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Antifogging additives for greenhouse covers – effects on phytochemicals and nutritional quality of lettuce

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Summary

Antifogging additives are commercially used in greenhouse films to prevent water droplet formation on these films. This can increase light transmission, and thus, improve crop yield. However, the effect of polytunnels with antifogging additives on phytochemical content in lettuce (*Lactuca sativa var. capitata*) is currently unclear. Here, polytunnels were chosen as a model to investigate the impact of antifogging additives in a completely randomized setting. Analysis by means of chromatographic methods coupled with mass spectrometry revealed a general influence of polytunnel cultivation compared to lettuces grown without a polytunnel on the content of phenolic compounds, photosynthetic pigments and fatty acids. The use of antifogging additives does not lead to significant differences in phenolic compounds and fatty acids. However, significant differences were observed for carotenoids and chlorophylls by both polytunnel cultivation and the use of antifogging additives. These differences probably occurred predominantly due to differences in light and temperature regimes related to polytunnel cultivation. Thus, due to polytunnels in general and the use of antifogging additives in particular, environmental conditions are created that impact valuable compounds and alter nutritional quality of crops.

Keywords: Polytunnel, phenolic compounds, carotenoids, fatty acids, plastic additives, light transmission, *Lactuca sativa*

Introduction

Plastic films are widely used to cover greenhouses and polytunnels to produce horticultural crops. It is estimated that 5,630,000 ha of land was used for protected agriculture worldwide in 2019 (World Greenhouse Vegetable Statistics, 2019). In Germany, these protected agricultural area covers 1,279.3 ha (Statistisches Bundesamt, 2019). The advantages of using protected cultivation compared to open field conditions are improved yield and productivity since farmers can produce off-season or start growing ahead of the season (Gruda, 2005). Moreover, in hotter climates, it is possible to conserve water, and thus, improve the efficiency of crop production (Irusta et al., 2009). Of note is that low material costs make plastic greenhouse films more favorable than glass greenhouses and this is reflected in Southeast European countries (SEE countries) where the greenhouse surface made of glass compared to plastic is about 8,305 ha and 46,280 ha, respectively (Baudoin et al., 2017).

The benefits of using greenhouses or polytunnels result from the control of environmental factors, such as light and temperature, enabling optimal growing conditions to be created for the cultivated crops. The materials used for greenhouse covers provide different light transmittances and thermal efficiencies, so the selection of different materials can influence both crop yield and nutritional quality. Although several studies have investigated the impact of such materials on plant growth and crop production (Papadopoulos et al., 1997; Hao et al., 1999; Gruda, 2005; Cemek et al., 2006), those on how greenhouse materials affect nutritional quality are rare (Petropoulos et al., 2019; Ahmadi et al., 2019). In addition, plastic greenhouse films can be modified with various plastic additives to generate beneficial properties. For example, UV-blockers can prevent UV-light transmission and protect plants against damage (Katsoulas et al., 2020). Some studies have demonstrated that UV-blocking greenhouse films have an effect on crop yield and nutritionally valuable compounds such as plant secondary metabolites, like phenolic compounds, carotenoids and chlorophylls (reviewed by Katsoulas et al., 2020). However, research on the effect of other plastic additives, such as antifogging additives, is currently lacking. Antifogging additives are used to prevent the formation of water droplets on the inside of the greenhouse. This has several advantages, such as improved light transmission through the films, prevention of microbiological contamination as well as heat retention in the greenhouse (Ren et al., 2018).

Here, we investigated the impact of polytunnels with antifogging additives on the nutritional quality of lettuce (*Lactuca sativa var. capitate* cultivars 'Veronique' and 'Attractie'). Lettuce is often grown under protective covers. In Germany, 2,331.04 t of lettuce were produced under protected conditions on an area of 61.57 ha in 2019 (Statistisches Bundesamt, 2019). Due to its high water content, the nutritional value of lettuce has been underestimated although cultivars with favorable nutrient content are known (Kim et al., 2016). Besides essential vitamins and minerals, lettuce also provides several phytochemicals with potential health-promoting effects such as flavonoids, carotenoids and polyunsaturated fatty acids (Kim et al., 2016). Importantly, these phytochemicals are associated with positive health effects such as a reduced risk of noncommunicable diseases like cancer, cardiovascular disease or age-related functional decline (Kim et al., 2016; Clifton et al., 2017; Eisenhauer et al., 2017; Milani et al., 2017; Kim et al., 2018; Rees et al., 2018; Kopustinskiene et al., 2020).

We hypothesized that the use of antifogging additives in polytunnels will affect nutritional quality of lettuce due to changes especially in the light regime. To test this hypothesis, we cultivated lettuce under polytunnels with and without antifogging additives. To determine the general impact of microclimate induced by polytunnels, we cultivated lettuce cover-free (without a polytunnel). Climatic conditions were monitored throughout the experiment. Valuable compounds such as flavonoids and phenolic acids, carotenoids and chlorophylls as well as fatty acids were determined by chromatographic methods coupled with mass spectrometry.

A step towards Sustainable Development Goal 2 "zero hunger"

Current food production systems undergo transformation in terms of productivity, resource use and environmental impacts. Greenhouses

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and polytunnels provide favorable growth conditions for vegetables and are thought to be a possible pathway towards sustainable production (ZHOU et al., 2021). Increased productivity can be achieved e.g. by off-season production and target for instance the target 2.1 'ensure access by all people to safe and nutritious food all year around' of the SDG 2 – "end hunger, achieve food security and improved nutrition and promote sustainable agriculture". In addition, crop losses could be reduced using, for example, antifogging additives by preventing crop spoilage. Finally, the selection of useful covering materials can contribute to the improvement of nutritional quality and thus to the achievement of the SDG 2 "zero hunger".

Material and methods

Plant cultivation, preparation and covering material

The experiment was conducted from 19th September to 28th October 2019 and repeated from 16th January to 28th February 2020. A glass-coated greenhouse was used for the experiments located at the Leibniz Institute of Vegetables and Ornamental crops (Großbeeren, 52°20'5N 13°18'35.3"E). The greenhouse temperature and the relative humidity was set to 22 °C and 70%, controlled by open vents. Additional artificial light (SON T AGRO 400 W, Phillips) was applied once the outer light intensity was lower than 50 klx for a maximum of 10 h per day. Eight polytunnels (58 × 50 × 50 cm, $L \times W \times H$, Supplemental Figure S1) were built for a sufficient number of experimental repetitions. The covers must be completely closed to generate high humidity conditions that the antifogging additives become active. A completely randomized setup was chosen to minimize the impact due to position of plants and polytunnels. The plants under the polytunnels were randomized twice a week, and the polytunnel position was randomized once halfway through the experiment. Commercially available three-layered polyethylene film (low-density/linear low-density polyethylene/14% ethylene butyl acrylate (middle layer), 180 µm thickness, CONSTAB polyolefin additives GmbH, Rüthen, Germany) was used as covering material. Half of the films contained a mixture of antifogging additives (Sabostat A 300 and Atmer 103, 0.35%) embedded in the plant-facing side. The other half was without additives. Transmission spectra were measured for both films using a V-670 photospectrometer (Jasco Deutschland GmbH, Pfungstadt, Germany). Seven plants were grown under each polytunnel or without being covered by a polytunnel, corresponding to a total of 28 plants per treatment (84 plants in total, Supplemental Figure S2). For the first experiment, two different cultivars were chosen ('Veronique' and 'Attractie'), corresponding to 16 repetitions for 'Veronique' and 12 repetitions for 'Attractie'. The cultivars were randomly placed under the polytunnels and in the trays. The second experiment was performed with cultivar 'Veronique' only.

Lettuce seeds were germinated in a climate chamber under the following conditions: 12 °C temperature, 75% relative humidity, 12/12 h day/night period and 350 µmol m^{-2} s^{-1} light intensity. Eleven (experiment 2019) and 18 (experiment 2020) days after sowing when the plants reached the two-leaf stage, they were transplanted in 13 cm pots with soil (the pH of the soil was 5.9, N was 183 mg L^{-1}, P_2O_5 was 135 mg L^{-1}, K_2O was 212 mg L^{-1}, and salinity was 1.23 g L^{-1}, Einheitserde classic, Einheitserde Werkverband e.V., Sinntal-Altengronau, Germany) and transferred into the experimental setup. The edible part of the plants was harvested after 38 to 43 days with a fresh weight of 11.4 ± 4.2 g. Half of each plant was taken as one sample. The samples were immediately frozen in liquid nitrogen, lyophilized and stored vacuum-packed at ambient temperature in the dark until further analysis. Before analysis, samples were ground to a fine powder with a mill (Retsch® MM 400, 45 sec, 2 repetitions at 25 1 s^{-1}).

Climatic condition measurements

During the experiments, temperature, relative humidity and photosynthetic active radiation (PAR) were monitored. For this purpose, two sensors (LI-190R Quantum Sensor, LI-COR Biosciences GmbH, Germany; sensor type KPC 1/5, PT - 100 type B sensor, Galltec Mess- und Regeltechnik GmbH, Bondorf, Germany, MELA Sensortechnik GmbH Mohlsdorf-Teichwolframsdorf, Germany) were placed under two polytunnels of each treatment. To determine climatic conditions in the greenhouse chamber, an aspiration psychrometer (Type ELAU KlimaExpert, KE-PTFF-8024-OF, Elektro- und Automatisierungsanlagen Pierre Ambrozy, Gatersleben, Germany) was used. The greenhouses had PAR sensors on the roof (PAR-Quantumsensor DK-PHAR 2, deka Sensor u. Technologie, Entwicklungs u. Vertriebs GmbH, Teltow, Germany), which were used to determine the value inside. To determine the transmittance of the greenhouse roof and thus calculate the light intensity in the chamber, a light meter (Model LI-250 Light Meter, LI-COR Biosciences GmbH, Germany) was used. The measurements indicated a 50% reduction of light intensity (PAR) through the glass roof.

Analysis of flavonoid glycosides and caffeic acid derivatives by HPLC-DAD-MS/MS

The analysis was performed according to NEUGART et al. (2019). Freeze-dried and powdered samples (10 mg) were extracted with methanol/water (3:2, v/v) and analysed via HPLC-DAD-ESI-MS/MS using a series 1260 Infinity II HPLC chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with an Ascentis® Express F5 column (150 mm × 4.6 mm, 5 µm, Supelco, Sigma Aldrich Chemical Co., St Louis, MO, USA), a degaser, binary pump, autosampler, column oven and a photodiode array detector (DAD). Compounds were detected in negative polarity with a Bruker amazon SL ion trap mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). The tentative identification of the compounds was based on the comparison of absorption maxima, mass spectra and fragmentation pattern in MS^3 with reference compounds (when available) or with literature data (Supplemental table S1). External calibration with standards (PhytoLab GmbH & Co. KG, Vestenbergsgreuth, Germany) was used to quantify flavonoid glycosides at wavelength 370 nm and caffeic acid derivatives at 330 nm.

Analysis of carotenoids and chlorophylls by UHPLC-DAD-ToF-MS

For the analysis, 5 mg of freeze-dried and powdered lettuce material were extracted with tetrahydrofuran/methanol (1:1, v/v), as previously described by FREDE et al. (2018) with some modifications. Analysis was performed via UHPLC-DAD-ToF-MS using an Agilent Technologies 1290 Infinity UHPLC with separation on a C30 column (YMC Co. Ltd, Kyoto, Japan, YMC C30, 100 × 2.1 mm, 3 µm). Compounds were detected with a multimode ion source in positive polarity with an Agilent Technologies 6230 ToF LC/MS. The gas temperature was set to 300 °C with a flow rate of 8 L min^{-1}, whereas the vaporizer was set to 200 °C using a nebulizer pressure of 35 psig. The voltage was set to 3500 V and a fragmentor voltage was set to 175 V, with corona current application of 4.0 µA. The (tentative) identification of the compounds was based on the comparison of retention time, absorption maxima and mass spectra with standards or with the literature (Supplemental table S2). External calibration with chlorophyll and carotenoid standards (Sigma-Aldrich, St Louis, MO, USA; CaroteNature GmbH, Münsingen Switzerland) was used for quantification at detection wavelength 450 nm.

Analysis of fatty acids as fatty acid methyl esters by GC-MS

Fatty acids were extracted and derivatized to methyl esters using a modified method by BROWSE et al. (1986). Fifteen mg of freeze-dried

and powdered material was mixed with 1 mL methanolic-hydrochloric acid reagent (3 M HCl/methanol 1:2 v/v, added with 5% 2,2-dimethoxypropane, Merck KGaA, Darmstadt, Germany). As an internal standard, 500 µL heptadecanoic acid (0.2 mg/mL, Merck KGaA, Darmstadt, Germany) was added. For the extraction and derivatization procedure, samples were shaken for 60 min at 80 °C under nitrogen atmosphere to protect unsaturated fatty acids. After samples cooled to room temperature, 750 µL hexane and 1000 µL saturated sodium chloride solution were used to extract fatty acid methyl esters in the upper phase. Samples were centrifuged for 5 min at 2560 *g* at 20 °C. A total of 500 µL of the upper hexane phase was filtered with sodium sulphate (anhydrous). Samples were immediately analyzed with GC-MS using an Agilent 6890 GC equipped with a J&W DB-23 GC Column (Agilent Technologies Germany GmbH & Co. KG, Waldbronn, Germany, 30 m × 0.25 mm × 0.25 µm). Samples were injected splitless at an injector temperature of 230 °C. Helium carrier gas had an initial flow of 1.2 mL min^{-1}. The following temperature program was used for elution: 80 °C for 2 min, 80 °C to 120 °C with 5 °C min^{-1}, 120 °C to 220 °C with 2 °C min^{-1}, held at 220 °C for 5 min. Compounds were detected with an Agilent 5973 mass selective detector. The source temperature was set to 230 °C, the quadrupole temperature was set to 150 °C and the voltage was set to 953 V. Analysis was performed in scan mode using a mass range between *m/z* 90 to 400. Fatty acids were identified as their methyl esters by comparing retention time and mass spectra with those of standards (Merck KGaA, Darmstadt, Germany, Supplemental table S3). For quantification the internal standard was used and response factors of the fatty acids of interest were determined.

Statistical analysis

The statistical analysis was performed using SigmaPlot 14 (Systat Software GmbH, Erkrath, Germany). Differences in the treatments were analyzed with a one-way ANOVA followed by Tukey's HSD *post hoc* test assuming normal distribution. In the case of non-normally distributed data, a Kruskal-Wallis test was applied. A *p*-value of $p \leq 0.05$ was considered a significant difference. Data are represented as mean ± standard error.

Results

Measurement of climatic conditions

During both experiments, the climatic conditions of temperature, relative humidity and PAR were monitored in the greenhouse chamber and under the polytunnels (Tab. 1). In 2019, the average temperature was 4.47 °C higher than in the 2020 experiment, regardless of cultivation condition. A temperature difference of 1.1 was found when comparing the temperature in the greenhouse with the temperature under the polytunnels. Between the polytunnels (with and without antifogging additives), no differences could be measured. A 1.6-fold higher relative humidity was measured under polytunnels compared with the greenhouse chamber in both experiments. No difference was detected for both polytunnels with or without additives. The measured PAR was similar in both experiments. Therefore, it is assumed that additional artificial light was able to compensate possible differences between the two experiments. Lettuce cultivated cover-free (without a polytunnel) in the greenhouse chamber were exposed to a 1.9-fold higher light intensity followed by lettuce grown under polytunnel with additives (1.2-fold), both compared to lettuce grown under additive-free polytunnels.

Determination of the lettuce fresh weight

The fresh weight of each lettuce (edible part, including leaf and stem) was determined directly after the harvest (Fig. 1). In 2019, the fresh weight of polytunnel-grown lettuce was significantly higher compared to lettuce grown without a polytunnel for both cultivars 'Attractie' (1.4-fold) and 'Veronique' (1.9-fold). Incorporated antifogging additives did not lead to differences in the fresh weight of both cultivars. However, there was a significant difference (1.2-fold) in fresh weight of lettuce grown under polytunnels with and without antifogging additives, while there is no difference comparing cover-free grown lettuce and lettuce grown under additive-containing polytunnels in 2020. For the experiment conducted in 2020, the overall fresh weight of the lettuce was 2.0-times lower compared with the experiment in 2019.

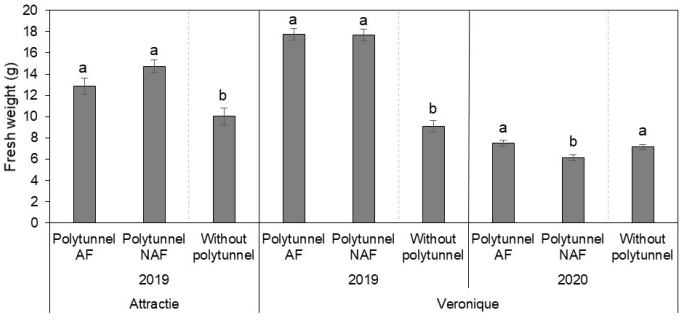

Fig. 1: Lettuce fresh weight (g) grown under polytunnel with (AF) and without antifogging additives (NAF) and without polytunnel. The fresh weight is expressed as mean ± SE (n = 4). Significant differences ($p \leq 0.05$) for each experiment and cultivar are indicated by different letters.

Flavonoid glycosides and caffeic acid derivatives

In both cultivars, three flavonoid glycosides and five caffeic acid derivatives were tentatively identified (Supplemental table S1) and quantified (Fig. 2 and 3). Quercetin and luteolin flavonoids, both conjugated with glucuronide moieties and a quercetin glucoside bound with a malonylic acid moiety were found in lettuce. Notably, the individual flavonoid glycosides as well as total flavonoid glycosides had

Tab. 1: Monitored climatic conditions in the greenhouse chamber (without polytunnel), under polytunnels with (AF) and without antifogging additives (NAF). Temperature (°C) and relative humidity (%) are expressed as daily averages ± SD. The light intensity (photosynthetic active radiation PAR) is expressed as averaged daytime ± SD (6 AM to 6 PM, µmol m^{-2} s^{-1}) and daily light integral ± SD (mmol m^{-2} s^{-1}). Calculated values are marked by †.

	2019			2020		
	Polytunnel AF	Polytunnel NAF	Without polytunnel	Polytunnel AF	Polytunnel NAF	Without polytunnel
Temperature	22.93 ± 1.44	22.96 ± 1.38	20.82 ± 0.67	18.56 ± 1.04	18.27 ± 1.03	16.60 ± 0.56
Relative humidity	94.12 ± 4.55	95.46 ± 2.45	61.15 ± 6.05	95.76 ± 3.71	96.21 ± 3.58	60.62 ± 4.81
Averaged photosynthetic active radiation (PAR)	87.96 ± 32.25	68.61 ± 23.56	131.74 ± 45.50†	84.14 ± 32.00	70.34 ± 24.20	127.80 ± 35.72†
Daily light integral (DLI)	13.60 ± 6.12	10.69 ± 4.15	15.93 ± 5.46†	12.58 ± 5.52	10.48 ± 3.83	13.24 ± 4.45†

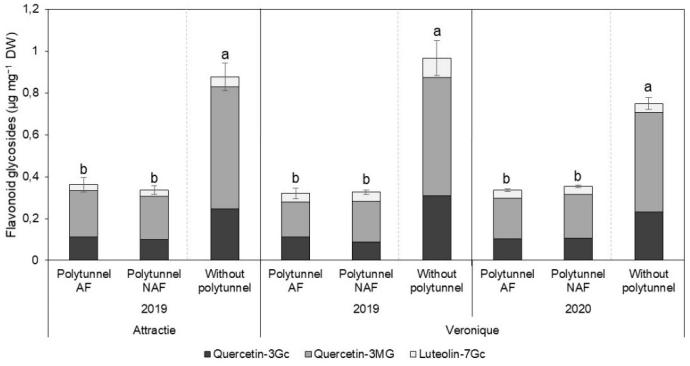

Fig. 2: Content of flavonoid glycosides (µg mg^{-1} DW) in lettuce grown under polytunnel with (AF) and without antifogging additives (NAF) and without polytunnel. The total flavonoid glycoside content is expressed as mean ± SE (n = 4). Significant differences ($p \leq 0.05$) of total flavonoid glycosides for each experiment and cultivar are indicated by different letters. Abbreviations, Gc: glucuronide, MG: malonyl glucoside.

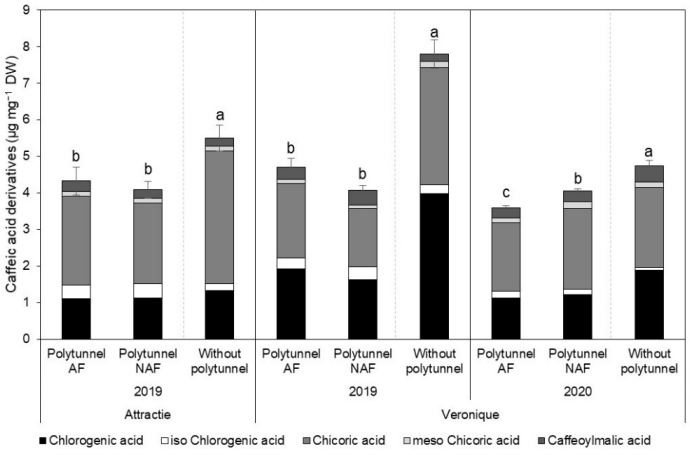

Fig. 3: Content of caffeic acid derivatives (µg mg^{-1} DW) in lettuce grown under polytunnel with (AF) and without antifogging additives (NAF) and without polytunnel. The total caffeic acid content is expressed as mean ± SE (n = 4). Significant differences ($p \leq 0.05$) of total caffeic acids for each experiment and cultivar are indicated by different letters.

a 2.4-fold higher content in the lettuce grown cover-free compared with the lettuce grown under polytunnels, regardless of the content of antifogging additives. However, no significant differences were detected for flavonoid glycosides in lettuce grown under polytunnels with antifogging additives compared with the additive-free polytunnels. This pattern was found in both experiments conducted in 2019 and 2020.

In both cultivars, the two main caffeic acid derivatives, chlorogenic acid and chicoric acid, were tentatively identified (Supplemental table S1). They also contained three derivatives namely *iso*-chlorogenic acid, *meso* chicoric acid and caffeoylmalic acid – albeit in minor amounts. Total caffeic acid content was highest for cover-free lettuce (1.4 fold compared to polytunnel-grown lettuce), whereas no significant differences were observed for lettuce grown under polytunnels with additives compared to without additives in 2019. In contrast, for the 2020 experiment a significant 1.1-fold higher content was found in lettuce under additive-free polytunnels compared those with antifogging (Fig. 3). However, a closer look at the content of individual caffeic acid derivatives revealed some differences. In detail, the chlorogenic acid content in cultivar 'Veronique' was 1.9-fold higher in cover-free lettuce, but in cultivar 'Attractie' no differences were observed. There were also no significant differences between both polytunnel cultivation conditions. Moreover, in both cultivars grown in 2019, the chicoric acid content in the cover-free lettuce was also 1.6-fold higher compared to the lettuce grown under polytunnels. In cultivar 'Veronique' grown under polytunnels with additives, a significant 1.3-fold higher content was detected compared to those grown additive-free. The lettuce grown in 2020 also showed a significant difference for both polytunnel cultivation conditions. What is remarkable, is the high content of chicoric acid in lettuce grown under polytunnels without additives, which was comparable to the cover-free grown lettuce. The minor-content caffeic acids showed predominantly lower contents in the cover-free lettuce compared to polytunnel cultivation. Finally, significant differences were detected for both polytunnel cultivation conditions (1.2-fold) for all minor-content caffeic acids in lettuce in the 2020, but not the 2019 experiment.

Carotenoids and chlorophylls

The analysis revealed chlorophyll a, chlorophyll b and lutein as the main pigments in both cultivars. The lettuces also contained the xanthophylls neoxanthin as well as zeaxanthin in small amounts. Beta-carotene and a lettuce-specific carotenoid lactucaxanthin were also identified (Supplemental table S2, Fig. 4 and 5). A significantly lower amount of total carotenoids occurred in cover-free grown lettuce of 'Veronique' compared with lettuce grown under additive-free polytunnels for both experiments in 2019 (1.4-fold) and 2020 (1.1-fold). In 2019, this is also reflected in the individual carotenoids neoxanthin (1.7-fold), lactucaxanthin (1.5-fold), lutein (1.3-fold) and β-carotene (1.3-fold) as well as the chlorophylls (1.5-fold). In the 2020 experiment, for the individual carotenoids lutein (1.2-fold), neoxanthin (1.1-fold) and chlorophyll b (1.2-fold) such differences were detected. Moreover, in the 2020 experiment, zeaxanthin and

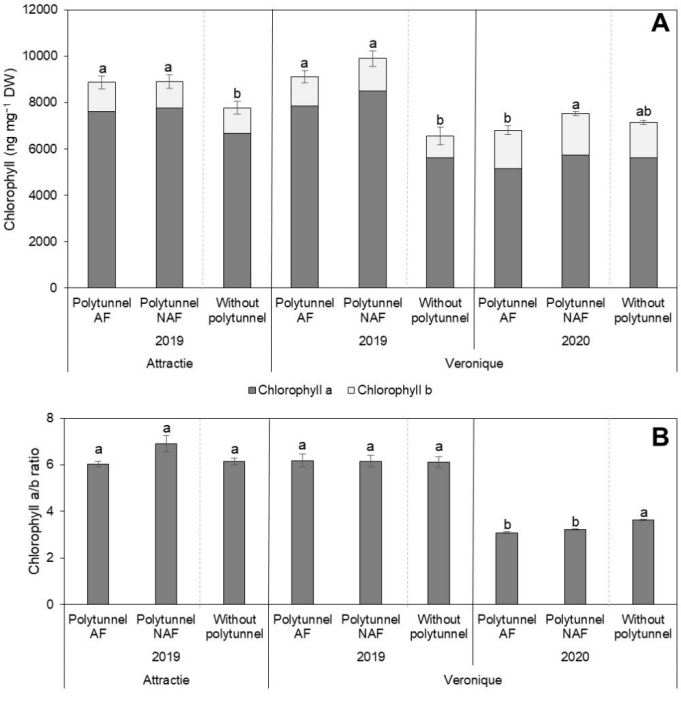

Fig. 4: (A) Chlorophyll content (ng mg^{-1} DW) and (B) chlorophyll a/b ratio of lettuce grown without polytunnel and lettuce grown under polytunnel with (AF) and without antifogging additives (NAF). Ratios and total chlorophylls are expressed as mean ± SE (n = 4). Different letters indicate significant differences ($p \leq 0.05$) of total chlorophyll content and chlorophyll a/b ratios for each experiment and cultivar.

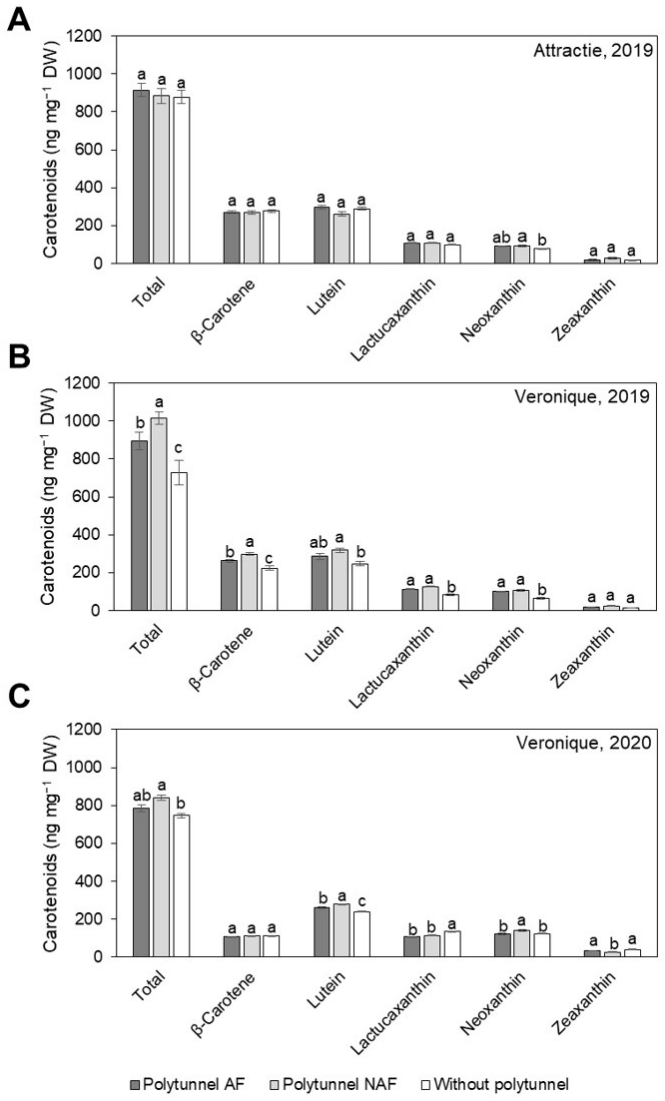

Fig. 5: Carotenoid content (ng mg^{-1} DW) of cultivar 'Attractie' (A) and 'Veronique' (B) from the 2019 experiment and 'Veronique' from the 2020 experiment (C) grown under polytunnel with (AF) and without antifogging additives (NAF) and without polytunnel. Values show means ± SE (n = 4). Different letters indicate significant differences ($p \leq 0.05$) of individual carotenoids for each experiment and cultivar.

lactucaxanthin were significantly higher in cover-free lettuce than in the additive-free polytunnel grown lettuce by 1.5-fold and 1.2-fold, respectively. Here, the β-carotene content was not affected at all. When comparing total carotenoids in cultivar 'Veronique' for both polytunnel treatments, a significant 1.1-fold higher amount was observed in additive-free polytunnel grown lettuce, only in the 2019 experiment. Additionally, a significant effect due to the use of additives in the polytunnels occurred for β-carotene (1.1-fold) in 2019 and for zeaxanthin (1.3-fold), lutein (1.1-fold), neoxanthin (1.1-fold) and chlorophyll a (1.1-fold) in 2020. In general, the use of antifogging additives in polytunnels leads to lower carotenoid contents in the cultivar 'Veronique' compared to lettuce grown under additive-free polytunnels.

For cultivar 'Attractie', some differences to 'Veronique' were found. At first, no significant differences were observed for total and individual carotenoids, except neoxanthin. Cover-free grown lettuce had a 1.2-fold significantly lower neoxanthin and chlorophyll a content compared to lettuce grown under additive-free polytunnels. No effect due to the use of additives were detected for both pigments. No differences in chlorophyll b content between cover-free lettuce and additive-free polytunnel grown lettuce were observed. However, lettuce grown under polytunnels with additives showed a significant 1.1-fold higher chlorophyll b content compared with both. Thus, the differences between cultivars indicate a cultivar-specific effect, both through the use of polytunnels in general and antifogging additives in particular.

Fatty acids

Palmitic acid followed by linolenic acid and linoleic acid were the main fatty acids determined in lettuce extracts. Furthermore, palmitoleic, stearic and oleic acid were identified in both cultivars (Supplemental table S3). Although there are few differences in total fatty acid content for all cultivation conditions, closer examination revealed some differences (Tab. 2). In cultivar 'Veronique', the amounts of total fatty acids were 2.9-fold higher in 2020 than in 2019. Lower palmitic acid content was observed in cover-free grown lettuce compared with both polytunnel treatments for cultivar 'Veronique' (1.2-fold) and 'Attractie' (1.1-fold). In addition, in 'Veronique', cover-free grown lettuces had significantly lower amounts of stearic acid (1.1-fold) and oleic acid (3.0-fold) in the 2019 experiment and linoleic acid (1.4-fold) in the 2020 experiment compared to polytunnel cultivation. Nevertheless, most individual fatty acids were unaffected and were present in similar amounts, regardless of cultivation conditions. No effect was detected for usage of antifogging additives.

Tab. 2: Composition of saturated and unsaturated fatty acids (µg mg^{-1} DW) in lettuce grown without a polytunnel and lettuce grown under polytunnels with (AF) and without antifogging additives (NAF). Values shows means ± SE (n = 4). Different letters indicate significant differences ($p \leq 0.05$) for each experiment and cultivar.

	Attractie 2019			Veronique 2019			Veronique 2020		
	Polytunnel AF	Polytunnel NAF	Without polytunnel	Polytunnel AF	Polytunnel NAF	Without polytunnel	Polytunnel AF	Polytunnel NAF	Without polytunnel
Total	21.15 ± 0.65^{a}	22.40 ± 1.52^{a}	21.16 ± 1.01^{a}	18.46 ± 1.36^{ab}	22.47 ± 1.37^{a}	18.03 ± 1.07^{b}	60.75 ± 3.43^{a}	59.05 ± 3.55^{a}	49.80 ± 4.69a
Saturated									
Palmitic acid	12.15 ± 0.13^{a}	11.99 ± 0.30^{a}	10.72 ± 0.19^{b}	11.90 ± 0.22^{a}	12.24 ± 0.22^{a}	9.66 ± 0.27^{b}	11.50 ± 0.16^{a}	11.59 ± 0.03^{a}	10.72 ± 0.08^{b}
Stearic acid	0.87 ± 0.03^{a}	0.85 ± 0.08^{a}	0.91 ± 0.02^{a}	0.83 ± 0.04^{a}	0.89 ± 0.03^{a}	0.76 ± 0.02^{b}	0.87 ± 0.29^{a}	0.57 ± 0.01^{a}	0.60 ± 0.04^{a}
Unsaturated									
Palmitoleic acid	1.03 ± 0.04^{a}	0.98 ± 0.06^{a}	1.04 ± 0.04^{a}	0.95 ± 0.04^{a}	1.10 ± 0.04^{a}	0.89 ± 0.05^{a}	1.64 ± 0.17^{a}	1.49 ± 0.01^{a}	1.46 ± 0.03^{a}
Oleic acid	0.32 ± 0.04^{a}	0.35 ± 0.06^{a}	0.28 ± 0.04^{a}	0.20 ± 0.03^{ab}	0.38 ± 0.05^{a}	0.10 ± 0.03^{b}	1.35 ± 0.22^{a}	1.06 ± 0.08^{a}	0.86 ± 0.11^{a}
Linoleic acid	3.04 ± 0.24^{a}	3.68 ± 0.53^{a}	3.15 ± 0.32^{a}	2.55 ± 0.18^{a}	3.35 ± 0.42^{a}	2.22 ± 0.31^{a}	15.57 ± 1.11^{a}	15.05 ± 1.10^{a}	11.03 ± 1.26^{b}
Linolenic acid	3.16 ± 0.32^{a}	4.08 ± 0.67^{a}	4.02 ± 0.47^{a}	2.70 ± 0.24^{a}	3.99 ± 0.65^{a}	2.68 ± 0.46^{a}	28.36 ± 2.40^{a}	28.01 ± 2.38^{a}	23.66 ± 3.18^{a}

Discussion

Experimental setup and general restrictions of the study

Several studies indicate an impact of greenhouse covering materials on plant yield, however, few have focused on nutritionally important metabolites. Papadopoulos et al. (1997) showed differences in marketable tomato yields among three greenhouse covering materials, namely D-poly, acrylic and glass. Furthermore, Petropoulos et al. (2019) investigated the impact of three different polyethylene greenhouse covering materials with differently structured layers on tomato fruit yield and quality. They found that yield and valuable compounds such as tocopherols, carotenoids and chlorophylls were affected by different cultivation conditions. A difference of the polytunnel films with and without incorporated antifogging additives was only observed for the experiment in 2020, but not in 2019. Polytunnel cultivation generally resulted in significantly higher fresh weights of the lettuce plants in the 2019 experiment compared to cover-free grown lettuce, while no differences in fresh weight of lettuce grown under polytunnel with antifogging additives compared to cover-free grown lettuce were observed in the 2020 experiment. This is probably more an effect of the temperature difference of 4.47 °C between the 2019 and 2020 experiments and would reflect the overall 2.0-fold higher fresh weight of lettuce in the 2019 experiments compared to 2020.

However, it is difficult to reconcile the dimensions of a greenhouse with the necessary number of replicate greenhouses to generate statistically significant results. In this context, Petropoulos et al. (2019) used three separate greenhouses covered with three different materials albeit for one repetition. In contrast, in this study, we used multiple polytunnels due to their reduced size (58 × 50 × 50 cm, $L \times W \times H$). Cemek et al. (2006) highlighted the problem of greenhouse size with repetitions. To overcome it, they built greenhouses in smaller dimensions (9 × 3 × 2.5 m, $L \times W \times H$) with two replicates per treatment. Moreover, Papadopoulos et al. (1997) reduced the greenhouses size to 6.2 × 7.2 × 3 m ($L \times W \times H$), in order to ensure three replicates per covering material. However, not only the number of repetitions, but also the placement of plants could cause bias.

To overcome this experimental challenge, we used polytunnels. Lettuce under the polytunnel were randomized regularly and the polytunnels themselves were randomized halfway through the experiment to prevent spatial influences. Cemek et al. (2006) and Petropoulos et al. (2019) pointed out that as a solution they used a randomized complete block experimental design to ensure reproducibility of subsequent experiments.

It must be noted that in this study, the selected polytunnel sizes might have had an impact on the microclimate since the temperature inside the polytunnels was 1.1-fold higher compared with the greenhouse chamber in both experiments. These differences, however, are comparable with the results of Cemek et al. (2006). In their study, the temperatures varied from 15.9 °C (outside greenhouses) to 21.3 °C (D-Poly greenhouse). Hao et al. (1999) did not observe significant differences for the temperature inside greenhouses with different covering materials (D-Poly, acrylic and glass). The optimal temperature for lettuce cultivation is between 16 °C and 18 °C (Sanders, 2019). This temperature range corresponds to the conditions in the 2020 experiment. In contrast, in 2019, temperatures were about 4 °C above this optimum, which was not due to the polytunnel microclimate but rather due to the general climate in that month. However, high relative humidity was monitored under the polytunnels compared with the greenhouses used in the study by Cemek et al. (2006). Therefore, lettuce was selected for this study because it is a crop with high water demand (Sanders, 2019). Nevertheless, some caution should be exercised in interpreting the results since the microclimate and greenhouse conditions may also affect the plant response.

Fadel et al. (2016) highlights temperature and light as the most important greenhouse controlled environmental factors. Plants respond to these changing environments by adapting their metabolite profiles. Of note is that such a response could result in altered nutritional value of plants grown in greenhouses or polytunnels. Temperature varied in both experiments, light intensity was similar.

Flavonoid glycosides and caffeic acid derivatives

In this study, flavonoid glycosides as well as main caffeic acid derivatives showed the highest content in cover-free grown lettuce. No significant differences were observed for flavonoid glycosides in lettuce ('Attractie' and 'Veronique') grown under polytunnels with and without additives in both experiments and main caffeic acid derivatives in the 2019 experiment. However, a significant difference was detected for most caffeic acid derivatives in the 2020 experiment. Flavonoids provide several health-promoting effects for humans. As free-radical scavenging antioxidants, they have been associated with a lower risk of cardiovascular disease, various types of cancer and in addition, they tend to have anti-inflammatory and immunomodulatory properties (Rees et al., 2018, Kopustinskiene et al., 2020). This study demonstrates a negative effect on the content of these phenolic compounds in lettuce by using polytunnels, independent of whether antifogging additives were used.

Ahmadi et al. (2019) found that only individual but not total flavonoids and phenolic acids in tomato fruits varied due to growth under different polyethylene-covered greenhouses. In contrast to the present results, where no differences were observed between the two cultivars, they showed cultivar-specific differences. In agreement with our study, lettuces grown in greenhouses showed lower levels of flavonoids compared to open-field conditions (Romania et al., 2002). Moreover, the use of UV-blocking covering materials for greenhouses had a negative effect on phenolic compounds in leafy vegetables, including rocket and lettuce (Katsoulas et al., 2020). In this study, UV-light transmission was partly reduced by the polyethylene films with and without antifogging additives (Supplemental Figure S3), whereas the cover-free lettuce was grown in a UV-transmissible greenhouse chamber, which resulted in the highest contents of flavonoid glycosides and caffeic acid derivatives.

Becker et al. (2013) observed significantly higher levels of flavonoid glycosides in lettuce treated with higher light (410 µmol m^{-2} s^{-1}) compared with lower light intensities (225 µmol m^{-2} s^{-1}). Although the light intensities were slightly higher in polytunnels with antifogging additives compared to polytunnels without additives, no significant differences were observed for flavonoid glycosides and few differences were observed for some caffeic acid derivatives in both cultivars in the 2019 experiment. This observation might be due to the differences in the light regime being too small to cause significant effects. Interestingly in the study of Becker et al. (2013), the caffeic acid derivatives were not affected at all, which is in contrast to the findings of the 2020 experiment. However, chlorogenic acid as well as *meso* chicoric acid content in lettuce 'Attractie' was not altered due to polytunnel cultivation. The high content of minor caffeic acid derivatives in lettuce grown under polytunnels without additives in general and the high amount of chicoric acid in lettuce grown under polytunnels in particular cannot be currently explained. However, it should be borne in mind that an increase in temperature from 25 °C to 33 °C can cause higher flavonoid accumulation in lettuce (Sublett et al., 2018). Thus, a possible reason for the previous observation would be the different temperature regime.

Carotenoids and chlorophyll

While carotenoids are largely unaffected by polytunnel cultivation with and without antifogging additives in the cultivar 'Attractie', differences are evident in 'Veronique'. This suggests a cultivar-specific response to different cultivation conditions, which Ahmadi et al. (2019) also found for greenhouse-grown tomato fruits covered with

different polyethylene materials. In the same study, they found that lycopene, but not lutein or total carotenoids were affected by different covering materials. This is in contrast with this study, where individual and total carotenoids differ due to the use of antifogging additives in polytunnels. Finally, PETROPOULOS et al. (2019) showed a possible impact of polyethylene covering materials on carotenoids and also chlorophyll degradation associated with tomato fruit ripening.

Light is an important factor for biosynthesis of carotenoids and chlorophylls as they are photosynthetic pigments. Light regime differs when comparing open-field conditions and greenhouses and greenhouse covering materials can also affect light transmission and light quality. For example, OHASHI-KANEKO et al. (2007) showed that different light qualities using colored LEDs resulted in altered levels of carotenoids and chlorophylls in spinach and lettuce. COZZOLINO et al. (2020) compared how clear and diffuse greenhouse films affect valuable compounds in lamb's lettuce and observed no significant differences for carotenoids and chlorophylls. However, it is known that light intensity can influence the content of photosynthetic pigments in plants. For example, KOSMA et al. (2013) detected a positive correlation between total chlorophyll content and reduced PAR intensities (26, 47 and 73% of incident light intensity) in hydroponically cultivated lettuce. In the present study, differences in light intensities (PAR) were detected for all three cultivation conditions. In agreement with the aforementioned studies, chlorophyll contents were higher in lettuce grown under polytunnels compared to cover-free grown lettuce. Furthermore, significantly higher chlorophyll a and b contents of lettuce grown under additive-free compared to additive-containing polytunnels were observed in the 2020 experiment. In their review, SHAFIQ et al. (2021) showed that the behavior of plants in terms of chlorophyll content seems to be different under low light conditions (shade), while some studies showed lower chlorophyll contents in shade-grown plants, some also found the opposite. Therefore, SHAFIQ et al. (2021) hypothesized that chlorophyll content tends to increase in shade tolerant cultivars in response to enhanced light harvesting. In fact, comparable photosynthetic rates in lettuce grown under polytunnels with and without additives compared to cover-free grown lettuce were observed for the 2020 experiment (Supplemental Figure S4). Presumably, the adaptation of the photosynthetic pigments in the lettuce led to an efficient light harvesting and did compensate the lower light intensities under the polytunnels. In addition, a significantly higher photosynthesis rate was measured in lettuce grown under polytunnels with additives than in additive-free polytunnels, which is probably also related to the different light intensity.

The influence of different light intensities, ranging from 125 to 620 µmol m^{-2} s^{-1}, on major carotenoids (β-carotene and lutein) and chlorophylls was examined by LEFSRUD et al. (2006) in kale and spinach. The highest pigment contents tended to be found at 335 µmol m^{-2} s^{-1} in kale and at 200 µmol m^{-2} s^{-1} in spinach. SONG et al. (2020) treated lettuce with different light intensities (150 to 450 µmol m^{-2} s^{-1}) and nutrient solution concentrations. Comparing the carotenoids in lettuce at different light intensities and same nutrient solution treatments (1/4 and 3/4 nutrient solution level), no significant differences for carotenoid contents between these treatments were found, which is in line with our findings for 'Attractie'. Interestingly, the chlorophyll a/b ratio of their treated lettuce was highest under higher light intensities (at same nutrient solution levels). This is consistent with the results of the 2020 experiment, but not with 2019, where no differences in chlorophyll ratios were determined. It should be noted that the 2019 and 2020 experiments were conducted in different months of the years and some differences in the content of photosynthetic pigments were observed. This could possibly be due to the different spectral qualities of the light in these months. In detail, the plant photosystems PSI and PSII exhibit different absorption maxima due to their carotenoid and chlorophyll compositions, resulting in differing responses depending on the light quality (CAFFARRI et al., 2014). In addition, not only the light quality but also the light quantity may stimulate the two photosystems differently. This could also have led to a different adaptation of the photosystems during the experiments (BALLOTTARI et al., 2007). Thus, the altered chlorophyll a/b ratio in 2019 compared to 2020 might be an indication of this altered adaptation of the photosystems.

However, not only light, but also other factors can impact the photosynthetic pigments in lettuce grown under polytunnels. For example, temperature can potentially affect the adaptation of the photosystems (BALLOTTARI et al., 2007). In this context, the 4.47 °C temperature difference in the 2019 and 2020 experiments is remarkable. LEFSRUD et al. (2005) cultivated kale and spinach at different air temperatures (from 10 to 30 °C) and showed that β-carotene, lutein and chlorophyll contents for both vegetables tended to be the highest at 30 °C, when calculated on a dry weight basis. This observation could also explain the differences in carotenoid and chlorophyll content comparing the 2019 and 2020 experiments. In particular, carotenoids showed the highest levels in polytunnel-grown lettuce in 2019, while these differences were observed only for a few carotenoids in the 2020 experiment. This might be a result of the 4.47 °C higher temperatures in 2019 than in 2020. The changing spectrum of sunlight and photoperiod in the different months of the experiments could also have an influence on the carotenoids. The changes in zeaxanthin indicate a temperature-dependent difference in accumulation in both experiments. Zeaxanthin protects plant membranes against reactive oxygen species under high light and high temperature conditions (DAVISON et al., 2002). Under lower light conditions, zeaxanthin decreases and converts to violaxanthin, as part of the violaxanthin-zeaxanthin cycle (JAHNS et al., 2009). This is consistent with the observations in both experiments. While in 2019, zeaxanthin tended to have higher amounts in lettuce under polytunnels, the opposite was found in the 2020 experiment. There were significantly lower amounts of zeaxanthin in lettuce grown under polytunnels compared to cover-free grown lettuce. It therefore appears that the effect of greenhouse covering materials on the plants grown below is a complex interaction of various environmental factors to which the plant adapts, presumably to optimize the photosynthetic process under the given environmental conditions. Such adaptations also affect the nutritional quality of cultivated vegetables, which has implications on human health since carotenoids and chlorophylls have potential health-promoting effects. Carotenoids and chlorophylls as well as derivatives exert antioxidant activities that have been associated with a reduced risk of cardiovascular disease and cancer, as well as eye disease (FERRUZZI et al. 2007; MILANI et al., 2017). Especially, the carotenoids lutein and zeaxanthin have shown preventive effects against age-related macular degeneration, while other carotenoids can act as provitamin A (EISENHAUER et al., 2017; MILANI et al., 2017). As shown in this study, the use of polytunnels (protected cultivation) revealed an accumulation on these compounds in lettuce.

Fatty acids

In general, it can be seen that polytunnel cultivation and the incorporation of antifogging additives have a negligible effect on the fatty acid profiles of lettuce. The differences found for fatty acids in lettuce when comparing growing conditions might be caused by the higher temperatures generated under the polytunnels. FALCONE et al. (2004) investigated changes in membrane fatty acid profiles of *Arabidopsis thaliana* due to elevated temperatures (from 17 to 36 °C) and found increased levels of some unsaturated fatty acids (oleic and linoleic acids) and saturated palmitic acid, a finding that is consistent with the present study. In contrast, they also showed decreased levels of linolenic acid, which was not observed in this study. Based on their

finding they hypothesized that plant membranes might require certain levels of distinct fatty acids for photosynthetic thermostability and acclimation. Petropoulos et al. (2019) found some variations in the polyunsaturated/saturated fatty acid ratios of tomatoes grown under different polyethylene cover materials. They emphasized the good nutritional value of the polyunsaturated fatty acids present in tomatoes. Kim et al. (2016) studied the nutritional value of different lettuce cultivars and detected the essential fatty acids linolenic and linoleic acid as the main fatty acids in both cultivars. Notably, cultivars 'Veronique' and 'Attractie' also contained both essential fatty acids in predominant amounts.

Conclusion

The impact of antifogging additives from greenhouse covering materials and polytunnel cultivation (protected cultivation) on valuable phytochemicals in lettuce was investigated in this study. Both, the polytunnel cultivation and the additives can alter the phytochemical content. This is due to a complex interaction of different environmental conditions, especially light and temperature. Since, antifogging additives slightly alter the light transmission through the polytunnels compared to those without additives, differences were presumably only detected for pigments related to photosynthesis. Nevertheless, the highest levels of these phytochemicals were detected under polytunnels without additives. A negative effect on flavonol glycosides as well as main caffeic acid derivatives was shown by the utilization of polytunnels, probably due to the shielding effect of such films. However, the use of antifogging additives did not cause any changes in these compounds. Antifogging additives are not only used to improve light transmission, but also to prevent plant damage and microbiological contamination by condensed water. In this study, the lettuce had a short growing period, and thus, such factors are of less importance within the experimental time. Even though the use of antifogging additives in greenhouse films did not have an overall positive impact on phytochemicals, they do protect crops with a longer growing period from spoiling. In addition, the effect of polytunnel cultivation and additive use on plant metabolite profiles was shown to be cultivar-specific. To conclude, with regard to the nutritional value of plants, the selection of a greenhouse covering material and the incorporation of useful additives could be a factor to improve the quality of horticultural crops and thus contributes to the implementation of SDG2 "zero hunger". However, as a limitation of this study remains the size of the polytunnels, future studies should therefore address non-model conditions.

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Conflict of interest

No potential conflict of interest was reported by the authors.

Note by the editor

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References

Ahmadi, L., Haob, X., Tsaoc, R., 2018: The effect of greenhouse covering materials on phytochemical composition and antioxidant capacity of tomato cultivars. J. Sci. Food Agric. 98, 4427-4435. DOI: 10.1002/jsfa.8965

Ballottari, M., Dall'Osto, L., Morosinotto, T., Bassi, R., 2007: Contrasting behavior of higher plant photosystem I and II antenna systems during acclimation. J. Biol. Chem. 282(12), 8947-8958. DOI: 10.1074/jbc.M606417200

Baudoin, W., Nersisyan, A., Shamilov, A., Hodder, A., Gutierrez, D., De Pascale, S., Nicola, S., 2017: Good agricultural practices for greenhouse vegetable production in the south east European countries. Principles for sustainable intensification of smallholder farms. Food and Agriculture Organization of the United Nations (FAO), Rome.

Becker, C., Kläring, H.P., Kroh, L.W., Krumbein, A., 2013: Temporary reduction of radiation does not permanently reduce flavonoid glycosides and phenolic acids in red lettuce. Plant Physiol. Biochem. 72, 154-160. DOI: 10.1016/j.plaphy.2013.05.006

Browse, J., Mccourt, P.J., Somerville, C.R., 1986: Fatty acid composition of leaf lipids determined after combined digestion and fatty acid methyl ester formation from fresh tissue. Anal. Biochem. 152, 141-145. DOI: 10.1016/0003-2697(86)90132-6

Caffarri, S., Tibiletti, T., Jennings, R.C., Santabarbara, S., 2014: A comparison between plant photosystem I and photosystem II architecture and functioning. Curr. Protein Pept. Sci. 15, 296-331. DOI: 10.2174/1389203715666140327102218

Cemek, B., Demir, Y., Uzun, S., Ceyhan, V., 2006: The effects of different greenhouse coveringmaterials on energy requirement, growth and yield of aubergine. Energy 31, 1780-1788. DOI: 10.1016/j.energy.2005.08.004

Clifton, P.M., Keogh, J.B., 2017: A systematic review of the effect of dietary saturated and polyunsaturated fat on heart disease, Nutr. Metab. Cardiovasc. Dis. 27(12), 1060-1080. DOI: 10.1016/j.numecd.2017.10.010

Cozzolino, E., Di Mola, I., Ottaiano, L., El-Nakhel, C., Mormile, P., Rouphael, Y., Mori, M., 2020: The potential of greenhouse diffusing cover material on yield and nutritive values of lamb's lettuce grown under diverse nitrogen regimes. Italus. Hortus. 27(1), 55-67. DOI: 10.26353/j.itahort/2020.1.5567

Davison, P.A., Hunter, C.N., Horton, P., 2002: Overexpression of β-carotene hydroxylase enhances stress tolerance in Arabidopsis. Nature 418, 11. DOI: 10.1038/nature00861

Eisenhauer, B., Natoli, S., Liew, G., Flood, V.M., 2017: Lutein and zeaxanthin-food sources, bioavailability and dietary variety in age-related macular degeneration protection. Nutrients 9(2), 120. DOI: 10.3390/nu9020120

Fadel, M.A., Hammad, B.A.B., Alhosany, F., Iwaimer, O., 2016: Greenhouses covering materials: A comparative study. Agr. Eng. Int. CIGR. J. 18, 48-57.

Falcone, D.L., Ogas, J.P., Somerville, C.R., 2004: Regulation of membrane fatty acid composition by temperature in mutants of Arabidopsis with alterations membrane lipid composition. BMC. Plant Bio. 4, 17. DOI: 10.1186/1471-2229-4-17

Ferruzzi, M.G., Blakeslee, J., 2007: Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. Nutr. Res. 27, 1-12. DOI: 10.1016/j.nutres.2006.12.003

Frede, K., Schreiner, M., Zrenner, R., Graefe, J., Baldermann, S., 2018: Carotenoid biosynthesis of pak choi (*Brassica rapa ssp. chinensis*) sprouts grown under different light emitting diodes during the diurnal course. Photochem. Photobiol. Sci. 17, 1289. DOI: 10.1039/C8PP00136G

Gruda, N., 2005: Impact of environmental factors on product quality of greenhouse vegetables for fresh consumption. Crit. Rev. Plant Sci. 24(3),

227-247. DOI: 10.1080/07352680591008628

Hao, X., Papadopoulos, A.P., 1999: Effects of supplemental lighting and cover materials on growth, photosynthesis, biomass partitioning, early yield and quality of greenhouse cucumber. Sci. Hortic. 80, 1-18. DOI: 10.1016/S0304-4238(98)00217-9

Hortidaily, 2019: World greenhouse vegetable statistics updated for 2019. Retrieved 22th January 2021, from https://www.hortidaily.com/article/9057219/world-greenhousevegetable-statistics-updated-for-2019/.

Irusta, L., González, A., Fernández-Berridi, M.J., Iruin, J.J., Asúa, J.M., Albizu, I., Ibarzabal, A., Salmerón, A., Espi, E., Fontecha, A., García, Y., Real, A.I., 2009: Migration of antifog additives in agricultural films of low-density polyethylene and ethylene-vinyl acetate copolymers. J. Appl. Polym. Sci. 111, 2299-2307. DOI: 10.1002/app.29280

Jahns, P., Latowski, D., Strzalka, K., 2009: Mechanism and regulation of the violaxanthin cycle: The role of antenna proteins and membrane lipids. Biochim. Biophys. Acta 1787, 3-14. DOI: 10.1016/j.bbabio.2008.09.013

Katsoulas, N., Bari, A., Papaioannou, C., 2020: Plant responses to UV blocking greenhouse covering materials: a review. Agronomy, 10(7), 1021. DOI: 10.3390/agronomy10071021

Kim, D.-E., Shang, X., Assefa, A.D., Keum, Y.-S., Saini, R.K., 2018: Metabolite profiling of green, green/red, and red lettuce cultivars: Variation in health beneficial compounds and antioxidant potential. Food Res. Int. 105, 361-370. DOI: 10.1016/j.foodres.2017.11.028

Kim, M.J., Moon, Y., Tou, J., Mou, B., Waterland, N.L., 2016: Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa L.*). J. Food Compos. Anal. 49, 19-34. DOI: 10.3390/foods_2020-07709

Kopustinskiene, D.M., Jakstas, V., Savickas, A., Bernatoniene, J., 2020: Flavonoids as anticancer agents, Nutrients 12(2), 457. DOI: 10.3390/nu12020457

Kosma, C., Triantafyllidis, V., Papasavvas, A., Salahas, G., Patakas, A., 2013: Yield and nutritional quality of greenhouse lettuce as affected by shading and cultivation season. Emir. J. Food Agric. 25(12), 974-979. DOI: 10.9755/ejfa.v25i12.16738

Lefsrud, M.G., Kopsell, D.A., Kopsell, D.E., Curran-Celentano, J., 2005: Air temperature affects biomass and carotenoid pigment accumulation in kale and spinach grown in a controlled environment. HortScience 40(7), 2026-2030. DOI: 10.21273/HORTSCI.40.7.2026

Lefsrud, M.G., Kopsell, D.A., Kopsell, D.E., Curran-Celentano, J., 2006: Irradiance levels affect growth parameters and carotenoid pigments in kale and spinach grown in a controlled environment. Physiol. Plant 127, 624-631. DOI: 10.1111/j.1399-3054.2006.00692.x

Milani, A., Basirnejad, M., Shahbazi, S., Bolhassani, A., 2017: Carotenoids: biochemistry, pharmacology and treatment. Br. J. Pharmacol. 174, 1290-1324. DOI: 10.1111/bph.13625

Neugart, S., Tobler, M.A., Barnes, P.W., 2019: Different irradiances of UV and PAR in the same ratios alter the flavonoid profiles of *Arabidopsis thaliana* wild types and UV-signalling pathway mutants. Photochem. Photobiol. Sci. 18, 1685. DOI: 10.1039/C8PP00496J

Ohashi-Kaneko, K., Takase, M., Kon, N., Fujiwara, K., Kurata, K., 2007: Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. Environ. Control Biol. 45(3), 189-198. DOI: 10.2525/ecb.45.189

Papadopoulos, A.P., Hao, X., 1997: Effects of three greenhouse cover materials on tomato growth, productivity, and energy use. Sci. Hort. 70, 165-178. DOI: 10.1016/S0304-4238(97)00054-X

Petropoulos, S.A., Fernandes, Â., Katsoulas, N., Barros, L., Ferreira, I.C.F.R., 2019: The effect of covering material on the yield, quality and chemical composition of greenhouse-grown tomato fruit. J. Sci. Food Agric. 99, 3057-3068. DOI: 10.1002/jsfa.9519

Rees, A., Dodd, G.F., Spencer, J.P.E., 2018: The effects of flavonoids on cardiovascular health: A review of human intervention trials and implications for cerebrovascular function, Nutrients 10(12), 1852. DOI: 10.3390/nu10121852

Ren, S., Wang, L., Yu, H., Haroon, M., Ullah, R.S., Haq, F., Khan, R.U., Fahad, S., 2018: Recent progress in synthesis of antifogging agents and their application to agricultural films: a review. J. Coat. Technol. Res. 15(3), 445-455. DOI: 10.1007/s11998-018-0051-x

Romania, A., Pinellia, P., Galardia, C., Sanib, G., Cimatob, A., Heimler, D., 2002: Polyphenols in greenhouse and open-air-grown lettuce. Food Chem. 79, 337-342. DOI: 10.1016/S0308-8146(02)00170-X

Sanders, D., 2019: Lettuce, Horticulture Information Leaflets. NC State Extension Publications. Retrieved 22th January 2021, from https://content.ces.ncsu.edu/lettuce.

Shafiq, I., Hussain, S., Raza, M.A., Iqbal, N., Asghar, M.A., Raza, A., Fan, Y.F., Mumtaz, M., Shoaib, M., Ansar, M., Mana, A., Yang, W., Yang, D., 2021: Crop photosynthetic response to light quality and light intensity. J. Integr. Agric. 20(1), 4-23. DOI: 10.1016/s2095-3119(20)63227-0

Song, J., Huang, H., Hao, Y., Song, S., Zhang, Y., Su, W., Liu, H., 2020: Nutritional quality, mineral and antioxidant content in lettuce affected by interaction of light intensity and nutrient solution concentration. Sci. Rep. 10, 2796. DOI: 10.1038/s41598-020-59574-3

Statistisches Bundesamt (DESTATIS), 2019: Gemüseerhebung – Anbau und Ernte von Gemüse und Erdbeeren. Fachserie 3 Reihe 3.1.3.

Sublett, W.L., Barickman, T.C., Sams, C.E., 2018: Effects of elevated temperature and potassium on biomass and quality of dark red 'Lollo Rosso' lettuce. Horticulturae 4, 11. DOI: 10.3390/horticulturae4020011

Zhou, D., Meinke, H., Wilson, M., Marcelis, L.F.M., Heuvelink, E., 2021: Towards delivering on the sustainable development goals in greenhouse production systems. Resour. Conserv. Recycl. 169, 105379. DOI: 10.1016/j.resconrec.2020.105379

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Supplemental material

Figure S1: Pictures of the polytunnels and lettuce grown without a polytunnel in the greenhouse chamber used in this study.

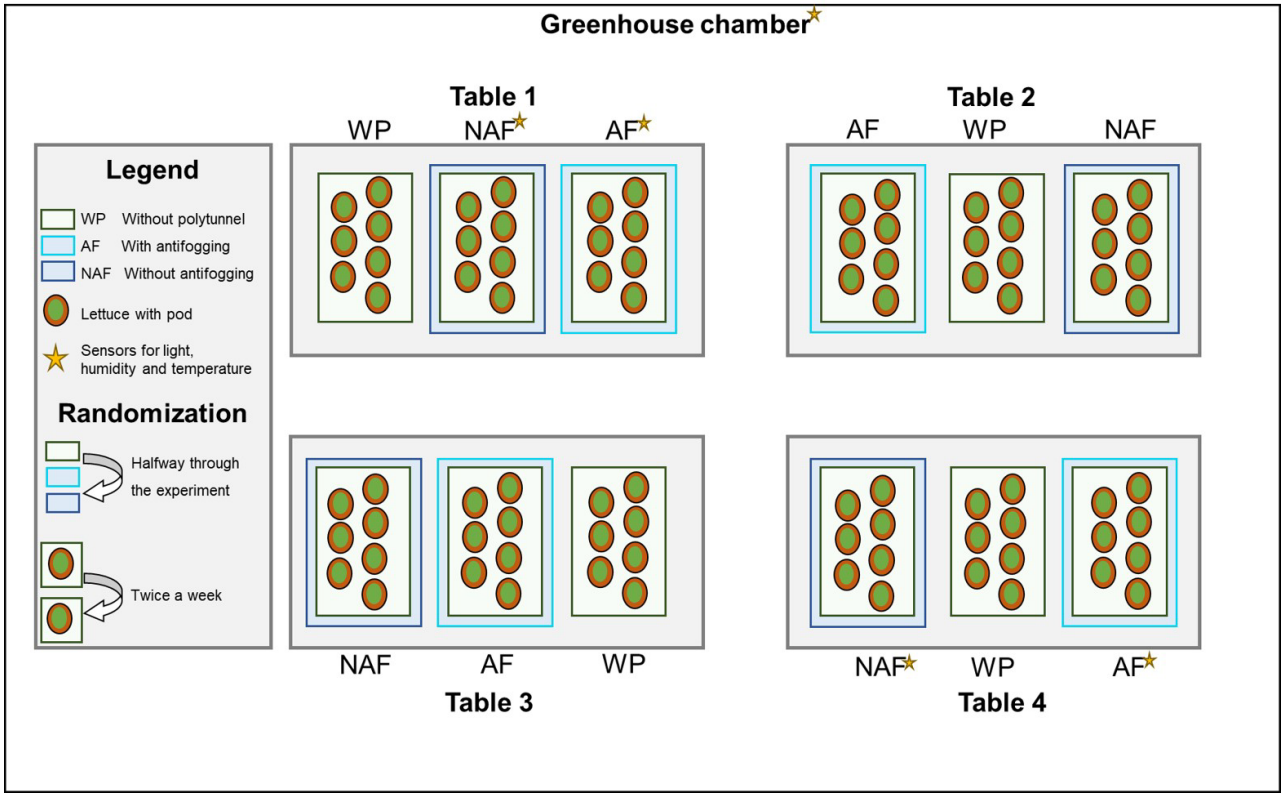

Figure S2: Experimental setup and randomization procedure of the experiments in 2019 and 2020.

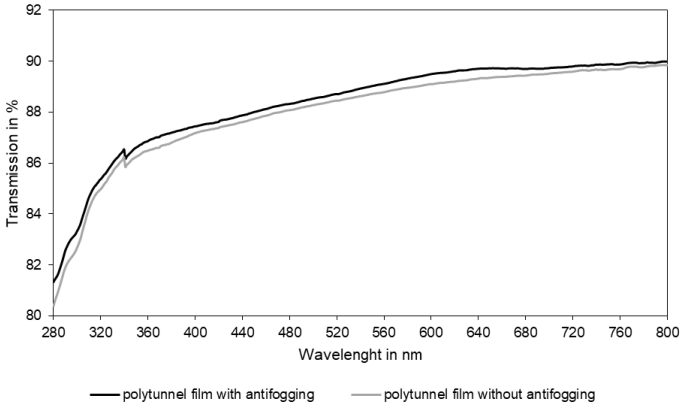

Figure S3: Light transmission (%) of polytunnel films with and without anti-fogging additives before use.

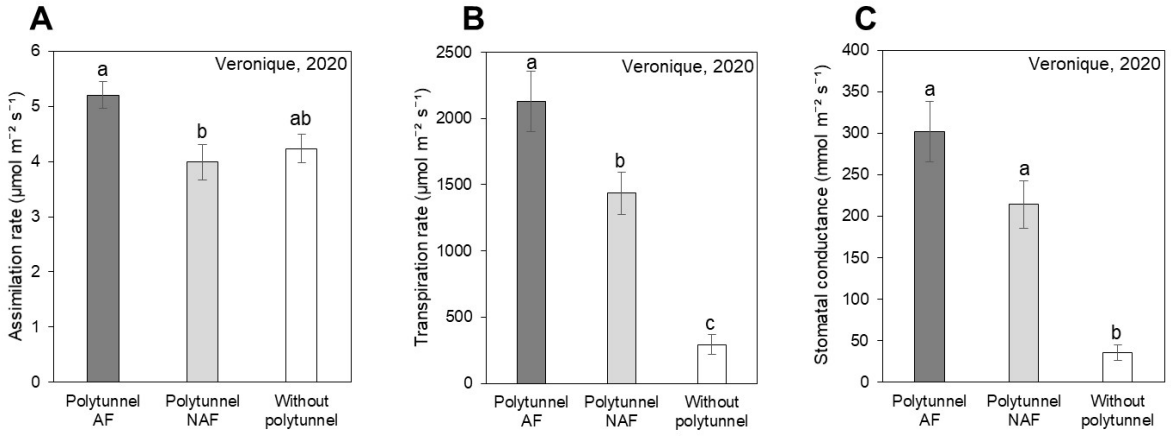

Figure S4: Physiological measurements (assimilation rate A, µmol m^{-2} s^{-1}; transpiration rate B, µmol m^{-2} s^{-1} and stomatal conductance C, mmol m^{-2} s^{-1}) of cultivar 'Veronique' from the 2020 experiment grown under polytunnel with (AF) and without antifogging additives (NAF) and without polytunnel. Values show means ± SE (n = 4). Different letters indicate significant differences ($p \leq 0.05$) between the cultivation conditions.

Measurement of physiological plant parameters

The measurements were performed with the LI-6800 gas exchange system (LI-COR Biosciences GmbH, Germany) for lettuce only in the 2020 experiment in the afternoon, one day before the harvest. The measurements were conducted at eight plants of each cultivation condition (two plants per table, Figure S2). The measurement conditions were PAR 290 µmol m^{-2} s^{-1}, temperature at 22 °C and 70 % relative humidity. The carbon dioxide concentration in the chamber was set to 400 µmol mol^{-1} with a flow rate of 500 µmol s^{-1}. Fan speed was set to 8000 rpm.

Table S1 Identification parameters for phenolic acids and flavonoid glycoside compounds in lettuce based on the literature. Compounds verified with authentic standard compounds are marked by †.

Compound	Retention time in min	MS^1 *m/z* $[M-H]^-$	MS^2 *m/z* $[M-H]^-$	MS^3 *m/z* $[M-H]^-$	Absorption maxima in nm
Chlorogenic acid†	7.36	354	191 179		246, 300, 340
iso-Chlorogenic acid†	23.21	515	191, 179		242, 326
Chicoric acid†	13.77	473	311	149, 179	244, 300, 342
meso Chicoric acid	13.01	473	311	149, 179	242, 328
Caffeoylmalic acid	9.60	295, 133	353, 179		242, 328
Quercetin-3-glucuronide†	17.88	477	301		256, 350
Quercetin-3-malonylglucoside†	21.58	549, 505	463, 301		256, 352
Luteolin-7-glucuronide†	20.49	461	285		222, 252, 342

Tentative identification based on the literature by: BECKER, C. et al., 2015: PLoS One 10, 11 e0142867, LLORACH R et al., 2008: Food. Chem. 108(3), 1028-1038.

Table S2 Identification parameters for chlorophylls and carotenoids in lettuce based on the literature. Compounds verified with authentic standard compounds are marked by †.

Compound	Retention time in min	Ion	MS *m/z*	Absorption maxima in nm
β-Carotene†	48.39	$[M+H]^+$	537.45	424 452 480
Lutein†	18.65	$[M+H-H2O]^+$	551.43	420 444 472
Lactucaxanthin	16.55	$[M+H-H2O]^+$	551.43	414 438 468
Neoxanthin (9-*Z*)†	12.16	$[M+H-H2O]^+$	583.42	410 434 464
Zeaxanthin†	20.60	$[M+H]^+$	569.43	426 452 478
Chlorophyll a†	22.47	$[M+H]^+$	893.54	432
Chlorophyll b†	18.02	$[M+H]^+$	907.52	468

Tentative identification based on the literature by: DIOP NDIAYE, N. et al., 2011: J. Agric. Food Chem. 59(22), 12018-12027, BRITTON, G. et al., 2004: Carotenoids: Handbook, Birkhäuser, GOPAL et al., 2017: Food Funct. 8, 1124.

Table S3 Identification parameters for fatty acids in lettuce. All compounds were verified with authentic standard compounds.

Compound	Retention time in min	MS *m/z* methylated fatty acid
Palmitic acid	25.18	270
Palmitoleic acid	25.38	268
Stearic acid	32.30	298
Oleic acid	32.82	296
Linoleic acid	34.44	294
Linolenic acid	36.44	292

Table S4: Determined phenolic compounds (µg mg^{-1} DW) in lettuce grown without a polytunnel and lettuce grown under polytunnels with (AF) and without antifogging additives (NAF). Values shows means ± SE (n = 4). Different letters indicate significant differences ($p \leq 0.05$) for each experiment and cultivar. Abbreviations, Gc: glucuronide, MG: malonyl glucoside.

	Attractie 2019			**Veronique 2019**			**Veronique 2020**		
	Polytunnel AF	Polytunnel NAF	Without polytunnel	Polytunnel AF	Polytunnel NAF	Without polytunnel	Polytunnel AF	Polytunnel NAF	Without polytunnel
Caffeic acid derivatives									
Total	4.327 ± 0.379^{b}	4.086 ± 0.230^{b}	5.499 ± 0.347^{a}	4.700 ± 0.243^{b}	4.068 ± 0.135^{b}	7.796 ± 0.383^{a}	3.825 ± 0.071^{c}	4.329 ± 0.060^{b}	4.905 ± 0.160^{a}
Chlorogenic acid	1.110 ± 0.056^{a}	1.121 ± 0.113^{a}	1.329 ± 0.102^{a}	1.917 ± 0.152^{b}	1.628 ± 0.068^{b}	3.975 ± 0.264^{a}	1.122 ± 0.021^{b}	1.212 ± 0.024^{b}	1.888 ± 0.089^{a}
Iso chlorogenoic acid	0.359 ± 0.056^{a}	0.400 ± 0.031^{a}	0.178 ± 0.027^{b}	0.305 ± 0.040^{a}	0.342 ± 0.020^{a}	0.239 ± 0.024^{b}	0.182 ± 0.008^{a}	0.144 ± 0.006^{b}	0.069 ± 0.003^{c}
Chicoric acid	2.431 ± 0.254^{b}	2.201 ± 0.125^{b}	3.629 ± 0.240^{a}	2.029 ± 0.095^{b}	1.608 ± 0.051^{c}	3.212 ± 0.123^{a}	1.872 ± 0.053^{b}	2.221 ± 0.037^{a}	2.195 ± 0.064^{a}
Meso chicoric acid	0.133 ± 0.016^{a}	0.127 ± 0.009^{a}	0.132 ± 0.026^{a}	0.110 ± 0.005^{a}	0.082 ± 0.004^{b}	0.159 ± 0.016^{a}	0.143 ± 0.005^{b}	0.176 ± 0.006^{a}	0.150 ± 0.005^{b}
Caffeoylmalic acid	0.293 ± 0.020^{a}	0.238 ± 0.013^{ab}	0.229 ± 0.017^{b}	0.339 ± 0.023^{a}	0.408 ± 0.022^{a}	0.210 ± 0.015^{b}	0.263 ± 0.008^{c}	0.300 ± 0.005^{b}	0.429 ± 0.008^{a}
Flavonoid glycosides									
Total	0.362 ± 0.034^{b}	0.336 ± 0.020^{b}	0.876 ± 0.066^{a}	0.320 ± 0.025^{b}	0.326 ± 0.010^{b}	0.966 ± 0.084^{a}	0.336 ± 0.007^{b}	0.354 ± 0.006^{b}	0.750 ± 0.029^{a}
Quercetin-3-Gc	0.113 ± 0.012^{b}	0.101 ± 0.006^{b}	0.245 ± 0.018^{a}	0.111 ± 0.007^{b}	0.088 ± 0.003^{b}	0.309 ± 0.023^{a}	0.104 ± 0.002^{b}	0.107 ± 0.002^{b}	0.231 ± 0.010^{a}
Quercetin-3-MG	0.221 ± 0.022^{b}	0.205 ± 0.015^{b}	0.584 ± 0.061^{a}	0.169 ± 0.023^{a}	0.196 ± 0.006^{a}	0.564 ± 0.088^{a}	0.194 ± 0.004^{b}	0.208 ± 0.004^{b}	0.475 ± 0.019^{a}
Luteolin-7-Gc	0.029 ± 0.002^{b}	0.030 ± 0.002^{b}	0.047 ± 0.005^{a}	0.040 ± 0.004^{b}	0.042 ± 0.001^{b}	0.093 ± 0.007^{a}	0.038 ± 0.001^{b}	0.038 ± 0.001^{b}	0.044 ± 0.001^{a}

Table S5: Carotenoids, chlorophylls (ng mg^{-1} DW) and chlorophyll a/b ratio in lettuce grown without a polytunnel and lettuce grown under polytunnels with (AF) and without antifogging additives (NAF). Values shows means ± SE (n = 4). Different letters indicate significant differences ($p \leq 0.05$) for each experiment and cultivar.

	Attractie 2019			**Veronique 2019**			**Veronique 2020**		
	Polytunnel AF	Polytunnel NAF	Without polytunnel	Polytunnel AF	Polytunnel NAF	Without polytunnel	Polytunnel AF	Polytunnel NAF	Without polytunnel
Carotenoids									
Total	914.03 ± 23.37^{a}	883.92 ± 22.22^{a}	877.91 ± 19.39^{a}	894.20 ± 32.43^{b}	1015.76 ± 22.72^{a}	726.98 ± 39.51^{c}	783.53 ± 18.77^{b}	840.87 ± 12.70^{a}	745.96 ± 11.06^{b}
Beta-carotene	271.08 ± 7.08^{a}	269.37 ± 7.83^{a}	276.42 ± 5.94^{a}	262.64 ± 8.35^{b}	299.07 ± 7.06^{a}	223.82 ± 11.96^{c}	107.90 ± 2.53^{a}	111.13 ± 1.61^{a}	112.10 ± 1.58^{a}
Lutein	295.54 ± 8.57^{a}	261.27 ± 12.81^{a}	288.72 ± 6.36^{a}	286.85 ± 16.48^{ab}	317.98 ± 13.16^{a}	247.79 ± 12.85^{b}	262.38 ± 5.14^{b}	277.60 ± 3.75^{a}	239.55 ± 3.74^{c}
Lactucaxanthin	109.12 ± 3.10^{a}	108.16 ± 3.14^{a}	99.01 ± 3.21^{a}	113.81 ± 3.86^{a}	126.52 ± 2.67^{a}	84.88 ± 5.78^{b}	106.54 ± 2.92^{b}	113.83 ± 1.68^{b}	135.55 ± 2.17^{a}
Neoxanthin	91.00 ± 3.33^{ab}	93.30 ± 4.50^{a}	78.64 ± 2.67^{b}	100.55 ± 3.62^{a}	107.74 ± 4.66^{a}	64.94 ± 5.12^{b}	123.21 ± 3.55^{b}	141.00 ± 3.04^{a}	124.29 ± 2.61^{c}
Zeaxanthin	18.18 ± 3.08^{a}	27.92 ± 4.63^{a}	17.53 ± 2.50^{a}	17.25 ± 2.02^{a}	25.90 ± 3.84^{a}	14.47 ± 1.43^{a}	34.82 ± 1.70^{a}	26.04 ± 1.87^{b}	40.03 ± 1.83^{a}
Chlorophylls									
Total	8876.83 ± 931.91^{a}	8902.02 ± 942.97^{a}	7776.72 ± 826.78^{b}	9119.07 ± 839.53^{a}	9893.03 ± 944.88^{a}	6557.33 ± 634.76^{b}	6801.80 ± 190.98^{b}	7524.05 ± 97.55^{a}	7151.17 ± 96.72^{ab}
Chlorophyll a	7613.32 ± 235.79^{ab}	7776.01 ± 275.76^{a}	6688.05 ± 245.00^{b}	7849.85 ± 230.91^{a}	8510.29 ± 301.48^{a}	5634.00 ± 330.86^{b}	5139.95 ± 155.82^{b}	5741.45 ± 77.64^{a}	5605.56 ± 76.56^{a}
Chlorophyll b	1263.51 ± 36.14^{a}	1126.01 ± 38.31^{b}	1088.67 ± 26.99^{b}	1269.22 ± 55.00^{a}	1382.74 ± 36.22^{a}	923.33 ± 53.97^{b}	1661.85 ± 37.56^{b}	1782.60 ± 21.86^{a}	1545.61 ± 21.50^{b}
Chlorophyll a/b ratio	6.03 ± 0.11^{a}	6.91 ± 0.34^{a}	6.14 ± 0.15^{a}	6.18 ± 0.27^{a}	6.15 ± 0.24^{a}	6.10 ± 0.23^{a}	3.09 ± 0.04^{b}	3.22 ± 0.02^{b}	3.63 ± 0.02^{a}