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Sesquiterpene Lactone Content and Overall Quality of Fresh-Cut Witloof Chicory (*Cichorium intybus* L. var. *foliosum* Hegi) as Affected by Different Washing Procedures

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ABSTRACT: Fresh-cut chicory was produced by four different processing lines testing cold and warm water (4/45 °C, 120 s) and cold water containing cysteine (0.5 mM) against an unwashed control. Levels of sesquiterpene lactones (SLs), sensory, and color attributes as well as O_2 and CO_2 levels in the modified atmosphere of the fresh-cut produce stored in consumer-sized bags (4 °C, 10 days) were monitored. All washing procedures applied significantly reduced the SL contents and bitterness in fresh-cut chicory. Warm water washing was most effective, reducing the total SL levels by 60.9−64.5%. Sensory and color attributes were also retained best by warm water washing, suggesting such treatments to be suitable measures to improve the quality and reduce the bitterness of fresh-cut chicory. SL profiles changed substantially in all samples during storage, revealing lactucopicrin to be the major SL directly after processing, whereas 11(*S*),13-dihydrolactuin prevailed at the end of storage.

KEYWORDS: *chicory, Cichorium intybus L. var. foliosum Hegi, fresh-cut, washing procedure, cysteine, warm water, sesquiterpene lactone, sensory evaluation, color*

INTRODUCTION

The cultivation of chicory (*Cichorium intybus* L. var. *foliosum* Hegi; Asteraceae) is widespread in Europe, North Africa, and parts of Asia.[1] The use of both, the roots and leaves, is manifold. While the roots are utilized as a source of inulin or fructose, cooked as vegetable or roasted as coffee surrogate, the leaves serve as livestock forage especially for ruminants and as a vegetable for the human diet.[2,3] While in many countries such as Italy chicory is largely cultivated as a green vegetable, in Western Europe mainly witloof chicory is consumed. Its apical buds (chicons) are usually forced by hydroponic growing in the dark, thus displaying white and pale-yellowish color due to the lack of chlorophyll.[1] The bitter taste of chicory originates from sesquiterpene lactones (SLs) mainly present in the latex.[4] They are taxonomically characteristic secondary metabolites of the *Asteraceae*. In chicory roots and leaves, the guaiane-type SLs lactucin, 8-deoxylactucin, lactucopicrin, and their 11(*S*),13-dihydroderivatives are prevailing.[5,6] Within the plant, they occur freely, as glycosides, 15-oxalates and 8-sulfates.[4] The SL levels in chicory vary strongly between cultivars[7] and were shown to depend on cultivation conditions such as nitrogen manuring, phosphorus availability, or forcing times[7−9] as well as climate, season, and state of growth.[10,11] While SLs play an important physiological role in plant defense against herbivores and pathogens,[11] their bitter taste is disliked by many consumers. In fact, according to Drewnowski et al.,[12] bitter taste is the main reason for the rejection of diverse foods. The preference of less bitter chicory by the consumers prompted the breeding of cultivars with very low levels of SLs.[7] However, reduced contents of SLs lower the disease resistance of the plants and hence may result in lower crop yield or more intense application of pesticides.[7]

Soaking of cut lettuce in cold or warm water prior to consumption is a homespun remedy used to debitter lettuces such as chicory, endive (*Cichorium endivia* var. *latifolium*), or sugarloaf lettuce (*Cichorium intybus* var. *foliosum*). However, although applied for centuries, to the best of our knowledge the effect of washing on the levels of SLs in lettuces has so far not been investigated.

As modern lifestyle is accompanied by an increasing demand for convenience products, the production of fresh-cut chicory as an admixture to lettuce blends seems to be promising. Because of wounding and decompartmentalization of plant cells by shredding of fresh-cut produce, such products are especially prone to undesired browning, catalyzed by oxidative enzymes such as polyphenol oxidase (PPO). Furthermore, the lack of an intact epidermis and release of cellular fluids on cut edges facilitates microbial growth and accelerates spoilage.[13] Therefore, the washing step, representing the only step that reduces microbial contamination within the production chain of such products, is most crucial with regard to the quality of the end products. Up to now, chlorine-releasing agents are used in many countries to decontaminate process water in the production of fresh-cut produce. However, due to the growing organic sector where chemical disinfectants are prohibited, and due to restrictions regarding the contamination with haloform byproducts,[14] more compatible alternatives are highly desired. Recently, numerous studies have been conducted concerning the efficacy of alternative washing and sanitizing treatments to improve the quality and shelf life of fresh-cut lettuces.[15−18] Warm water treatments (45 °C, 2 min) were shown to be suitable measures to better retain the quality of fresh-cut iceberg lettuce and endive.[18] The effect of warm water

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treatments was mainly ascribed to a better wash-off of bacterial biofilms and to the formation of heat shock proteins, thus preventing the synthesis of wound-induced phenylalanine ammonia-lyase (PAL), a key enzyme of the phenylpropanoid pathway.[18]

L-Cysteine has been shown to effectively prevent PPO catalyzed browning in different fruits and vegetables.[19,20] The effect was ascribed to the direct inhibition of polyphenol oxidase by cysteine[20] and the trapping of enzymatically formed *o*-quinones by cysteine to form colorless adducts acting as competitive inhibitors.[21]

Therefore, the aim of the present study was to investigate the efficacy of washing with cold and warm water as well as with cold water containing cysteine to reduce bitterness and retain the overall quality of fresh-cut chicory. Furthermore, the SL contents in the course of a 10 days refrigerated storage of fresh-cut chicory in the dark should be studied.

MATERIALS AND METHODS

Solvents and Reagents. All reagents and chemicals were of analytical or HPLC grade. L-Cysteine was purchased from Sigma Aldrich (St. Louis, MO, USA), and Novozym 33095 and Celluclast 1.5L were supplied by Novozymes A/S (Bagsvaerd, Denmark). All other chemicals were from VWR (Darmstadt, Germany). Solutions and eluents were prepared with Milli-Q system (Millipore, Bedford, MA, USA) ultrapure water.

Plant Material. Chicory (*Cichorium intybus* L. var. *foliosum* Hegi cv. Joker) was purchased from a local processor (Birkenhof Gemüse, Köngen, Germany) in November 2012. According to common practice, the chicory was packaged lightproof in dark plastic bags and reached the pilot plant at Hohenheim University in cardboard boxes on the day of harvest. The raw material was stored refrigerated (4 °C) until processing within less than 24 and 48 h for series 1 and 2, respectively.

Processing and Experimental Set Up. Prior to processing, production facilities, and equipment were carefully sanitized. Laboratory coats, disposable gloves, hairnets, and masks were worn throughout the chicory processing to ensure hygienic standards of good manufacturing practice. Each washing experiment was carried out in duplicate (series 1 and 2) with lots of 16 kg of chicory per processing line. During trimming, damaged outer leaves were manually removed. Subsequently, the chicory heads were cut into halves, and the core was excised using sharp stainless steel knives. Shredding of the trimmed chicory heads was conducted using a GS 10 multipurpose belt cutting machine (Kronen, Kehl, Germany). Within a series, each of the following four processing lines (I–IV) with different experimental arrangements was investigated (Figure 1):

(I) without washing
(II) washing in tap water (4 °C, 120 s), subsequent immersion in cold tap water (4 °C, 30 s)
(III) washing in warm tap water (45 °C, 120 s), subsequent immersion in cold tap water (4 °C, 30 s)
(IV) washing in tap water containing 0.5 mM L-cysteine (4 °C, 120 s), subsequent immersion in cold tap water (4 °C, 30 s)

The shredded chicory was washed in a stainless steel tank (500 L filling volume) with 150 L of process water per processing line. A rotary pump with flexible hoses (SIPLA S-10, Hilge, Bodenheim, Germany) connected the inflow with the outflow at the bottom of the tank, thus allowing water circulation (~100 L/min) with vigorous hydrodynamic agitation at adjusted temperatures inside the tank. At the beginning of each washing procedure, chicory was immersed into the water by means of a stainless steel grid and removed in the same way at the end of the washing process, thus ensuring an equal retention time of 120 s. After each washing operation, the shredded chicory was dipped into cold tap water (4 °C, 30 s) to cool down the produce that was washed with warm water and for rinsing off added cysteine that might affect the sensory properties of the end product.

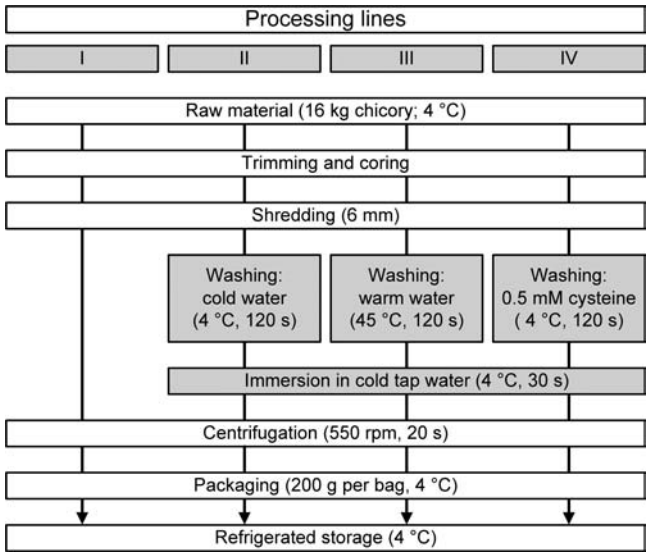

Figure 1. Flowchart of fresh-cut chicory production according to four different processing lines (I–IV).

Cold water washed chicory was subjected to the same process step to ensure consistent treatment for each processing line. To remove adherent water, chicory was subsequently centrifuged (550 rpm, 20 s) using a centrifuge K50-100s (Kronen, Kehl, Germany). The pooled and blended fresh-cut chicory was then packaged into consumer-sized film bags (Amcor Flexibles, Bristol, UK; 25 cm × 25 cm) in 200 ± 2 g quantities at 4 °C. The bags exhibited O_2 and CO_2 transmission rates at 23 °C and 0% relative humidity of 40 and 160 $cm^3 \cdot m^{-2} \cdot d^{-1} \cdot atm^{-1}$, respectively. After heat sealing using a C300 packaging machine (Multivac, Wolfertsschwenden, Germany), the bags were stored in a dark cooling chamber at 4 °C.

Headspace Analysis. Prior to further sample preparation, headspace O_2 and CO_2 concentrations in the chicory packages were monitored on storage days 0, 2, 4, 6, and 10 using a CheckMate 9900 O_2/CO_2 gas analyzer (PBI-Dansensor, Ringsted, Denmark). Headspace gas samples were taken by means of a hypodermic needle through an adhesive septum previously fixed on the bags. On each sampling day, five separate bags per processing line (I–IV) were analyzed.

Sample Preparation. For the extraction of SLs and color measurements lyophilized samples were used. On each sampling day, the contents of 2 bags per processing line were pooled and rapidly frozen by immersion into liquid nitrogen. The samples were stored at −20 °C in sealed plastic containers until freeze-drying using a Lyovac GT 4 (AMSCO Finn-Aqua, Hürth, Germany). The freeze-dried samples were finely ground in a Grindomix GM 200 laboratory mill (Retsch, Haan, Germany) and subsequently stored at −20 °C until analysis.

Sample Analysis. *Extraction of Free and Bound Sesquiterpene Lactones (SLs).* Aliquots of 1 g of lyophilized samples were exactly weighed into Erlenmeyer flasks, rehydrated by adding 20 mL of deionized water, and liquefied using 100 μL of each, Celluclast 1.5L and Novozym 33095. Liquefaction took place under continuous stirring for 1.5 h at 37 °C and pH 5. After filtration through Macherey-Nagel MN 615 1/4 paper (Düren, Germany) and addition of 20 mL of NaCl (10% w/v), the solution was twice extracted in a separation funnel with 40 mL of ethyl acetate. The combined supernatants were dried over sodium sulfate and filtered through Macherey-Nagel MN 615 1/4 paper. The organic solvent was evaporated to dryness (<30 °C), and the residue was dissolved in 2 mL of MeOH, membrane-filtered (0.45 μm, Macherey-Nagel) and used for HPLC. All extraction protocols were carried out in duplicate.

Preparative Isolation of SL References. Lactucin (LAC), 11(*S*),13-dihydrolactucin (DLAC), 8-deoxylactucin (8-DL), 11(*S*),13-dihydro-8-deoxylactucin (D8-DL), lactucopicrin (LCP), and 11(*S*),13-dihy-

drolactucopicrin (DLCP) were quantified with standard compounds isolated by preparative HPLC from chicory stems. For this purpose, lyophilized and powdered chicory stems were extracted as described above with 5 g sample powder and 5-fold quantities of all chemicals and reagents.

Standards were isolated using a Bischoff HPLC system controlled by McDAcq32 control software (version 2.0; Bischoff, Leonberg, Germany) equipped with an LCCaDI 2241 controller, two solvent delivery modules, a 2250 HPLC compact pump and a SPD 10AVVp UV/vis detector (Shimadzu Corporation, Kyoto, Japan). The column used was a preparative Phenomenex Aqua C18 reversed phase column (250 mm × 21.1 mm i.d., particle size 5 μm; 125 A pore size). The mobile phase consisted of 5% formic acid in water (eluent A) and methanol (eluent B) using a linear gradient as follows: 35−50% B (40 min), 50−100% B (5 min), 100% B isocratic (5 min), 100−35% B (2 min), 35% B isocratic (3 min) at a constant flow rate of 6 mL per min at 20 °C. For each HPLC run, aliquots of 500 μL of the chicory stem extract were injected and UV detection was performed at 258 nm.

HPLC-DAD Analysis of SLs. SL analyses were performed using a series 1100 HPLC system (Hewlett-Packard, Waldbronn, Germany) equipped with ChemStation software, a G1322A degasser, a G1312A binary gradient pump, a G1329/1330A thermoautosampler, a G1316A column oven, and a G1315A diode array detector (DAD). The column used was an analytical Phenomenex (Torrance, CA, USA) C18 Synergi 4u Hydro-RP 80 Å pore size (150 mm × 3.0 mm i.d.) column fitted with a Phenomenex (Torrance, CA, USA) security guard column (4 mm × 3.0 mm) operated at 25 °C. The mobile phase consisted of 5% formic acid in water (eluent A) and methanol (eluent B). The gradient program was optimized as follows: 20% B isocratic (15 min), 20−50% B (35 min), 50−100% B (5 min), 100% B isocratic (5 min), 100−20% B (5 min), 20% B isocratic (5 min). A constant flow rate of 0.5 mL per min at 25 °C was applied, and the injection volume for all samples was 6 μL. LAC, DLAC, 8-DL, D8-DL, LCP, and DLCP were quantified by external standard calibration using calibration curves of the respective reference compound isolated as described above. 8-DL and D8-DL were quantified as their sum. Assuming equal absorption coefficients for both derivatives, the concentrations of these two compounds were calculated proportionately to the ratios of their peak areas in the calibration curve.

Bitter Activity Value. Bitter activity values were calculated as described by Seo et al.[22] using bitter thresholds of LAC, DLAC, 8-DL, D8-DL, LCP, and DLCP according to van Beek et al.[5]

Identification of Individual SLs by HPLC-DAD/MS^n. SLs were identified by their UV and mass spectrometric data. HPLC-DAD/MS^n analyses were performed with an HPLC system (Agilent, Waldbronn, Germany) identical to the system described above. The HPLC system was coupled online with a Bruker (Bremen, Germany) model Esquire 3000+ ion trap mass spectrometer fitted with an electrospray ionization (ESI) source. Data acquisition and processing were performed using Esquire Control software. Positive ion mass spectra of the column eluate were recorded in the range of m/z 50−600. Nitrogen was used both as the drying gas at a flow rate of 9.0 L/min and as the nebulizing gas at a pressure of 40.0 psi. The nebulizer temperature was set at 365 °C, and a potential of 4.00 kV was used on the capillary. Collision-induced dissociation spectra were obtained with a fragmentation amplitude of 1.0 V.

Sensory Evaluation. Evaluation of chicory appearance and odor took place on storage day 6, representing the end of commercial shelf life according to the recommendations of the DGHM.[23] The evaluation was conducted according to Baur et al.[17] with modifications. It was exclusively carried out within series 2. The sensory panel consisted of 27 members of the Hohenheim University, Institute of Food Science and Biotechnology, all experienced in sensory evaluation. A 5-point rating scale, were 5 and 1 were the best and worst scores, respectively, was used to evaluate chicory quality. Panelists were asked to evaluate the chicory samples using the following parameters:

(1) Overall visual quality (OVQ; 5 = very good/fresh appearance, 3 = moderate, 1 = poor/no fresh appearance)
(2) Cut edge vascular tissue browning (CEVTB; 5 = none, 3 = moderate/slight, 1 = severe)
(3) Typical, fresh chicory odor (TFCO; 5 = very fresh/full characteristic, 3 = moderate, 1 = poor/none/atypical)
(4) Moldy and earthy off-odor (MEO; 5 = none; 3 = moderate; 1 = severe)
(5) Fermentative and alcoholic off-odor (FAO; 5 = none; 3 = moderate; 1 = severe)

After measurement of the gaseous atmospheres to ensure air-tightly sealed seams, two bags of each processing line (I−IV) coded with three-digit random numbers were presented for visual evaluation (OVQ, CEVTB) under white light. For odor and off-odor assessment (TFCO, MEO, FAO), chicory of three bags per processing line was pooled and filled into glass jars correspondingly coded. To evaluate odors and off-odors, panelists were asked to briefly remove the screw closure of the glass jars.

Color Measurements. Color measurements were performed on lyophilized and finely ground samples on storage days 0, 2, 4, 6, and 10 with a CR-300 chroma meter (Minolta, Osaka, Japan) using illuminant D_{65} and a 10° observer angle. Prior to measurements, the instrument was calibrated to a standard white tile (L* = 97.43, a* = −0.01, b* = 1.64). A glass Petri dish containing the samples was placed below the light source. Measurements were conducted 10-fold at various pieces.

Determination of Dry Matter. Dry matter contents of the chicory samples were determined thermogravimetrically using a MA 40 electronic moisture analyzer at 105 °C (Sartorius, Göttingen, Germany). The determination was carried out in duplicate with 2 ± 0.2 g of homogenized chicory tissue.

Statistical Evaluation. Data were subjected to one-way analysis of variance (ANOVA) using the SAS software package (SAS Institute, Cary, NC, USA; v. 9.1) for different washing procedures and separately for each series. In the case of significant differences, individual washing procedures were compared using the Tukey test for differences between independent samples (α = 0.05).

RESULTS AND DISCUSSION

Process Data. Chicon weights amounted to 216 ± 8 g and 200 ± 8 g for series 1 and 2, respectively. Per each of the four processing lines (Figure 1), total processing time was below 1 h. The overall average waste rates (discarded cores and damaged outer leaves) were 22.0 ± 0.7% and 21.4 ± 5.1% for series 1 and 2, respectively, thus confirming consistent trimming and good chicon quality.

Headspace Analysis. Chicory processed in series 1 and 2 exhibited virtually the same response to the different washing procedures (I−IV) with respect to their gas composition. Therefore, changes in headspace O_2 and CO_2 levels from series 1 are shown exemplarily in Figure 2. As expected, due to the respiratory activity of the plant tissue, O_2 and CO_2 concentrations showed an inverse course with proceeding storage time in the bags of all washing treatments (I−IV). Compared to cold or warm washed variants (processing lines II−IV), unwashed chicory (processing line I) exhibited a noticeably higher respiration rate indicated by significantly higher CO_2 concentrations in the bags on storage days 2−10 and significantly lower O_2 concentrations on storage days 2 and 4. This may be due to a more vigorous growth of aerobic bacteria, as described elsewhere on iceberg lettuce and endive.[18] Whereas bags of unwashed chicory were virtually devoid of O_2 from storage day 4 on, cold water treated chicory did not fully consume oxygen until storage day 6. Warm water washed chicory reached complete anaerobic conditions on storage day 10, showing a slightly reduced respiration with significantly higher O_2 and lower CO_2 levels on storage day 6. Baur et al.[17] reported an increased respiration as a reaction to heat stress when trimmed iceberg lettuce heads were exposed to a

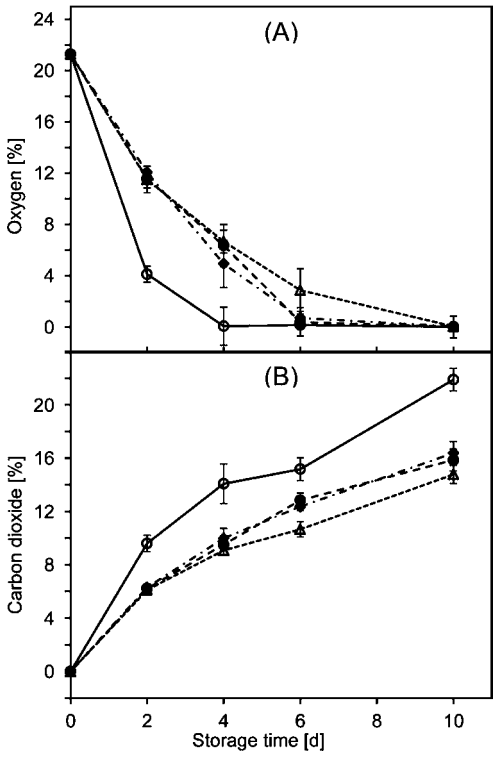

Figure 2. Changes in headspace O_2 (A) and CO_2 (B) levels in packages of shredded chicory during storage at 4 °C: (○) line I, no washing; (●) line II, washing in cold tap water; (Δ) line III, washing in warm tap water; (◆) line IV, washing in cold tap water containing 0.5 mM cysteine. Each data point represents the mean of five separate bags ± standard deviation from series 1.

prewashing step with warm water (50 °C, 60 s). However, the warm water treatment applied in the present study (45 °C, 120 s) apparently did not trigger the respiration rate of the plant tissue, thus confirming previous reports on iceberg lettuce and endive.[18] Bags with lettuces processed in lines II (cold water) and III (cold water with cysteine) exhibited virtually the same course of their headspace composition. Hence, the addition of cysteine to the washing water obviously did not affect the respiratory activity of chicory tissue.

Whereas in a previous study CO_2 concentrations in bags of unwashed, equally packaged and stored iceberg lettuce and endive increased to 12% and 17%, respectively,[18] bags with unwashed chicory exhibited up to 22% CO_2 in their headspace at the end of storage, thus reflecting a considerably higher respiratory activity.

A modified atmosphere containing 0.5−3% O_2 and 5−20% CO_2 is recommended to prolong the shelf life of shredded lettuce.[24,25] While CO_2 levels in excess of 5% exhibit bacteriostatic properties, CO_2 concentrations ≥10% may control the sporulation and growth of many fungal decay organisms.[26,27] In the present study, CO_2 concentrations above 5% were achieved on storage day 2 for all processing lines. O_2 concentrations <3% were achieved on storage day 4 and 6 for the unwashed and washed variants (II−IV), respectively.

Extraction, Analysis, and Characterization of Individual SLs. In the present study, SLs were extracted after enzymatic liquefaction of the lyophilized and powdered chicory and subsequent extraction with ethyl acetate. In preliminary trials, the enzymatic liquefaction step was replaced by boiling of the lyophilized sample with MeOH under reflux for 1 h as described by Tamaki et al.[28] However, when compared to the enzymatic liquefaction applied in the present study, by refluxing with MeOH only 37%, 33%, and 56% of LAC, 8-DLAC, and LCP, respectively, were extracted (data not shown). The considerably lower SL yields by hot MeOH extraction may most likely result from their thermal instability[29] and possibly also from an incomplete cell disruption. In contrast, enzymatic liquefaction of the plant tissue facilitated SL extraction because of the complete breakdown of cell walls, as earlier stated by Peters et al.[30]

In further preliminary trials, incubation times during enzymatic liquefaction were varied between 1.5 and 2.5 h. As the amount of extracted SLs did not vary depending on these extraction times (data not shown), an extraction time of 1.5 h was selected. Peters et al.[30] reported an incubation time ≤2 h to be optimal with respect to the extraction yield of lactucin-like SLs and lactucopicrin. Furthermore, the water phase after ethyl acetate extraction was shown to be devoid of free lactucopicrin and only contained a minor proportion of lactucin-like SLs.[30] As a 3-fold ethyl acetate extraction did not increase SL yields in preliminary trials (data not shown), a 2-fold extraction of the liquefied sample with ethyl acetate was applied in the following to achieve an almost exhaustive SL extraction from chicory.

The chemical structures of the SLs investigated and the HPLC separation of SLs in extracts of unwashed chicory on storage day 0 and 10 are shown in Figure 3 and 4, respectively. Peak identification was carried out by HPLC-DAD/ESI(+)-MS^n analysis. Corresponding characteristic UV−vis and mass spectrometric data as well as peak assignments of the separated compounds are specified in Table 1.

Compounds **1** and **2** were identified as DLAC and LAC with $[M + H]^+$ ions at m/z 279 and 277, respectively. They revealed

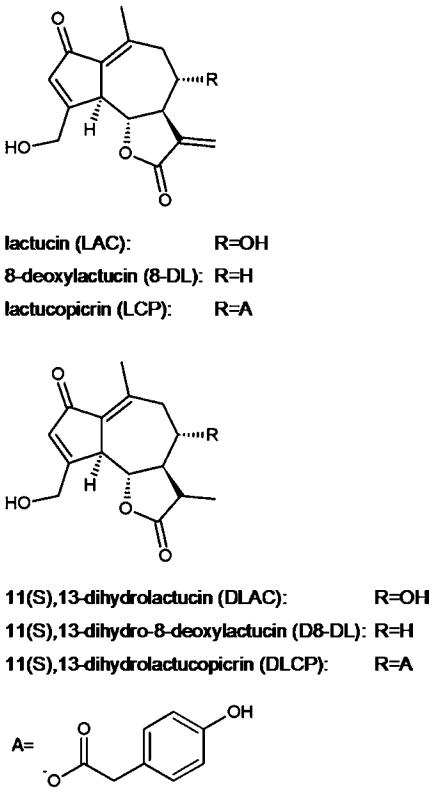

Figure 3. Structures of the main sesquiterpene lactones (SLs) in chicory.

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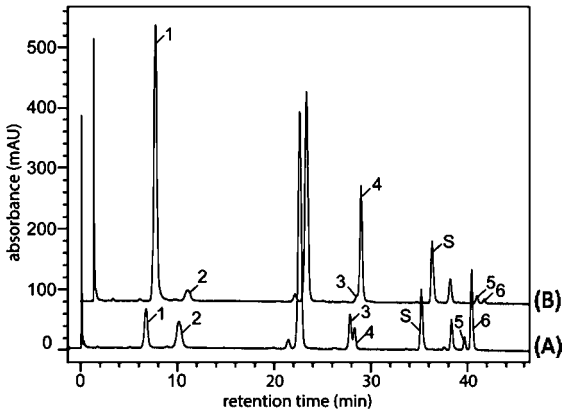

Figure 4. HPLC-DAD chromatograms (258 nm) of sesquiterpene lactones (SLs) in unwashed chicory on storage day 0 (A) and storage day 10 (B). For peak assignment, see Table 1.

a most abundant fragment at *m/z* 215 (DLAC) and 213 (LAC), representing an ion fragment formed by the loss of formic acid and water (64 atomic mass units). Compounds **3** and **4**, revealing $[M + H]^+$ ions at *m/z* 261 and 263, respectively, were identified as 8-DL and D8-DL. The most abundant daughter ions at *m/z* 215 (8-DL) and 217 (D8-DL) were ascribed to the loss of formic acid (46 atomic mass units).The latter fragments were determined correspondingly by Ferioli et al.[6] For compounds **5** and **6**, unambiguously identified as DLCP and LCP, the predominant positive fragments with *m/z* 261 and 259, respectively, resulted from the loss of the *p*-hydroxyphenylacetic acid moiety (152 atomic mass units), being consistent with earlier findings.[4,6] All SLs analyzed showed characteristic absorption maxima between 257 and 259 nm.

Influence of the Washing Procedure and Refrigerated Storage on the SL Level. As chicory of both series exhibited similar levels of the SLs investigated (LAC, DLAC, 8-DL, D8-DL, LCP, and DLCP), only data of series 1 is shown in Figure 5. The levels of SLs in fresh-cut chicory varied considerably between the different washing procedures applied (lines I–IV) and were also shown to be strongly affected by refrigerated storage over 10 days (Figure 5). Directly after processing (storage day 0), LCP was the predominant SL in chicory samples of all processing lines and both series. This is in accordance with the findings of Peters et al.[31] in the central axis and the leaves of uncut chicons of cv. Focus and cv. Monitor. However, in the present study, the SL profiles of shredded, packaged chicory changed drastically during storage, revealing DLAC to be the major SL at the end of storage.

Throughout storage, unwashed chicory (line I) exhibited significantly higher levels of all SL investigated compared to the washed variants (lines II–IV). At storage day 0, total SL levels in unwashed chicory amounted to 723.7 mg/kg of dry weight (DW) and 718.6 mg/kg DW in series 1 and 2, respectively. Foster et al.[9] reported considerably higher total SL levels ranging from 1550 to15240 mg/kg DW in green leaves of forage chicory. SL contents of chicory have been shown to vary considerably among cultivars[7] depending on the cultivation conditions,[7−9] climate, season, and state of growth.[10,11] In the present study, commercial witloof chicory was processed. As consumers prefer less bitter chicory, cultivars with lower SL levels are commonly used for forcing.[7] Therefore, as expected, the produce under investigation being destined for human consumption and forced under exclusion of light contained lower SL levels than chicory used as forage.

By washing with cold water (line II), 39.5% and 37.1% of the total SLs were leached for series 1 and 2, respectively. When cysteine was added to the washing water (line IV), the SL reduction comprised 33.4% (series 1) and 46.2% (series 2). Obviously, although LAC, 8-DL, and LCP exhibit exocyclic α-methylene functions that may conjugate with the sulfhydryl group of cysteine by a Michael addition,[32] cysteine did not substantially contribute to decrease the levels of bitter SLs from chicory. Possibly, longer contact times (>120 s) and higher cysteine concentrations are required to get an effect.

The highest SL reduction could be achieved by using warm water (line III). Hereby, 60.9% and 64.5% of the total SLs were leached in series 1 and 2, respectively. It is assumed that better solubility of SLs at increased temperature was responsible for the improved effect of warm water. In agreement with our findings, Peters et al.[8] reported SL levels to be significantly lower in cooked than in raw chicory. Besides a partial degradation of the SLs due to high temperatures, thermal destruction of the plant tissue, and thus enhanced transition of SLs into the cooking water was considered responsible for the lower SL levels of cooked chicory in the latter study.

Table 1. UV/Vis Spectra and Mass Spectrometric Characteristics of Major Sesquiterpene Lactones (SLs) Extracted from Chicory

peak no.	compd[a]	t_R (min)	HPLC-DAD λ_{max} (nm)	$[M + H]^+$ *m/z*	HPLC/ESI(+)-MS^2 and MS^3 *m/z* (% base peak)
1	DLAC	7.4	259	279	MS^2[279]: 215(100), 187(28), 261(22), 159 (17), 243(17) MS^3[279→215]: 187(100), 197(71), 169(67), 143(21), 179(11)
2	LAC	11.6	258	277	MS^2[277]: 213(100), 241(38), 185(36), 167 (22), 259 (20) MS^3[277→213]: 185(100), 157(52), 143(41), 141(37), 167 (25)
3	8-DL	29.2	257	261	MS^2[261]: 215(100), 243(75), 169(36), 187(35), 197(34) MS^3[261→215]: 187(100), 188(23), 131(19), 197(18), 145(17)
4	D8-DL	29.7	257	263	MS^2[263]: 217(100), 245(68), 189(35), 227(17), 171(15) MS^3[263→217]: 189(100), 199(79), 159(70), 171(66), 161(57)
5	DLCP	42.2	258	413	MS^2[413]: 261(100), 215(19), 262(12), 187(17), 143(12) MS^3[413→261]: 187(100), 215(91), 159(72), 177(42), 216(42)
6	LCP	42.8	258	411	MS^2[411]: 259(100), 260(34), 185(14), 213(14), 195(11) MS^3[411→259]: 213(100), 214(36), 157(24), 231(22), 241(21)

[a]DLAC, 11(*S*),13-dihydrolactucin; LAC, lactucin; 8-DL, 8-deoxylactucin; D8-DL, 11(*S*),13-dihydro-8-deoxylactucin; DLCP, 11(*S*),13-dihydrolactucopicrin; LCP, lactucopicrin.

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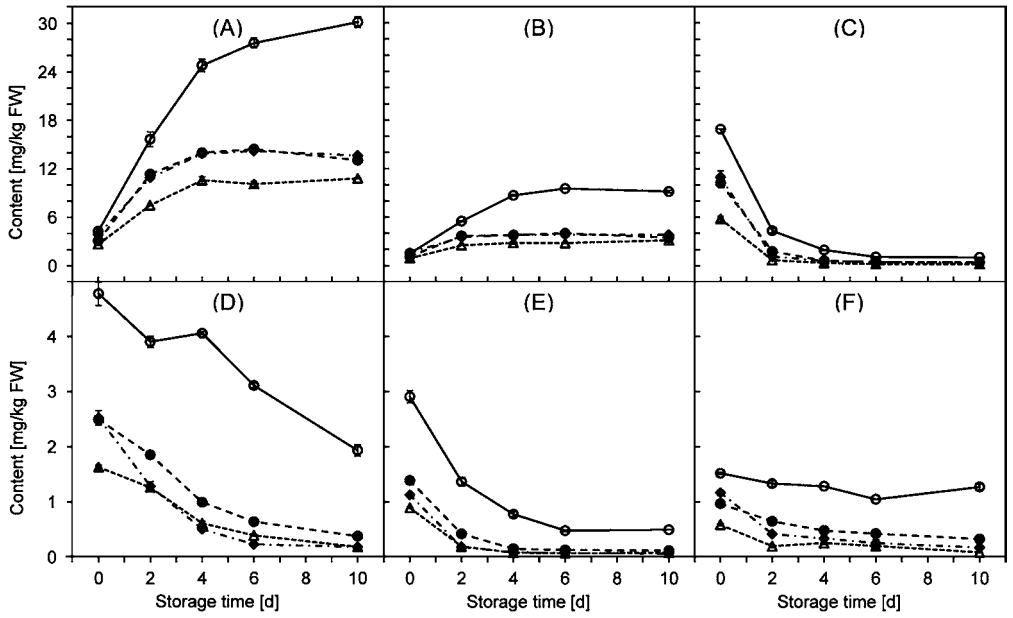

Figure 5. Content of individual sesquiterpene lactones (SLs) in fresh-cut chicory during storage at 4 °C: (A) 11(*S*),13-dihydrolactucin (DLAC), (B) 11(*S*),13-dihydro-8-deoxylactucin (D8-DL), (C) lactucopicrin (LCP), (D) lactucin (LAC), (E) 8-deoxylactucin (8-DL), (F) 11(*S*),13-dihydrolactucopicrin (DLCP); (○) line I, no washing; (●) line II, washing in cold tap water; (Δ) line III, washing in warm tap water; (◆) line IV, washing in cold tap water containing 0.5 mM cysteine. Each data point represents the mean of two values ± standard deviation from series 1.

In the present study, the contents of DLAC and D8-DL were less affected by washing than those of LAC, 8-DL, LCP, and DLCP. Regarding processing line III (washing with warm water), between 61.8 and 69.4% and 65.9−74.37% of LAC, 8-DL, LCP, and DLCP were leached for series 1 and 2, respectively, whereas D8-DL and DLAC levels were only reduced by 38.0−39.7% (series 1) and 33.5−35.0% (series 2). The latter might be due to the lower polarity of D8-DL and DLAC due to the missing exocyclic methylene group (Figure 3). The comparatively high losses of DLCP by washing may be caused by its enhanced water solubility due to the presence of a polar *p*-hydroxyphenylacetic acid moiety.

SL profiles in fresh-cut chicory samples of all processing lines unexpectedly changed during storage at 4 °C for 10 days. DLAC and D8-DL increased during storage in samples of all processing lines. For DLAC, the increment was particularly pronounced in unwashed chicory (line I), where the initial content was augmented by factor 7.0 and 6.5 during storage for series 1 and 2, respectively. Regarding the washed chicory variants (lines II−IV), contents of DLAC increased only by factor 3.5−4.0 and 3.4−4.2 for series 1 and 2, respectively. Similar tendencies could also be observed for D8-DL.

In contrast, contents of LAC, 8-DL, LCP, and DLCP in fresh-cut chicory of all processing lines declined. The strongest decline was observed for LCP, which was virtually absent in all samples from storage day 6 on.

While total SL contents stayed rather constant throughout storage for the cold water washed variants (processing lines II and IV), they increased in unwashed chicory (processing line I) by 38.8% and 45.3% for series 1 and 2, respectively. A slight increase in total SL levels in the course of storage was also observed for warm water washed chicory samples (processing line III), where total SL levels increased by 16.3% (series 1) and 28.2% (series 2). Regarding the rise in total SL levels, a de novo synthesis may be assumed. SLs play an important role in plant defense against herbivores and pathogens.[11] Consequently, triggered de novo synthesis may be due to wounding stress of the plant tissue caused by shredding and due to the growth of microorganisms. However, as the quantities of individual SLs changed drastically during storage, which cannot be consistently explained by de novo synthesis, probably also the conversion of SLs during refrigerated storage may be considered.

In the biosynthesis of the guaianolide SLs present in chicory, (+)-costunolide is an intermediate.[33] The formation of 11(*S*),13-dihydrocostunolide was shown to be independent of O_2 but to be strongly enhanced by the presence of NADPH. The hydrogenation of the C11−C13 exocyclic double bond was considered to be catalyzed by enoate reductases, occurring in many microorganisms and cell cultures.[33]

Because of the respiration of the plant tissue, anaerobic conditions were reached in bags of all processing lines throughout storage (Figure 2). As NADPH accumulates in anaerobic metabolism due to glycolysis, thus creating a reductive environment, LAC and 8-DL might be converted into their 11(*S*),13-dihydro derivatives in a similar reaction as described for their precursor (+)-costunolide. Such a conversion may be responsible for the decline in LAC and 8-DL and the increase in DLAC and D8-DL during storage under anaerobic conditions. However, although LCP contents decreased rapidly in all chicory samples, the levels of DLCP also decreased slightly during storage. Possibly, hydrolysis of LCP into LAC and *p*-hydroxyphenylacetic acid might be due to esterase activities originating from the plant tissue and/or the microorganisms.

According to Van Beek et al.,[5] bitterness threshold values vary among SLs, with DLAC and LAC possessing the highest intensity of the SLs investigated in the present study. The relative importance of individual SLs with regard to their overall bitterness was assessed by comparing bitter activity values (BAVs), calculated by dividing the concentration of each individual SL by its bitter threshold in water[5,22] (Table 2). Although total SL contents increased (lines I and III) or stayed rather constant (lines II and IV), BAVs decreased at the

Table 2. Bitterness Based on Bitter Activity Values as Affected by Different Washing Procedures (I–IV) and Refrigerated Storage[a]

	processing line[b]			
day	I	II	III	IV
	Series 1			
0	50.8 ± 0.2	30.9 ± 0.0	18.9 ± 0.7	34.4 ± 1.7
2	35.0 ± 0.3	19.7 ± 0.3	10.8 ± 0.3	18.2 ± 1.2
4	37.9 ± 0.3	17.6 ± 0.0	11.5 ± 0.3	16.5 ± 0.3
6	37.9 ± 0.2	17.6 ± 0.3	11.6 ± 0.7	16.3 ± 0.1
10	38.4 ± 0.4	15.3 ± 0.1	11.6 ± 0.0	15.2 ± 0.2
	Series 2			
0	52.3 ± 0.1	33.5 ± 1.7	16.4 ± 0.3	26.4 ± 1.4
2	35.2 ± 1.1	17.4 ± 0.1	12.6 ± 0.2	16.6 ± 0.8
4	37.3 ± 0.4	18.5 ± 0.1	12.3 ± 0.3	17.2 ± 0.1
6	33.4 ± 0.0	17.6 ± 0.1	11.8 ± 0.3	15.9 ± 0.0
10	42.7 ± 0.4	16.4 ± 0.5	12.5 ± 0.0	16.9 ± 0.1

[a]Each data point represents the mean of two values ± standard deviation. [b](I) No washing; (II) washing in cold tap water; (III) washing in warm tap water; (IV) washing in cold tap water containing 0.5 mM cysteine.

beginning of storage due to the rapid decline of the LCP content within the first two days of storage. From storage day 2 on, BAVs kept rather constant until the end of storage (Table 2). BAVs of unwashed chicory (line I, series 1 and 2) were significantly higher than the washed variants throughout their entire storage, while warm water treated chicory (line III) exhibited the lowest BAVs within the storage time considered. Hence, all washing procedures applied (lines II–IV), but particularly washing with warm water, effectively reduced the bitterness of fresh-cut chicory.

Sensory Attributes As Affected by Different Washing Procedures. Sensorial evaluation of fresh-cut chicory was performed on storage day 6, representing the end of commercial shelf life according to the recommendations of the DGHM.[23] The mean scores assigned by 27 panelists for the sensory attributes of chicory processed in four different lines (lines I–IV) are shown in Table 3. For all sensory properties investigated, scores of unwashed chicory were significantly lower than those of the washed variants (lines II–IV). Hence, in accordance with previous reports, the removal of cellular fluids by washing after shredding is most crucial for quality retention during storage.[15,34] Significant differences in sensory properties between the washing procedures applied were found for the parameters "overall visual quality" (OVQ) and "cut edge vascular tissue browning" (CEVTB). Browning, the most serious defect of visual appearance of lettuces, is caused by the induction of the phenylpropanoid metabolism upon wounding, being a decisive factor limiting shelf life and marketability of fresh-cut products.[35] The ratings for OVQ and CEVTB practically paralleled, which is in accordance with former studies,[15,17] demonstrating warm water treated chicory (line I) to be significantly superior to the cold water variants (lines II and IV). Confirming the results of previous studies,[17,36] the aroma of fresh-cut chicory was affected by the different washing procedures to a lesser extent. Except for the unwashed control (line I), the ratings for "typical fresh chicory odor" (TFCO), "moldy and earthy off-odor" (MEO), and "fermentative and alcoholic off-odor" (FAO) were not substantially affected by the different washing treatments applied (lines II–IV). However, although insignificant, aroma scores of stored chicory that had been subjected to washing with warm water were superior to lettuce washed with cold water. By trend, quality retention of chicory treated with cold water and added cysteine (line IV) was slightly enhanced with regard to browning (OVQ, CEVTB) and aroma properties (TFCO, MEO, FAO) compared to chicory solely washed in cold water. Obviously, the cysteine concentration applied in the present study (0.5 mM) did not adversely affect the sensory properties of fresh-cut chicory as may be assumed for higher doses.[20,37]

Table 3. Effect of Different Processing (Lines I–IV) on Mean Scores for Sensory Attributes of Fresh-Cut Chicory on Storage Day 6[a]

	processing line[b]			
parameter	I	II	III	IV
overall visual quality (OVQ)[c]	1.5z	3.4y	4.7x	3.7y
cut edge vascular tissue browning (CEVTB)[d]	1.4z	3.6y	4.5x	3.6y
typical, fresh chicory odor (TFCO)[e]	1.5y	3.2x	3.8x	3.4x
moldy and earthy off-odor (MEO)[f]	2.0y	3.4x	3.9x	3.7x
fermentative and alcoholic off-odor (FAO)[g]	2.4y	4.1x	4.7x	4.3x

[a]Each value represents the average of 27 observations. x, y, z: within a row for a given parameter, different letters indicate significant differences (α = 0.05). [b](I) No washing; (II) washing in cold tap water; (III) washing in warm tap water; (IV) washing in cold tap water containing 0.5 mM cysteine. [c]Scoring system: 5 = very good/fresh appearance, 3 = moderate, 1 = poor/no fresh appearance. [d]Scoring system: 5 = none, 3 = moderate/slight, 1 = severe. [e]Scoring system: 5 = very fresh/full characteristic, 3 = moderate, 1 = poor/none/atypical. [f]Scoring system: 5 = none; 3 = moderate; 1 = severe. [g]Scoring system: 5 = none; 3 = moderate; 1 = severe.

Color Development in the Course of Storage As Affected by Different Washing Procedures. CIE L*a*b* values were similar in both processing series. Therefore, data of series 2 are shown exemplarily in Table 4. Shredded lettuce comprises a heterogeneous blend of vascular and photosynthetic tissues with vascular tissue being more susceptible to brown discoloration than photosynthetic tissue.[38] Baur et al.[15] described poor correlation between instrumental color measurement and visual evaluation of shredded lettuce pieces due to considerable heterogeneity of the produce. Therefore, in the present study, color measurements were conducted on lyophilized and powdered samples to ensure maximum sample homogeneity.

Because of the appearance of browning, lightness values (L*) uniformly decreased significantly in the course of storage for all processing lines and both series. Consistent observations were reported by Kenny et al.[39] for sliced iceberg lettuce during storage. Compared to the washed variants (lines II–IV) of both series, significantly lower L* values and higher a* values were observed for unwashed chicory samples (line I) throughout the entire storage time. The latter findings suggest an accelerated quality loss due to enzymatic browning and the breakdown of chlorophyll, consistent with the ratings of the sensorial evaluation (OVQ, CEVTB).

By comparing all washing procedures applied (lines I–IV), samples of chicory subjected to warm water (line III) exhibited the highest lightness values (L*) toward the end of storage (significant on storage day 6 for series 1 and on storage days 4–10 for series 2). Obviously, by the warm water treatment applied in the present study (45 °C, 120 s), a good quality retention of fresh-cut chicory with respect to reduced browning

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Table 4. Color Characteristics as Affected by Different Washing Procedures (I–IV) and Refrigerated Storage (n = 10)[a]

		processing line[b]			
parameter	day	I	II	III	IV
L*	0	91.6 ± 0.2c	94.3 ± 0.1a	93.7 ± 0.1b	94.3 ± 0.1a
	2	91.1 ± 0.2c	93.2 ± 0.2b	93.2 ± 0.3b	93.5 ± 0.1a
	4	91.3 ± 0.2c	93.1 ± 0.2b	93.4 ± 0.1a	92.9 ± 0.1b
	6	89.3 ± 0.4c	92.1 ± 0.1b	92.7 ± 0.1a	92.2 ± 0.1b
	10	89.4 ± 0.2c	90.9 ± 0.1b	92.2 ± 0.2a	91.2 ± 0.2b
a*	0	−0.52 ± 0.03a	−1.23 ± 0.03c	−1.14 ± 0.02b	−1.49 ± 0.06d
	2	0.01 ± 0.04a	−0.90 ± 0.05b	−1.10 ± 0.11c	−1.29 ± 0.05d
	4	0.05 ± 0.05a	−0.69 ± 0.56b	−1.01 ± 0.07b	−0.93 ± 0.05b
	6	−0.09 ± 0.05a	−1.20 ± 0.02c	−1.11 ± 0.06b	−1.21 ± 0.05c
	10	−0.52 ± 0.05a	−1.27 ± 0.04c	−0.97 ± 0.03b	−1.40 ± 0.05d
b*	0	13.2 ± 0.5b	12.2 ± 0.2d	12.8 ± 0.2c	13.8 ± 0.4a
	2	12.2 ± 0.3c	11.9 ± 0.4c	12.9 ± 0.4b	13.4 ± 0.3a
	4	11.6 ± 0.3c	11.9 ± 0.3c	12.2 ± 0.3b	12.6 ± 0.1a
	6	12.7 ± 0.5a	11.2 ± 0.3b	12.5 ± 0.3a	12.3 ± 0.3a
	10	13.5 ± 0.3a	11.6 ± 0.2c	11.9 ± 0.3b	12.1 ± 0.3b

[a]Each data point represents the mean of 10 values ± standard deviation. Different letters (horizontally) indicate significant differences ($p < 0.05$). [b](I) No washing; (II) washing in cold tap water; (III) washing in warm tap water; (IV) washing in cold tap water containing 0.5 mM cysteine.

reactions was achieved, being in good accordance with the sensory evaluation of OVQ and CEVTB. In preliminary studies, warm water treatments (45 °C, 120 s) were consistently shown to be suitable measures to improve the quality of iceberg lettuce and endive.[18] The effect of a moderate heat treatment to prolong the shelf life of fresh-cut lettuces was ascribed to the prevention of wound-induced PAL. This key enzyme, activated upon wounding, catalyzes the formation of *trans*-cinnamic acid from its precursor phenylalanine.[40] *trans*-Cinnamic acid may be converted into various caffeic acid derivatives,[16,41,42] which are substrates of enzymatic browning. Cells exposed to wound and heat preferentially synthesized heat shock proteins decreasing the capacity to synthesize PAL.[35] Another reason for the improved quality retention of warm water washed lettuces was shown to be a better wash-off of bacteria, presumably due to the enhanced disintegration of bacterial biofilms by warm water.[18]

In both series, cysteine treated chicory samples exhibited significantly lower initial a* values on storage days 0 and 2 than those of the other processing lines, indicating a more intense green color at the beginning of storage. This effect might be ascribed to the inhibitory effect of cysteine on PPO and thus enzymatic browning. During enzymatic oxidation by PPO, cysteine was shown to trap the *o*-quinones by forming colorless cysteinyl adducts, thus preventing plant tissue discoloration.[21] Furthermore, such cysteine–quinone adducts were shown to competitively inhibit apple PPO because of their affinity for the enzyme higher than that of their precursors.[21] Accordantly, for lettuce PPO the inhibitory effect of cysteine was also explained by a competitive model.[19] Further studies reported a direct irreversible inhibition of strawberry and palmito PPO by cysteine.[20,43] In the present study, significant differences were only found for lower a* values within the first two days of storage. Presumably, due to rinsing after washing, cysteine has mostly been removed from the fresh-cut products, which is corroborated by the sensorial evaluation where none of the panel members noticed off-odor caused by cysteine. Therefore, the inhibitory effect on browning by cysteine did not persist during storage.

The b values of the chicory samples were not substantially affected by the different washing procedures applied and remained rather constant throughout storage, the latter being consistent with the findings of Hisaminato et al.[41] for cut iceberg lettuce.

The results of the present study demonstrate that washing procedures as well as subsequent refrigerated storage strongly affect the quality of fresh-cut chicory. The application of warm water proved to be most suitable to improve the quality of fresh-cut chicory regarding its sensory attributes including color and bitterness. Further research is required to elucidate the biochemical processes responsible for the observed alteration of the SL profiles of fresh-cut chicory during storage under modified atmosphere.

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