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Quantification of chicory root bitterness by an ELISA for 11β,13-dihydrolactucin

Philippe Hance, Yoann Martin, Jacques Vasseur, Jean-Louis Hilbert *, Francis Trotin

UMR 1281, Stress Abiotiques et Différenciation des Végétaux Cultivés, USTL, INRA, ERT1067, IFR147 Université des Sciences et Technologies de Lille, F-59655 Villeneuve d'Ascq, France

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Abstract

Chicory root (*Cichorium intybus* L. var. *sativum*) is an important foodstuff appreciated for its bitter taste, which is caused by sesquiterpene lactones. These compounds represent a quality parameter for monitoring the raw material. Using polyclonal antibodies, an enzyme-linked immunosorbent assay (ELISA) was developed to quantify the bitter compound 11β,13-dihydrolactucin in chicory root. Assay linearity ranged from 4.6 to 300 ng/ml, with intra- and inter-assay variations of 4.9% and 7.2%, respectively. An IC_{50} of 2 ng/ml and a detection limit of 0.16 ng/ml were obtained. No or little cross-reactions with other sesquiterpene lactones occurred. Roots of three different chicory varieties were evaluated for their bitter taste and were investigated by the ELISA. Distinct concentrations of 11β,13-dihydrolactucin ranging from 485 to 1720 mg/kg dry matter were correlated with the bitterness degree ($r = 0.9$). The ELISA appeared sensitive, selective, accurate and may serve as screening tool in breeding of chicory roots for bitterness.

Keywords: Chicory (*Cichorium intybus* L. var. *sativum*); Root; Bitterness; 11β,13-Dihydrolactucin; Quality; Sensitive ELISA; Breeding

1. Introduction

The root of chicory (*Cichorium intybus* L.) is an important agricultural crop in the North of France, used for the production of ingredients for the food industry (roasted products, chicory flour) or eaten roasted for consumers (coffee subsitute). One of the principal quality attributes of chicory root is bitterness, which is linked to the presence of sesquiterpene lactones (Peters & Van Amerongen, 1998; Price, Du Pont, Shepherd, Chan, & Fenwick, 1990; Pyrek, 1985; Rees & Harborne, 1985; Seto et al., 1988; Van Beek et al., 1990), e.g. lactucin, 8-deoxylactucin, lactucopicrin and their 11β,13-dihydro derivatives (Fig. 1).

Particularly abundant in the root (Poli et al., 2002; Van Beek et al., 1990), a high concentration of sesquiterpene lactones can be a barrier to consumption. Another problem in the production of chicory roots is the wide variation in the content of the sesquiterpene lactones, causing a significant difference in taste between different varieties as well as between different harvests. To respond to the increasing quality demands of consumers, chicory producers in the North of France developed a breeding program to obtain chicory varieties with different levels of bitterness. In this program, metabolites linked to root bitterness are evaluated as predictive markers for adapting the composition of the raw material, to give a root product which is acceptable for the food industry, and which satisfies consumers.

A rapid, sensitive and reproducible evaluation test is required. Many analytical methods have been applied to identify the sesquiterpene lactones in plants (Merfort, 2002), including an accurate high-performance liquid chromatography (HPLC) method for chicory (Leclercq, 1984;

* Corresponding author. Present address: UMR 1281, Stress Abiotiques et Différenciation des Végétaux Cultivés, IFR 118, UFR de Biologie, Université des Sciences et Technologies de Lille, 59655 Villeneuve d'Ascq, France. Tel.: +33 3 20 43 66 78; fax: +33 3 20 33 72 44.
E-mail address: jean-louis.hilbert@univ-lille1.fr (J.-L. Hilbert).

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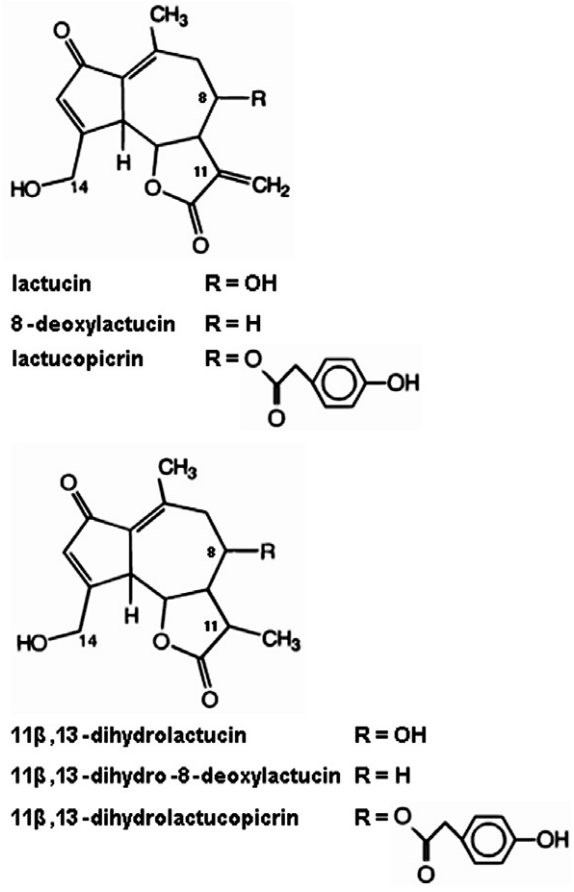

Fig. 1. Structures of the sesquiterpene lactones found in roots of chicory (*Cichorium intybus* L.).

Price et al., 1990; Van Beek et al., 1990). However, for small and medium size enterprises, HPLC represents an extensive investment, requiring highly qualified personnel, expensive equipment and high costs for sample analysis.

Immunological approaches for the detection and quantification of specific compounds can be simple, rapid, and economical (Feng-Yih, Yueh-Hui, & Ching-Chyuan, 2006; Ferreira & Janick, 1996; Lee et al., 2004; Marco, Gee, & Hammock, 1995). To evaluate bitterness in chicory endive, Peters, Haagsma, Gensch, and Van Amerongen (1996) used antibodies raised against lactucin and lactucopicrin in an enzyme-linked immunosorbent assay (ELISA), to determine sesquiterpene lactone levels in endive heads. Though good correlations ($r = 0.8$) were found between the levels of lactucin, and lactucin glucoside, and the bitterness perceived by a sensory panel (Peters & Van Amerongen, 1996; Peters & Van Amerongen, 1997), other studies in chicory indicated that 11β,13-dihydrolactucin is responsible for the bitter intensity (Poli et al., 2002; Van Beek et al., 1990).

In the present work, we developed a sensible, competitive, indirect ELISA for the evaluation of bitterness in industrial chicory root, using polyclonal antibodies against 11β,13-dihydrolactucin. Roots of three chicory varieties cultivated in the North of France were used to establish a high correlation ($r > 0.9$) between the content of 11β,13-dihydrolactucin as determined by ELISA, and bitterness as evaluated by a sensory panel.

2. Materials and methods

2.1. Plant material

For isolation of sesquiterpene lactone compounds, fresh chicory roots of *Cichorium intybus* L. var. *sativum*, grown in the North of France and harvested in November 2003, were supplied by the Florimond Desprez Company (Cappelle en Pévèle, France).

The application of ELISA as a screening tool in breeding programs was performed on three varieties of chicory roots (*Cichorium intybus* var. *sativum*) codified as RCO-0031, RCR-001 and RCA-002, harvested in 2003 and furnished by the Leroux-Finaler Company (Orchies, France).

2.2. Animals

Female New Zealand white rabbits were obtained from Agro-Bio (La Ferté St. Aubin, France).

2.3. Chemicals and immunochemicals

HPLC-grade solvents and high quality grade chemicals were used. Extraction and chromatographic solvents, salts and reagents were purchased from Elvetech (Mundolshein, France). Freund's complete and incomplete adjuvant, bovine serum albumin (BSA), keyhole limpet hemocyanin (KLH), goat anti-rabbit IgG conjugated to horseradish peroxidase (HRP) and 3,3′,5,5′-tetramethylbenzidine (TMB) were obtained from Sigma (St. Louis, MO). Pyridine and *p*-nitrophenyl chloroformate were obtained from Merck (Hohenbrunn, Germany). Goat anti-rabbit IgG conjugated to alkaline phosphatase (IgG–AP) was obtained from Chemicon International (Temecula, CA). *p*-Nitrophenylphosphate and diethanolamine buffer for alkaline phosphatase reaction detection were from Bio-Rad (Hercules, CA). Cellulase and pectinase enzymes Peclyve LI preparation was furnished by Peclive (Colombelles, France). Ultrapure water was produced with an Elga model system (Elga, High Wycombe, Bucks, England).

2.4. Equipment

Enzymatic liquefaction was achieved in four 5 l tankers mounted on a Polytest 20 automatic shaker (Bioblock, Illkirch, France). Samples were ground with a 38BL41 Waring blender (Torrington, CT). Nunc Maxisorp polystyrene microtitre plates were acquired from Dutscher (Brumath, France). ELISA washing steps were carried out using the microplate ImmunoWash 1575 from Bio-Rad (Richmond, CA). The Thermo Labsystems Multiskan microplate reader equipped with the Ascent Multiskan software

(version 2.6) for ELISA absorbance, the Thermo Spectra-Physics HPLC (high-performance liquid chromatography) system including a SCM400 degasser, a 8800 ternary pump and a UV/Visible Spectra 100 detector and the ChromJet integrator were from Thermo Electron Corporation (Courtaboeuf, France). Semi-preparative HPLC was accomplished on an LC10A Shimadzu System (Kyoto, Japan). The structures of pure and mixed haptens were confirmed by NMR spectra obtained with a Bruker DPX300 spectrometer (Bruker BioSpin, Wissembourg, France).

2.5. Extraction of sesquiterpene lactones and the 11β,13-dihydrolactucin antigen

Fresh chicory roots (14 kg) were washed, cut into small pieces and subjected to enzymatic hydrolysis in sodium acetate buffer (22 mM pH 4; 1:2, w/v) with 1 g/l of Peclyve LI for 24 h at 40 °C with shaking (Leclercq & Netjes, 1985). The suspension was filtered through a 500 μm nylon sieve and centrifuged (6000 rpm; 15 min). The supernatant was thrice extracted with ethyl acetate (1:2, v/v) for 15 min. The organic layers were combined, dried over Na_2SO_4 and reduced under vacuum at 40 °C. Five hundred millilitres of reduced ethyl acetate extract (2 kg fresh root equivalent) were subjected to flash chromatography on a silica gel 60 H (Merck) 80 × 2.5 cm column, using ethyl acetate as eluent at a 30 ml/min flow rate. Three fractions containing sesquiterpene lactones were evaporated under vacuum and dissolved in methanol. Six sesquiterpene lactones were identified, according to their absorbance at 254 nm and mass spectra, by HPLC on a Merck 250 × 4.5 mm 5 μm Lichrospher RP18 column (Darmstadt, Germany) at 40 °C with a 1 ml/min flow rate, using a water (A)/methanol (B) solvent gradient starting at 30% B, increasing to 60% in 14 min, up to 100% in 1 min and maintained at 100% for 4 min. The relevant methanolic fractions were further purified on a semi-preparative 250 × 10 mm 5 μm Kromasil C18 column (Eka Chemicals, Bohus, Sweden). Elution, at a total running time of 100 min at 4 ml/min, with 75:25, 62:38 and 53:47 water/methanol as mobile phases, yielded pure lactucin (20.7 mg), pure 11β,13-dihydrolactucin (56.1 mg), a mixture of 8-deoxylactucin and 11β,13-dihydro-8-deoxylactucin (ca. 33:7, 41.4 mg), and a mixture of lactucopicrin and 11β,13-dihydrolactucopicrin, respectively.

Physicochemical data (UV, mass, ^{1}H NMR, ^{13}C NMR) of 11β,13-dihydrolactucin, obtained as a sand-coloured powder, were in accordance with the published data (Kisiel & Zielińska, 2001; Pyrek, 1985; Seto et al., 1988).

2.6. Conjugation of haptens to proteins

11β,13-Dihydrolactucin was conjugated to KLH for immunogen and to BSA for coating antigen *via* the hydroxyl group on carbon 14 by the *p*-nitrophenyl chloroformate activation method, as previously described by Peters et al. (1996).

2.7. Buffers

Coating and assay buffer for antisera titration with HRP system was carbonate–bicarbonate buffer (CB; 50 mM, pH 9.6). Coating buffer and assay buffer were phosphate-buffered saline (PBS; 10 mM, pH 7.2). Blocking buffer (PBS-B) was made as PBS with 2% of milk powder (w/v) and washing buffer (PBS-W) was made as PBS with 0.1% Tween 20 (v/v).

2.8. Production of antisera and titration by indirect ELISA

Polyclonal antisera were obtained from two New Zealand white rabbits immunised subcutaneously. Each rabbit received 2 ml of an emulsion containing 0.25 mg/ml of KLH-11β,13-dihydrolactucin hapten conjugate in PBS and complete Freund's adjuvant (1:1, v/v). The rabbits were boosted four times within two-weeks intervals using the same amount of immunogen in emulsified incomplete Freund's adjuvant. Blood was collected 21 days after each injection. The two antisera, named DHLc1 and DHLc2, were obtained after coagulation at 4 °C overnight and after centrifugation at 10,000*g* for 5 min, and were stored at −20 °C.

For titration, microwell plates were coated with 100 μl of BSA-11β,13-dihydrolactucin (2.5 μg/ml) in CB at 4 °C overnight. Plates were rinsed three times with 300 μl/well PBS-W. The wells were blocked with 200 μl/well PBS-B for 1 h at 37 °C. Serial dilutions (1:125–1:32,000; 100 μl/well) of antiserum in PBS were added to the wells and incubated for 1 h at 37 °C. After a washing step, 100 μl/well of goat anti-rabbit IgG–HRP conjugate (1:1000) in PBS were added. After 1 h at 37 °C, wells were washed and 100 μl/well of TMB in CB were added. After 30 min at 37 °C, the reaction was stopped with 50 μl/well of 1 M H_2SO_4 and the absorbance was read at 450 nm.

2.9. General development of indirect ELISA

The optimal concentration for antiserum and BSA-11β,13-dihydrolactucin conjugate was determined by ELISA through a two-dimensional titration matrix, where each well of a plate received a single combination of coating antigen concentration (5–50 ng/well) and antiserum dilution (1:10–1:10,000). Coating of 100 μl/well of conjugate solution was made in PBS at 4 °C overnight. The wells were blocked with 200 μl/well PBS-B for 1 h at 37 °C. The serial dilutions of antiserum (100 μl/well) in PBS were added and incubated for 1 h at 37 °C. Subsequently, 100 μl/well of goat anti-rabbit IgG–AP conjugate (1:1000) in PBS were added. After 1 h at 37 °C, 100 μl/well of *p*-nitrophenylphosphate (1 mg in 200 μl of diethanolamine buffer and 800 μl of ultra-pure water) were added. Colour development was stopped with 50 μl/well of 0.4 M NaOH after 45 min at 37 °C, and the absorbance was read at 405 nm. A combination antigen/antiserum resulting in an absorbance of 1.5 ± 0.3 (data not shown) was chosen for further studies on competitive assays.

2.10. Competitive ELISA for quantification of 11β,13-dihydrolactucin and sesquiterpene lactones

The ability of free 11β,13-dihydrolactucin and other sesquiterpene lactones to bind antibodies was investigated by the general protocol (described above) except for the primary immunoreaction; each well received 50 μl of analyte (purified sesquiterpene lactones or samples) followed by 50 μl of antiserum, both reagents at the appropriate dilution.

2.11. Evaluation of ELISA: calibration, specificity, sensitivity and accuracy

All standard curves were obtained by plotting absorbance against the logarithm of analyte concentration. For the calibration curve, concentrations of 11β,13-dihydrolactucin ranging from 0.02 to 20,000 ng/ml (0.001–1000 ng/well) were used and absorbance normalised as:

$$A/A_0 = (A_{an}/A_{DHLc}) \times 100,$$

where A_{an} is the absorbance value in presence of competitor, and A_{DHLc} is the absorbance value at zero concentration of 11β,13-dihydrolactucin. The concentration of each analyte producing a 50% inhibition of antiserum binding compared to the zero analyte control (IC_{50}) was directly read from its curve.

The specificity was assessed by determining the cross-reactivity (CR) of related compounds. Stock solutions of lactucin, 11β,13-dihydrolactucin, as well as the mixtures of 11β,13-dihydro-8-deoxylactucin and 8-deoxylactucin, and of lactucopicrin and 11β,13-dihydrolactucopicrin, were serial diluted in PBS and used in the competitive assay. The CR was expressed according to the relation (IC_{50DHLc}/IC_{50an}) × 100, where $IC_{50DHLc} = IC_{50}$ of 11β,13-dihydrolactucin and $IC_{50an} = IC_{50}$ of competitor.

The sensitivity of the assay was established by the evaluation of the detection limit (DL) of 11β,13-dihydrolactucin from 0.02 ng/ml to 20,000 ng/ml and determined as the lowest detectable analyte concentration, statistically different from that of the zero dose, minus three standard deviations.

Accuracy was determined using replicates of the assay standard curve. Intra-assay variation analyses were determined on the same microplate using three replicates, and repeated four times (once per week), providing an estimate of the inter-assay variation.

2.12. Samples preparation for ELISA application

Freshly harvested chicory roots were washed, ground and centrifuged at 20,000*g* for 15 min. The supernatant was filtered and subjected at appropriate dilution to the competitive ELISA.

2.13. Sensory analysis

The sensory evaluations were performed by the Leroux-Finaler Company, based on AFNOR V 09-105 and V 09-001 norms (AFNOR, 1983; AFNOR, 2002). Panel members were trained in chicory root bitterness assessment. All samples of *C. intybus* were crushed and infused for 20 min with boiling mineral water (1:10, w/w). Extracts were filtered and filtrates were diluted 5- or 10-fold (w/w) before tasting in two different sessions with random presentation. Bitterness was scored, according to 11β,13-dihydrolactucin content, employing a scale graded from 1 (the least bitter) to 4 (the most bitter).

Friedman's test and pairwise comparison were performed to denote significant differences among mean values with a level of significance at 5% (AFNOR, 1983).

3. Results and discussion

3.1. Characteristics of antisera polyclonal antibodies and development of ELISA

Immunisations were performed with the 11β,13-dihydrolactucin hapten conjugated with a KLH carrier protein. Two female rabbits were immunised with the conjugate to generate two antisera, DHLc1 and DHLc2. The titres of the antisera were estimated by indirect ELISA using the BSA-11β,13-dihydrolactucin conjugate as coating antigen. Over 3 months no significant increase in titres was observed and the animals showed differences in their antiserum titre ranging from 1:400 to 1:2000. DHLc1, having a higher titre than DHLc2 for all dilutions tested, was subsequently assayed for its ability to recognise free 11β,13-dihydrolactucin by competitive indirect ELISA. Inhibition of alkaline phosphatase product formation higher than 50% started from 2 ng/well of free 11β,13-dihydrolactucin, in presence

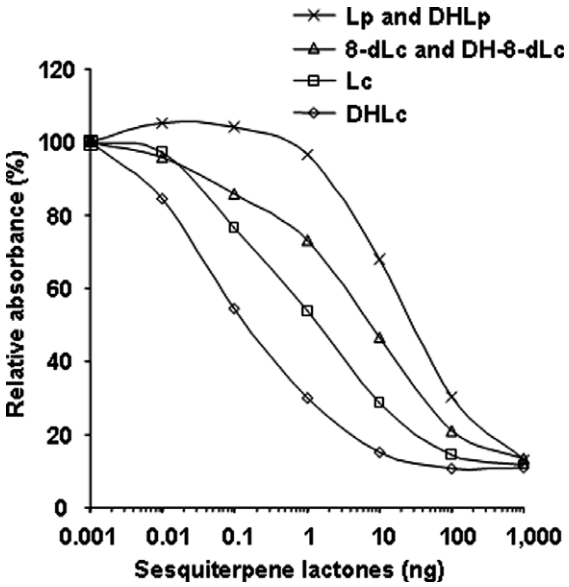

Fig. 2. Cross reactivity of sesquiterpene lactones in competitive indirect ELISA. Assays were carried out on five replicates. DHLc: 11β,13-dihydrolactucin; Lc: lactucin; 8-dLc and DH-8-dLc: 8-deoxylactucin and 11β,13-dihydro-8-deoxylactucin mixture; Lp and DHLp: lactucopicrin and 11β,13-dihydrolactucopicrin mixture.

Table 1
IC_{50} and cross-reactivity of antiserum DHLc1 with sesquiterpene lactones isolated from chicory root

Sesquiterpene lactones	IC_{50} (ng/ml)[a]	Cross-reactivity (%)
DHLc[b]	2 ± 0.3	100.00
Lc[c]	30 ± 8.0	6.67
DH8dLc + 8dLc[d]	100 ± 20.0	2.00
DHLp + Lp[e]	440 ± 46.0	0.45

[a] Values are means ± SD ($n = 5$).
[b] DHLc, 11β,13-dihydrolactucin.
[c] Lc, lactucin.
[d] DH8dLc + 8dLc, 11β,13-dihydro-8-deoxylactucin and 8-deoxylactucin mixture.
[e] DHLp + Lp, lactucopicrin and 11β,13-dihydrolactucopicrin mixture.

of various dilutions of DHLc1 (optimal at 1:3200). Upon a checkerboard titration of immunoreagents using the 11β,13-dihydrolactucin coating antigen, the optimal conditions for competitive indirect ELISA were 100 ng/ml of 11β,13-dihydrolactucin coating concentration and an antiserum dilution of 1:800 per well. The lowest IC_{50} was obtained with 5 ng/ml of free 11β,13-dihydrolactucin.

3.2. Specificity

Immunoassay specificity was evaluated by preparing standard curves of different purified sesquiterpene lactones using dilution series ranging from 0.001 to 1000 ng/well (Fig. 2). Cross-reactivity values are listed in Table 1. All standards were measured in five replicates, and results showed no variation higher than 5% for each concentration tested. The assay is quite specific towards the 11β,13-dihydrolactucin hapten, since only a 6% cross-reactivity was observed with lactucin. The mixtures of 8-deoxylactucin and 11β,13-dihydro-8-deoxylactucin, lactucopicrin and 11β,13-dihydrolactucopicrin were very weakly recognised (<2%). As illustrated in Fig. 1, lactucin contains a methylene moiety on carbon 11 instead of a methyl group as in 11β,13-dihydrolactucin. The absence of the hydroxyl group on carbon 8, or the presence of *p*-hydroxyphenyl acetic acid ester moiety, corresponded to a lack of recognition. These results suggest that the methyl group is a significant epitope for the specificity of the polyclonal antibodies. 11β,13-Dihydrolactucin as immunogen seemed to have induced the production of antibodies more specific than lactucin, used as antigen by Peters et al. (1996), yielding a DHLc1 antiserum highly 11β,13-dihydrolactucin-selective.

3.3. Sensitivity and accuracy

IC_{50} values reported in Table 1 also showed differences depending on the structure assayed, resulting in a gradual increase of recognition the closer the structure was to that of 11β,13-dihydrolactucin. On the linear detection range, determined in Fig. 2 between concentrations producing 10% and 80% inhibition (0.2–200 ng/ml), the detection limit was 0.2 ng/ml. Enhancement of measurement points

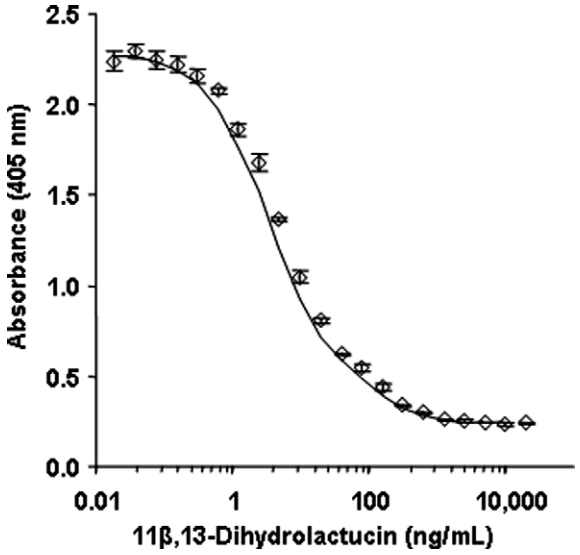

Fig. 3. Standard competitive inhibition curve of 11β,13-dihydrolactucin in indirect ELISA. Assays were carried out in triplicate.

on this first inhibition curve furnished the standard curve for 11β,13-dihydrolactucin ELISA (Fig. 3), characterised by good repeatability, exhibiting a coefficient of variation (CV) of 2.2%, and a maximal deviation from concentration mean of 3% at a confidence interval of 1%. From this new curve, the assay working range (Fig. 4) was definitely established between 5 and 300 ng/ml (0.23 and 15 ng/well). The limit of detection was improved, reaching 0.16 ng/ml, and the IC_{50} value of 2 ng/ml was confirmed.

Assay reproducibility and repeatability (Table 2) were studied over 4 weeks with fresh buffer solutions each time, except for DHLc1 and conjugate solution. On average, variability increased at lower 11β,13-dihydrolactucin concentrations but never exceeded 10%. The week-to-week

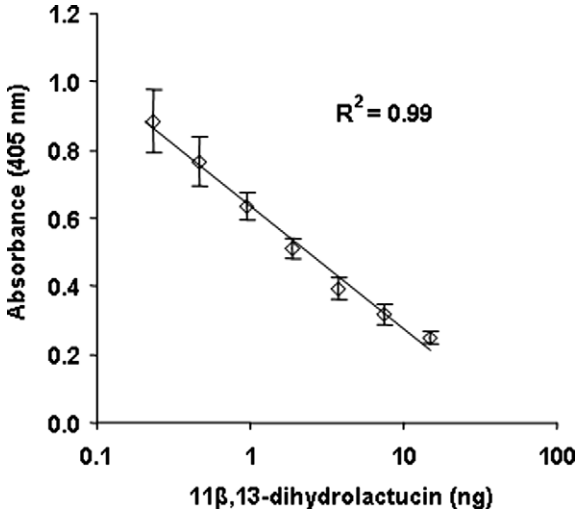

Fig. 4. Working range assay calibration line for 11β,13-dihydrolactucin quantification in competitive indirect ELISA. Assays were carried out four times in triplicate during 4 weeks. Each point represent the mean of 12 measurements.

Table 2
Precision of 11β,13-dihydrolactucin measurement in immunoassay

	Sample[a]						
	1	2	3	4	5	6	7
Dose (ng/ml)	300	150	75	37.5	18.8	9.4	4.7
Repeatability[b]	4.3	4.0	4.7	4.7	4.3	4.8	7.6
Reproducibility[b]	7.1	9.8	6.9	4.7	5.3	8.6	8.2

[a] 11β,13-Dihydrolactucin in assay buffer determined in triplicate for each assay during four weeks.
[b] Coefficient of variation in percent.

reproducibility ($n = 12$) was ensured since no significant variation was observed higher than 5.8% from the mean concentration, with a confidence interval of 1%. For the intra-assay analyses made in triplicate, all CV values were below 8% with a mean of 4.9%. Despite the daily changes of the buffer solutions, the 11β,13-dihydrolactucin ELISA was reproducible with a total CV of 6.1% ($n = 84$) for all concentrations tested. This reproducibility suggested the high stability of immunoreagents, DHLc1 and BSA-11β,13-dihydrolactucin conjugate, and ensured the robustness of the test.

3.4. Application of the competitive indirect ELISA

Two roots from different harvests were crushed and centrifuged. Supernatants diluted 1:10,000–1:2,560,000 were compared with 11β,13-dihydrolactucin standard curve (Fig. 5) from 0.001 to 15 ng/well (0.02–300 ng/ml). The curves shared a common range between dilutions of 1:10,000–1:160,000, inside the linearity limits of the assay working range, corresponding to 11β,13-dihydrolactucin concentrations of 5.8 and 291 ng/ml. Superimposition of curves in this range indicated the absence of a matrix effect. These data indicated that the ELISA allowed specific determination of 11β,13-dihydrolactucin content in chicory root extracts diluted 1:10,000 by a direct comparison to the calibration curve.

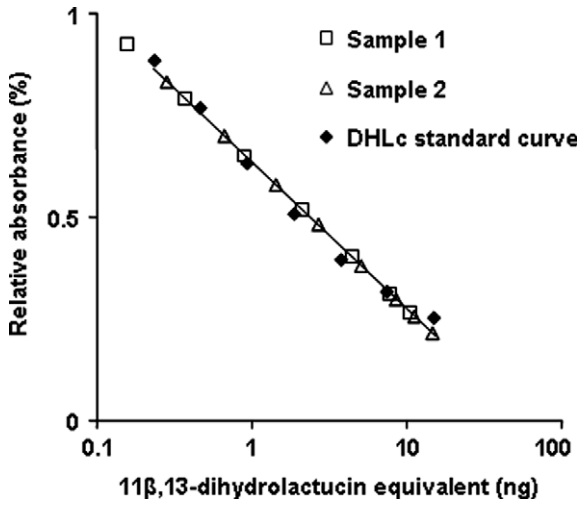

Fig. 5. Competitive indirect ELISA of 11β,13-dihydrolactucin in aqueous extracts of chicory roots.

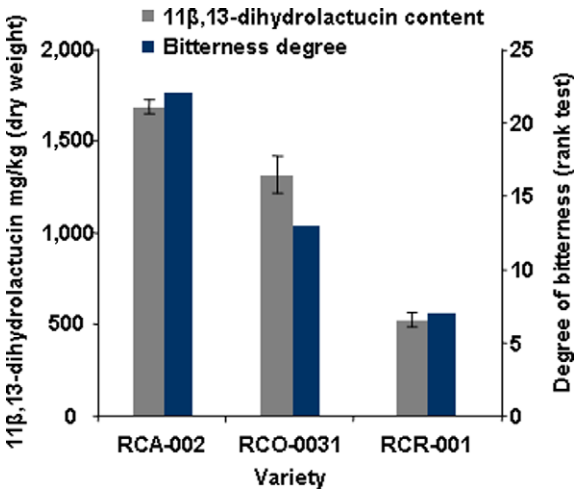

Fig. 6. Concentrations of 11β,13-dihydrolactucin in aqueous root extracts of three different chicory varieties and their degree of bitterness as determined by sensory evaluation. Assays were carried out on six replicates. Friedman's and pairwise treatments were performed to reveal differences among varieties ($P < 0.05$).

Three roots from the RCO-0031, RCR-001 and RCA-002 varieties, collected at harvesting stage, were investigated with the developed competitive ELISA for their degree of bitterness. Sensory assay was performed using a preparation of three roots from each variety, and was repeated two times at two different dilutions to avoid the dilution effect on bitter taste appreciation. Trained panelists evaluated the bitterness degree of samples compared with a four point sensorial reference scale: from 1 to 4, the most bitter one being 4. Data analyses by Friedman's and pairwise treatments showed that the mean ranks for each of the three samples were significantly different ($P \leqslant 0.05$); bitterness increased in the order RCR-001, RCO-0031 and RCA-002 (Fig. 6). The 11β,13-dihydrolactucin contents of the samples, assessed by competitive ELISA, were clearly different too. The repeatability and reproducibility values were equivalent to that obtained in the validation procedure, intra- and inter-assay mean variations being 5.9% and 8.9%, respectively. A correlation coefficient of 0.9 ($y = 53.2 \times -0.85$) between the rank mean values and sesquiterpene lactone levels was found, revealing a positive relationship between the total 11β,13-dihydrolactucin content and bitterness.

4. Conclusion

The ELISA based on polyclonal antibodies against the sesquiterpene lactone 11β,13-dihydrolactucin appears highly suitable to predict and monitor the bitterness quality of industrial chicory root (*Cichorium intybus* L. var. *sativum*). The sensitivity of the competitive assay, 12-fold more sensitive than an immunoassay against lactucin-like sesquiterpene lactones (Peters et al., 1996), allows a

detection of the target analyte at a concentration lower than 0.2 ng/ml. The present ELISA system thus provides a reproducible and useful tool, with high accuracy, to discriminate variations of 11β,13-dihydrolactucin concentrations between root samples from different chicory varieties. The easy procedure, and instruction, for ELISA, and the simple preparation of the root extracts for analysis, respond to the requirements for a low-cost, high throughput screening method that allows both the breeders and food industry to assess crop and processing conditions.

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