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FATTY ACIDS IN POLAR LIPIDS FROM ETIOLATED *CICHORIUM INTYBUS*

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Key Word Index—*Cichorium intybus*; Compositae; witloof; Belgian endive; etiolation; fatty acids; postharvest.

Abstract—Changes in the fatty acid (FA) content of polar lipids were studied during the postharvest period of Belgian endive (*Cichorium intybus* var. *foliosum* cv. Final). The major fatty acid present in these lipids was linoleic (33–62%) followed by palmitic (24–36%). Changes in the FA composition were observed after harvest in both leaves and flowering stalks. The most pronounced changes occurred in the younger leaves within the first 4 days postharvest. Older leaves showed the greatest amounts of total FA. Younger leaves had higher saturated to unsaturated FA ratios. Floral stalks and leaves showed a decrease in total FA and saturated to unsaturated FA ratios with time. The results are discussed in the relation to the diagnostic value in respect of plant tissue age determination. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The total lipid content in plant cells is *ca* 5–10% of the dry weight. In plant tissues, most of the lipids are membrane components. The fatty acids (FAs) present normally have a carbon chain length of 16 or 18, varying in the number of *cis*-double bounds (0–3) [1]. Physico-chemical changes in the lipid phase of the membranes modifies membrane permeability, energy transduction capacity and activities of membrane-bound enzymes [2]. These changes are linked with the saturation level of FA in the lipids. Lipid peroxidation is associated with senescence in living biological systems; polyunsaturated FAs are preferentially degraded. It has been reported that during *in vitro* senescence of microsomal membranes from harvested carnation flowers, an increase in the saturated to unsaturated FA ratio was observed owing to a reduction in unsaturated FA (linoleic and linolenic), resulting in a decrease in membrane fluidity [3]. During ripening of mature green tomatoes, the saturation level of FA increased slightly, whereas phospholipids and galactolipids dropped by *ca* 20 and 35%, respectively. During chilling of the harvested fruit, the unsaturation increased slightly, phospholipid content did not change and glycolipids decreased by *ca* 15% [4]. A significant rise in unsaturated C_{18} FA in total polar lipids was reported during development of peach fruits, together with a decrease in total lipid content [5].

In plasma membranes from mung bean hypocotyl segments of different physiological age, variation of phospholipid content was found with a decrease from top (young) to the lower segment [6].

For market, it is important to define good quality parameters of endive heads in relation to shelf-life. The aim of the present study was to identify the FA composition of endive heads and follow changes with physiological age in leaves and flower stalks.

RESULTS

Leaves

In all leaves comprising the endive head, linoleic (18:2) was the major FA, ranging from 33 to 62%, followed by palmitic (16:0; 24–36%), linolenic (18:3; 8–26%) and then by stearic (18:0; 2–17%). Oleic acid (18:1) and palmitoleic acid (16:1) were both present in amounts lower than 5% and they were included only in the total FAs (Table 1). During shelf-life, full-grown endives showed dry weight losses of several percentage. For the outside (older) leaves there was a 1.5–2.0% dry weight loss. From position 5, the dry weight losses become even more important; the inner (younger) leaves especially lost up to 6% (Fig. 1). These changes in dry weight may arise from differing losses in water and/or organic material. In endives kept for 8 days at 21°, 18:2 and 18:3 are still the major FA isolated from the polar lipids. This is reflected in the saturated to unsaturated FA ratio (Fig. 2). The content of 16:0 and 18:0 in leaves of different physiological age did not change. But from 6 days after harvest, the content of these two FA decreases in all leaves (Table

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Table 1. Fatty acid contents (mg g^{-1} dry wt) of Belgian endive

Time (days)	Fatty acid	Leaf position 1	2	3	4	5	6	7	8
0	16:0	5.2±1.0	5.0±0.3	5.1±0.3	5.0±0.7	3.8±0.1	4.8±1.0	3.5±0.3	3.6±0.2
	18:0	1.3±0.2	1.5±0.2	1.4±0.1	1.6±0.3	1.1±0.1	1.4±0.1	1.4±0.1	1.5±0.3
	18:2	6.5±0.5	7.3±0.9	6.5±1.3	5.9±0.2	4.8±0.4	4.8±0.5	3.2±0.3	3.5±0.3
	18:3	3.7±0.1	3.3±0.7	2.7±1.1	2.5±0.6	1.9±0.4	1.8±0.5	0.8±0.2	1.0±0.2
	Total	17.6±1.8	18.0±1.8	16.3±2.8	16.0±0.5	12.4±0.7	13.7±1.7	9.5±0.6	10.8±1.1
2	16:0	4.2±0.9	4.1±0.5	3.5±0.5	3.5±0.6	3.8±0.9	3.8±0.7	3.2±0.4	3.2±0.6
	18:0	1.2±0.8	2.1±0.5	1.0±0.7	1.4±0.5	2.0±0.4	1.6±0.5	1.6±0.3	1.6±0.4
	18:2	6.2±0.3	7.2±0.4	6.2±0.3	5.5±0.5	6.0±0.6	5.8±0.5	4.9±0.5	5.2±0.8
	18:3	4.0±0.2	4.2±0.3	3.3±0.1	2.6±0.1	2.8±0.3	2.6±0.3	2.1±0.1	2.9±0.3
	Total	15.5±2.4	16.5±1.5	13.9±1.3	12.6±1.3	13.5±2.5	13.2±1.5	10.8±1.3	11.9±2.1
4	16:0	4.3±0.4	4.1±0.4	3.9±0.4	3.8±0.5	3.8±0.1	3.6±0.5	4.7±0.2	3.9±0.4
	18:0	1.5±0.5	1.4±0.2	1.1±0.1	1.7±0.4	1.7±0.3	1.2±0.6	2.6±0.2	1.8±0.6
	18:2	6.0±0.9	5.6±1.1	5.8±0.9	4.8±0.9	4.9±0.7	6.0±0.5	6.2±0.8	5.3±0.4
	18:3	3.6±0.8	2.7±0.6	2.4±0.4	2.1±0.4	2.3±0.5	2.2±0.4	2.6±0.5	2.3±0.5
	Total	16.7±2.3	15.4±2.1	14.6±1.3	13.8±2.2	14.0±1.0	13.2±1.9	15.2±0.4	13.2±1.3
6	16:0	2.0±0.2	2.7±0.5	2.5±0.4	2.6±0.4	2.0±0.3	2.1±0.2	2.8±1.2	2.6±0.6
	18:0	0.4±0.2	0.5±0.3	0.5±0.3	0.5±0.3	0.5±0.3	0.2±0.0	0.6±0.1	0.3±0.1
	18:2	3.8±0.4	5.2±0.8	5.0±0.6	4.8±0.6	3.6±0.3	4.1±0.8	5.4±1.8	4.6±1.1
	18:3	1.4±0.5	1.8±0.2	1.6±0.1	1.6±0.2	1.1±0.1	1.5±0.4	2.0±0.9	1.8±0.5
	Total	7.5±0.9	10.3±1.8	9.3±1.3	9.3±1.5	7.1±0.9	7.9±1.3	10.5±4.5	9.5±2.3
8	16:0	1.8±0.1	1.7±0.2	1.5±0.1	1.8±0.3	1.5±0.2	1.7±0.3	2.1±0.4	1.8±0.4
	18:0	0.1±0.01	0.1±0.01	0.1±0.01	0.2±0.03	0.1±0.03	0.1±0.02	0.2±0.03	0.2±0.04
	18:2	3.8±0.2	4.0±0.5	3.5±0.4	4.6±0.6	3.3±0.5	3.5±0.5	4.0±0.6	2.7±0.4
	18:3	1.4±0.2	1.1±0.2	1.0±0.1	1.3±0.2	1.1±0.2	1.2±0.2	1.8±0.2	1.5±0.1
	Total	7.3±0.4	6.8±0.9	6.1±0.6	7.3±1.6	6.0±0.9	6.7±1.0	8.6±1.8	7.4±1.3

Mean values for $n = 3$, ±s.e.
Position 1 = two outer leaves, position 2 = leaves 3 and 4, in sequence til the inner ones = position 8.

1). The total amount of FA at the time of harvest is the lowest in young leaves (positions 7 and 8) and highest in old leaves (positions 1 and 2), ranging from 10.8 to 17.6 mg g^{-1} dry weight (Fig. 3). This difference was the result of an increase of 30% in 16:0, 50% in 18:2, 72% in 18:3 and 3% in 18:0 (Table 1).

The unsaturated FA from the polar lipids of the younger leaves show an initial increase (up to day 4) and than a decrease. For the same FA in older leaves, only a decrease from day 4 onwards was observed. Both young and old leaves from day 4 onwards showed a clear decrease in saturated FA (Table 1). The saturated to unsaturated FA ratio was high in young leaves. At harvest, the ratio was *ca* 87% higher in young leaves than in old ones. This difference declined with time, with the amounts being almost equal at day 8. The younger leaves showed a more pronounced decline in the saturated to unsaturated FA ratio as a function of time, being *ca* 64% compared with 44% in older leaves at day 2 (Fig. 2). Total FA content was

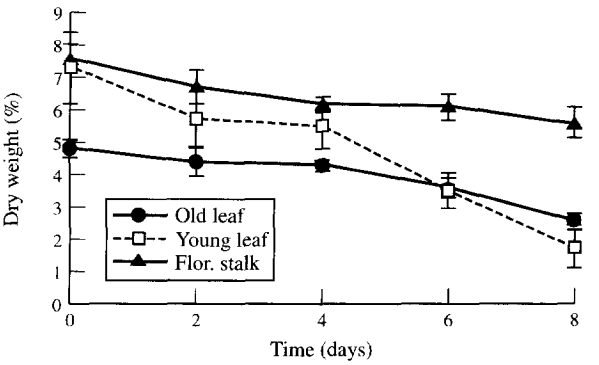

Fig. 1. Variation of per cent dry weight in endive leaves of different physiological ages (old and young) and floral stalk with time after harvest. Error bars indicate s.e. for $n = 3$.

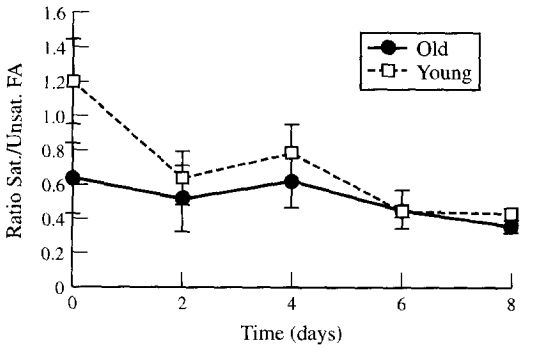

Fig. 2. Variation of saturated to unsaturated FA ratio in endive leaves at different physiological ages (old and young) as a function of time after harvest. Error bars indicate s.e. for $n = 3$.

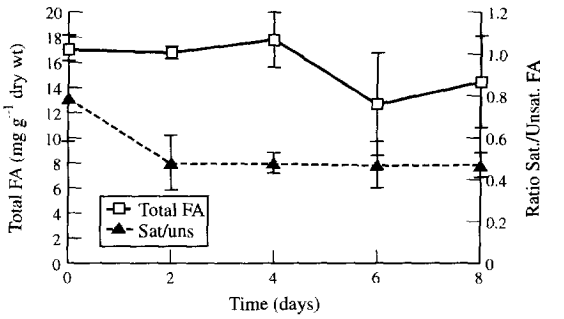

Fig. 4. Variation of total FA and saturated to unsaturated FA ratio in the floral stalk of endives at different times after harvest. Error bars indicate s.e. for $n = 3$.

lower in young leaves than in old leaves until day 4. Total FA decreased with shelf-life time for all leaves from day 4 onwards (Fig. 3).

Flowering stalk

The size of the flowering stalk from the endives studied was considered high, having, at harvest, a relative length of 62% against the total size of the endive and after 8 days, up to 120%, varying from 9 up to 14.5 cm high. The dry weight of the floral stalk decreased almost linearly from 7.6 to 5.6% after 8 days shelf-life (Fig. 1). At harvest, the floral stalk was characterized by a FA content similar to that of the fresh outer leaves. The total FA content decreased by only *ca* 15% after 8 days shelf-life, whereas the mean decrease of FA in leaves was *ca* 51% after this period (Tables 1 and 2). At harvest, 18:2 and 18:3 percentages were, respectively, 42 and 10% of the total FA. After 2 days, these contents change to 53% 18:2 and 15% 18:3, and then remained constant. In general, saturated FAs in the floral stalk tissues tended to decrease with time and unsaturated FAs had a tendency to increase (Table 2).

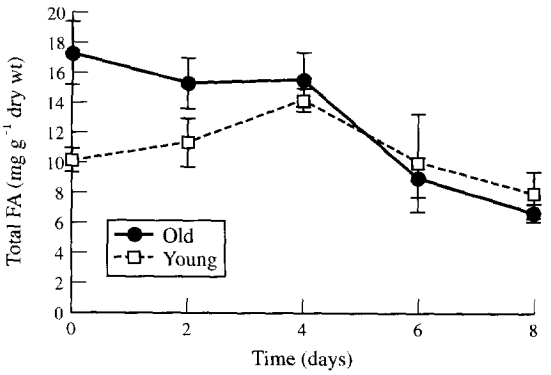

Fig. 3. Variation of total FA content in old and young endive leaves at different times after harvest. Error bars indicate s.e. for $n = 3$.

Total FAs decreased slightly, whereas the saturated to unsaturated ratio only shifted from 0.8 to 0.4 within the first 2 days after harvest (Fig. 4). All FAs found were present in roughly equal amounts compared with the composition of the leaves, where 18:2 is the major FA. However, the decrease in total FA is associated more with a drop in 16:0 content (Table 2).

Membrane permeability

Water permeability through cell membranes is lower in young than in old leaf tissue. During the shelf-life period, this transport in older tissue does not change very much. For young tissue, an increase in water permeability occurs at the end, similar to the old leaf

Table 2. Fatty acid content (mg g^{-1} dry wt) and variation with shelf-life of floral stalks of Belgian endives

	Time after harvest (days)				
Fatty acid	0	2	4	6	8
16:0	5.4±0.5	4.6±0.3	4.6±0.7	3.6±1.3	3.8±0.9
18:0	1.9±0.3	1.1±0.1	1.2±0.1	0.6±0.2	1.2±0.1
18:2	7.3±0.4	9.0±0.1	9.5±1.0	7.1±1.9	8.3±1.8
18:3	1.6±0.2	2.5±0.2	2.5±0.2	1.7±0.4	2.1±0.3
Total	17.1±1.4	16.8±0.7	17.8±2.1	12.8±3.9	14.6±3.2

Mean values for $n = 3$, ±s.e.

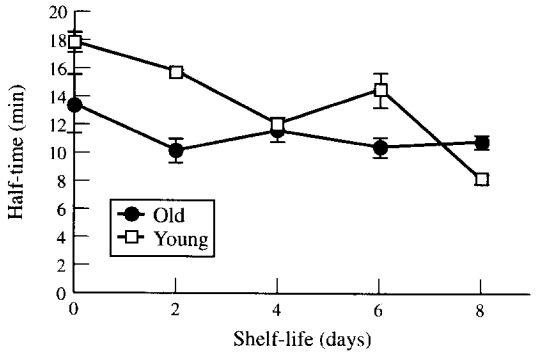

Fig. 5. Variation of half-time for efflux of tritiated water out of cells in young and old leaves of endives. Error bars indicate s.e. for $n = 3$.

permeability. We have observed an increase of up to 55% (Fig. 5).

DISCUSSION

Belgian endive is produced as an etiolated vegetable, thus lacking chloroplasts and, consequently, photosynthetic activity. Glycolipids and phospholipids are the main structural lipids of chloroplasts and plasma membranes, respectively [2, 7]. This accounts for the FA composition being higher in 16:0 and 18:2, the most important FAs of phospholipids [8]. The major FAs found in endive heads are similar to those found in plasma membranes and tonoplasts isolated from etiolated mung bean seedlings, [9]. In endive, the highest FA content was 18:2 (up to 7.2 mg g^{-1} dry wt), instead of 16:0 (up to 5.2 mg g^{-1} dry wt), as repeated in other plant material. By the method used, only polar lipids will be transmethylated. It is expected that endive does not accumulate triacylglycerols and, being an etiolated material (glycolipids are the major component of chloroplasts), the FA analysed originate mainly from phospholipids [10].

Considering the physiological age of leaves, we expected that at harvest older leaves would have a lower total FA content and also a higher saturated to unsaturated FA ratio than younger leaves [6, 11, 12]. For endives, the opposite was found (Figs 2 and 3). This might be linked with leaf expansion. In primary bean leaves, an increase in phosphatidylglycerol during leaf development was found until 4 days after full expansion and then its content decreased. Phosphatidylcholine was constant during the starting period of leaf development and decreased after full expansion; it rose again at senescence. Phosphatidylethanolanine steadily decreased [13]. This might be the reason for the higher FA content in the older but not fully expanded endive leaves, since we are dealing with etiolated material.

At harvest, total FA content in older leaves was higher than in younger leaves. For older leaves, almost no change in total FA occurred until 4 days after harvest. At the same period, younger leaves still showed an increase in FA content. The difference might be explained by the fact that young leaves are still expanding, while the older ones are already fully developed (in terms of etiolated material). After 4 days, both old and young leaves showed a similar decrease in their total FA contents. This indicates that at 4 days after harvest, the young leaves have probably started their 'aging' process, along with the old leaves. From 8 days after harvest, there was no difference in total FA content between different leaves (Table 1 and Fig. 3).

Degradation of unsaturated fatty acids in senescent membranes is well documented and is attributed to the activity of lipoxygenases, which attack 18:2 and 18:3 FAs [2, 12]. A general decrease in the total amount of FAs was registered in endive, but also a decrease in the saturated to unsaturated FA ratio (Table 1, Figs 2 and 3). A lowering as a function of time occurred only for 18:3 and 18:2, specifically in older leaves. A decrease in 16:0 and 18:0 was found in both old and young leaves (Table 1). During storage of pollen, there was no large selective loss of polyunsaturated FA, suggesting another unknown mechanism of lipid degradation, different from peroxidation, operating during aging of pollen [14]. There is little information about FA kinetics in etiolated material available in literature.

Lipid breakdown might also be induced by exterior factors such as light and temperature. Research with the moss *Sphagnum magellanicum* in dark conditions showed a decline in 18:3 FAs from glycolipids [15]. The change is probably linked with the breakdown of the thylakoids. Again, in darkness, an increase of 16:0 in glycolipids and 18:2 in phospholipids was found in another moss (*Physcomitrella patens*), together with a decline in 18:3 in both lipid classes [7]. In cyanobacteria, light and temperature-lowering induced desaturation of FAs [16]. The authors (Wada and Murata **16**) suggested that a change in desaturation is connected with reactions involved in the photosynthetic electron transport. The crop studied by us was etiolated leaf material produced at 15–16° and stored at 21°, and kept in darkness at all times. This might account for the lack of desaturation.

Cauliflower treated with gamma-irradiation showed an increase in the ratios of sterols to phospholipids and of free fatty acids to phospholipids during storage. The depletion of polyunsaturated FA occurred in the free FA fraction, not in the polar lipid fraction [17]. The free FA fraction of endive, during a period of 8 days after harvest, did not show any appreciable changes (data not shown). The floral stalk continues to grow even in the postharvest period (Table 2, Figs 1 and 4).

Although we obtained unexpected results for the breakdown of unsaturated FA, we observed a decrease in their content in older leaves as a function of time. Total FA content decreased in all leaves with time, indicating a relation with membrane degradation. Although at harvest a difference in water permeability was observed between younger and older leaves, the lack of differences in old (expanded) leaves with time makes such measurements an unsuitable parameter for characterizing leaf senescence.

Further research is necessary to unravel the change in dry weight composition of, especially, inner leaves, that might be used as additional parameter for shelf-life control. Sudden changes in dry weight and the decrease in unsaturated and total FA content between days 4 and 6 might be of diagnostic value for the quality control of the endive crop.

EXPERIMENTAL

Plant material. Belgian vernalized endive roots *Cichorium intybus* L. var. *foliosum* cv. Final were grown in hydroponic culture in darkness at 16–18° and 90–100% r.h. After 3 weeks, 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±1° and 60% r.h. Sampling was done at harvest day and every 2 days after until 8 days harvest. Three endives were sampled per day and the leaves separated into groups of two to three pieces, according to their position in the crop. Each group represents one specific physiological age dependent upon the position, outer leaves (groups 1–3) being the oldest and inner leaves (groups 7–8) the youngest. The floral stalk enclosed in the leaves was also analysed. The material was freeze-dried. For H_2O permeability expts, fr material was used, taking two outer and two inner leaves from each crop, and repeating the measurements during different periods of shelf-life.

Fatty acid analysis. Base-catalysed transmethylation of FAs bound to phospholipids (and triacylglycerols) was done in 5% KOH in MeOH. All solvents contained 5 ml l^{-1} butylated hydroxytoluene. Dry powdered tissue (10 mg) was sonicated (1 min) and extracted in 1 ml iso-PrOH containing Me 17:0 (10 μg ml^{-1}) as int. standard, by vigorously shaking during 30 min at 0° in the dark. After centrifugation, the pellet was extracted a second time with $CHCl_3$–MeOH (2:1) for 1 hr at 0°, while continuously shaking. Both extracts were combined and evap in a vacuum centrifuge at room temp. The lipid extract was dissolved in 100 μl toluol and sonicated (30 sec). Then, 100 μl 5% KOH in dry MeOH was added and, after exactly 4 min at room temp., 200 μl H_2O was added. The FA Me esters were extracted in 1 ml pentane, that was pipetted off, washed with 200 μl H_2O and evapd in a vacuum centrifuge. As breakdown of samples might occur during storage, capillary GC analysis always immediately followed sample prepn. During GC analysis, the samples dissolved in 50 μl pentane, were stored on ice. Under these conditions of base-catalysed transesterification, free FA are not esterified and steryl esters are hardly influenced, as they are only transesterified very slowly [10]. GC conditions: Bio-Rad 30 m × 0.32 μm bonded FSOT RSL-500 (polyphenylcyanopropyl-methylsiloxane), df = 0.25 μm; oven 190°; detector: FID at 250°; split-injector (1:100) at 220°; injection vol. 3 μl.

Membrane permeability. Tissue permeability for ^3H_2O was measured on 5 mm segments of the central nerve of the leaf, according to ref. [18]. Each expt was done in duplicate with five segments of the outer and five of the inner leaves. The water permeability of the ± issue satd with ^3H_2O was measured. The efflux of the ^3H_2O was followed during 30 min using a flow-through cell, taking samples every 30 sec. A home-made computer program permitted differentiation between the various diffusions occurring out of different compartments and calculation of the half-times of diffusion through the membranes [18, 19].

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