic was grown at two localities – at Lednice (Břeclav district) from planting on November 12th 1999 and at Pravčice (Kroměříž district) from planting on November 6th 1999, all in 4 replications. The plant spacing was 0.3 × 0.1 m and the planting depth 80 mm.

The common growing technology was applied. Garlic was harvested on July 20th 2000, and then dried at 25–30°C for 6 weeks. The storage temperature of 5–10°C was maintained during 7 months of storage, until the time of analysis.

### Sample preparation

Each sample consists of 10 g of garlic cloves in fresh weight. These cloves were collected from different plants. All samples were prepared in four replications. After mixing with mobile phase at a ratio 1–10 the samples were filtered through mull, centrifuged in EBA 12 centrifuge (Hettich, Germany) at 3,800 rpm for 10 minutes and filtered through Milipore PTFE 0.45 μm filter. The prepared samples were stored in dark at 5°C for maximally 2 hours until the analysis. The injected sample volume was 20 μl.

### Analytical conditions

Analysis by the method of RP-HPLC was performed on Ecom apparatus (Ecom, Czech Republic) in isocratic mode with analytical pump LCP 4000.11. The mobile phase contains 0.01 M tetrabutylammonium hydroxide (TBAH), 0.1 M oxalic acid and deionised water (10:20:70 – v/v/v). All chemicals were of analytical or HPLC grade. The mobile phase flow rate was 0.5 ml per minute.

The column CGC Separon SGX C18 (Tessek, Czech Republic) of the size 150 × 4.6 mm and pre-column Tessek 30 × 3 mm were filled with the identical sorbent sized to 7 μm. In-line filter 2 μm Tessek was included before the column system. Detection was performed at 254 nm by UV-VIS Ecom detector LCD 2082.2. The column oven Ecom was set to 30°C. The whole system was equilibrated 4 hours before start of analytical works except the detector lamp. Each analysis was performed twice. A blank analysis was measured at each sample set.

A standard solution of L-ascorbic acid (of analytical grade) dissolved in the mobile phase was prepared. Calibration curve was set from solutions of 10, 20, 50, 100 and 150 mg/kg of vitamin C. The sample with exactly 50 mg/kg of vitamin C for stability evaluation was stored at 5° and 20°C and analysed in 1-hour intervals.

Evaluation of analyses was performed by software application CSW version 17 (DataApex, Czech Republic).

Table 1. Mean retention time and vitamin C content in garlic (mg/kg f.m.)

| Locality | Lednice | | Pravčice | |
|----------|---------|---------|----------|---------|
| Variety | Rt (min) | vitamin C | Rt (min) | vitamin C |
| Alan | 3.28 | 351.5 bc | 3.23 | 358.3 b |
| Lukan | 3.29 | 320.3 ab | 3.26 | 307.7 a |
| P10E | 3.24 | 343.3 abc | 3.22 | 279.8 a |
| Ropal | 3.20 | 299.5 ab | 3.25 | 251.3 a |
| Vekan | 3.25 | 400.5 c | 3.22 | 406 b |
| Vladan | 3.28 | 276.5 a | 3.25 | 269 a |
| Mean | 3.26 | 328.0 | 3.24 | 302.8 |

Legend: Rt – retention time
Means followed by different letters are significantly different at $P = 0.05$ (Tukey's test)
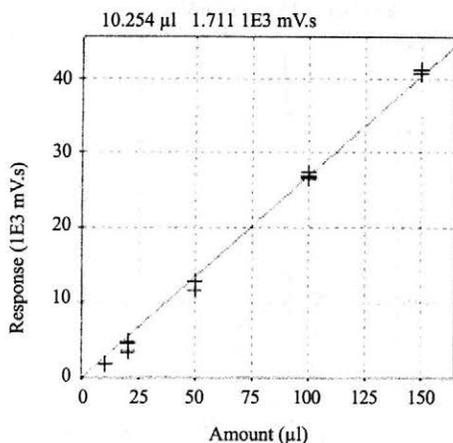


10.254 μl   1.711 1E3 mV.s
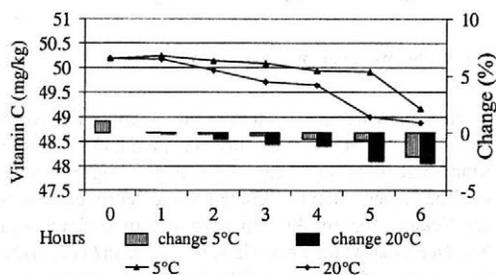
Fig. 1. Calibration curve of vitamin C



Fig. 2. Vitamin C stability in samples

Reproducibility (RSD – relative standard deviation) was calculated from sets of 20 analyses in 4 replications.

## RESULTS

The calibration graph based on 5 different concentration levels of vitamin C results in a linear model ($y = 269.132x$) with correlation coefficient 0.999 (Fig. 1). The minimal limit of detection was 1 mg/kg of vitamin C. Evaluation of L-ascorbic acid stability showed the good ability of oxalic acid to protect it up to 6 hours without any significant decrease in vitamin C content in sample. Fig. 2 shows average change of vitamin C content in per cent and average percentage decrease in vitamin C content. No significant effect of storage temperature was observed.

A chromatogram (Fig. 3) displays a typical record of garlic analysis. The second (the highest) peak is represented by oxalic acid from mobile phase and the third peak is vitamin C. The other peaks remain unidentified. The mean retention time of vitamin C was 3.25 ± 0.05 minute and its total variability was 3% only. The total elution time of analysis was 18 minutes. Reproducibility of the tested method showed a very good value, with RSD = 1.9%.
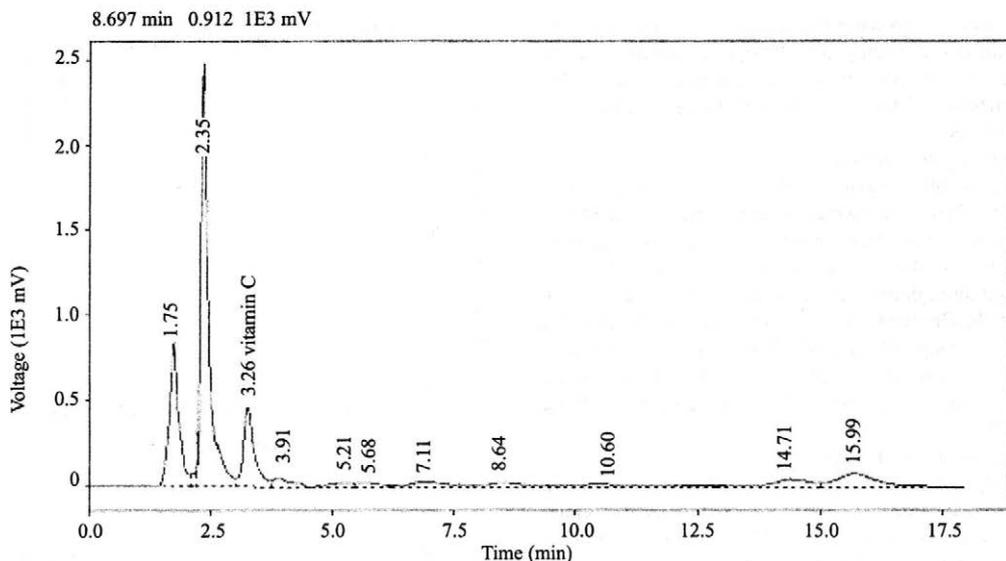
8.697 min   0.912   1E3 mV



Fig. 3. Chromatogram of garlic analysis

The mean vitamin C content in garlic varieties is shown in Table 1. No effect of locality was found. On the other hand, there was a significant difference among the varieties. The highest vitamin C content was determined in variety Vekan (403 mg/kg f.m.) from both Lednice and Pravčice locality; the lowest in variety Vladan (277 mg/kg f.m.) from Lednice and variety Ropal (251 mg/kg f.m.) from Pravčice.

## DISCUSSION

HPLC methods with detection at 254 nm are commonly used for vitamin C determination (PERÉZ et al. 1997). The same wavelength was also used in our experiments. The tested analytical approach is fully comparable with other approaches published previously (CZERWIECKI, WILCZYNSKA 1999). The sensitivity of this method is sufficient for analysis of vegetable samples (ALBRECHT, SCHAFER 1990; ORUNA-CONCHA et al. 1998).

Samples were prepared by centrifugation and filtering through millipore filters. Such approach shortened the necessary time and contributed to vitamin C preservation. The evaluated method of preparation showed sufficient stability of L-ascorbic acid. No significant vitamin C loss within 6 hours was observed if samples were stored at 5° or 20°C. A similar effect was reported by GENNARO and BERTOLO (1990) in the case of phosphoric acid use.

The total elution time is influenced by sample type; 18 minutes were sufficient for garlic (PERÉZ et al. 1997). The obtained retention time of vitamin C (mean 3.25 minutes) is lower in comparison with ROMERO et al. (1992). The use of column oven at 30°C increased stability of results and also limited the variability of retention time (PERÉZ et al. 1997).

The chosen calibration curve led to exact and highly reproducible results. No serious interferences were observed in analysed samples. The calibration was highly precise, but due to the low vitamin C stability it is recommended to make a new calibration for each new set of samples.

No significant effect of locality was found. Variety is an important factor influencing the vitamin C content. The experimental garlic varieties showed good vitamin C content in comparison with literature (KOPEC 1998; ANONYMOUS 1999).

## CONCLUSION

The experiments demonstrated that the method of vitamin C determination by help of SGX C18 column and mobile phase consisting of tetrabutylammonium hydroxide, oxalic acid and water is sufficiently sensitive. The isocratic RP-HPLC UV detection at 254 nm results in high reproducibility. The used extraction method with oxalic acid in extraction solution was sufficient with regard to the vitamin C stability in samples. The mean vitamin C content of 315 mg/kg of f.m. represents a high nutritive value of garlic and it was influenced by garlic variety.

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# Využití HPLC pro rychlé stanovení vitaminu C v česneku

**ABSTRAKT**: Stanovení vitaminu C se provádí různými metodami, mezi kterými je významná kapalinová chromatografie. V laboratorních pokusech byla ověřována izokratická RP-HPLC metoda stanovení kyseliny L-askorbové v česneku při použití kolony SGX C18 o rozměru 150 × 3 mm a zrnitosti sorbentu 7 μm. Mobilní fáze byla složena ze směsi tetrabutylamonium hydroxid, kyselina šťavelová a voda. Bylo hodnoceno šest odrůd česneku ze dvou pěstebních lokalit. Vzorky byly mixovány s mobilní fází a po přečištění bezprostředně analyzovány. Z pokusů vyplynulo, že detekční limit metody je 1 mg/kg, dosažený retenční čas vitaminu C byl průměrně 3,25 min, reprodukovatelnost metody byla 1,9 %. Vitamin C byl v použitém extrakčním roztoku kyseliny šťavelové stabilní až šest hodin od přípravy vzorku. Dosažený průměrný obsah vitaminu C v česneku byl 315 mg/kg č.h. Nejnižší obsah byl doložen u odrůdy Vladan (273 mg/kg č.h.), zatímco nejvyšší u odrůdy Vekan (403 mg/kg č.h.). Testovaná metoda vykazovala dostatečnou citlivost pro analýzu zeleniny, přičemž stanovení vitaminu C bylo provedeno během 18 minut analýzy.

**Klíčová slova**: HPLC; vitamin C; analýza; česnek

*Corresponding author:*

Ing. ROBERT POKLUDA, PhD., Mendelova zemědělská a lesnická univerzita, Brno, Zahradnická fakulta, Valtická 337, 691 44 Lednice na Moravě, Česká republika
tel.: + 420 627 34 01 05, fax: + 420 627 34 01 59, e-mail: pokluda@zf.mendelu.cz

# Artichoke and cardoon – vegetables of the future

J. Stano[1], D. Lišková[2], R. Slavik[3], K. Mičieta[4], A. Valent[1], H. von Barth[5], M. Koreňová[1]

[1]*Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic*
[2]*Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovak Republic*
[3]*Seeds Royal Corp., Toronto, Canada*
[4]*Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic*
[5]*PROBMOL Ltd., Halle, Federal Republic of Germany*

**ABSTRACT**: The review summarizes information and knowledge of biologically active substances, predominately with hepatoprotective and hypocholesterolemic effects on the human body, of the genus artichoke (*Cynara*). This contribution should draw attention to this pharmaceutically interesting plant, and initiate its utilization in the phytotherapy in the countries where this plant is less known.

**Keywords**: artichoke; biologically active substances; inulin; vegetables; drugs

Artichoke (family *Asteraceae*), reported already in the Herbarium by Mathioli (AMBROŽ 1931), is an ancient cultural plant with beneficial effects on the liver diseases. This, in many countries less known plant was a favourite vegetable in ancient Egypt 2,500 years ago. In antique Greece and Rome it was considered as an aphrodisiac. In the 15th century the artichoke became popular in Italy and one century later also in Germany and Spain (MÜLLER 1867; LÁNSKÁ, ZEMINA 1998).

Artichokes are vigorous perennial herbs, growing up to the height of 1.5 m and more in the temperate climate. The plants have well developed roots and upright ribbed stalks. Leaves have incised leaf margins and are alternately arranged on the stem, or they form leaf rosettes. On the top of branched flower stems are large anthodia with blue-violet or white flowers blooming until August. This vigorous herb resembling the thistle is not only a delicacy, but also an attractive ornamental plant (PAVORDOVÁ 1997).

Artichoke probably originates from north Africa. This thermophilous plant, sensitive to frost and mostly grown in Spain, is possible to cultivate also in the temperate climate, where the winter temperature falls deeply under the freezing point. It needs to be cultivated at warm and sunny places protected from frost and wind. Artichokes need well cultivated soil, rich in nutrients. They can be propagated vegetatively by cuttings or generatively by seeds. In optimal conditions they produce heads during 4–6 years mostly in August (HÖHNE, WILHELM 2000).

The flower heads of *Cynara scolymus* L. and leaves of *C. cardunculus* L. are not only popular vegetables but also sources of numerous biologically active substances. The most important are derivatives of caffeic acid, flavonoids, sesquiterpene lactones, coumarins, sterols, pentacyclic triterpenes, anthocyanins (MÜLLER 1867; PANIZZI, SCARPATI 1954; CONSTANTINESCU et al. 1967; BOMBARDELLI et al. 1977; GRANČAI et al. 1994a,b).

From the pharmacological point of view the most important group of biologically active substances is represented by derivatives of caffeic acid. In 1954 cynarine from leaves of *C. scolymus* L. was isolated. The molecule of this substance is composed of caffeic and quinic acids (PANIZZI, SCARPATI 1954). Cynarine was later identified also in *C. cardunculus* L. (GRANČAI et al. 1994a,b). Besides cynarine, in artichoke there are present caffeic, 3-O-caffeylquinic (neo-chlorogenic acid), 4-O-caffeylquinic (cryptochlorogenic), 5-O-caffeylquinic (neo-chlorogenic), 1,3-dicaffeylquinic, and 3,5-di-O-caffeylquinic acids. The content of benzylcarboxy-acids and flavonoids in leaves is dependent on their position and collection time of leaves between 0.35–2.65% and 0.1–1.0%, respectively. The decrease of flavonoids is characteristic of the blooming time and formation of heads (CONSTANTINESCU et al. 1967).

## Flavonoids

A simple synthesis of cynarine stimulated the intensive research of its pharmacological effects. The fact that cynarine is effective only in higher concentrations and its con-

tent in plants is relatively low, indicates that artichoke leaves contain also other biologically active substances. This finding initiated the interest in substances similar to cynarine – flavonoids (CONSTANTINESCU et al. 1967; EL-NEGOUMY et al. 1987). In the leaves of *C. scolymus* L. luteolin glycosides are present: luteolin-7-O-ß-glucoside (cynaroside), luteolin-7-O- ß-rutinoside (scolymoside), and luteolin-7-O-rutinozyl-4-O-glucoside (EL-NEGOUMY et al. 1987).

**Sesquiterpene lactones**

Quantitatively sesquiterpenes are the most abundant metabolites of artichoke (2.43%). The main components isolated from *Cynara scolymus* and *Cynara cardunculus* are guaianolide and cynaropicrine (SUCHÝ et al. 1960; SCHNEIDER, THIELE 1974a,b). Sesquiterpenes are present only in green parts of the plant. However, their content in the apical part of the plant, in leaf petiole and in buds is lower (0.6% of dry weight) as compared with leaf lamina (0.5–4.5% of dry weight). The level of these metabolites depends also on the leaf ontogenesis. In young leaves sesquiterpenes are more abundant than in older ones (SCHNEIDER, THIELE 1974). Sesquiterpene glycosides of guaiane type (cynaroside A and 11 ß, 13-dihydrodesacylcyanopicrine-8-ß-D-glucoside) in *C. scolymus* are additionally present in *C. scolymus* (SHIMIZU et al. 1988). Besides cynaropicrine, in the aboveground part of *C. humilis* L. were ascertained also aquerine B, solstitialine, 13-acetylsolstitialine, 13-chlorosolstitialine, 3-acetyl-chlorosolstitialine, and 11,13-epoxysolstitialine (RHEIS et al. 1992).

**Coumarins**

Coumarins represent a minor group of secondary compounds of artichoke. In the leaves of *C. scolymus* L. were found esculin and scopoletin (HINOU et al. 1989), and in unopened flowers or buds of *C. cardunculus* L. scopoletin and scopoletin glycoside (GRANČAI et al. 1994a,b).

**Steroids and pentacyclic triterpenes**

Steroids and triterpenes in higher plants belong to substances present in bound forms. Artichoke roots contain pentacyclic triterpenes – taraxasterol, taraxasteryl-3ß-acetate, and steroid ß-sitosterol. In buds sitosteryl-3ß-acetate and sitosteryl-3ß-glucoside are also present, and in the aboveground part of *C. humilis* L. campesterol, ß-sitosterol, sitosteryl-3ß-glucoside, stigmasterol, triterpenes, taraxasterol, lupeol, ß-amyrin, ursolic and oleanolic acids were identified (RHEIS et al. 1992).

**Saponins**

The confirmation of saponins in the family Asteraceae was proved by Japanese authors. In roots of *C. cardunculus* L. saponins of ursolic and oleanolic type were ascertained (*Cynara* saponins A-J) (SHIMIZU et al. 1988).

**Anthocyanins**

In flowers as well as in leaves of *C. scolymus* L. anthocyanins (cyanidol-3-sophoroside, cyanidolglucoside, cyanidol-5,3-diglucoside, cyanidol-5-glucoside, and cyanidol-3-caffeylsophoroside) were identified (FOURY, AUBERT 1977).

**Other substances**

Besides the described substances, the genus *Cynara* is rich in different acids: palmitic, stearic, linolenic and arachidic, further in tannins, mucilages, enzyme ascortinase, and provitamin A and vitamin B (ELGAMAL et al. 1991).

## PHARMACOLOGICAL EFFECTS

As already mentioned in the Natural Pharmacy, artichoke has hepatostimulative, choleretic, hepatoprotective, and hypocholesterolemic effects, and also positive effects on liver regeneration. The water deproteinized extract from *C. scolymus* L. leaves, perorally applied for 3 weeks after partial hepatectomy, stimulated the increase in the weight of residual liver of rats, division and development of cells (MAROS et al. 1966, 1968). The hepatoprotective properties of artichoke extracts were observed on isolated rat hepatocytes (HOŘEJŠÍ 1989). Among the tested polyphenolic substances only cynarine and caffeic acid showed hepatoprotective effects. All other derivatives of caffeic acid-chlorogenic and iso-chlorogenic acid were ineffective. The hepatoprotective effect can possibly be explained by antioxidative properties of polyphenols (ADZET et al. 1987).

Chlorogenic and caffeic acids inhibit the production of triglycerides (TG), lipid peroxides (LP), total cholesterol content (TC), glutamate-oxalacetate transaminase (GOT), and glutamate-pyruvate transaminase (GPT) in the serum, and also LP in the liver of rats fed with peroxidic maize oil. High fat diet containing this oil induced hyperlipemia with the increase in TC, TG, LP, free fatty acids (FFA), accumulation of TC, TG, LP in the liver, and liver damage with the increase in serum GOT and GPT. The results showed two possible mechanisms of action of the caffeic acid derivatives in the protection process. The inhibition of LP production, and the inhibition of destructive processes of lipid peroxides in liver cells. Chlorogenic and caffeic acid cause a decrease in serum and liver lipids, therefore their effect on lipid metabolism is supposed, e.g. by inhibition of absorption in the intestine, by inhibition of triglyceride biosynthesis in the liver, and by acceleration of lipid transport in muscles (KIMURA et al. 1985).

The application of artichoke (*C. scolymus* L.) extract induced choleretic and hypocholesterolemic effects in rats. A significant increase in gall secretion after extract application could explain the hypocholesterolemic effect (LIETTI 1977).

However, the content of cynarine in artichoke extracts is low and it is not present at effective therapeutic doses (BOMBARDELLI et al. 1977).

Further studies showed the responsibility of taraxasterol and ß-sitosterol present in artichoke extract for the hypolipemic effect in guinea-pigs with hypercholesterolemia induced by the nutrition (SCHMIDTOVÁ et al. 1998). Favourable hypolipidemic and choleretic effects of cynarine were proved in clinical studies. Therefore the prophylactic and therapeutic use of cynarine (2 × 250 mg daily) is recommended in the case of different types of dyslipoproteinemia (MONTINI et al. 1975).

The effect of artichoke extract against acute and chronic metabolic diseases was observed. When perorally ap-

plied (capsules with 320 mg of artichoke extract per day), a positive choleretic effect and an increase in the portion of all gall compounds were observed. The therapy in which the artichoke extract is used for dyspepsia connected with diskinesia of bile-ducts is recommended also for long-term application (KIRCHHOFF et al. 1994).

Besides pharmacological effects the caffeic acid derivatives play an important role in the protection of plants against pathogens. It was ascertained that their concentration is changing very quickly, e.g. the content of chlorogenic acid from coffee (*Coffea*) can be doubled within 4 days after the infection (MOLGAARD, RAVN 1988).

Artichoke is a source of the reserve polysaccharide – inulin used predominately by diabetics, because after the consumption the level of glucose in blood is not raised. Maybe the last but so far not mentioned characteristic of the artichoke is its diuretic effect. We consider as a great success of gene engineering the potatoes, the reserve polysaccharides of which are starch and inulin. Inulin is the reserve polysaccharide of the family Asteraceae. The vegetables of this family (chicory, sweet potato, etc.) together with potatoes containing inulin as well, are a good supplement to diabetic diet (ČEŠKA, ŠOBRA 1992; PAVORDOVÁ 1997; HELLWEGE et al. 2000). The preparation, meeting also the requirements of a gourmet, was described by numerous authors (MÜLLER 1867; PAVORDOVÁ 1997; LÁNSKÁ, ZEMINA 1998; FRASEROVÁ 1999).

**Drugs and food supplements**

Significant pharmaceutical effects of artichoke have stimulated many firms to produce drugs. Known for the preparation of drugs with artichoke extract are: Klosterfrau Artischocken Dragees, Kneipp Artischocke, Hepar-SL, etc., in the Slovak Republic is available Cynarosan and Cynalip, a novelty in Slovak pharmacies is Botadyn. The use of artichoke in the production of liqueurs, e.g. the liqueur Cynar, is also well-known.

## CONCLUSIONS

Hypercholesterolemia (together with smoking and hypertension) is one of the most risk factors for the origin of atherosclerosis. Increased levels of cholesterol LDH, apolipoprotein B-100, decrease in cholesterol HDL and apolipoprotein A-1 are the factors accelerating the process of atherogenesis. An effective decrease in the cholesterol level leads to less frequent occurrence of ischemic disease of the heart as well as to the regress of atherosclerosis (ČEŠKA, ŠOBRA 1992).

At present, besides synthetic remedies natural products are used in the therapy. Besides their hypolipidemic effect, these natural products often show also hepatoprotective antioxidative effects demonstrated in experiments on animals with liver damaged by different types of toxins (FILIP 1994).

The study of secondary compounds of the genus *Cynara* shows the great importance of caffeic acid derivatives and flavonoids – compounds of polyphenolic type for the therapy.

The results of studies focused on the pharmacodynamic effects of the tested compounds, predominately of caffeylquinic and dicarboxyl derivatives, document their possible use for the treatment of cardiovascular system (atherosclerosis, dislipoproteinemia), in the case of metabolic failures making use of their choleretic effect, and in the prevention of liver damage caused by hepatotoxic compounds.

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## Artičoky – zelenina budúcnosti

**ABSTRAKT**: Prehľadný článok prináša informácie a poznatky o biologicky aktívnych látkach, najmä s hepatoprotektívnym a hypocholesterolemickým účinkom na ľudský organizmus, rodu artičoka (*Cynara*). Cieľom príspevku je obrátiť pozornosť na farmaceuticky zaujímavú rastlinu a na jej využitie vo fytoterapii v krajinách, kde je táto rastlina menej známa.

**Kľúčové slová**: artičoka; biologicky aktívne látky; inulín; zelenina; liečivo

*Corresponding author:*

RNDr. JÁN STANO, CSc., Záhrada liečivých rastlín, Farmaceutická fakulta, Univerzita Komenského, Odbojárov 10, 832 32 Bratislava, Slovenská republika
tel.: + 421 2 50 25 92 75, fax: + 421 2 555 72 20 65, e-mail: stano@fpharm.uniba.sk

# K OSMDESÁTINÁM ING. FRANTIŠKA MAREČKA
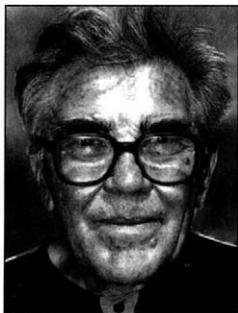
Když se někdo dožije 80 let, připomeneme jeho obdivuhodnou svěžest a přejeme mu hodně zdraví a elánu do dalších let. Málokomu se však podaří, aby aktuálnost svého pevného kořínku a činorodosti prokázal naprosto nezpochybnitelným, úplně čerstvým důkazem. To se bezesporu podařilo Ing. Františku Marečkovi, který své životní jubileum oslavil 30. července.

My všichni, kteří s ním přicházíme po léta do styku, víme, že je „zahradníkem tělem i duší". Šíře jeho znalostí ze všech zahradnických oborů nemá v naší generaci, směřující k čím dál větší specializaci, obdoby. Jeho univerzálnost a široké praktické zkušenosti z něho učinily uznávanou osobnost, která usiluje o všestranné povznesení nejen českého zahradnictví. Jeho ochota a neúnavná pracovitost v kolektivu i v řídící práci byla vždycky pověstná.

Připomeňme jen v hlavních rysech jeho bohatou činnost z dob, kdy řídil různé odborné komise, byl členem ČSAV a ČSAZ, ředitelem Výzkumného ústavu rostlinné výroby v Praze-Ruzyni a potom Výzkumného ústavu zelinářského v Olomouci, kdy organizoval řadu domácích konferencí a účastnil se řady konferencí zahraničních. Přitom stačil publikovat mnoho článků, vydat několik knih a vyšlechtit 11 zeleninových odrůd.

Předpokladem k této všestranné činnosti mu dal jeho původ i životní zkušenosti. Pochází ze známé rodiny se zahradnickou tradicí v Kuklenách u Hradce Králové, jejímž se cítil být pokračovatelem. Zděděné vlastnosti a rodinná výchova ho vedly od mládí k širokému zájmu o většinu zahradnických odvětví, citu pro aktuální technologické problémy oboru a k účasti na jejich řešení. Jeho otec byl nejen známým novátorem v oblasti balíčkování sadby, předpěstování sazenic na podložkách, konstruktérem sázecího stroje pro zeleninu, autorem rychlírenských staveb z pařeništních oken, ale i tvůrcem sortimentové školky růží a jejich šlechtitelem. Byl také zakladatelem místní družstevní organizace pro odbyt zeleniny a výrobní pomoc zelinářům.

František Mareček syn šel ve šlépějích svého otce se stejnou vynalézavostí, prozíravostí a užitečnou fantazií, pokud jde o technologie budoucnosti. Ve VÚRV v Praze-Ruzyni, kde prožil téměř celý svůj život výzkumníka, byl první, kdo postřehl význam fólií pro krytí zeleninových kultur a mulčování. Založil tu také první pokusný jabloňový sad s hustou výsadbou vhodných odrůd na slabě rostoucích podnožích. Patřil k těm, kdo mezi prvními poznali význam netypických odrůd zelenin, umožňujících žádoucí prodlužování sklizňového období, jako je ledový salát Pražan, skladovatelná kapusta Langendijská, pozdní kedlubna typu Gigant. Zabýval se nejen jejich šlechtěním, ale také semenářstvím, které bylo v tehdejší době pro zavedení nových odrůd rozhodující.

Vraťme se však k dnešku a době nedávné. F. Mareček kdysi prohlásil, že jeho životním cílem je založit vědecký zahradnický časopis a vydat Zahradnický slovník. První český vědecký zahradnický časopis se mu podařilo založit v r. 1973 – byl to Sborník ÚVTIZ Zahradnictví, dnešní Horticultural Science – Zahradnictví, který přežil různá obtížná období a letos vychází v 28. ročníku. Ani s druhým cílem to nebylo jednoduché. Zahradnický slovník naučný, který měl navázat na tradici po generace výborně sloužícího Kavinova Ovocnicko-vinařského a zahradnického slovníku z let 1934–1942, začal vznikat už v r. 1990. F. Mareček mu vtiskl širší koncepci, která základní botaniku obohatila především o moderní technologie a o poznatky z biologie. Využil svých neobyčejně bohatých osobních kontaktů a autority k vytvoření kolektivu koordinátorů jednotlivých oborů, kteří zpracovali hesláře a vytvořili užší kolektivy autorů jednotlivých hesel a obrazové dokumentaci. Po celou dobu práce na slovníku byl F. Mareček odborníkem, jehož rukama prošla všechna hesla a všechny obrázky a na němž spočinula také odpovědnost za rozhodování v konečné fázi zpracování slovníku v silné časové tísni.

Právě několikaletá práce na slovníku dokládala celému kolektivu spolupracovníků, jak Marečkovy vlastnosti – neúnavná pracovitost, trpělivost a zodpovědnost, záviděníhodná paměť a mimořádné stylistické schopnosti – vydržely až do současnosti, kdy se v polovině roku 2001 podařilo dílo uzavřít a vydat. Jeho rozhodnost a cílevědomost, spojená s citlivostí v jednání s lidmi, umožnila dosáhnout jednotné koncepce a dát vznik dílu, na které se může dnes dívat s oprávněným zadostiučiněním.

Zahradnický slovník naučný vznikal v době mimořádně komplikované, v zahradnickém oboru plné zásadních proměn. Po prvních jednáních s tehdejším Zemědělským nakladatelstvím a následném několikaletém odkladu se vydávání uskutečnilo v dnešním Ústavu zemědělských a potravinářských informací. Jedno štěstí však přitom slovník měl: práce na něm se jako hlavní redaktor ujal čerstvý penzista, který se rozhodl svou nezdolnou energii plně věnovat svému dávnému životnímu cíli. Na svou aktivní výzkumnou činnost tedy navázal desetiletou úspěšnou činností na Zahradnickém slovníku naučném.

Při pohledu na Marečkův životní styl lze věřit tomu, že právě činorodost je základem zdraví i ve vysokém věku. Jeho nejbližší spolupracovníci vědí, že jeho životní zásadou je heslo „neduhy se léčí pohybem". A jiná kategorie jeho přátel může i v letošním roce dosvědčit, v kolika zahradách provedl kompletní zimní řez stromů, kolik vlastnoručně naroubovaných stromků rozdal a kolik zeleninových, ovocných a okrasných výpěstků roznesl přátelům.

Přejeme oslavenci, aby plně využil uspokojení z právě dokončeného záslužného díla. O nedostatek dalších odborných kontaktů nemusí mít žádné obavy – jako jedinečný pamětník a zkušený praktik i vzdělaný publicista zůstane stále vyhledávaným rádcem a společníkem.

*Eva Pekárková*

# INSTRUCTIONS FOR AUTHORS

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The manuscript should be typed on standard A4 paper. A PC diskette with the complete text and including references, tables and figure legends of graphical documentation should be provided with each manuscript, indicating the used editor program.

Manuscript should consist of the following sections: Title page, Abstract, Keywords, Introduction, Materials and Methods, Results, Discussion, References, Address of corresponding author, Tables, Legends to figures.

The Title page must contain an informative title, complete name(s) of the author(s), the name(s) and address(es) of the institution(s) where the work was done.

The **Abstract** should state in a short and concise form what was done and how, and should contain basic numerical and statistical data representative of the results. It should be submitted in English and, if possible, also in Czech.

**Keywords** follow the abstract; they are ranked from general to specific terms, and are written in lower case letters and separated by semicolons.

The **Introduction** (without a subtitle) should consist of a short review of literature relevant to and important for the study. The reason(s) for carrying out the work may be included.

In **Materials and Methods**, the description of experimental procedures should be sufficient to allow replication. Abbreviations can be used if necessary; first use of an abbreviation should be just after its complete name or description. The International System of Units (SI) and their abbreviations should be used.

**Results** should be presented clearly and concisely. In this section figures and graphs should be used rather than tables for presentation of quantitative values. A statistical analysis of recorded values should be summarized in tables.

The **Discussion** should interpret the results, without unnecessary repetition. Sometimes it is possible or advantageous to combine Results and Discussion in one section.

If **Acknowledgments** are needed, they come next.

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**Tables and Figures** shall be enclosed separately. Each of them must be referred to in the text. Figures should be restricted to material essential for documentation and understanding of the text. Duplicate presentation of data in both tables and figures is not acceptable. All illustrative material must be of publishable quality. Both line drawings and photographs are referred to as figures. Photographs should have high contrast. Each figure should be accompanied by a concise, descriptive legend.

**Reprint**: Ten (10) reprints of each published paper are supplied free of charge.

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Autor je plně odpovědný za původnost práce a za její věcnou i formální správnost. O uveřejnění práce rozhoduje redakční rada se zřetelem k lektorským posudkům, vědeckému významu a přínosu i kvalitě práce.

Rukopis musí být zaslán vytištěný na papíru formátu A4. K rukopisu je vhodné přiložit disketu s textem práce, popř. grafickou dokumentací pořízenou na PC (uvést použitý program).

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**Klíčová slova** (keywords, index terms) se připojují po vynechání řádku pod souhrn. Řadí se směrem od obecných výrazů ke speciálním; začínají malým písmenem a oddělují se středníkem.

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**Materiál a metody**: Model pokusu musí být popsán podrobně a výstižně. Popis metod by měl umožnit, aby kdokoli z odborníků mohl práci opakovat. Zkratky jsou používány jen pokud je to nutné; první použití zkratky musí být uvedeno úplným popisem nebo vysvětlením. Používané měrové jednotky musí odpovídat soustavě měrových jednotek SI.

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# HORTICULTURAL SCIENCE

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