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Variation in the content of the main guaianolides and sugars in *Cichorium intybus* var. "Rosso di Chioggia" selections during cultivation

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Abstract

Cichorium intybus var. "Rosso di Chioggia" is a chicory variety cultivated predominantly in Northeastern Italy and highly appreciated for its bitter taste. The commercial portion of the plant, used as food, comprises the innermost leaves (head) of plants harvested during the first year of growth; the outermost leaves and the roots are considered wastes. Three selections belonging to this variety have been analyzed for guaianolide and sugar contents and compared with the commercial product (head), roots and outermost leaves. The qualitative and quantitative evaluations of both classes of compounds were achieved by densitometric HPTLC. The analytical data showed a high variability during the stages of growth, both among the parts of the plant and among the three selections of chicory considered. With particular reference to the heads, a sensory bitterness evaluation method was set up in order to find a correlation between the analytical data and the bitter taste. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Chicory; Sesquiterpene lactones; 8-Deoxylactucin; 11β,13-Dihydrolactucin; HPTLC densitometry; Bitter sensorial analysis

1. Introduction

Throughout Europe, many varieties of *Cichorium intybus* L. (*Asteraceae*) chicory are quite important agricultural crops, highly appreciated for their bitter taste. This bitterness is due to the presence of large quantities of sequiterpene lactones, such as lactucin, 8-desoxylactucin, lactucopicrin and 11β-dihydro-derivatives (Peters & Van Amerongen, 1998, and references therein), particularly in the roots (van Beek, Maas, King, Leclerq, Voragen, & De Groot, 1990).

One of the general problems encountered in the production of chicory, is that the sequiterpene lactone content varies widely, not only from one cultivar to another, but also within a single cultivar. Besides determining the exact moment for harvest by defining the exact point at which each cultivar ripens (Peters & Van Amerongen, 1996), the sequiterpene lactone content causes wide differences in flavour from one harvest to the next. Indeed, for a single cultivar, such flavour differs greatly, depending on what period of the year the crop is harvested (Chillemi, 1998; Pimpini & Chillemi, 1993). To alleviate this problem, numerous attempts have been made to correlate the guaianolide content of *C. intybus* plants with the bitter taste determined by sensorial testing (Dirinck, Van Poucke, Van Acker, & Schamp, 1985; Peters & Van Amerongen, 1998; Price, Du Pont, Shepherd, Chan, & Fenwick, 1990; Price, Du Pont, Shepherd, Chan, & Fenwick, 1990; Van Mazijk-Bokslag, Cramwinckei, Essers, & Hollman, 1991).

The present work studies three selections of *C. intybus* L. var. "Rosso di Chioggia", a variety cultivated predominantly in Northeastern Italy and highly appreciated for its bitter taste. The commercial portion of the plant, used as a food, comprises the tender, innermost

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leaves or "head" of plants, harvested during the first year of growth; the outermost leaves, which are easily removed, and the roots are considered wastes. Analysis was performed to determine the content of the main guaianolides and sugars in plants from three selections of "Rosso di Chioggia", taken during the first year of growth. In comparison to the commercial product, an attempt was made to determine whether there were any differences between the three selections in terms of the quality of the bitter taste and to correlate sensorial data with the analytical results. In addition, the extraction method was optimized, using water as the solvent of choice rather than organic solvents (Kisiel & Barszcz, 1997; Leclercq, 1984; Peters & Van Amerongen, 1996; Pyrek, 1985; Seto, Miyase, Umehara, Ueno, Hirano, & Otani, 1988; Van Beek et al., 1990).

2. Materials and methods

2.1. Plant material

Plants belonging to three selections of *C. intybus* L. var. "Rosso di Chioggia" (kindly provided by the Centro Sperimentale "Veneto Agricoltura" of Po di Tramontana, Rovigo, Italy) were used in this study. The commercial product—the head or epigeal portion (i.e. the innermost portion of the head)—was taken from plants during their first year of growth. In particular, the selections analyzed were line "chp c 54/3-8-2-4-7" (C1), a so-called "early" variety; line "ea 1° p 5a/38-7-7-6" (C2), having a slightly longer than average growth span; line "ea 2° p IV ep 24/6-6-5-3" (C3), a "later" variety than the other two. The selections were planted in the nursery and, after 30 days, the plantules were transplanted to the open field, following the established cultivation techniques (Chillemi, 1998; Pimpini & Chillemi, 1993). From the time of sowing until 30 days after, the ripe product was harvested (over-ripening), a sample of each selection was taken every 15 days. For the first 30 days the analyses were performed on the whole plantule; thereafter, separate samples and analyses were performed on the roots, heads and outer leaves, taking 100 g of product for each sample. For the plants cultivated in the open field, the roots, heads and outer leaves were collected and separated manually. A total of 50 plants of each selection were considered at each stage.

2.2. Preparation of chicory extracts

At each harvest (for the entire period of cultivation), samples of the three selections of *C. intybus* var. "Rosso di Chioggia" were separated (outer leaves, head and roots), weighed (fresh weight) and immediately stored in the freezer (−20 °C). Then, in an onmimixer, samples of the plantules, outer leaves, heads and roots were homogenized with water (1:1.5 w/v) and steeped for 30 min in an ultrasound bath. This treated plant material was then steeped in the dark for 24 h under constant agitation. Finally, the extract was centrifuged for 20 min at 2000 rpm, filtered and subjected to chromatography.

2.3. Sesquiterpene lactones

The main guaianolides in *C. intybus* L. var. "Rosso di Chioggia" were isolated from the roots because they are extremely rich in these bitter principles (Van Beek et al., 1990). The procedures proposed by Pyrek (1985) were followed in extracting, isolating and identifying these compounds. The predominant sesquiterpene lactones, present in the first-year-growth samples examined, were 8-deoxylactucin and 11β,13-dihydrolactucin. The chemical-physical characteristics (UV, MS, NMR, TLC, HPLC) determined for the two compounds (data not shown) are comparable to those reported in the literature (Barton & Narayanan, 1958; Nishimura, Miyase, Ueno, Noro, Kuroyanagi, & Fukushima, 1986; Pyrek, 1977; Savona & Bruno, 1983). The 8-deoxylactucin and 11β,13-dihydrolactucin were then used as standards for HPTLC screening of the samples from the three selections.

2.4. Sugars

For the qualitative and quantitative HPTLC analysis of the sugars, commercial standards for glucose, fructose and sucrose (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) were used.

2.5. Qualitative and quantitative HPTLC analysis

Qualitative and quantitative HPTLC analysis, of the guaianolides 8-deoxylactucin and 11β,13-dihydrolactucin, was performed by chromatographing the aqueous extracts on silica gel HPTLC plates using a fluorescence indicator (Silica Gel 60 F_{254}, Merck, Darmstadt, Germany) and isocratically developed (first step: ethyl acetate, 8 cm; second step: hexane, 9 cm). The bands obtained in this manner were compared with those from 8-deoxylactucin and 11β,13-dihydrolactucin standards.

For analysis of the sugars, on the other hand, the aqueous extracts were chromatographed on Diole HPTLC plates 10x10 (Merck, Darmstadt, Germany). An elution system was set up for development of the plates so that the results obtained would be comparable with those obtained with automated multiple development-high performance thin layer chromatography (AMD-HPTLC; Lodi, Bighi, Brandolini, Menziani, & Tosi, 1997).

The elution solvent was made up of five acetonitril, mixtures: acetone (1:1) and water in varying percentages

used for the five steps in development: first step = acetonitrile/acetone (85%):water (15%), 1.5 cm; second step = acetonitrile/acetone (86.875%):water (13.125%), 3 cm; third step = acetonitrile/acetone (88.75%):water (11.125%), 4.5 cm; fourth step = acetonitrile/acetone (90.625%):water (9.375%), 6 cm; fifth step = acetonitrile/acetone (92.5%):water (7.5%), 7.5 cm. Before each step, the chromatographic chamber was conditioned for 50 s with ammonia vapour, produced from a 1 M solution of NH_4OH under a flow of nitrogen. Upon completion of the chromatographic development, the plates were derivatized with α-naphthol.

"Quali-quantitative" evaluations of the guaianolides and sugars in the chicory extracts were obtained by densitometry, using a Camag TLC Scanner II densitometer, and dedicated software (Cats 3, Camag) at the following wavelengths: 254 nm for guaianolides; 550 nm for sugars.

2.6. Sensorial analysis

To evaluate the bitter taste of the samples from the ripe commercial heads of *C. intybus* L. var. "Rosso di Chioggia", from a pool of 50 volunteers, 30 individuals (age range: 20–55 years) were selected on the basis of their ability to perceive the bitter taste of a 0.058 mg solution of quinine hydrochlorate (Pharmacopoea Italica, 1991). To construct a reference curve of the bitterness vs. guaianolide and sugar contents in the chicory, these individuals were subsequently asked to taste 20 aqueous solutions of "Rosso di Chioggia" chicory containing different guaianolide and sugar concentrations and to evaluate the bitter taste on a scale from 0 to 10: 0–4 "not particularly bitter", 4–7 "bitter" and 7–10 "very bitter". The solutions tasted were prepared using 50 ml of uncarbonated commercial mineral water with no taste of its own, presented in a plastic cup at room temperature, as reported by Leclercq (1992).

During selection of the taste-testers, the bitterness-inducing 8-deoxylactucin and 11β,13-dihydrolactucin were determined following the method laid out by the Pharmacopoea Italica (1991), through comparison with a known quinine hydrochlorate solution:

$$\frac{c \times 2000}{a \times d}$$

where a = mg of the current sample (bitter solution) in 10 ml of the solution at a limit concentration determined by tasting; c = mg of quinine hydrochlorate contained in 10 ml of the solution at the limit concentration; d = mg of the product in one ml of the current sample; 2000 = bitterness capacity of quinine expressed in bitterness-inducing units.

3. Results

The main guaianolides in selections C1, C2 and C3 of *C. intybus* var. "Rosso di Chioggia" during the first year of growth were: 8-deoxylactucin and 11β,13-dihydrolactucin as well as traces of lactucin and lactucopicrine. The main sugars were: fructose, glucose and sucrose. The compounds belonging to the two chemical classes were quantitatively determined using HPTLC densitometry, applied at the various stages of plant growth. The growth of the plants belonging to the three selections was monitored from the plantule stage all the way through to over-ripening of the commercial product.

3.1. Plantules

The study of the plantules corresponds to the first 30 days of controlled growth in the nursery. This period of growth was monitored with two samplings: 10 (stage I) and 30 (stage II) days after sowing. The data obtained refer to the guaianolide (8-deoxylactucin and 11β, 13-dihydrolactucin) and sugar (glucose, fructose, sucrose) contents (Figs. 1 and 2). On the whole, it was found that the guaianolide content in selections C1 and C2 progressively decreased—respectively, by 43.2 and 32.7%—while the stage II values for selection C3 were slightly higher than at the first sampling (11.2%). Moreover, while in C1 the trend in total sugar content paralleled that of the guaianolides (decreasing by 14.4%), in selection C2 and C3 it increased, most significantly in C2 (50.1 and 9.8%, respectively).

After the first two stages of growth and transplanting to the open field, plant morphology was such that the inner leaves (commercial product) or heads and the roots and outermost leaves (the wastes) could be considered separately.

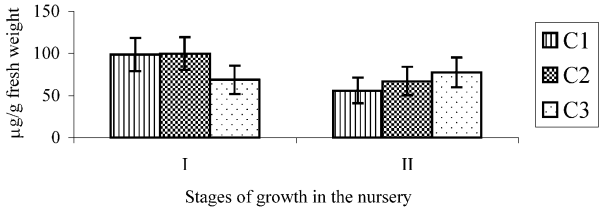


Fig. 1. Guaianolide contents in the plantules of the three selections of *Cichorium intybus* var. "Rosso di Chioggia".

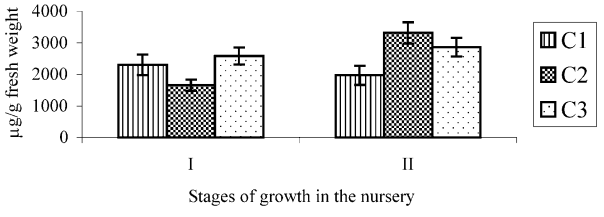


Fig. 2. Sugar contents in the plantules of the three selections of *Cichorium intybus* var. "Rosso di Chioggia".

3.2. Selection C1

In the field, the overall growth of the plants belonging to this selection could be broken down into five stages (III–VII) at 15-day intervals (Figs. 3–8). The stage V growth of this selection constitutes the period when the commercial product (head) is harvested.

3.2.1. Roots

In the plants of selection C1, the 8-deoxylactucin content was always higher than 11β,13-dihydrolactucin and both showed similar trends throughout all stages of growth, with a relative maximum at stage V (when the commercial product is harvested; Fig. 3).

Sucrose was always the predominant sugar, exceeding fructose and glucose at all stages of growth examined (Fig. 4). Considering the sugars and guaianolides as a whole, the trend was qualitatively quite similar, both classes of products presenting a relative maximum at the moment the commercial product was harvested (stage V) and peaking at over-ripening (stage VII).

3.2.2. Inner leaves (head)

In the heads of selection C1, 8-deoxylactucin and 11β,13-dihydrolactucin presented similar values and progressively increased up to stage V (Fig. 5). At this stage of growth, both guaianolides peaked with 8-deoxylactucin, in particular, being much higher than 11β,13-dihydrolactucin. During the subsequent stages, while 11β,13-dihydrolactucin decreased progressively, 8-deoxylactucin showed a slight increase upon over-ripening after having dropped significantly at stage VI. It is notable that, at this stage of growth, the total guaianolide content was approximately 6 times lower in the head than in the roots. From the moment when the product is ripe (stage V) to over-ripening (VII), the guaianolide content progressively declined (60.5%) until it reached values similar to those found at stage III growth.

At every stage of growth, the total sugar content in the heads was always greater than that found in the roots (Fig. 6). In particular, at all stages of growth, there was always a greater amount of glucose than fructose or sucrose. Considering the sugars as a whole, there was a slight drop (22.5%) at the time the commercial product was harvested vs. the previous stage; this was due to the strong drop in glucose, the dominant sugar.

3.2.3. Outer leaves

At all stages of growth, except the period at which the commercial product ripened (V), the amount of 11β,13-dihydrolactucin was always greater than that of 8-deoxylactucin, as previously observed in the heads (Fig. 7). This trend, however, led to a slight drop in total guaianolide content at stage V (8.9% lower than at stage IV growth), contrary to what was found in the head and, in particular, in the roots. It must, nevertheless, be pointed out that, in all cases, the guaianolide content in the outer leaves was always higher (21.6%) that in the heads.

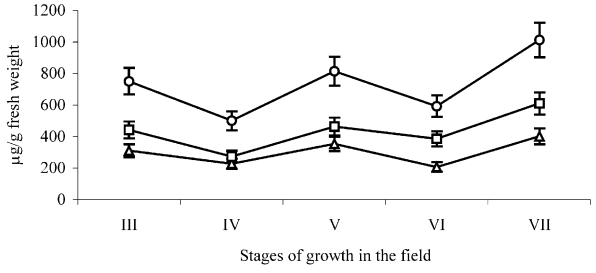


Fig. 3. Guaianolide contents in the roots of the *Cichorium intybus* var. "Rosso di Chioggia" C1 selection. Total guaianolides: ○; 8-deoxylactucin:□; 11β,13-dihydrolactucin: △.

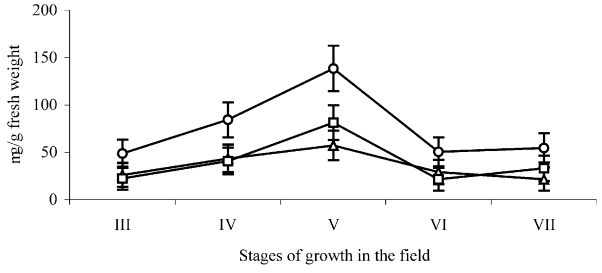


Fig. 5. Guaianolide contents in the inner leaves (head) of the *Cichorium intybus* var. "Rosso di Chioggia" C1 selection. Total guaianolides: ○; 8-deoxylactucin: □; 11β,13-dihydrolactucin: △.

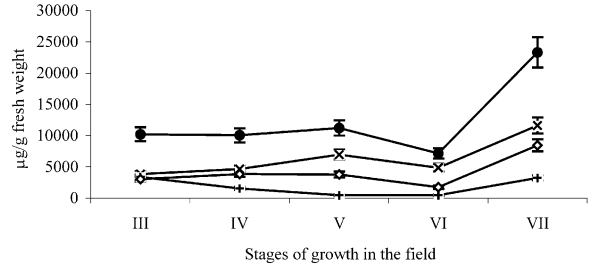


Fig. 4. Sugar contents in the roots of the *Cichorium intybus* var. "Rosso di Chioggia" C1 selection. Total sugars: ●; glucose: +; fructose: ◇; sucrose: x.

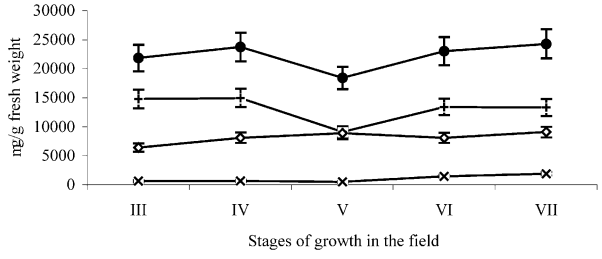


Fig. 6. Sugar contents in the inner leaves (head) of the *Cichorium intybus* var. "Rosso di Chioggia" C1 selection. Total sugars: ●; glucose: +; fructose: ◇; sucrose: x.

At the stage prior to ripening (IV) and at the time of ripening (V), the sugar content in the outer leaves was generally lower than in the heads and greater than in the roots (Fig. 8). The relationship between the various compounds is analogous to what was found in the heads with glucose being present in greater amounts than fructose and sucrose at all stages of growth.

3.3. Selection C2

In the field, the overall growth of the plants of this selection could be broken down into six stages (III–IIX) at 15-day intervals (Figs. 9–14). The stage VI growth of this selection constitutes the period when the commercial product is harvested.

3.3.1. Roots

In the plants of this selection, there were always greater amounts of 8-deoxylactucin than 11β,13-dihydrolactucin, except at stages V and VI (Fig. 9). On the whole, while the guaianolide content was at its peak at stage III and at a minimum at over-ripening (stage IIX), there were no significant quantitative variations throughout ontogenesis.

Among the sugars, glucose showed values analogous to those of sucrose and fructose although, after peaking at stage IV, it declined progressively until the stage of over-ripening (IIX; Fig. 10). Sucrose and fructose followed generally similar trends. On the whole, the sugar pool recorded an 11.6% drop during the stage at which the commercial product was harvested for market.

3.3.2. Inner leaves (head)

In the heads, the 11β,13-dihydrolactucin content was always higher than the 8-deoxylactucin content, except during stage III growth (Fig. 11).

Glucose was always the dominant sugar, showing greater values than either fructose or sucrose (Fig. 12). On the other hand, the glucose content increased until stage V and thereafter significantly decreased at the time the commercial product was harvested. During subsequent stages, the glucose values were slightly higher

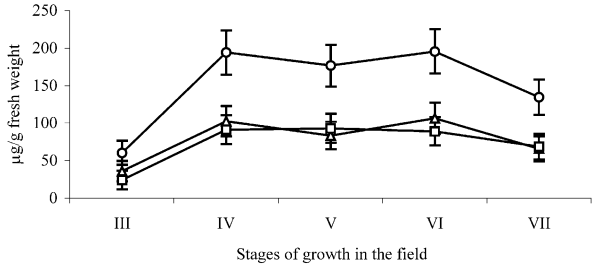


Fig. 7. Guaianolide contents in the outer leaves of the *Cichorium intybus* var. "Rosso di Chioggia" C1 selection. Total guaianolides: ○; 8-deoxylactucin: □; 11β,13-dihydrolactucin: △.

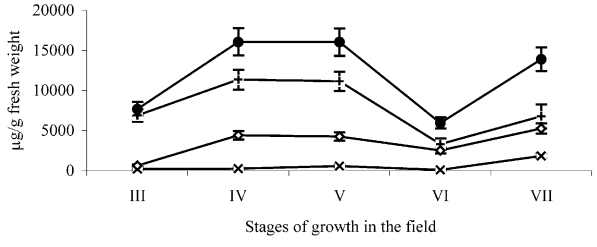


Fig. 8. Sugar contents in the outer leaves of the *Cichorium intybus* var. "Rosso di Chioggia" C1 selection. Total sugars: ●; glucose: +; fructose: ◇; sucrose: x.

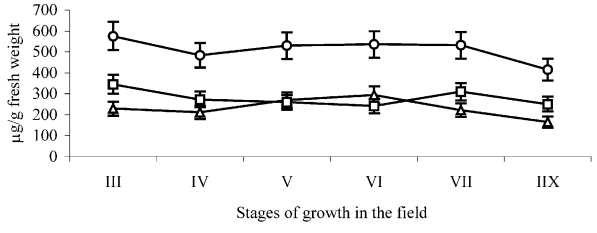


Fig. 9. Guaianolide contents in the outer leaves of the *Cichorium intybus* var. "Rosso di Chioggia" C2 selection. Total guaianolides: ○; 8-deoxylactucin: □; 11β,13-dihydrolactucin: △.

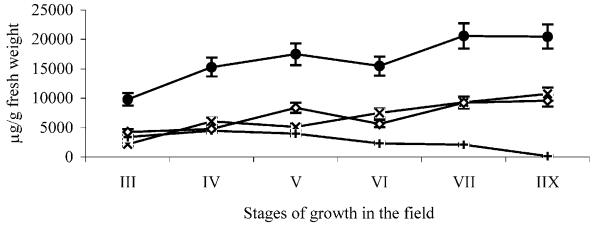
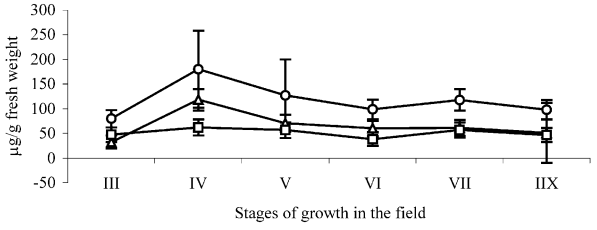


Fig. 10. Sugar contents in the outer leaves of the *Cichorium intybus* var. "Rosso di Chioggia" C2 selection. Total sugars: ●; glucose: +; fructose: ◇; sucrose: x.



Figs. 11. Guaianolide contents in the inner leaves (head) of the *Cichorium intybus* var. "Rosso di Chioggia" C2 selection. Total guaianolides: ○; 8-deoxylactucin: □; 11β,13-dihydrolactucin: △.

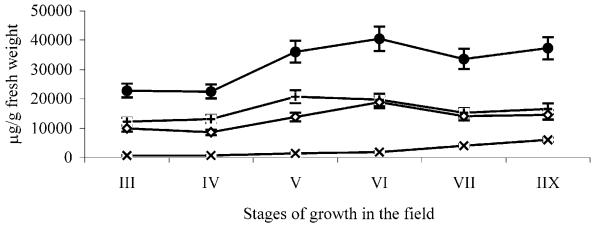


Fig. 12. Sugar contents in the inner leaves (head) of the *Cichorium intybus* var. "Rosso di Chioggia" C2 selection. Total sugars: ●; glucose: +; fructose: ◇; sucrose: x.

than those for fructose. On the whole, at the time of harvest, the sugars were at maximum values while the total guaianolides were at a minimum.

3.3.3. Outer leaves

The trend in the guaianolides, 11β,13-dihydrolactucin and 8-deoxylactucin during the various stages of growth, is quite similar to what was found in the head (Fig. 13). Likewise, from the qualitative point of view, both compounds showed concentrations similar to those found in the heads, particularly at the moment the commercial product was harvested (Stage VI).

As in the head, there was always more glucose than fructose and sucrose (Fig. 14). From the qualitative point of view, however, the total sugar concentration reached an absolute minimum in the ripe product (stage VI).

3.4. Selection C3

In the field, the overall growth of the plants belonging to this selection could be broken down into nine stages (III–XI) at 15-day intervals (Figs. 15–20). The stage IX growth of this selection constitutes the period when the commercial product (head) is harvested.

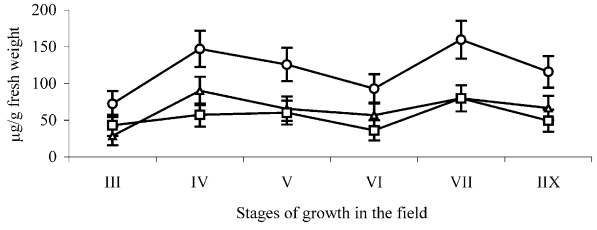


Fig. 13. Guaianolide contents in the outer leaves of the *Cichorium intybus* var. "Rosso di Chioggia" C2 selection. Total guaianolides: ○; 8-deoxylactucin: □; 11β,13-dihydrolactucin: △.

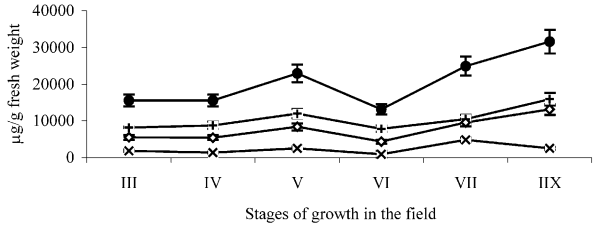


Fig. 14. Sugar contents in the outer leaves of the *Cichorium intybus* var. "Rosso di Chioggia" C2 selection. Total sugars: ●; glucose: +; fructose: ◇; sucrose: x.

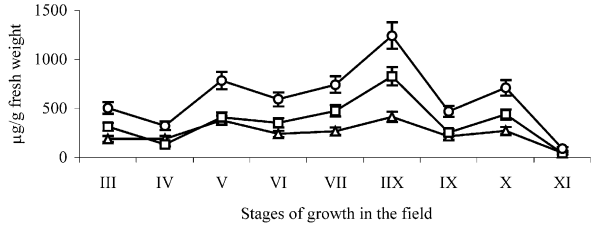


Fig. 15. Guaianolide contents in the roots of the *Cichorium intybus* var. "Rosso di Chioggia" C3 selection. Total guaianolides: ○; 8-deoxylactucin: □; 11β,13-dihydrolactucin: △.

3.4.1. Roots

8-Deoxylactucin was always present in greater quantity than 11β,13-dihydrolactucin, except at stages IV and XI where the two compounds showed quite similar values (Fig. 15). For both guaianolides, the peak value was recorded at stage IIX while, at the time the commercial product was harvested, they were both at a relative minimum, showing very similar lower values.

Among the sugars, glucose presented peak values at the initial stage of in-field cultivation, higher than those of sucrose and fructose, and then progressively decreased until over-ripening (XI) although it did show a slight increase at the time of ripening (IX; Fig. 16). In this selection, ripening coincided with the maximum sugar and minimum total guaianolide contents.

3.4.2. Inner leaves (head)

8-Deoxylactucin was always present in greater quantity than 11β,13-dihydrolactucin except at stage VI where both showed quite similar concentrations (Fig. 17). Significant values were recorded at growth stages V and VII, declining progressively thereafter until stage X and then increasing again until over-ripening (XI). Overall, at the time the commercial product was harvested, there was a strong drop in the guaianolides with concentrations being seven times lower than that found in the roots.

Of the sugars, only until growth stage V was glucose more abundant than fructose and sucrose (Fig. 18). In

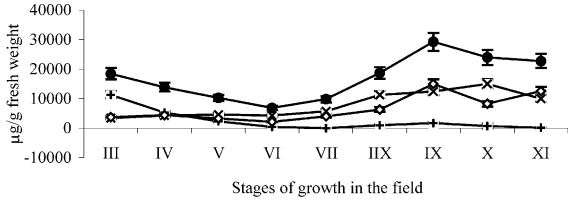


Fig. 16. Sugar contents in the roots of the *Cichorium intybus* var. "Rosso di Chioggia" C3 selection. Total sugars: ●; glucose: +; fructose: ◇; sucrose: x.

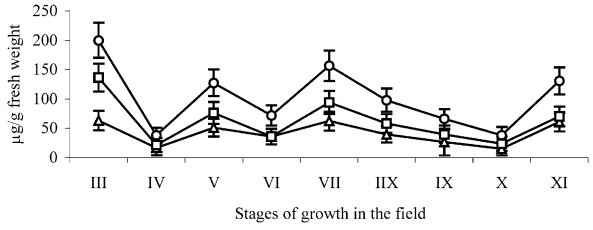


Fig. 17. Guaianolide contents in the inner leaves (head) of the *Cichorium intybus* var. "Rosso di Chioggia" C3 selection. Total guaianolides: ○; 8-deoxylactucin: □; 11β,13-dihydrolactucin: △.

the subsequent stages, fructose was always the most abundant compound until it peaked at the time the commercial product ripened (IX). It is notable that all three of these sugars showed a decrease during the stage preceding ripening. At the time of harvest (IX), however, the total sugar concentration was at its peak, higher than at any other growth stage, while the guaianolides were lower than at the previous stages.

3.4.3. Outer leaves

The 8-deoxylactucin concentration was always higher than 11β,13-dihydrolactucin and, at all stages of growth, their trend generally paralleled what found in the heads (Fig. 19). However, in particular at the stage where the heads ripened (IX), the guaianolide content was higher than in the heads.

The trend in sugar content during the various growth phases proved highly variable, with absolute minimum and maximum achieved, respectively, at growth stages VI and VII (Fig. 20). On the whole, at the time of ripening, the overall sugar content peaked at the time of ripening although the quantities were lower than those found in the heads at the same stage of growth.

3.5. Sensorial analysis

A selected sample of volunteers was asked to taste aqueous solutions of chicory "Rosso di Chioggia" extract containing known amounts of guaianolides and sugars and make a qualitative evaluation of the bitter taste using a scale from 0 to 10: "not particularly bitter" (0–4), "bitter" (4–7) and "very bitter" 7–10 (Fig. 21). In fact, in the solutions tasted, the correlation between guaianolide content and bitterness was always a positive 0.97 (limit of significance 1% = 0.59). On the other hand, the sugar concentrations were not homogeneous, and thus the index of correlation with the sensorial data was below the limit of significance (0.25).

This evaluation instrument made it possible to predict the degree of bitterness of each selection on the basis of the guaianolide content. Indeed, taking the guaianolide content, expressed in μg/g of commercial product, it was possible to hypothesize that, at the corresponding stage of harvesting the commercial product, selections C1 (total guaianolides at the V stage = 138 ± 23.9 μg/g fresh weight) and C2 (total guaianolides at the VI stage = 98.6 ± 19.9 μg/g fresh weight) both yield "very bitter" products while selection C3 (total guaianolides at the IX stage = 66.2 ± 16.7 μg/g fresh weight) produced a "bitter" product.

Then the bitterness-producing capacity of "Rosso di Chioggia" chicory was determined in the same manner as used to select the volunteer tasters. The result was that an aqueous solution of "Rosso di Chioggia" chicory, at a known concentration showed a bitterness capacity of 895 units, corresponding to approximately 45% that of quinine (2000 bitterness units).

$$\frac{c \times 2000}{a \times d} = \frac{0.042 \times 2000}{1 \times 0.09386} = 895$$

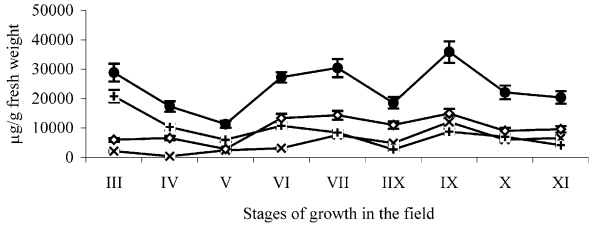


Fig. 18. Sugar contents in the inner leaves (head) of the *Cichorium intybus* var. "Rosso di Chioggia" C3 selection. Total sugars: ●; glucose: +; fructose: ◇; sucrose: x.

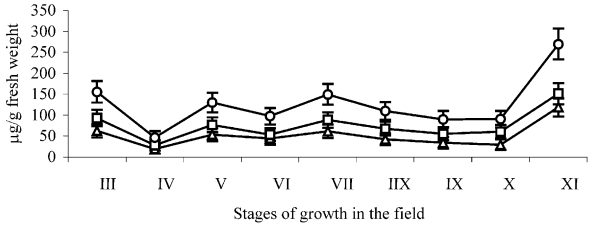


Fig. 19. Guaianolide contents in the outer leaves of the *Cichorium intybus* var. "Rosso di Chioggia" C3 selection. Total guaianolides: ○; 8-deoxylactucin: □; 11β,13-dihydrolactucin: △.

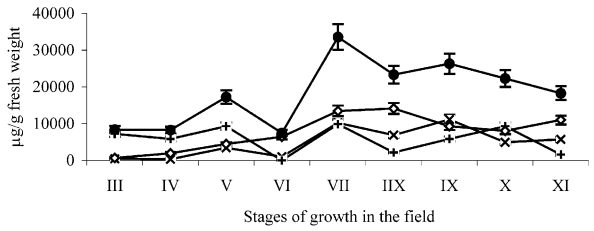


Fig. 20. Sugar contents in the outer leaves of the *Cichorium intybus* var. "Rosso di Chioggia" C3 selection. Total sugars: ●; glucose: +; fructose: ◇; sucrose: x.

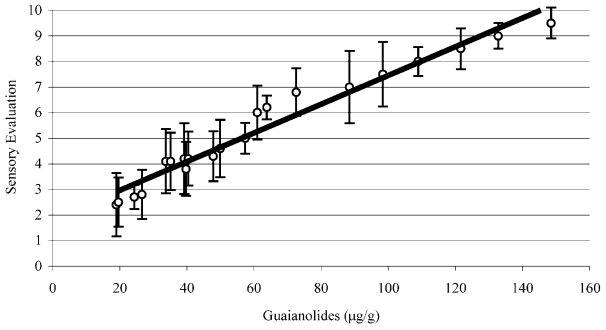


Fig. 21. Point intensity bitterness scale: 0–4 "not particularly bitter", 4–7 "bitter" and 7–10 "very bitter".

4. Discussion

In the three selections of *C. intybus* var. ''Rosso di Chioggia'', monitored during the first year of cultivation, the guaianolide and sugar contents of the commercial product (head) varied greatly throughout the various phases of growth, both within plants of a single selection and between selections. In fact, significant differences in the contents of these two classes of compounds were seen at the very outset, at the plantule stage. Likewise, during the subsequent stages of growth, there were no factors, whether guaianolide (8-deoxylactucin, 11β,13-dihydrolactucin) or sugar (sucrose, fructose and glucose), that proved characteristic of all three selections.

In the commercial product (heads), while the C3 8-deoxylactucin content was always greater than the 11β,13-dihydrolactucin, at all stages of in-field growth, selection C2 showed the opposite situation, except at stage III, just prior to transfer to the open field. In selection C1 the situation was again different, with 8-deoxylactucin greater than 11β,13-dihydrolactucin only when the product was ripe for harvest and when overripe. However, it must be pointed out that, at the moment of harvest, only C1 presented a peak of absolute maximum in total guaianolide concentration, 28.7 and 52.1% higher than in C2 and C3, respectively. Such extreme variability has also been found in other varieties of *C. intybus* at the time the ripe product was harvested for market (Peters & Van Amerongen, 1996).

Qualitative and quantitative screening of the fundamental sugars, in the head, at the time of ripening, showed that the highest total sugar concentration was in C2 while the concentration in C3 was 11.5% lower and in C1 a full 54.5% lower. The guaianolide and sugar contents encountered in the three selections at the time the heads were harvested for market were compared with the constructed 20 point bitterness intensity scale. After checking that the sugar content encountered seemed not to affect the perception of bitterness in the guaianolide-containing solutions, it is possible to predict that the product from selection C3 would be ''bitter'' while that of C1 and C2 could be ''very bitter''. This sensorial scale could also make it possible not only to better characterize the ideal moment to harvest the product, but also to obtain and market products with different degrees of bitterness, simply by varying the moment of harvest, even slightly. The correlation between the guaianolide content and the bitter taste, obtained using the constructed bitterness intensity scale, appeared particularly reliable for the extraction method used. It has been reported that extraction by soaking in water, and particularly the use of an ultrasound bath, optimizes extraction times and yields total sesquiterpene lactones (free and glycoside compounds) which better correlate with the sensorial bitterness. The use of an ultrasound bath—which enhances extraction yield by breaking down the cell walls and vacuolar membranes— releases sesquiterpenes, which would otherwise remain entrapped and enzymes that catalyze the transformation of glycosilates (multi-polar) into free compounds (Peters & Van Amerongen, 1997, and references therein). This type of extraction would, therefore, yield a pool of sesquiterpene lactone compounds that are qualitatively and quantitatively closer to what would be perceived as a bitter taste if the product were chewed. Other extraction methods suggested in the literature (Kisiel & Barszcz, 1997; Leclercq, 1984; Peters & Van Amerongen, 1996; Pyrek, 1985; Seto et al., 1988; Van Beek et al., 1990), using organic solvents, have given results comparable to those obtained with the extraction method used here. The method used, however, takes on greater importance in view of the principles of so-called ''green chemistry'', advancing methods that reduce environmental impact and reduce the use of organic solvents, the cost of which is steadily rising (Anastas, 1999, and references therein). Likewise the analysis method used, HPTLC densitometry, not only enabled us to analyze a large number of samples, but also gave results comparable to those obtained with HPLC, reducing analysis times and costs.

Application of this model, set up for the extraction and analysis of guaianolides and sugars, could prove of interest in the exploitation of *C. intybus* var. ''Rosso di Chioggia'' wastes. Monitoring the 8-deoxylactucin and 11β,13-dihydrolactucin contents throughout all stages of growth of the three selections, even in the roots and outer leaves, showed the same high degree of variability as seen in the heads. As other authors have reported for other varieties of *C. intybus*, the guaianolide content in the roots was much higher than in the head and outer leaves (Van Beek et al., 1990).

The data on the guaianolide content in the waste products, obtained from working ''Rosso di Chioggia'' chicory for the market, suggest that the roots, and possibly even the outer leaves, could be exploited as a renewable resource. In fact, the biomass discarded could be used as a source of bitterness principles to be used in several fields. The bitterness capacity of an aqueous solution of ''Rosso di Chioggia'' proves to be 45% that of quinine, the reference bitterness principle (Pharmacopoea Italica, 1991), and could justify the flavourings industry's use of ''Rosso di Chioggia'' chicory in the in the production of bitter-tonics, aperitifs and, soft-drinks. (Van Beek et al., 1990).

The known biological properties of sesquiterpene lactones (Picman, 1986) also suggest their use as pure compounds in such fields as pharmaceuticals for the production of over-the-counter products. On the other hand, in agriculture, they could be used in the production of ecological pest control products since they have also been reported to have a significant effect as

anti-feedant and repellants of phytopathogens and herbivores (Picman, 1986; Rees & Harborne, 1985). The potential importance of these products as renewable resources is also associated with the low-cost means of extraction used and its reduced environmental impact. This combination would lower the costs for the disposal of wastes and biomass. Eliminating the wastes normally left on fields after harvest would also be an appropriate preventative phytosanitary measure as it would reduce the incidence of infections in the next year's crop (Chillemi, 1998; Pimpini & Chillemi, 1993).

The roots of some varieties of *C. intybus* are already used as foodstuffs in some areas of northern France and Belgium; moreover, with the high free sugar and polyfructoside (inulin) contents, they could be used as a resource for the sweetener industry and in the preparation of products for diabetics. Indeed, significant amounts of sugars were also found in the roots and outer leaves of the three selections examined at the various stages of growth. In the roots of all three selections the sucrose and fructose concentrations were generally higher than the glucose concentration.

In conclusion, the analyses performed on three selections of "Rosso di Chioggia" revealed that the time of harvest does not always correspond to the peak guaianolide content. The sensorial test related to the guaianolide content could be used to determine the ideal harvest time to meet the market demand for products with different degrees of bitterness. Likewise, the significant guaianolide and sugar contents found in the wastes after working the commercial product make the prospective use of this biomass quite interesting.

Acknowledgements

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