Changes of Furanocoumarins Content in Vegetables during Storage

P. BOTEK, V. SCHULZOVÁ*, R. PEROUTKA and J. HAJŠLOVÁ*

*Department of Food Chemistry and Analysis, Institute of Chemical Technology, Prague, Czech Republic, *E-mail: vera.schulzova@vscht.cz, petr.botek@vscht.cz*

Abstract: Long term experiments, which simulated storage of celery (*Apium graveolens*) under industrial (air-conditioned store)/household (common cellar) conditions, were carried out. Several celery cultivars from both organic and/or conventional production were monitored for furanocoumarins levels for 16–26 weeks. The increase of furanocoumarin concentrations during the storage of all the tested celery cultivars was observed, nevertheless, the extent of toxicants accumulation differed among tested cultivars. The changes of furanocoumarin levels occurring during processing of vegetables were studied, too. Levels of both linear (psoralen, bergapten, xanthotoxin, trioxsalen, isopimpinellin) and angular (angelicin, sphondin, isobergapten) furanocoumarins in tested vegetable were determined by validated GC/MS (SIM) method. The detection limits (LODs) obtained by this analytical method were around 0.003 mg/kg.

Keywords: furanocoumarins; storage; vegetable; gas chromatography

INTRODUCTION

Furanocoumarins are present in several plant families such as *Apiaceae, Rutaceae* and *Moraceae* [1]. The most widely consumed vegetable with high content of these natural toxins are celery, parsley and parsnip. Considering their chemical structure (Figure 1, Table 1), two groups of these biologically active compounds, linear and angular furanocoumarins, can be recognized.

Furanocoumarins are classified as phototoxic compounds, after exposure to UV light formation of adducts with DNA can take place [1–3]. In addition, mutagenic and carcinogenic effects were demonstrated in experimental animals when exposed to high doses of furanocoumarins.

Figure 1. General structures of linear (A) and angular (B) furanocoumarins

Table 1. Structure of common furanocoumarins

Linear:	Angular:
Psoralen (R^1 = H, R^2 = H)	Angelicin (R^1 = H, R^2 = H)
Bergapten (R^1 = OCH_3, R^2 = H)	Isobergapten (R^1 = OCH_3, R^2 = H)
Xanthotoxin (R^1 = H, R^2= OCH_3)	Sphondin (R^1 = H, R^2 = OCH_3)
Isopimpinellin (R^1 = OCH_3, R^2 = OCH_3)	Pimpinellin (R^1 = OCH_3, R^2 = OCH_3)

As regards humans, the dietary intake largely depends not only on a variety of celery consumed but also on its storage and processing. As shown in our study, concentrations of these toxins in respective crop may increase significantly under stress conditions (attack of insects or fungi, mechanical damage, unfavourable climatic/storage factors etc.) [1, 4, 5] and no significant decrease can be expected as a result of cooking since furanocoumarins are relatively stable under common heat treatment conditions [5].

EXPERIMENTAL

Material. *Stored celery* – Two storage experiments (starting in October) were carried out:

(*i*) Four celery cultivars (Maxim, Radiant, Diamand, Neon) were purchased from a private producer. Samples were stored at household conditions in a cellar for 26 weeks at 2–13°C. Dry matter of celery bulbs at the beginning of experiment was around 12% (year 2002 – high rainfall). Relative standard deviation (sum of furanocoumarins) for bulbs was 7% at the beginning of storage experiment and 53% at the end of storage experiment.

(*ii*) Six cultivars (3 root celery – Albin, Kompakt, Maxim, 2 petiolate celery – Malachit, Avalon and 1 leafy celery – Jemny) both of organic and conventional origin were purchased from the Czech University of Agriculture in Prague. Samples were stored at industrial (air-conditioned store) conditions at 4°C. Bulbs were stored for 16 weeks, relative standard deviation (sum of furanocoumarins) for bulbs was 32% at the beginning of experiment, 49% at the end. Dry matter of bulbs at the beginning of experiment was around 25% (year 2003 – low rainfall during growing period). Leaves were stored for 4 weeks, dry matter at the beginning of experiment was 21–34%, at the end 31–76%.

Grated celery – Samples were purchased from the retail market. Grated celery and parsnip root were stored ten days in refrigerator at 4°C. Samples were packed in food stretch film, dry matter was constant during the experiment.

Processing of celery – Samples (cultivar President) were purchased from various phase of sterilization processing from industrial producer. Product was a grated pickled celery (pH 4).

Chemicals and methods. *Standards* – Standards of psoralen, angelicin, xanthotoxin, bergapten, trioxsalen were purchased from Sigma-Aldrich (Germany), isopimpinellin was obtained from Indofine (USA). Isobergapten and sphondin were identified on the basis of mass spectra, and quantified using bergapten and xanthotoxin for calibration. Standards were dissolved in ethyl acetate, stock solution at concentration 50 μg/ml was stored at 4°C.

Chemicals – Ethyl acetate for residue pesticide analysis was obtained from Scharlau (Spain), sodium sulphate was from Penta (Czech Republic).

Methods – Five bulbs (washed, bottom and upper parts removed) or 100 g of celery leaves from five plants were homogenized to obtain representative sample. 10 g of homogenized sample was extracted with ethyl acetate (2 × 40 ml) by shaking for 30 min. Combined extracts were filtered through anhydrous sodium sulphate and transferred into 100 ml volumetric flasks.

GC/MS analyses of extracts were carried out using Agilent HP 6890 gas chromatograph with Mass Selective detector HP 5973 (Agilent, USA) operated in SIM mode. GC conditions were as follows: fused silica capillary column DB5-MS 60 m × 0.25 mm × 0.25 μm; column temperature program 68°C (hold for 2 min) to 250°C at 20°C/min, and hold for 5 min, then 20°C/min to 285°C (hold for 15 min); carrier gas He with constant flow 2 ml/min; injection temperature 250°C; injection volume 1 μl using splitless injection mode (splitless time 2 min). For detection characteristic m/z values were used: angelicin (186), psoralen (186), sphondin (216), xanthotoxin (216), isobergapten (216), bergapten (216), trioxsalen (228), isopimpinellin (231).

Methods performance characteristic – detection limits were in the range 0.001–0.008 mg/kg, recovery 89–97% (spiking level 10 mg/kg), relative standard deviation 3.9–4.7%

RESULTS AND DISCUSSION

In celery samples the following components were determined: psoralen, xanthotoxin, bergapten, isopimpinellin; in parsnip angelicin, psoralen, sphondin, xanthotoxin, isobergapten, bergapten, trioxsalen, isopimpinellin. In the next results, the sum of these furanocoumarins is presented.

Storage of celery at household conditions

As shown in Figure 2 successive increase of furanocoumarin levels occurred during storage in all celery varieties, the most pronounced was recognized in the cultivar Maxim: after 10 weeks their

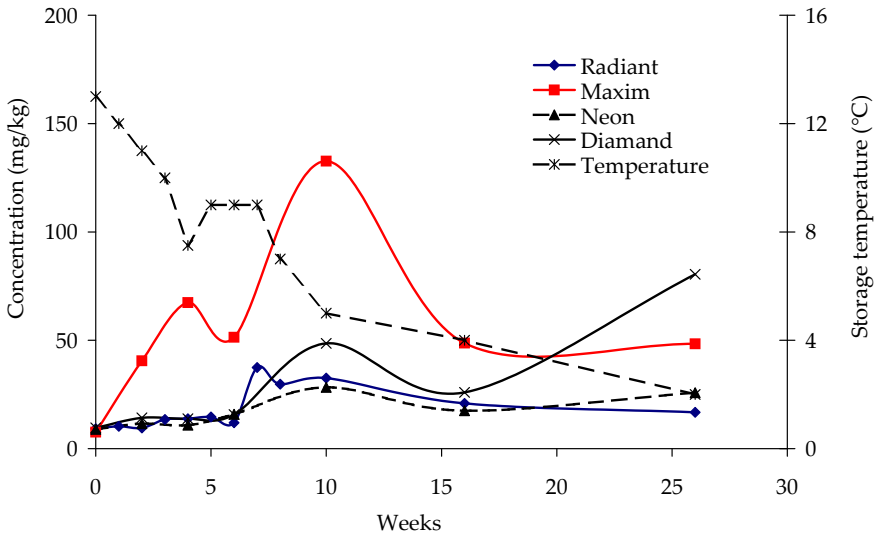

Figure 2. Storage of celery bulbs at household conditions (total furanocoumarins in fresh matter)

content was even 17 times higher as compared to that determined after harvest.

Storage of celery under industrial conditions (air conditioned store held at 4°C)

Similarly to the previous experiment the gradual increase of furanocoumarins was observed during the storage period (Figure 3). The dynamics of toxicants in conventionally grown cultivar Maxim was rather different as compared to other samples. Rather surprisingly, the content of furanocoumarins in the same cultivar obtained from the organic farming system was distinctly lower. The maximal accumulation (14 times higher concentrations than in bulbs after harvest) of furanocoumarins occurred in conventionally grown cultivar Maxim after 16 weeks. Expressed on dry matter the highest levels of furanocoumarins were found after 12 weeks of storage. Conventionally grown cultivars Albin (63.7 mg/kg) and Kompakt (38.0 mg/kg) were analyzed only after harvesting. Content of furanocoumarins after harvest was higher (1.3–2.8 times) in conventional grown bulbs compared to organic ones.

In celery leaves higher accumulation of furanocoumarins was found after 4 weeks of storage (14 times higher expressed on fresh matter, 9 times higher on dry matter). Expressed on dry matter in some samples the levels of the furanocoumarins did not change. The influence of growing conditions on furanocoumarin levels was not significant.

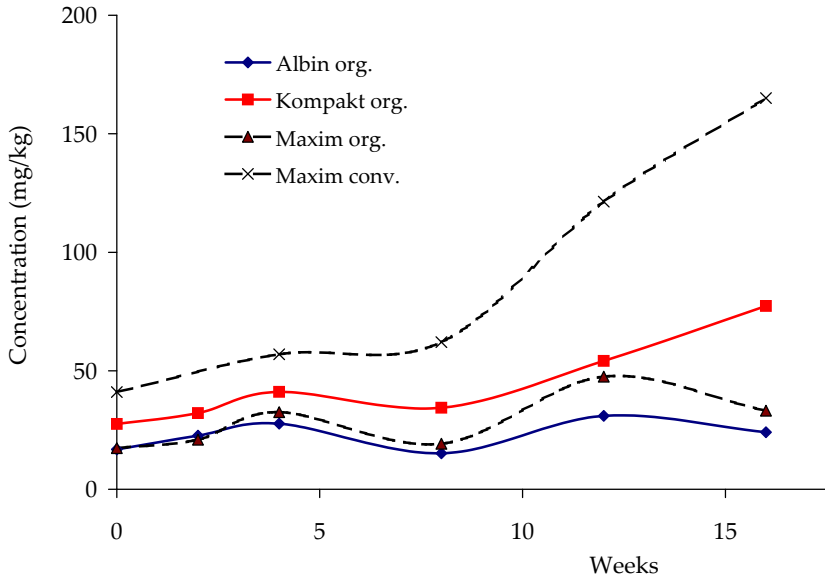

Figure 3. Storage of celery bulbs in refrigerator (total furanocoumarins on fresh matter)

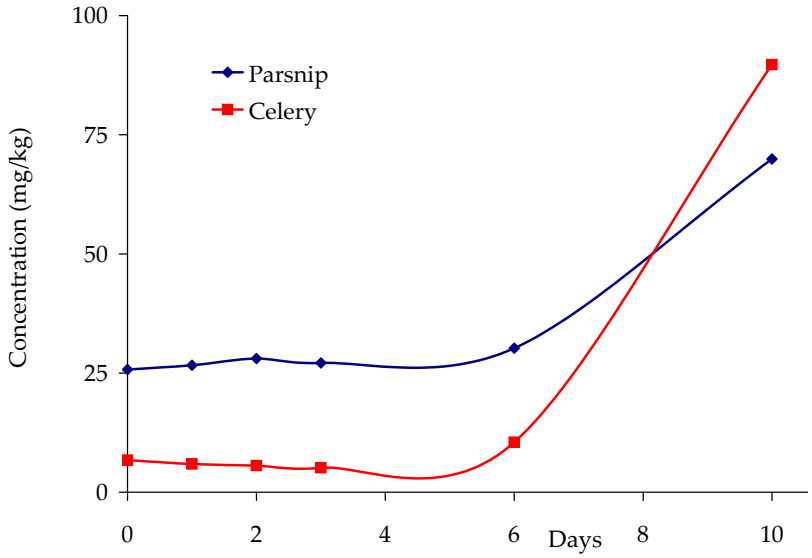

Figure 4. Thermodynamics of furanocoumarins in grated celery and parsnip stored at 4°C (total furanocoumarins on fresh matter)

Storage of grated celery and parsnip

Rapid increase of furanocoumarin levels occurred 6th day of storage of grated vegetables at 4°C (Figure 4); in day 10 the levels of toxicants were 270% in parsnip and 1330% in celery storage as compared to the original content (day 0).

Thermo sterilized celery

No change of furanocoumarins occurred as a consequence of thermo sterilization process and during subsequent storage. Significant reduction of toxicants content was obtained by peeling of bulbs (c.a. 47%) and their blanching (23%).

CONCLUSIONS

Changes of furanocoumarins may occur during storage, their dynamics depends on storage conditions. Generally higher content of furanocoumarins in bulbs was found in conventionally grown crops, the increase was as high as 1700% of the original levels. Regarding celery leaves, the way of farming did not result in differing content of furanocoumarins.

Processing of both celery and parsnip may lead to changes of furanocoumarins. The main way of their reductions in a diet is peeling and blanching of bulbs, the breakdown during thermo sterilization does not take place. Since successive increase of furanocoumarins may occur in grated celery and parsnip, the period before processing or consumption of these vegetables should be shortened as much as possible.

Acknowledgement: This study was carried out within the project MSM No. 223300004 supported by the Ministry of Education, Youth and Sports of the Czech Republic.

References

[1] Søborg I., Andersson C., Gry J. (1996): TemaNord: Furanocoumarins in Plant Food. Nordic Council of Ministers, Kopenhagen.

[2] Llano J., Raber J., Eriksson L.A. (2003): J. Photochem. Photobiol. A, **154**: 235.

[3] Ostertag E., Becker T. Ammon J., Bauer-Aymanns H., Schrenk D. (2002): J. Agric. Food Chem., **50**: 2565.

[4] Beier R.C., Oertli E.H. (1983): Phytochemistry, **22**: 2595.

[5] Lombaert G.A., Siemens K.H., Pellaers P., Makotia M., Ng W. (2001): J. AOAC Int., **84**:1135.