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Polyamines and sterols in *Cichorium* heads

Evelin O. Krebsky[a,*], Jan M. C. Geuns[b], Maurice De Proft[a]

[a]*Lab. Plantenteelt, KULeuven, De Croylaan 42, B-3001 Heverlee, Belgium*
[b]*Lab. Plantenfysiologie, KULeuven, Kard. Mercierlaan 92, B-3001 Heverlee, Belgium*

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Abstract

Free sterol and polyamine contents were investigated in chicory leaves of different physiological ages and throughout a postharvest period. The major polyamine present was putrescine (put), especially in the oldest leaves. Spermidine (spd) was present in considerable amounts, showing a tendency to decrease with the increasing physiological age of the leaves. At harvest, the put content in the floral stalk was similar to that in older leaves and it increased during postharvest. The opposite was found for spd, being similar to younger leaves and being constant during postharvest. Free sterol content increased with postharvest and also with physiological age of the leaves. Sitosterol was always the major free sterol present, followed by stigmasterol and campesterol. The stigmasterol to sitosterol ratio rose with time after harvest, older leaves showing higher ratios than younger leaves.

Keywords: *Cichorium intybus*; Compositae; Chicory; Postharvest; Free sterols; Polyamines

1. Introduction

Chicory (Belgian endive), *Cichorium intybus* L. var. *foliosum* cv. Final, is a biennial plant, being grown in the field for root production during its first year. After harvest, roots are vernalised and then forced on a nutrient solution in darkness (De Proft, De Greef, Van Nerum & Goffings, 1986). Membrane deterioration is one of the first steps in senescence. Membrane permeability and fatty acid composition have therefore been investigated (Krebsky, Geuns & De Proft, 1996), but other membrane compounds were not studied at that time.

Polyamines (PAs) interact with nucleic acids and with membranes (Galston, 1983). Endogenous PAs show antisenescence effects by sharing *S*-adenosylmethionine as a common precursor for ethylene biosynthesis (Mattoo & Aharoni, 1988). Exogenous PAs are known to bind to membranes, prevent lipid peroxidation and scavenge free radicals involved in the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene (Evans & Malmberg, 1989). PAs are also associated with cell division, and a decline in PA content in many plant organs is correlated with senescence (Galston, 1983; Ludford, 1995). Sterols are structural and functional elements of membranes. Many investigations have shown an increase in the stigmasterol to sitosterol ratio during aging of plant tissues (Grunwald, 1975; Brown & DuPont, 1989; Whitaker, 1988, 1994; Geuns, Van Loenhout, Valcke, Van Loven, Redig et al., 1997).

In addition to the previous study in *Cichorium* plants on fatty acids from lipids (Krebsky et al., 1996), an analysis of PAs and free sterols should allow us to better evaluate the quality of the crop during the postharvest period.

2. Results and discussion

The etiolated chicory head contains 20–26 leaves. These leaves enclose a floral stalk elongating during chicory growth in darkness. This elongation depends on the cultivar used, growth conditions, root quality, etc. In this research, the length of the floral stalk was about 60% of the total chicory height at harvest and the stalk continued to elongate during shelf life.

In the different plant parts that we analysed, spermine (spm) levels were very low and did not change

* Corresponding author.

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Table 1
Polyamine ($\mu M\ g^{-1}$ dry wt)

Polyamine ($\mu M\ g^{-1}$ dry wt)		Days After Harvest				
		0	2	4	6	8
outer leaves	put	4.3 ± 0.4	3.7 ± 0.9	5.8 ± 1.9	4.5 ± 0.7	7.1 ± 1.3
	spd	3.2 ± 0.4	2.9 ± 0.3	3.4 ± 0.5	2.7 ± 0.1	3.2 ± 0.3
inner leaves	put	1.7 ± 0.1	2.1 ± 0.2	2.5 ± 0.3	1.9 ± 0.2	2.2 ± 0.2
	spd	4.2 ± 0.4	3.0 ± 0.5	4.0 ± 0.3	4.2 ± 0.2	4.4 ± 0.3
floral stalk	put	4.0 ± 0.4	5.3 ± 0.1	7.6 ± 1.5	6.3 ± 0.4	13.6 ± 1.3
	spd	4.2 ± 0.2	5.2 ± 0.2	4.6 ± 0.2	4.0 ± 0.4	4.5 ± 0.2

during postharvest. Therefore, data were not included in Table 1. From harvest day until 8 days after harvest, there was an increase of putrescine in the outer leaves of *Cichorium* heads, from about 4.2 to about 7.1 $\mu M\ g^{-1}$ dry wt. Spd level was constant over the 8 days after harvest (Table 1). In the inner leaves (young), spd and put levels were quite stable during the postharvest period. The floral stalk showed the highest PA content found in the endive. Until 2 days after harvest, the put and spd contents were similar but after this period put started to increase and spd stayed constant (Table 1).

The ratio of put to spd + spm increased with shelf life in a similar way for outer leaves and floral stalk, more sharply in the last one. For the inner leaves this ratio was constant during this period after harvest [Fig. 1(a)].

We considered 3 different types of plant tissues, namely outer and inner leaves and the floral stalk. During shelf life, we found increasing put levels in the outer leaves, the spd level remaining constant. These leaves are fully expanded and are the physiologically oldest part of the endive head. These results were in agreement with an observed accumulation of put when elongation growth takes place (Galston, 1983; Evans & Malmberg, 1989). Similar increases in put, while spd and spm were unaffected, were observed in cut Carnation flowers (Roberts, Walker, Thompson & Dumbroff, 1984) and in older leaves of *Petunia* (Gerats, Kaye, Collins & Malmberg, 1988). The increment in outer leaves during the postharvest period could also be a reaction to harvest stress (wounding). Different types of stresses (like mineral, osmotic, acid) can affect PA levels, especially the accumulation of put (Galston, 1983; Evans & Malmberg, 1989; Das, Bose & Ghosh, 1995).

In the inner, not fully expanded, leaves, spd was the major PA present (51–62%) (Table 1). However, its level and that of put stayed constant, possibly because no elongation growth took place.

The floral stalk had the highest put content during the whole shelf life (Table 1). This should be expected as PA levels increase during elongation growth taking place during this period (Galston, 1983; Evans & Malmberg, 1989; Palavan & Galston, 1982; Lee, Shieh & Chou, 1996). Although the crop visually showed signs of senescence 8 days after harvest, the total PA content did not decrease. In chicory heads, the ratio of put to spd plus spm was highest in the outer leaves until 6 days after harvest [Fig. 1(a)]. It increased during shelf life for outer leaves and floral stalk; inner leaves did not show a change in this ratio. It has been suggested that an increase of this ratio might also indicate the presence of a detrimental process (Tiburcio, Masdeu, Dumortier & Galston, 1986).

Membrane fluidity decreases during senescence of plants (Leshem, Halevy & Frenkel, 1986; Fobel, Lynch & Thompson, 1987). PAs were linked to the prevention of lipid peroxidation (Evans & Malmberg, 1989) and lipids are believed to be primarily linked to membrane fluidity. Besides phospholipids and galactolipids, sterols are also present in plant membranes with free sterols representing the major part of the sterol moiety. Sterols have been shown to maintain structural integrity of membranes, to regulate membrane stability and to reduce the mobility of phospholipid acyl chains (Grunwald, 1975).

In inner and outer leaves, the major free sterol present was sitosterol, followed by stigmasterol and campesterol (Table 2). Cholesterol was also found but in trace amounts (lower than 30 $\mu g\ g^{-1}$ dry wt) and not in all leaves or floral stalk (data not shown). In the leaves, all three free sterols increased with time but the highest levels were found in the outer leaves. Eight days after harvest, in the outer leaves, sitosterol increased about 54%, stigmasterol 200% and campesterol 42%. In the floral stalk, total free sterol content was similar to that in the outer leaves. Sitosterol was the major sterol present, followed by stigmasterol and campesterol. All three sterols increased with time in this tissue (Table 2). In the leaves the stigmasterol to sitosterol ratio increased with time. This ratio was lower in the floral stalk than in the leaves, but it also increased during shelf life of the crop [Fig. 1(b)].

The sitosterol content was found to be two times higher than that of stigmasterol in etiolated barley

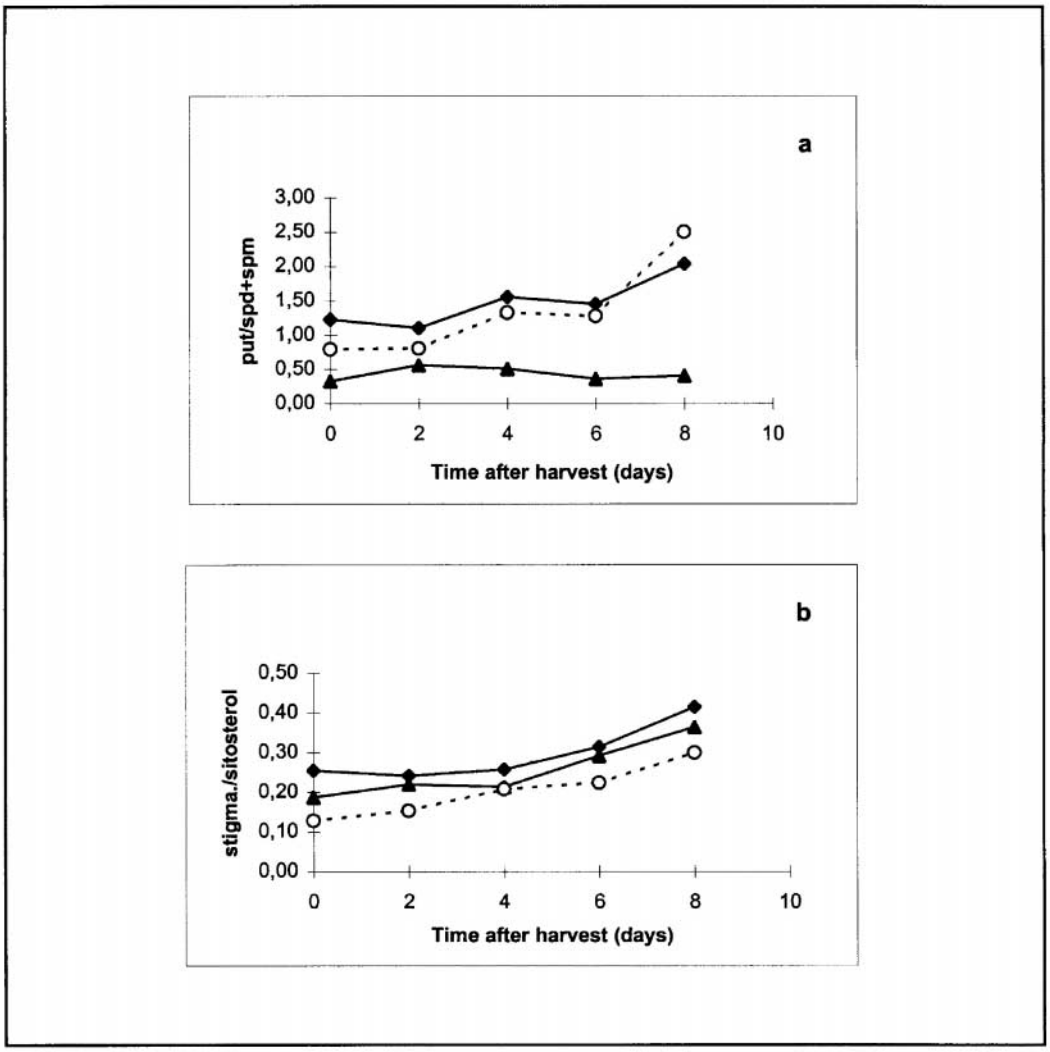

Fig. 1. Variation (a) of the ratio putrescine to spermidine plus spermine and (b) of the stigmasterol to sitosterol ratio in inner (▲) and outer (◆) leaves and in the floral stalk (fs, ○) of chicory as a function of time after harvest. Crops were stored at room temperature.

Table 2
Free sterol content (mg g^{-1} dry wt) in chicory stored at room temperature. Mean values for $n = 3$, ± s.e.

Free Sterols (mg g^{-1} dry wt)		Days After Harvest				
		0	2	4	6	8
Sitosterol	outer leaves	1.7 ± 0.18	2.0 ± 0.12	2.3 ± 0.21	2.4 ± 0.15[a]	2.7 ± 0.19[b]
	inner leaves	1.3 ± 0.19	1.6 ± 0.16	2.0 ± 0.21	1.7 ± 0.12	2.1 ± 0.11
	floral stalk	2.0 ± 0.02	2.2 ± 0.29	2.4 ± 0.09	1.7 ± 0.07	3.0 ± 0.40
Stigmasterol	outer leaves	0.4 ± 0.07	0.6 ± 0.04[a]	0.8 ± 0.11[a]	0.8 ± 0.02[b]	1.3 ± 0.14[b]
	inner leaves	0.2 ± 0.05	0.4 ± 0.08	0.4 ± 0.07	0.5 ± 0.03	0.8 ± 0.07
	floral stalk	0.3 ± 0.02	0.3 ± 0.05	0.5 ± 0.09	0.4 ± 0.01	0.8 ± 0.08
Campersterol	outer leaves	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.01	0.2 ± 0.02[b]
	inner leaves	0.1 ± 0.01	0.1 ± 0.03	0.1 ± 0.01	0.1 ± 0.03	0.1 ± 0.02
	floral stalk	0.2 ± 0.01	0.2 ± 0.04	0.2 ± 0.02	0.2 ± 0.02	0.3 ± 0.05

[a] significant at 95% level of confidence
[b] significant at 99% level of confidence

shoot tissue. In green material, the level of sitosterol equalled that of stigmasterol (Bush, Grunwald & Davis, 1971). In etiolated chicory leaves sitosterol was found as the major sterol (Table 2). The free sterol content was reported to decline with maturation of peach fruit (Izzo, Scartazza, Masia, Galleschi, Quartacci et al., 1995) or with senescence of *Sphagnum* (Karunen, Mikola, Linko & Euranto, 1979). On the contrary, during ripening of tomatoes, a large increase in the amount of steryl lipids was found, together with a rise in the stigmasterol to sitosterol ratio which was ascribed to fruit ripening rather than to aging (Whitaker, 1988).

The increase in the stigmasterol to sitosterol ratio has been linked with aging and ripening processes in *Phaseolus* (Geuns, 1973), in *Nicotiana* (Davis, 1972), in barley roots (Brown & DuPont, 1989) and in ripening tomato fruits (Whitaker, 1988, 1994) in which at the same time there was a decrease in membrane fluidity (Legge, Cheng, Lepock & Thompson, 1986). The rise in free sterols with aging can be explained by the fact that in mature leaves, the free sterols are not only membrane components, but also part of a non-metabolic sterol pool (Grunwald, 1975). In cytosolic particles of bean cotyledons the proportion of stigmasterol rose with senescence (McKegney, Yao, Ghosh, Huff, Mayak et al., 1995).

The outer leaves had the highest total free sterol levels (similar to the floral stalk) and the highest stigmasterol to sitosterol ratio as well [Table 2, Fig. 1(b)]. The older leaves of *N. tabacum* showed a higher sterol content, with the change in free sterol content accounting for the largest increase during aging (Davis, 1972). However, Geuns et al. (1997) found a decrease of total free sterol content in older leaves of *N. tabacum*.

This study, combined with our previous results (Krebsky et al., 1996), permit us to evaluate the quality of chicory crops during postharvest. Senescence and deterioration of the crop can be observed by the increase of put, by the increase of the put to spd + spm ratio, by the increase of the stigmasterol to sitosterol ratio as well as by the increase of the total sterol content, and by the decrease of the total fatty acid content in the floral stalk and the outer (older) leaves.

3. Experimental

3.1. Plant material

Belgian vernalised endive roots [*Cichorium intybus* L. var. Final] were grown in hydroponic culture in darkness, at 16–18° and 90–100% relative humidity. After 3 weeks the chicory heads were harvested and those with the biggest floral stalk were separated and kept protected from light and desiccation with dark blue parafilm paper, inside cardboard boxes. These boxes were stored at $21 \pm 1°$ and 60% relative humidity. Sampling was done at harvest day and every 2 days, 4 times. Three endives were sampled per day and leaves were separated in groups of 6 outer and 6 inner, according to their position in the crop. Each group represents a specific physiological age dependent upon the location, outer leaves being the oldest and inner leaves the youngest. The floral stalk enclosed in the leaves was analysed separately.

3.2. Polyamine analysis

10 mg of dry material were homogenized in 1 ml 4% (v/v) $HClO_4$ containing 1,7-diaminoheptane-2HCl ($2\ mg\ l^{-1}$) as internal standard. Dansylation and analysis were done as described in Geuns et al. (1997).

3.3. Sterol analysis

Dry powdered tissue (50 mg) was extracted 4× with 3 ml hexane:MeOH (1/1) containing 20 μg coprostanol as internal standard. Analysis was as described in Geuns et al. (1997).

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