Journal of Photochemistry & Photobiology, B: Biology

Valorization of waste cabbage leaves by postharvest photochemical treatments monitored with a non-destructive fluorescence-based sensor

--Manuscript Draft--

Manuscript Number:	JPHOTOBIOL-D-20-00952R1
Article Type:	Full length article
Keywords:	Brassica oleracea L.; Chlorophyll fluorescence; flavonols; non-destructive sensors; postharvest irradiation; vegetable waste valorisation
Corresponding Author:	Giovanni Agati IFAC CNR Sesto Fiorentino, Firenze ITALY
First Author:	Artur Kowalski
Order of Authors:	Artur Kowalski
	Giovanni Agati
	Maria Grzegorzewska
	Ryszard Kosson
	Barbara Kusznierewicz
	Tomasz Chmiel
	Agnieszka Bartoszek
	Lorenza Tuccio
	Daniele Grifoni
	Ingunn M. Vågen
	Stanislaw Kaniszewski
Abstract:	The biosynthesis of polyphenolic compounds in cabbage waste, outer green leaves of white head cabbage (Brassica oleracea L. var. capitata subvar. alba), was stimulated by postharvest irradiation with UVB lamps or sunlight. Both treatments boosted the content of kaempferol and quercetin glycosides, especially in the basal leaf zone, as determined by the HPLC analysis of leaf extracts and by a non-destructive optical sensor. The destructive analysis of samples irradiated by the sun for 6 days at the end of October 2015 in Skierniewice (Poland) showed an increase of leaf flavonols by 82% with respect to controls. The treatment by a broadband UVB fluorescent lamp, with irradiance of 0.38 W m-2 in the 290-315 nm range (and 0.59 W m-2 in the UVA region) for 12 hours per day at 17 °C along with a white light of about 20 µmol m-2 s-1, produced a flavonols increase of 58% with respect to controls. The kinetics of flavonols accumulation in response to the photochemical treatments was monitored with the FLAV non-destructive index. The initial FLAV rate under the sun was proportional to the daily radiation doses with a better correlation for the sun global irradiance (R2=0.973), followed by the UVA (R2=0.965) and UVB (R2=0.899) irradiance. The sunlight turned out to be more efficient than the UVB lamp in increasing the flavonols level of waste leaves, because of a significant role played by UVA and visible solar radiation in the regulation of the flavonoid accumulation in cabbage. The FLAV index increase induced on the adaxial leaf side was accompanied by a lower but still significant FLAV increase on the unirradiated abaxial side, likely due to a systemic signaling by mean of the long-distance movement of macromolecules. Our present investigation provides useful data for the optimization of postharvest photochemical protocols of cabbage waste valorization. It can represent a novel and alternative tool of vegetable waste management for the recovery of beneficial phytochemicals.
Suggested Reviewers:	Monika Screiner

	Leibniz Institute of Vegetable and Ornamental Crops Großbeeren/Erfurt e.V., Germany. Schreiner@igzev.de She has decades of experience with the effects of elicitor treatments, including UV radiation, on crop plants, and is one of the leading experts in this topic. https://www.researchgate.net/profile/Monika_Schreiner/publications
	Diego Moreno C.E.B.A.S. - CSIC. Phytochemistry Lab, Food Science & Technology Department, Murcia, Spain. dmoreno@cebas.csic.es He has a capacity in aspects of plant secondary metabolites, linking to both nutrition, human health, crop production and food waste utilization. https://www.researchgate.net/profile/Diego_A_Moreno/publications
	Matthew T Robson University of Helsinki - City Centre Campus: Helsingin Yliopisto matthew.robson@helsinki.fi His research activity is focused on plant photobiology involving signalling from UV radiation and how plants detect and response to changes in the spectral quality of radiation. https://www.researchgate.net/profile/Thomas_Robson3/publications
	Javier Martinez Abaigar University of La Rioja: Universidad de la Rioja javier.martinez@unirioja.es Among other activities, he studies the plant responses to the light (mainly UVB, but also UVA and PAR), in particular the synthesis of phenolic compounds both under natural conditions and under artificial irradiation. He is a member of the UV4Plant association which is focused on the interaction between UV radiation and plants. https://www.researchgate.net/profile/Javier_Martinez-Abaigar/publications
	Rajeev Bhat Estonian Biocentre rajeev.bhat@emu.ee Expert on the sustainable utilization of agri-food wastes and by-products. https://www.researchgate.net/profile/Rajeev_Bhat/publications
Response to Reviewers:	

The manuscript entitled “Valorization of waste cabbage leaves by postharvest photochemical treatments monitored with a non-destructive fluorescence-based sensor”
by Kowalski et al.

has been revised according to the Editor and the Reviewers comments and requests.
We carefully addressed them and hope that now the manuscript could be suitable for publication in the Journal of Photochemistry and Photobiology B: Biology.

The changes/additions are highlighted in the manuscript text.

Reviewer #1

In general, the subject of the work is worthy of investigation and follows current trends to establish which wavelength and intensity of light are more appropriate for inducing valorisation of by-products. There are a number of issues to be addressed, as outlined below, which make the manuscript not yet ready for publication.

-In the material and methods the authors must report which is the % of UV-A wavelength emitted from the broadlamps used in this experiment because the results obtained and reported as a consequence of UV-B irradiation could be derived also from this component.

Reply:

The % of UVA (60%) and UVB /40%) to the total UV have been reported in the text (Lines 198-200).

Of course, the presence of UVA radiation may contribute to the treatment effects. This has been highlighted in the text. All over the manuscript, care has been taken to specify that broadband UVB lamp (containing UVA) and not specific UVB radiation was used in the treatments of leaves.

-In the fig 1 the authors have to explain why at time 0 of treatments the FLAV index of UVAB and CTRAB relating to apical part of the leaves are so different also if not statistically significant

Reply:

It is widely accepted that the FLAV index of leaves is very sensitive to sunlight exposure. In our study we used the cabbage leaves closest to the cabbage head. For those, the most sunlight exposed leaf part is the apical one. Since the leaves treated by the UVB lamp were chosen randomly, the high variability found in the FLAV index on the apical part, especially AB side, are likely due to rather different exposure of the part to sunlight. To note that the apical part of some cabbage leaves could assume a different orientation, more or less folded, exposing to sunlight the AD or the AB side. This explanation has been add in the text. (see Lines 292-293)

- In my opinion to improve the quality of the manuscript it should be better to insert a chromatogram of HPLC analysis to individuate the profiling of flavonols detected

A new figure (Fig. 2 in the revised version) reporting chromatograms of leaf extracts of apical and basal portion of cabbage leaves stored with and without UVB treatment is included.

-The authors have to explain the discrepancy between Fig 3 A and B. In fig 3A as the irradiance decreases, an increase in FLAV index is observed in the basal part of the leaves while in fig 3B the increase of the FLAV index in both AD and AB occurs in conditions of constant brightness. Indeed during the time period considered a reduction of UV-B and UV-A radiations was observed.

Reply:

Comparing the day-by-day levels of FLAV and global solar radiation is not easy since we do not know precisely what could be the lapse time between the radiation stimulus, the synthesis of flavonols and the localization of flavonols on the leaf surface. And if the stimulation of the flavonol accumulation is based on a threshold for irradiance. We can observe that during the first four days of the end of September experiment, global solar irradiance decreased from 10.85 to 7.5 MJ m-2 day-1, while at the end of Oct, global solar irradiance varied less between 4 and 5.4 MJ m-2

day-1. Therefore, even if global irradiance decreased in September, it likely remained above the threshold to synthetize new compounds.

We believe that comparing irradiance (both UV or global) integrated over a time period and the FLAV accumulation rate, as reported in Table 1, is more correct and informative on the FLAV-radiation correlation.

Reviewer #2

Reviewer #2: Artur Kowalski and co-workers prepare a manuscript describing the effects of postharvest photochemical treatments on waste cabbage leaves by as monitored by HPLC and by a non-destructive fluorescent method. I am not in favor of publication because of several reasons:

1. The manuscript is purely observational. Amount of flavonols are compared with or without irradiation and there is not any tentative description of the mechanisms involved. I understand the study could be published in JPPB if there is mechanistic information, explaining how and why light treatment postharvest will increase levels of polyphenols. The sole information that the irradiation can increase levels of phenols is not new.

Reply:

We thank the Reviewer for the request to introduce the indication about possible mechanisms of photochemical induction of leaf flavonols.

We added a new section "3.5 Mechanisms of photochemically-induced flavonols accumulation in waste leaves" reporting mechanisms of action and referring to a schematic representation of them in the a figure (Fig. 6).

We also highlighted the effect of the irradiation treatments in enhancing the flavonols content in the abaxial non-irradiated side. This is a quite new information in the field.

A novel aspect of the manuscript is also the comparison between two methods of photochemical treatments. And it provides quantitative parameters to optimize the treatment for an industrial level of application.

2. While the HPLC method is quantitative the indirect fluorescence decrease may the caused by a variety of factors, not only by the increase of polyphenols content. Though I agree that the fluorescence quenching method is valuable as a screening tool, I do not recognize its benefits as a quantitative method for the present publication.

Reply:

The chlorophyll fluorescence excitation filtering method is a widely accepted method to non-destructively quantify both flavonols and anthocyanins on leaf or fruit surfaces. It is based on the ratio between far-red chlorophyll fluorescence detected at two excitation wavelengths, one absorbed by the compound under detection and the other as reference. Therefore, it is very sensitive to the absorbance of leaf/fruit surface at a specific wavelength.

It has been applied by our group and others on several species finding good correlations between the optical indices and the actual amount of leaf/fruit compounds determined destructively.

We are very confident with that.

See for example:

Agati et al. J. Agric. Food Chem. 64 (2016) 85–94
Agati et al. Functional Plant Biol 33 (2008) 77-84
Rybarczyk-Plonska et al. Postharvest Biol. Technol. 116 (2016) 105–114
Zivcak et al. Planta 245 (2017) 1225-1229
Hagen et al. Postharvest Biol Tech 41 (2006) 156-163
Pinelli et al. Food Chem 244 (2018) 213-223

The use in the present study of the optical sensor to follow the leaf increase of flavonols during irradiation was fundamental to measure the rates of accumulation and compare different treatments. It allowed to follow in time the very same leaf, no possible with destructive analyses, limiting the biological variability and giving more precise evaluation of the effects.

3. The results revealing the significant role for solar UVA and visible radiation in the regulation flavonoids could be better exploited. Why is this the case? Can the authors provide a mechanistic explanation?

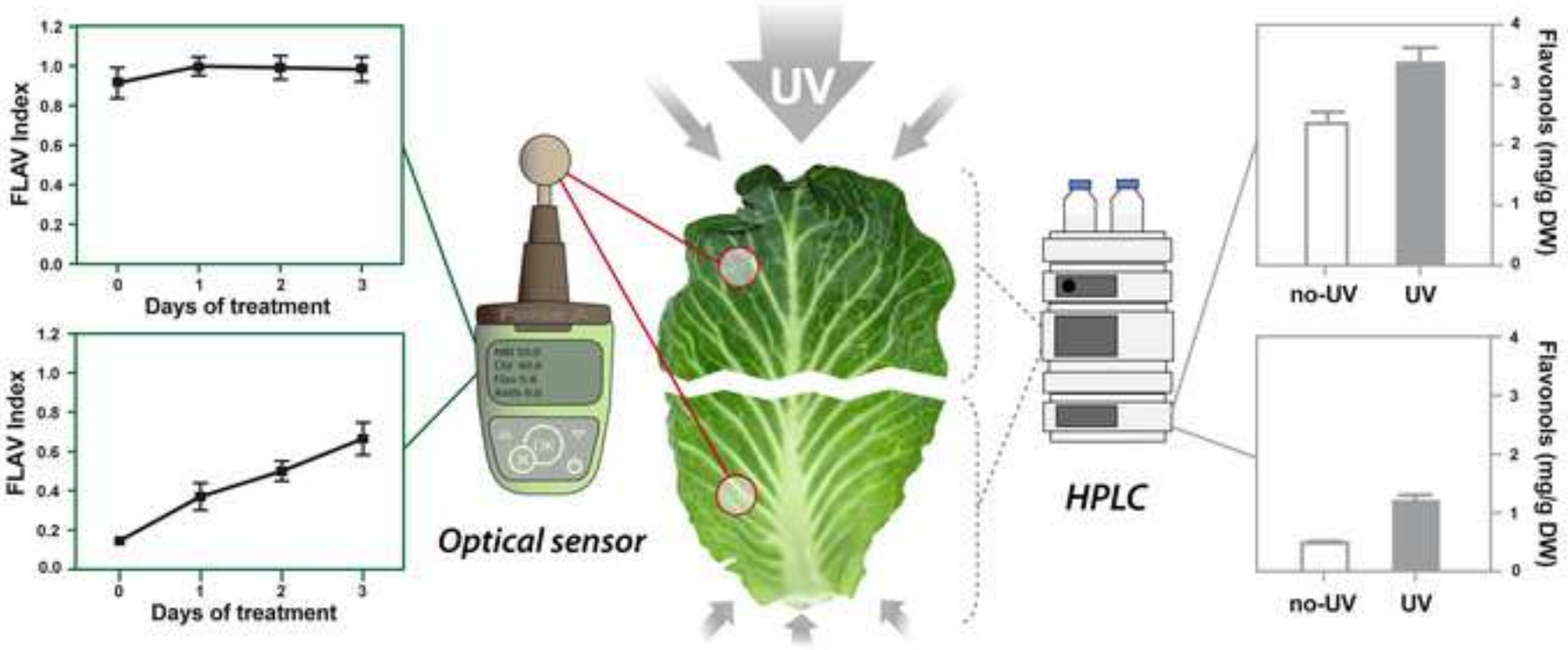
Reply:

These mechanisms are reported in the added 3.5 section.

Highlights

- Sunlight and UVB postharvest irradiation increase flavonols in waste cabbage leaves
- Non-destructive fluorescence sensors control bioactive compounds in cabbage waste
- Sunlight is an efficient and sustainable valorisation tool of cabbage waste leaves
- Accumulation of flavonols in the basal leaf parts is proportional to irradiance
- Signalling from irradiated leaf sides induces flavonols on unirradiated leaf sides

Graphical Abstract



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Author contributions

Artur Kowalski: Investigation. Giovanni Agati: Conceptualization, Methodology, Writing-Original draft preparation. Maria Grzegorzewska: Investigation, Data curation. Ryszard Kosson: Methodology. Barbara Kusznierewicz: Investigation, Data curation, Visualization. Tomasz Chmiel: Data curation, Validation. Agnieszka Bartoszek: Writing- Reviewing and Editing, Lorenza Tuccio: Investigation. Daniele Grifoni: Formal analysis. Ingunn M. Vågen: Conceptualization, Writing-Reviewing and Editing. Stanislaw Kaniszewski: Funding acquisition, Supervision.

Valorization of waste cabbage leaves by postharvest photochemical treatments monitored with a non-destructive fluorescence-based sensor

Artur Kowalski[a], Giovanni Agati[b,*], Maria Grzegorzewska[a], Ryszard Kosson[a], Barbara Kusznierewicz[c], Tomasz Chmiel[c], Agnieszka Bartoszek[c], Lorenza Tuccio[b], Daniele Grifoni[d,e], Ingunn M. Vågen[f], Stanislaw Kaniszewski[a]

[a]Research Institute of Horticulture, Konstytucji 3 Maja 1/3, Skierniewice, Poland.

[b]Istituto di Fisica Applicata 'N. Carrara' – CNR, Via Madonna del Piano, 10-50019 Sesto Fiorentino (Florence), Italy.

[c]Department of Food Chemistry, Technology and Biotechnology, Chemical Faculty, Gdansk University of Technology, Gdansk, Poland.

[d]Institute of Bioeconomy (IBE), National Research Council (CNR), Via Madonna del Piano 10, 50019, Sesto Fiorentino (FI), Italy.

[e]Laboratory of Monitoring and Environmental Modelling for the Sustainable Development (LaMMA Consortium), Via Madonna del Piano 10, 50019, Sesto Fiorentino (FI), Italy.

[f]Norwegian Institute of Bioeconomy Research – NIBIO, NO-1431 Ås, Norway.

*Corresponding author. E-mail address:g.agati@ifac.cnr.it (Giovanni Agati)

ABSTRACT

The biosynthesis of polyphenolic compounds in cabbage waste, outer green leaves of white head cabbage (*Brassica oleracea* L. var. *capitata* subvar. *alba*), was stimulated by postharvest irradiation with UVB lamps or sunlight.

Both treatments boosted the content of kaempferol and quercetin glycosides, especially in the basal leaf zone, as determined by the HPLC analysis of leaf extracts and by a non-destructive optical sensor.

The destructive analysis of samples irradiated by the sun for 6 days at the end of October 2015 in Skierniewice (Poland) showed an increase of leaf flavonols by 82% with respect to controls. The ~~UVB~~ treatment by a broadband UVB fluorescent lamp, with irradiance of 0.38 W m^{-2} in the 290-315 nm range (and 0.59 W m^{-2} in the UVA region) for 12 hours per day at 17 °C along with a white light of about 20 µmol m^{-2} s^{-1}, produced a flavonols increase of 58% with respect to controls.

The kinetics of flavonols accumulation in response to the photochemical treatments was monitored with the FLAV non-destructive index. The initial FLAV rate under the sun was proportional to the daily radiation doses with a better correlation for the sun global irradiance (R^2=0.973), followed by the UVA (R^2=0.965) and UVB (R^2=0.899) irradiance.

The sunlight turned out to be more efficient than the UVB lamp in increasing the flavonols level of waste leaves, because of a significant role played by UVA and visible solar radiation in the regulation of the flavonoid accumulation in cabbage. The FLAV index increase induced on the adaxial leaf side was accompanied by a lower but still significant FLAV increase on the unirradiated abaxial side, likely due to a systemic signaling by mean of the long-distance movement of macromolecules.

Our present investigation provides useful data for the optimization of postharvest photochemical protocols of cabbage waste valorization. It can represent a novel and alternative tool of vegetable waste management for the recovery of beneficial phytochemicals.

Keywords:
Brassica oleracea L.; chlorophyll fluorescence; flavonols; non-destructive sensors; postharvest irradiation; vegetable waste valorisation.

Abbreviations:
AB, Abaxial; AD, Adaxial; Chl, Chlorophyll; ChlF, Chlorophyll Fluorescence; DW, Dry Weight; FLAV, Flavonols index; ROS, Reactive Oxygen Species; UVBE, Ultraviolet Biologically Effective radiation; $UVBE_{ERYTH}$, Ultraviolet Biologically Effective radiation for Erythema induction; $UVBE_{GPD}$, Ultraviolet Biologically Effective radiation for Generalized Plant Damage; $UVBE_{flav}$, Ultraviolet Biologically Effective radiation for flavonols induction.

1. Introduction

Within the growing interest of a sustainable utilization of agri-food waste, significant care is now dedicated to the recovery of bioactive compounds [1]. This represents a worthwhile alternative to the most common utilization of vegetable and fruit wastes as bio-fuel, livestock feeds or as organic fertilizers.

The improvement of green-chemistry technologies permits now to extract high-value products from wastes and by-products of the agri-food sector [2].

This approach can generate benefits for the pharmaceutical, cosmetics as well as food industries as users of the bioactive natural compounds recovered from waste for the production of nutraceuticals and functional foods.

Moreover, in this way the negative environmental impact due to greenhouse gas emission from landfill vegetable and fruit residues would be reduced [3].

78 Fruit and vegetable wastes are rich in beneficial for humans and industrially valuable compounds as
79 recently reviewed [4]. Dietary fibers and plant secondary metabolites can be exploited for their
80 health properties such as antioxidant, anti-obesity, anti-inflammatory as well as cancer and
81 cardiovascular disease preventive [1].
82 Among different vegetable wastes, those deriving from Brassicaceae are of particular interest,
83 because of their significant content of beneficial phytochemicals [5] and large amount of waste
84 material. In broccoli (*Brassica oleracea* L. var. *italica*) for example, up to 90% of the above ground
85 plant parts become waste [6]. The non-edible parts of cauliflower (*Brassica oleracea* L. var.
86 *botrytis*), outer leaves, stems and pods, account for about 36% of the total biomass. Similarly, in
87 white cabbage (*Brassica oleracea* L. var. *capitata* subvar. *alba*), the percentage of discarded leaves
88 can reach about 35% of the whole plant [7]. Considering that the world production of cabbage for
89 2018 was estimated around 70 million of tons [8], cabbage waste can represent about 24 million
90 tons worldwide.
91 The amount of bioactive compounds contained in the inedible part of broccoli was found to be
92 superior to that of the edible florets [9,10] and comparable with other vegetables currently used as
93 food [6].
94 The outer leaves of white cabbage were characterized by a significantly greater nutritional value
95 compared to marketable heads, inclusive of higher content of total flavonoids, soluble polyphenols,
96 ascorbic acid and dry matter [7].
97 Wasted leaves are source of dietary antioxidant fiber powder as obtained by recently improved
98 processing [11]. White cabbage leaf powder was also proposed for the preparation of cakes with
99 particular qualitative characteristics [12]. Cabbage and cauliflower are likewise reported to be
0 important sources of isothiocynates, produced upon hydrolysis of glucosinolates, which are
1 associated with chemopreventive properties [13,14].
2 The chromatographic LC/MS analysis of aqueous methanolic leaf extracts revealed that flavonols
3 were the major group of phenolic compounds in cabbage waste. Fourteen quercetin and kaempferol

glycosides were detected and characterized [15]. Analogously, nineteen flavonoid glycosides mainly derived from kaempferol and quercetin were found in cauliflower waste [16]. These compounds are of special interest because of their anti-inflammatory and anti-carcinogenic properties [17,18].

There is now large evidence that low-dosage UVB radiation has a stimulatory effect on the plant secondary metabolism, determining the accumulation of phenolic compounds, in contrast to the distress damage induced by high-dosage UV [19].

Such non-destructive treatments were thus suggested to improve the nutraceutical value of edible products ensuring high content of health-promoting compounds, both during cultivation as well as in postharvest [20]. Besides, UVB radiation was found to be effective in increasing the total phenol content also in broccoli waste [10].

Harbaum-Piayda et al.[21] observed a significant increase in the content of quercetin-tri-glycoside and hydroxycinnamic acids in cabbage head leaves after postharvest low-dose UVB broadband treatments and low temperature (4 °C) storage.

Quantitative determination of bioactive compounds in plant material is usually achieved by high-performance liquid chromatography and spectrophotometric analysis of solvent extracts of samples. These methods are costly and time consuming, especially for industrial applications, and may produce artifacts due to compound instability and loss of material. Lately developed optical methods, instead, allow frequent and rapid monitoring of phytochemicals. The flavonoid content of leaves can be assessed in situ by non-destructive indirect methods developed within the past 20 years and based on the detection of chlorophyll (Chl) fluorescence [22].

The attenuation of the Chl fluorescence signals of leaves excited in the UV spectral range is proportional to the concentration of the epidermis-located flavonoids and therefore can be used as a proxy of their leaf content. Even if the method is limited to the detection of epidermal particles, it can represent a fast useful tool for the rapid screening of the presence of bioactive compounds in plant material.

The technique was previously applied to leaves and fruit skins of different plant species, even directly in the field by using portable sensors, as reviewed by Julkunen-Tiitto et al. [23]. In Brassicaceae, non-destructive fluorescence-based methods were previously used to estimate the flavonol content in curly kale (*Brassica oleracea* L. var. *acephala*) [24], broccoli flower buds [25,26] and white cabbage [15]. This last study showed that the content of flavonols in cabbage leaves was following a decreasing gradient from the apical to the basal part, due to the reduced (or absent) exposure of the basal leaf portion to sunlight.

In this work, we exposed waste cabbage leaves to postharvest irradiation by both sunlight and UVB artificial broadband lamps in order to heighten their phenolic content. Beside wet chemistry, the effect of the treatments was evaluated by non-destructive optical sensors allowing the monitoring with time of the leaf flavonol accumulation on the very same leaf material. The study provided basic data for the development of postharvest photochemical protocols dedicated to cabbage waste valorization.

2. Materials & Methods

2.1. Plant material

The white cabbage (*Brassica oleracea* L. var. *capitata* subvar. *alba*), cultivar Typhoon F1, plants were cultivated in the experimental fields of the Research Institute of Horticulture in Skierniewice, central Poland.

Transplants of cabbage were produced in greenhouse. Seeds were sown to multi-cell trays “Vefi” a’ 96 filled with prepared peat substrate “Select” from Klasmann-Deilman. Mean air temperature was maintained between 20 and 23°C (day) and 17 and 18°C (night) during the course of transplant production. Transplants at the stage of 5-6 leaves were transplanted to the field on a sandy-loam soil at a plant distance of 0.6 m and 0.5 m within and between rows, respectively, corresponding to 3.3 plants m^{-2}.

Nitrogen fertilization was applied at a rate of 200 kg N ha^{-1}. The phosphorus and potassium fertilization was applied according to the results of soil tests by bringing up the soil fertility to 80 mg P and 200 mg K per liter of soil. Irrigation was applied when soil moisture tension at the depth of 30 cm reached 0.04 MPa.

Cabbage heads harvest occurred on 20st October 2015 and 21st November 2016.

2.2. Non-destructive measurements of leaves

The non-destructive determination of leaf flavonols was performed by using the Dualex Scientific+ optical sensor (Force-A, Orsay, France) previously described by Goulas et al. [27]. It is a leaf-clip active device that uses LED radiation sources at 375 and 650 nm to excite chlorophyll fluorescence (ChlF) and photodiode detectors to indirectly estimate the in vivo absorbance of the leaf epidermis by using the ChlF screening method [22].

Flavonols present in the epidermis of leaves and absorbing at 375 nm attenuate the incident radiation, before this can reach the first chlorophyll layer present in the mesophyll. Therefore, the intensity of the ChlF induced by this radiation (ChlF_UV) will be inversely proportional to the epidermal flavonols concentration. Using a red light excitation, not attenuated by flavonols, a ChlF signal (ChlF_R) independent of the flavonols concentration is obtained. This signal is used as a reference. By comparing the ChlF signals from the two different excitations, the index of flavonols can be calculated (in accordance with the Beer−Lambert law) as the logarithm of the ratio between the ChlF under red light and that under UV radiation:

$$FLAV = \log(ChlF\_R/ChlF\_UV) \quad (1)$$

It is worth to remark that by definition the sensor limits the measurement to the leaf epidermis. It was used to measure both the adaxial and abaxial sides of the cabbage leaves on a spot of about 0.5 cm diameter. FLAV values were expressed as Dualex units.

The sensor provides also an index of Chl assessed as difference in the leaf light transmission between 710 and 850 nm.

2.3. UVB lamp treatment of detached leaves

Cabbage outer leaves were collected on October 27th 2016. Immediately after harvest, the detached cabbage leaves were kept in a storage room at the temperature of 15-17 °C and 70% relative humidity.

Part of them (12 leaves) were treated daily by ~~a~~ TL 100W/12 RS UVB Broadband Philips fluorescent lamp wrapped with cellulose acetate to remove residual UVC radiation.

The leaves were positioned with their adaxial surface facing the UVB lamp at a distance of about 140 cm and received an irradiance between 0.34 and 0.43 W m^{-2} in the 290-315 nm band.

The UVB radiation was measured by the OPHIR PD300-UV (Ophir Optronics Solutions Ltd, Jerusalem, Israel) power meter set at 313 nm and previously calibrated with a portable spectroradiometer (model SR9910-PC; Macam Photometrics Ltd., Livingstone, UK), on a Q-Panel (Q-Panel, Cleveland, OH) UVB lamp having a spectrum very close to that of the used lamp.

Twelve other leaves were positioned within the same storage room, outside the UVB lamp irradiation area, covered by the LEE 226 (Lee Filters, Andover, UK) plastic film that filter out all the UV radiation. These leaves were used as control.

The emission spectrum of a UVB lamp filtered by the cellulose acetate film and the transmittance spectrum of the LEE 226 film are reported in Fig. S1 of the online Supplementary data. From that, the percentage of UVB and UVA to the total UV was calculated to be about 40% and 60%, respectively.

The UVB lamp treatment consisted of a 12 hours period of irradiation and 12 hours of dark carried out for three consecutive days. During the irradiation period a supplementary sodium lamp was switched on.

The PPFD of the sodium lamp was measured by the SunScan sensor (Delta-T Devices, Cambridge, England) and was 19.7 µmol m^{-2} s^{-1} at the samples level. On the controls under the LEE filter it was 14 µmol m^{-2} s^{-1}.

All the leaves were measured by the Dualex sensor, described above, at two zones, apical and basal, on three points, adaxial and abaxial. The average of the three punctual measurements was calculated. Dualex measurements were taken before starting the UVB treatment and after each day of treatment.

At the end of the 3-days treatment, each leaf was divided into three parts, central part was discarded while apical and basal ones were collected and frozen in liquid N and then freeze-dried for successive extraction and analysis of phenolics by HPLC.

2.4. Sunlight irradiation of waste cabbage leaves

In a first trial, the cabbage leaves closest to the cabbage heads were harvested on Sep 22nd 2015 and left on the ground, with the adaxial side up, under solar irradiation until Sep 30th. During this period, the detached leaves were monitored by the Dualex sensor (Fig. S2 of Supplementary data).

In a second trial, the leaves were detached from the plants on Oct 27th 2015 and irradiated by sunlight in the field for 6 days. On Nov 2nd 2015, after the Dualex sensor measurements, 9 of the treated leaves were cut transversally into three parts, the central part was discarded while the apical and basal ones were then freeze-dried. Three more leaves were collected and treated the same on Oct 27th 2015 just before the irradiation period, and used as control.

Freeze-dried samples were extracted and analyzed for their content of polyphenols. In a third trial, cabbage heads were harvested on Oct 20th 2015 in a way to maintain the leaves closest to the heads attached to the stalk (Fig. S2 of Supplementary data). Twenty-four plants were used. On each plant, the most exposed leaf was monitored over time by measuring the apical and basal adaxial parts by the Dualex sensor up to 21 days, till Nov 10th.

During the trials, the average air temperature was ranging between 11.9 and 13.1 °C at the end of September and between 10.7 and 11.1 °C at the end of October. Daily average relative humidity was between 70% and 76% in September and between 83% and 97% in October. The daily mean global sun irradiance ranged between 0.86 MJ m^{-2} (Nov 10th) and 10.85 MJ m^{-2} (Sep 22nd).

The daily average values of UVB and UVA were obtained from AC SAF offline surface UV product database derived from the measurements of the operational polar orbiting Metop and NOAA satellites with a spatial resolution of 0.5°. Together with solar UVB and UVA, AC SAF makes also available the daily Ultraviolet Biologically Effective radiation (UVBE) calculated according to action spectra such as that for Erythema ($UVBE_{ERYTH}$, [28]) and that for the Generalized Plant Damage ($UVBE_{GPD}$, [29]). Unfortunately, AC SAF does not provide the solar UVBE daily dose calculated according to the ~~using the~~ biological weighting function for the induction of flavonols in *Mesembryanthemum crystallinum* ($UVBE_{flav}$) obtained by modelling the plant response to different cut-off filtered radiation between 280 and 360 nm [30] and considered more appropriate to evaluate the flavonol response in this study.

The STAR (System for Transfer of Atmospheric Radiation) model [31] was used to simulate clear sky solar spectral radiation at 30 min-time step intervals for the experimental site [32]. The resulting solar emission spectrum on Nov 1st 2015 under clear sky conditions is reported in Fig. S1. From the sun spectra available during the September-November 2015 period, the daily $UVBE_{GPD}$ and $UVBE_{flav}$ were calculated and their interrelationship under clear sky was defined. This was then used to estimate the solar $UVBE_{flav}$ over the experimental period from the $UVBE_{GPD}$ values obtained by AC SAF, in order to take into account the possible cloud attenuation.

The daily average values of UVB, UVA and $UVBE_{flav}$ doses are reported in Fig. S3 of online Supplementary data.

2.5. Chromatographic determination of leaf flavonols

The method based on RP-HPLC with UV and MS detections ~~was~~ used for the quantitative and qualitative determination of flavonols in freeze-dried cabbage leaves after acid hydrolysis of parent glycosides i~~a~~s described elsewhere [15]. The freeze-dried cabbage powders (60 mg) were extracted with the use of 1 mL of methanol/water (7:3, v/v). The extracts were sonicated (10 min), centrifuged (3000 rpm, 15 min) and clear supernatants collected. The extraction procedure was

repeated with a new portion of solvent (1 mL). The supernatants from two repeated extractions were combined. The portions of final extracts (1 mL) were used for acidic hydrolysis (90 °C for 120 min) by hydrochloric acid (3.233 M, 0.59 mL). The obtained hydrolysates were filtered through a 0.45 μm Millex HV filter (Merck Millipore, Warsaw, Poland) and immediately injected (20 μL) to HPLC-DAD-ESI-MS (1200 series with 6130 Quadrupole, Agilent Technologies, Santa Clara, CA). Phenomenex Kinetex XB-C18 100A column (150 mm x 4.6 mm, 5 μm) was used for chromatographic separation. Gradient elution was carried out using a mobile phase consisting of 0.1% formic acid in water (solvent A) and 0.1% formic acid in methanol (solvent B). The HPLC system was operated at flow-rate of 1 mL/min. The elution gradient profile used was 10-100% B in 30 min, followed by 5 min 100% B. The column was allowed to equilibrate between the injections for 5 min with the initial composition of mobile phase. Absorbance spectra were recorded between 190 and 700 nm, while the chromatograms were monitored at 350 nm. MS parameters were as follows: capillary voltage, 3000 V; fragmentor, 120 V; drying gas temperature, 350 °C; gas flow (N_2), 12 L/min; nebulizer pressure, 35 psig. The instrument was operated both in positive and negative ion modes, scanning from m/z 100 to 1200. The peaks were tentatively identified by comparison of UV and MS spectra with those for standards and literature data.

Total flavonols content in dry weight of cabbage leaves was calculated as a sum of aglycones and flavonol monoglycosides remaining after hydrolysis. The quercetin and kaempferol derivatives were quantitated based on external standards of respectively quercetin and kaempferol, respectively (Sigma Chemical Co., St. Louis, MO).

2.6. Data analysis

Statistical analysis and curve fitting were carried out with the SigmaPlot for Windows Version 12.5 software (Systat Software, Inc., San Jose, CA). Mean values of data underwent t-test or One Way Repeated Measures ANOVA and were compared by the All Pairwise Multiple Comparison Holm-Sidak Test. P-values <0.05 were considered statistically significant.

3. Results and Discussion

3.1 UVB lamp irradiation of waste cabbage leaves

In Figure 1, the flavonol FLAV index of harvested cabbage leaves monitored before and during storage with or without the UVB lamp treatment is reported. At harvest, the adaxial (AD) apical part of leaves showed FLAV values almost double that the abaxial (AB) apical part indicating that AD sides were more exposed to sunlight than AB sides (Fig. 1A).

The higher variability of FLAV observed on the AB leaf apical parts than the AD ones before the UVB lamp treatment, was likely due to their more or less folded orientation towards the sun.

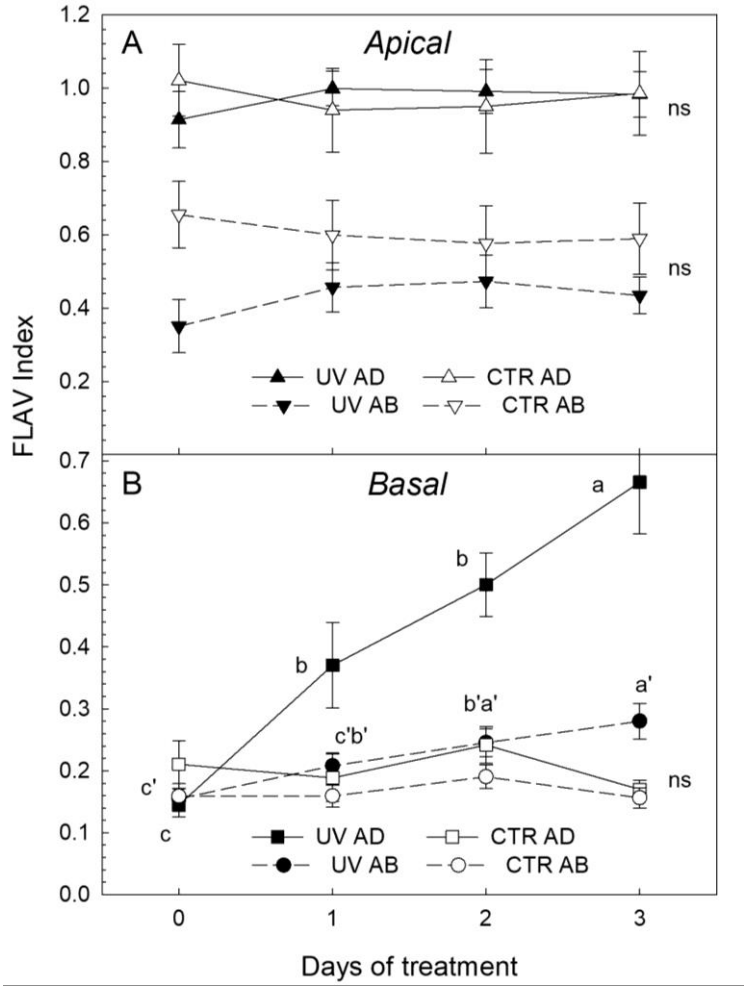


Figure 1. Kinetics of the FLAV index (mean ± SE, n=12) measured during storage of detached cabbage leaves with (closed symbols) or without (open symbols) the UVB lamp treatment (12 h d^{-1}) on the apical (A) and basal (B) leaf portion. In B, values of UV AD (adaxial leaf side) and UV AB

(abaxial leaf side) followed by a different letter and primed letter, respectively, are significantly different ($P<0.05$) according to the Holm-Sidak Test, ns = not significant.

Yet, on the basal part of leaves the lowest values of FLAV (around 015-0.2) were recorded (Fig. 1B). The difference in the FLAV index between apical and basal leaf zones is in agreement with the cabbage leaf longitudinal gradient of indices observed before [15].

During storage, no significant changes in FLAV were observed on the apical part of both UV~~B~~ treated and untreated leaves ($P>0.5$, one way Repeated Measures ANOVA). Instead, a marked increase in FLAV ($P <0.001$) was found on the basal AD sides of leaves (Fig. 1B). The increase in FLAV with UVB energy dose was linear with a rate of 0.0102 Dualex units per kJ m^{-2} (considering an average daily UVB dose of 16.6 kJ m^{-2}).

A slighter but still significant ($P<0.001$) increase in FLAV with UV~~B~~ treatment was also observed on the basal AB side of treated leaves, while flavonols content in controls did not change with storage on either leaf sides (Fig. 1B). A possible explanation for the enhancement of FLAV in the AB non-irradiated leaf surface in given below in the 3.5 section.

The HPLC analysis of leaf methanolic extracts confirmed that flavonols were the major group of phenolic compounds in cabbage leaves, as previously observed [15].

The chromatograms of apical and basal leaf extracts recorded at 350 nm shown in Figure 2 indicate the flavonols profile found in the UV-treated and control samples.

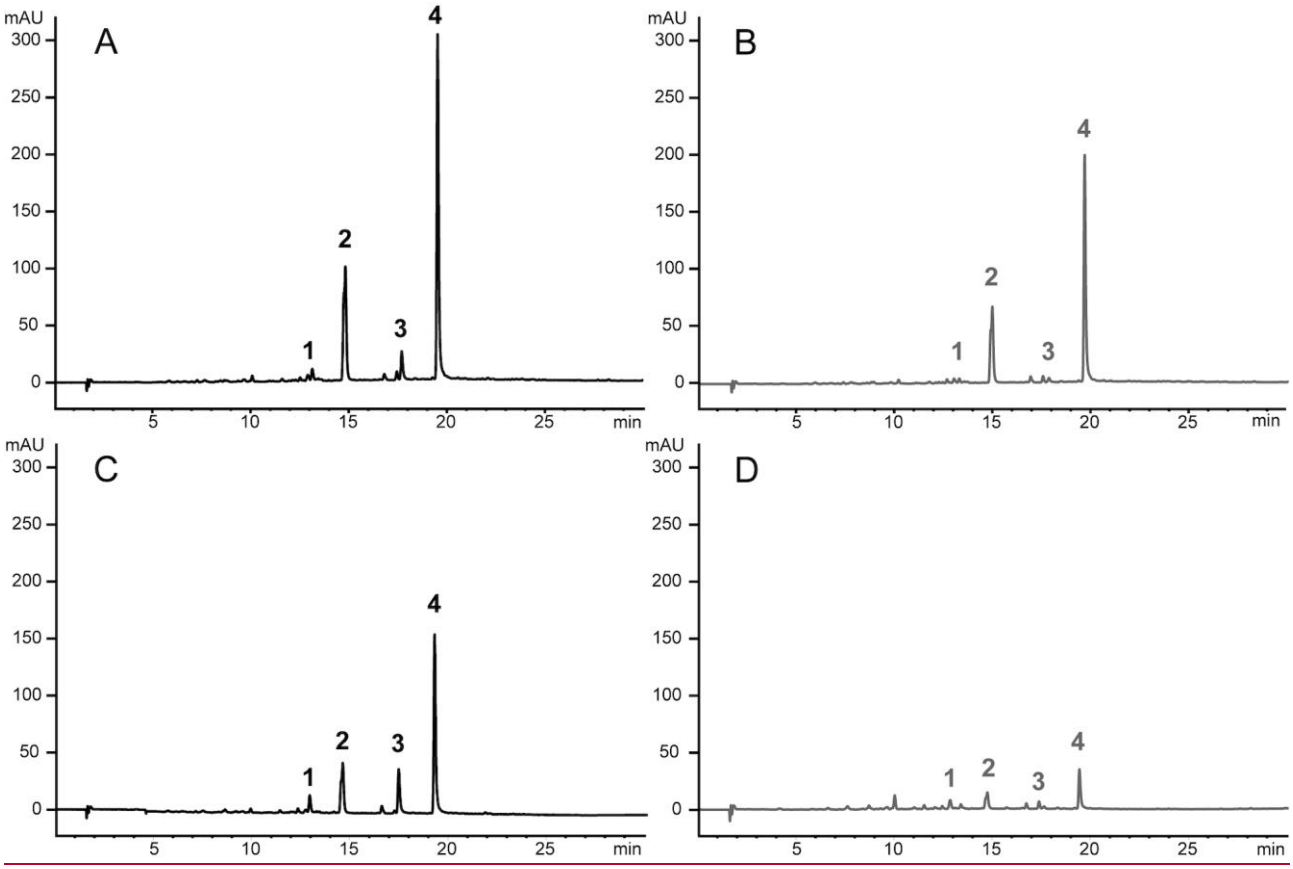


Figure 2. High performance liquid chromatography profiles registered at 350 nm of the flavonols liberated by acid hydrolysis from crude extracts of apical (A, B) and basal (C, D) portion of cabbage leaves stored with (A, C) and without (B, D) the UVB treatment. The four flavonols were identified as 1 quercetin glycoside, 2 kaempferol glycoside, 3 quercetin, and 4 kaempferol.

The identity of compounds remaining after acid hydrolysis of parent flavonols was confirmed based on MS fragmentation pattern characterized by major molecular ion peak, that in the case of positive ionization occurs as $[M+H]^+$ and in negative ionization as $[M-H]^-$. The presence of two flavonol aglycons: quercetin (303 Da $[M+H]^+$, 301 Da $[M-H]^-$) and kaempferol (287 Da $[M+H]^+$, 285 Da $[M-H]^-$) and two flavonol monoglycosides: quercetin glycoside (465 Da $[M+H]^+$, 463 Da $[M-H]^-$) and kaempferol glycoside (449 Da $[M+H]^+$, 447 Da $[M-H]^-$) was observed in cabbage hydrolysates.

The total content of flavonols in dry weight of cabbage leaves was calculated as a sum of these four compounds and with the use of calibration curves generated for standards of quercetin and kaempferol.

The flavonol content determined by the destructive (chromatographic) analysis in samples stored for three days with or without the UVB lamp radiation is reported in Fig. 3~~2~~. It showed that the ~~UVB~~ treatment increased the flavonol content compared to controls on both leaf zones, by 42% and 136% on the apical and basal leaf parts, respectively. On average, the flavonol content of cabbage leaves after 3-days of UVB lamp irradiation (at about 0.38 W/m^2) rose to 4.61 mg g^{-1} DW, and to 2.91 mg g^{-1} DW in untreated leaves.

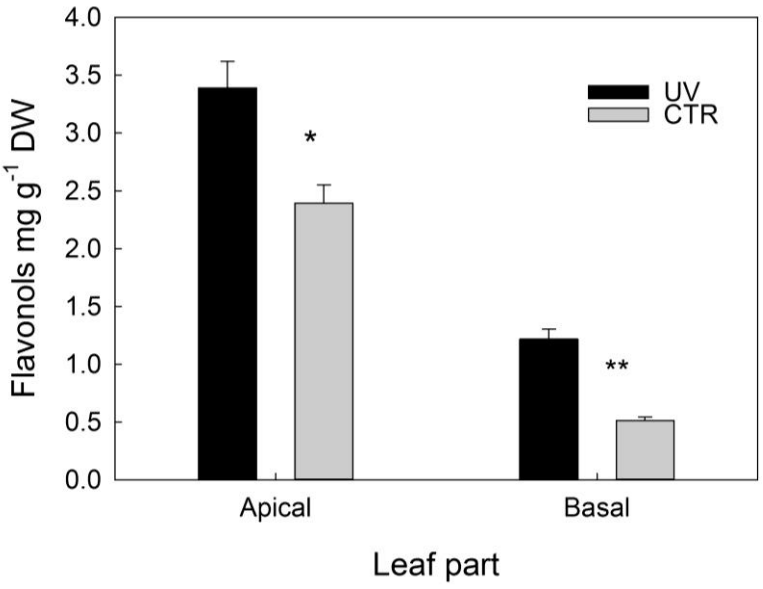


Figure 3~~2~~. Leaf flavonols concentration (mean ± SE, n=12) as determined by HPLC on extracts from the a~~A~~pical and b~~B~~asal parts of detached leaves stored for three days with (black bars, UV) or without (gray bars, CTR) the 12 h d^{-1} UVB lamp treatment. The t-test gave a significant difference of *P=0.002, **P<0.001.

While the results obtained by the HPLC analysis for the basal leaf parts agree with those observed by the Dualex sensor, the FLAV index failed to reveal the increase in the leaf flavonol content on the apical leaf part though it could be measured by HPLC following the leaves destruction.

This discrepancy suggests that the UVB lamp-induced accumulation of flavonols is not limited to the leaf outer layers but occurs also in the mesophyll. The amount of flavonols localized in the palisade and spongy tissues cannot be measured, by definition, by the Dualex, while they are quantified by the destructive analysis of the whole leaf extracts.

It is noteworthy that the FLAV index detected an increase in flavonols on the AB epidermis of basal leaf parts even if these were not directly exposed to the UV radiation. It is evident that a UV-induced stress signaling occurs through all the leaf tissue layers.

Postharvest UV irradiation effects on different plant materials were previously reported. The UVB broadband lamp treatment of tomato fruits at the dose of about 6 kJm^{-2} per day (1.69 Wm^{-2}) for 10 or 18 days, depending on the ripening stage at harvest, increased the level of ascorbic acid, carotenoids [33] and polyphenols [34]. As little as 3 min of 0.43 Wm^{-2} of $UVBE_{GPD}$ was sufficient to significantly increase the level of secondary metabolites in the flavedo of postharvest lemons [35].

Even a short UVB lamp irradiation of 5 min for 3 days inside a refrigerator induced the increment of flavonoids in fresh detached leaves of spinach, radish and parsley during the consecutive 3 days of storage [36]. This was related to the expression enhancement by UVB of all the genes involved in the flavonoid biosynthesis that reached its maximum at 12 h after irradiation. A similar delayed response to low-dosage UVB lamp pre-harvest treatment in increasing phenolic compounds during storage was observed in *Ocimum basilicum* [37].

In the *Brassica* species, an increase of flavonols in broccoli (*Brassica oleracea* L. var. *italica*) flower buds irradiated postharvest with a combination of visible light (19 µmol m^{-2} s^{-1}) and low-dose UVB (0.23 W m^{-2}) broadband lamp was observed by both destructive and non-destructive tools [26]. While Harbaum-Piayda et al. (2016) reported the induction by a low-dosage UVB lamp of quercetin-tri-glycoside in white cabbage heads.

Our results showed that the increase of flavonols induced by the postharvest UVB lamp treatment on the basal part, not exposed to sun, of waste leaves was 0.7 mg/g DW, somewhat higher than that

observed by Harbaum-Piayda et al. [21] in cabbage head leaves by a similar treatment. The difference can be due to the higher storage temperature (17 °C vs 4°C) used in the present study and/or the lack of supplemental white light in the previous study. In fact, maintaining the light/dark cycle during postharvest storage of Brassicaceae is important to preserve quality and phytochemicals [38].

Recently, a study related to the present one with postharvest UVB (1.4 W m^{-2}) broadband lamp in combination with white light treatments of leaf waste fractions from industrial trimming of white cabbage has showed an increased flavonol level of about 0.5 mg g^{-1} DW compared to untreated controls (Seljåsen and Vågen 2020, personal communication). Treatment with UV~~B~~ alone resulted in the formation of just half the amount of flavonols compared to treatments where UV~~B~~ was combined with white light. Interestingly, increasing the UVB dose (by 1.7 time) in combination with white light did not enhance the flavonol concentration.

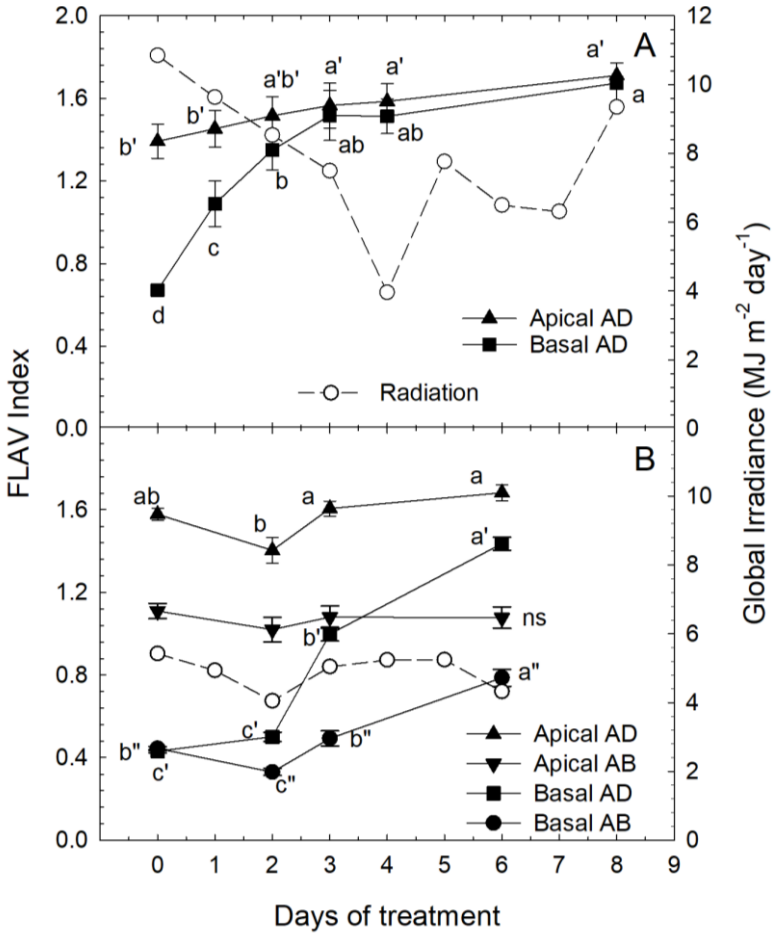


Figure 4~~3~~. Evolution of the mean (±SE) FLAV index in detached cabbage leaves exposed to sunlight from Sep 22 to Sep 30 (A, each point n=4) and from Oct 27 to Nov 2, 2015 (B, each point n=13-24). For each leaf part/side, values followed by a different letter are significantly different ($P<0.05$) according to the Holm-Sidak Test, ns = not significant.

All the above observations are ~~explained~~ consistent with ~~by~~ the UV-induced increase of the activity of phenylalanine ammonia-lyase and of other enzymes participating in the flavonoid synthesis such as chalcone synthase, chalcone isomerase and dihydroflavonol-4-reductase, as explained later (section 3.5). This process must take place in post-harvest the same way it occurs during pre-harvest UV treatments of plants [39].

3.2 Sun-exposure of waste cabbage leaves

Figure 43 reports the effect of sun irradiation on the FLAV index of detached cabbage leaves during two different periods, end of Sep (Fig. 43A) and end of Oct (Fig. 43B). In both cases, a large increase of flavonols in the basal leaf part was observed, while the apical part of leaves was only slightly affected by the irradiation. This result was expected since before harvest the apical parts of leaves were already acclimated to sunlight while the basal parts were sun-screened by the closest other leaves and heads.

As observed in the UVB lamp experiment, the FLAV index significantly increased even in the AB epidermis of basal leaf parts not directly exposed to sunlight, evoking again the occurrence of a stress signaling through the leaf tissues (see section 3.5).

The trial was repeated on leaves maintained attached to the plant after removing the cabbage heads to increase leaf irradiance (Fig. S2 of Supplementary data). In Fig. 54, the temporal average change of the leaf FLAV index from the start of exposure up to 3 weeks is reported. The flavonols content in the apical leaf part slightly decreased at 6 days of treatment but recovered the initial value at 10 days after treatment and maintained it for the next 11 days. On the leaf basal part, a continuous increase of flavonols was observed. This observation may suggest a saturation of plant capability of secondary polyphenolic metabolites biosynthesis in the sun exposed apical part of the leaves.

The effect of the different solar irradiation treatments was evaluated by comparing the initial rate of FLAV enhancement on the AD basal leaf parts. The highest slope of the linear phase of the FLAV evolution was observed for detached leaves irradiated at the end of September (0.28 Dualex units day^{-1}), while at the end of October, still on detached leaves, it was 0.18 Dualex units day^{-1}. The slowest in field treatment effect was recorded on the attached leaves at the end of October (0.09 Dualex units day^{-1}). These differences are consistent with the difference in the average global, UVA and UVB irradiance observed during the relative periods: 22-25 Sep, 20-26 Oct and 27 Oct-2 Nov, 2015, as reported in Table 1. Moreover, attached leaves may have been oriented in a way to reduce full exposure to sunlight.

A linear direct regression between the FLAV accumulation rate and any of the daily radiation doses was observed, however, the correlation was better for the sun global irradiance (R^2=0.973), followed by the UVA irradiance (R^2=0.965). The less accurate correlation (R^2= 0.899) was found for the UVB irradiance.

Accordingly, the FLAV rate correlated to $UVBE_{flav}$ similarly to UVA and better than to UVB doses (Table 1). This is because the action spectrum used in calculating $UVBE_{flav}$ attributes a biological effect, in inducing the synthesis of flavonoids, not only to the UVB, but also to the UVA component of solar radiation.

Table 1. Average daily sun radiation doses and initial rate of FLAV enhancement on the AD basal leaf parts.

2015 periods	Global MJ m^{-2} day^{-1}	UVA kJ m^{-2} day^{-1}	UVB kJ m^{-2} day^{-1}	$UVBE_{flav}$ kJ m^{-2} day^{-1}	FLAV rate Dualex units day^{-1}
22-25 Sep	9.1	646	10.7	5.05	0.28
20-26 Oct	2.7	215	2.4	1.20	0.09
27 Oct - 2 Nov	4.8	347	3.9	2.18	0.18
$R^{2,a}$	0.973	0.965	0.899	0.94	

[a]Coefficient of determination for the regression between the FLAV rate and the radiation dose of each column.

Our results indicate that detached leaves maintain the mechanisms to activate the biosynthesis of secondary metabolites in response to the sunlight irradiation, as it occurs in intact plants. The main difference in this effect between attached and detached leaves was likely due to the different sun irradiance of treatments that determined the rate of the process. At longer irradiation times, in fact, the extent of flavonol accumulation in both attached and detached leaves was similar (compare Figs. 5~~4~~ and 4~~3~~).

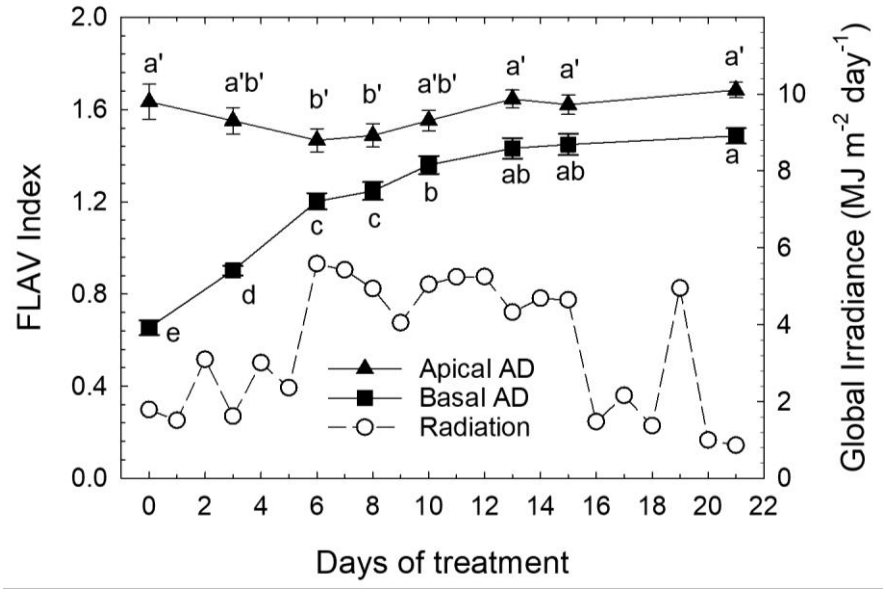


Figure 5~~4~~. Evolution from Oct 20th 2015 of the mean (±SE) FLAV index in cabbage adaxial leaves attached to the plant exposed to sunlight after head removal. Each point is the average of 24 different samples. For Basal and Apical samples, values followed by a different letter are significantly different (P<0.05) according to the Holm-Sidak Test.

The destructive HPLC analysis confirmed the effect of solar irradiation in increasing the flavonol content of waste cabbage leaves estimated by the Dualex sensor, mainly in the basal part (Table 2). The mean flavonol content in the apical part of irradiated detached leaves was 1.4 times higher than that of non-irradiated leaves. In the basal part, flavonol levels increased 4.1 times after irradiation. On average, the total flavonol content of cabbage leaves after 6-days of sun irradiation (at the end of October) increased to 5.0~~3~~2 mg g^{-1} DW with respect to 2.7~~7~~6 mg g^{-1} DW of untreated leaves. This effect was markedly similar to that obtained by exposing leaves to the artificial UVB lamp radiation under storage (Fig. 3~~2~~).

When cabbage is harvested in the field, a number of leaves surrounding the cabbage head will normally be left intact on the plant. A practical inference of our study is that these leaves, when left in the field for a time, will accumulate flavonols, and will represent a valuable valorized source of nutraceuticals.

Table 2. Leaf flavonols content (mean ± SE), mg/g DW, as determined by HPLC on extracts from the Apical and Basal parts of detached leaves exposed for 6 days to sunlight from Oct 27 to Nov 2, 2015 (n=9). Values are compared with those determined on just harvested not exposed leaves (n=3).

Leaf part	Postharvest sun-exposure (6-days)		At harvest
Apical	3.27 ± 0.14	*	2.34 ± 0.09
Basal	1.75 ± 0.07	**	0.42 ± 0.04

The t-test gave a significant difference between exposed and not exposed leaves, apical *(P=0.004) and basal **(P<0.001) values.

3.3 Comparison of treatments

The in field sun-induced increase rate in leaf FLAV under postharvest at the end of Oct 2015 on detached leaves was similar to that induced by the UVB lamp radiation under storage, 0.18 versus 0.17 Dualex units day^{-1} on the AD leaf basal zones. However, the proper comparison of the efficiency of the two photochemical treatments must be fulfilled considering the intensity and spectral emission of the irradiation sources.

The daily doses of the UV lamp were 16.6 kJ m^{-2} and 25 kJ m^{-2}, for UVB and UVA, respectively, that is about 1.6 times higher and 9 times lower than the maximal UVB and the minimal UVA amount of radiation, respectively, seen by the cabbage leaves during the sun treatments (see Table 1). The emission spectrum of the UV lamp was, of course, much different from that of the sun, especially late in the season at the latitude of the experimental site (Fig. S1 of the Supplementary material). The UVA/UVB ratio was 1.5 and around 89 for the UVB lamp and the Oct sun radiation, respectively. Therefore, their effectiveness in producing leaf flavonols should be evaluated by weighting the emission spectra by the action spectrum for the synthesis of flavonols in plants. For this, we used the $UVBE_{flav}$ biological weighting function defined by Idbah et al. [30] for the production of mesembryanthin, the main flavonol in *M. crystallinum*. The resulting $UVBE_{flav}$ daily dose for the UV lamp was 9.12 kJ m^{-2}, that is 80% higher than the maximal $UVBE_{flav}$ dose received

by the leaves during the sun-treated trials (5.05 kJ m^{-2}, Table 1). According to the linear regression between the FLAV rate and the sun $UVBE_{flav}$ (Table 1), we expected a FLAV rate induced by the UVB lamp about 2.8 folds higher than the 0.17 Dualex units day^{-1} actually observed. This discrepancy can be explained by the fact that the biological weighting function used does not properly take into account the role of solar UVA and visible radiation in the synthesis of the specific flavonols of cabbage. Yet, it has been shown that UVB radiation is not a prerequisite for the biosynthesis of flavonoids [22,40], and that blue light can be an inducer of quercetin and kaempferol compounds ~~even more~~as effective ~~than~~ as UVB radiation [41].

Our results, then, revealed the significant role for solar UVA and visible radiation in the regulation of the accumulation of flavonoids in cabbage leaves.

Although the sun photochemical treatment of waste leaves is largely dependent on the weather condition at cabbage harvest time, it could be considered superior to the UVB lamp treatment, because of it larger efficiency and sustainability.

3.4 Effect of leaf detaching on polyphenols

Additional stresses faced by waste leaves as cutting and desiccation [42] may have induced a further raise of polyphenols over that caused by UV and sunlight irradiation alone.

Wounding of fresh produce may elicit an increase in antioxidant capacity and phenolic compounds, however, this effect is markedly species-dependent [43]. In the case of short-term storage of broccoli, some contradictory data are reported into the literature. Total flavonoid content of five broccoli cultivars were observed to decrease during three days of storage of florets at 20°C in polyethylene bags [44]. While, Starzyńska et al. [45] found that short-term storage of broccoli heads at room temperature caused the significant increase of antioxidant activity and the accumulation of phenylopropanoids and flavonoids. The accumulation of phenolic compound during storage can also depend on the day time of broccoli harvest [46].

In white cabbage, cutting produced the increase of PAL-activity in detached leaves, but it had no significant effect on the total soluble phenolics during two days of storage at 15 °C [43].

No change in flavonols was observed in white cabbage heads stored for 4 months at 1-2ºC and at 80-85% of relative humidity [47].

The actual amount of phenolic compounds measured in plant material results from the balance between their synthesis and utilization rates. In this equilibrium, a fundamental role is played by the plant primary antioxidants, i.e., antioxidant enzymes and low molecular-weight compounds, such as ascorbic acid and glutathione, that constitute the first line of protection against oxidative stress. Generally, the primary antioxidants are able to detoxify the reactive oxygen species (ROS) produced under mild to moderate stresses, but they may result not enough to face severe or multiple stresses [48].

The consequent depletion of antioxidant enzymes can activate the biosynthesis of secondary antioxidants, including flavonoids [49]. Indeed, it was observed that vegetables with lower or higher levels of ascorbic acid were associated to an increase or reduction, respectively, of phenolic content after wounding [43].

Accordingly, we can expect that in white cabbage detached leaves studied here, the rates of phenolic synthesis and decrease due to mechanical injuries would be similar and that the increase of the flavonols production was induced when the irradiation stress was added.

Monitoring the leaf chlorophyll content can be considered a good indicator of early postharvest senescence [43,45]. In the studied cabbage samples, Chl evolution on the very same leaves was controlled non-destructively by the Dualex sensor, as reported in Table 3.

From the previously defined calibration curve for the Dualex sensor [15], the Chl concentration at harvest for the different trials was ranging between 81-113 and 15-19 µg cm^{-2} for the apical and basal parts of leaves, respectively.

The index of Chl of the basal leaf parts showed no significant changes during the irradiation treatments (Table 3). A small decrease in the apical leaf part Chl was only observed between time 0

and 3 days of sunlight irradiation at the end of September 2015 (P=0.024). A significant decrease of Chl was instead found in the apical part of leaves stored with or without the UVB lamp radiation in the 2016 experiment.

Table 3. Leaf Chl content (Dualex units) estimated non-destructively by the optical sensor on cabbage leaves under the different irradiation treatments.

	Sun-exposure				Storage Nov 7-10, 2016			
	Sep 22-30, 2015		Oct 27 – Nov 2, 2015		Under UVB		Without UVB	
Days of treatment	leaf part		leaf part		leaf part		leaf part	
	Apical	Basal	Apical	Basal	Apical	Basal	Apical	Basal
0	66.1±1.8a	14.8±2.6	61.5±1.3	14.8±1.3	54.5±2.0a	16.4±1.8	58.9±1.9a	17.7±1.4
1	59.8±1.9ab	13.0±1.5			56.6±2.1a	17.6±1.6	57.0±1.9ab	16.6±1.7
2	61.3±1.0ab	14.4±1.9	57.5±2.0	16.9±1.4	46.4±2.3b	13.9±1.0	49.8±2.8bc	18.4±1.2
3	57.7±0.8b	11.9±1.1	59.4±1.2	16.2±1.2	37.9±2.4c	16.7±1.4	47.2±2.7c	15.6±1.2
4	60.0±1.4ab	12.8±1.4						
6			57.4±1.1	12.4±0.9				
8	59.8±2.3ab	18.8±2.6						
P*	0.032	0.207	0.126	0.072	<0.001	0.324	0.002	0.495

*Probability of the ANOVA test. For each column, values followed by a different letter are significantly different (P<0.05) according to the Holm-Sidak Test.

These results suggest that there is not a direct correlation between the early postharvest senescence induced by leaf detaching and dehydration and the increase of the FLAV index (compare Figs 1,~~4~~3 and Table 3), confirming the main role of irradiation in determining the accumulation of flavonols compounds. On the other hand, during the 2015 open-field trials on detached leaves the average air temperature was ranging between 11.9 and 13.1 °C at the end of September and between 10.7 and 11.1 °C at the end of October, while relative humidity was always higher than 70%. Therefore, under these conditions leaf desiccation could have been rather limited, with a minimal effect on the production of secondary metabolites.

3.5 Mechanisms of photochemically-induced flavonols accumulation in waste leaves

A schematic representation of the mechanisms involved in the photo-induced accumulation of flavonols in the basal part of cabbage leaves is reported in Fig.6.

It is widely known that plants respond to stressful UV radiation activating protective mechanisms that combine the accumulation of UV-absorbing compounds in the outer epidermal layers and the enhancement of leaf antioxidants.

In the UVB range, the plant response is mediated by the UVR8 (UV RESISTANCE LOCUS 8) photoreceptor that absorbs radiation by mean of a tryptophan chromophore and then interacts with the CONSTITUTIVE PHOTOMORPHOGENIC 1 (CROP1) regulator, leading to the change of gene expression [50]. Besides controlling plant development, this process regulates the leaf accumulation of UV-B-absorbing flavonols.

The UVB-induced expression of genes encoding the key enzymes of the flavonol biosynthesis included PAL, phenylalanine ammonium lyase; C4H, cinnamate-4-hydroxylase; 4CL, 4-coumarate:CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3'H, flavonoid 3'-hydroxylase; FLS, flavonol synthase and UGT, UDP-dependent glycosyltransferase [50]. This process leads to a significant increase of flavonols in the adaxial epidermis.

UV-induced gene expression could be transferred to deeper leaf tissues, not directly illuminated, by the long-distance movement of microRNAs via the vasculature of the plant [51].

This transmitted signaling can be responsible for the production of a certain amount of flavonols in the abaxial epidermis, as shown by the FLAV index in Figs 1B and 4B.

A similar effect was observed by Bidel et al. [52] when the AD sides of *Centella asiatica* leaves cultivated in a growth chamber were exposed to UVB lamp or full sun radiation. They also observed an increase of flavonols in the mesophyll, by selective leaf tissue extractions, and a FLAV increase in the half leaf adjacent to the irradiated one and protected by an UVB filter [52].

Our and these evidences suggest that a systemic translocation of macromolecules [53] could determine the synthesis of flavonols into the deeper leaf tissues starting from the adaxial UVB initiated signaling. A direct effect of the radiation penetrating through the palisade and spongy cell layers is unlikely since the diffusion of both UVB and UVA wavelengths is limited to the first few tenths of micron of leaf [54].

Definition of the mechanisms of the phenylpropanoid biosynthetic pathway activation by solar radiation is more complex due to the presence of multispectral band effects.

The UVA radiation induction of leaf flavonoids is less studied than that of UVB radiation. UVA effects has been properly reviewed by Verdaguer et al. [55], highlighting contrasting changes and large variability according to the species, compound specificity, doses of radiation and co-presence of UVB radiation.

In field sun spectral band-exclusion experiments have clearly shown the positive contribution of UVA and blue radiation in inducing leaf flavonols accumulation [41,56,57].

Both UVA and blue radiation are involved in the expression of flavonoid biosynthesis genes and phenolic accumulation in leaves mediated by the cryptochromes (CRYs) photoreceptors [41,58]. This is likely to occur through the interaction between CRYs and UVR8, favored by partially overlapped absorption spectra, and their binding to COP1 [40].

Considering also that the UVA/UVB and Blue/UVB ratios of solar radiation under our experimental conditions, accounting for about 89 and 260, respectively, were much larger than those in the UVB lamp (UVA/UVB = 1.5 and Blue/UVB = 0.48), the above proposed mechanism can explain the higher efficiency of the sun photochemical treatment with respect to the UVB lamp in enhancing flavonols in waste cabbage leaves.

Additionally, because of the large number of evidences in favor to the antioxidant role of dihydroxy-B-ring substituted flavonoids (quercetin, luteolin) [59], it is possible that their biosynthesis be regulated by excess light-induced oxidative damage, irrespective of the proportion of solar wavelengths reaching the leaf [60].

Since the irradiation treatment had similar effects on stalk-attached and detached leaves, we can assume that the processes depicted in Fig. 6 occur both in on-plant leaves and postharvest. Indeed, flavonols were increased by an UVB lamp treatment in detached Ginkgo biloba leaves [61]. Yet, the UVB-induced rise of the CHS and PAL enzyme activity was observed in detached basil leaves [62] and harvested carrot roots [63], respectively.

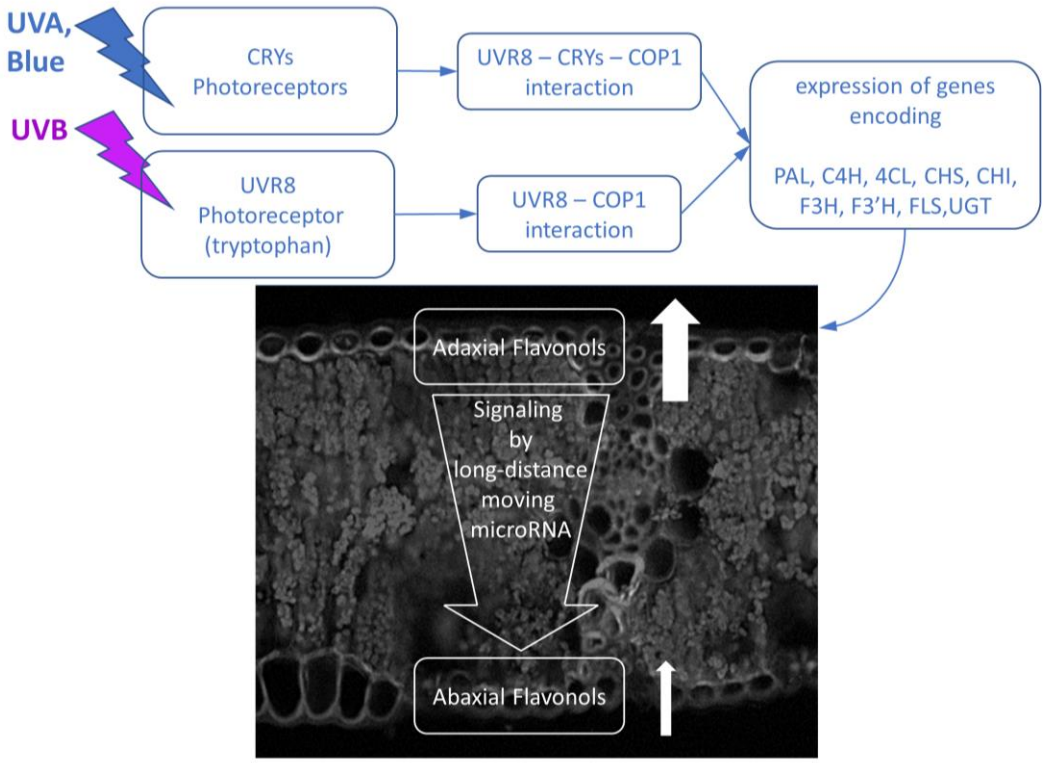


Figure 6. Schematic representation of the photochemically-induced mechanism of flavonols accumulation in the waste cabbage leaves. Abbreviations: UVR8: UV RESISTANCE LOCUS 8; CROP1: CONSTITUTIVE PHOTOMORPHOGENIC 1; CRYs: Cryptochromes; PAL: phenylalanine ammonium lyase; C4H: cinnamate-4-hydroxylase; 4CL: 4-coumarate:CoA ligase; CHS: chalcone synthase; CHI: chalcone isomerase; F3H: flavanone 3-hydroxylase; F3'H: flavonoid 3'-hydroxylase; FLS: flavonol synthase; UGT: UDP-dependent glycosyltransferase. The size of the white arrows indicate the extent of the increase in flavonols: higher in the AD and lower in the AB

leaf sides. The image refers to a cross section of a wheat leaf acquired by an epifluorescence microscope.

4. Conclusions

The present work indicated that exposing the left-over cabbage leaves after harvest of heads to sunlight in the field may be proposed as the sustainable method to improve the content of flavonols. Treatments of waste leaves by sunlight or by using an artificial source of UV (40% UVB and 60% UVA) radiation gave similar results regarding the increase in the total flavonols content.

The sunlight exposure at the end of October in Skierniewice (Poland) yielded an increase of 82% in total leaf flavonols, resulting in a significant valorization of cabbage waste. Certainly, the sun photochemical treatment markedly depend on the weather condition at harvest time, nevertheless it has been proved to be more efficient than the UVB lamp treatment, because of the additional contribution from UVA and blue light, and preferred for its sustainability.

Once the protocols of the treatment will be optimized for its application at an industrial level, we believe that this method can be useful as a novel and alternative management of vegetable waste for the recovery of beneficial phytochemicals.

Our study also confirmed the usefulness of a non-destructive device for a rapid control of flavonols accumulation during the treatments. It was fundamental to measure the rates of accumulation and compare different treatments on the very same leaf, no possible with destructive analyses, limiting the biological variability and giving more precise evaluation of the effects.

A significant result of the optical monitoring consisted in proving the induction of the flavonols synthesis in the abaxial unirradiated leaf tissue due to a signaling effect by systemic translocation of macromolecules originating from the adaxial irradiated leaf side.

Measuring the evolution of the FLAV index on the very same leaf samples allowed to evaluate the correlation between flavonols increase and irradiance.

667 The sensor can be also used for the optical sampling of leaf waste to select that with the highest
668 content in bioactive compounds.
669
670 **Acknowledgements**
671 This work was supported by the Daniel and Nina Carasso Foundation, France
672 (www.fondationcarasso.org), the Italian Ministry of Agriculture Food and Forestry Policies
673 (MIPAAF) (www.politicheagricole.it), the National Centre for Research and Development
674 (NCBR), Poland and the Research Council of Norway, through the SUSFOOD I ERA-NET
675 Programme, project SUNNIVA (project no. 29).
676 The authors thanks F. Sabatini (IBE-CNR, Italy) for performing the spectroradiometric calibration
677 of power meter and the AC SAF project of the EUMETSAT for providing surface UV timeseries
678 used in this paper.
679
680 **Funding**
681 This work was developed within the framework of the ERANET SUSFOOD Sunniva project,
682 "Sustainable food production through quality optimized raw material production and processing
683 technologies for premium quality vegetable products and generated by-products", and supported by
684 the Daniel and Nina Carasso Foundation, France (www.fondationcarasso.org), the Italian Ministry
685 of Agriculture Food and Forestry Policies (MIPAAF) (www.politicheagricole.it), and the National
686 Centre for Research and Development (NCBiR), Poland.
687
688 **Conflict Of Interest**
9 The authors declare no conflict of interest.
0
1 **Appendix A. Supplementary Data**
2 Supplementary data to this article can be find online at….

693

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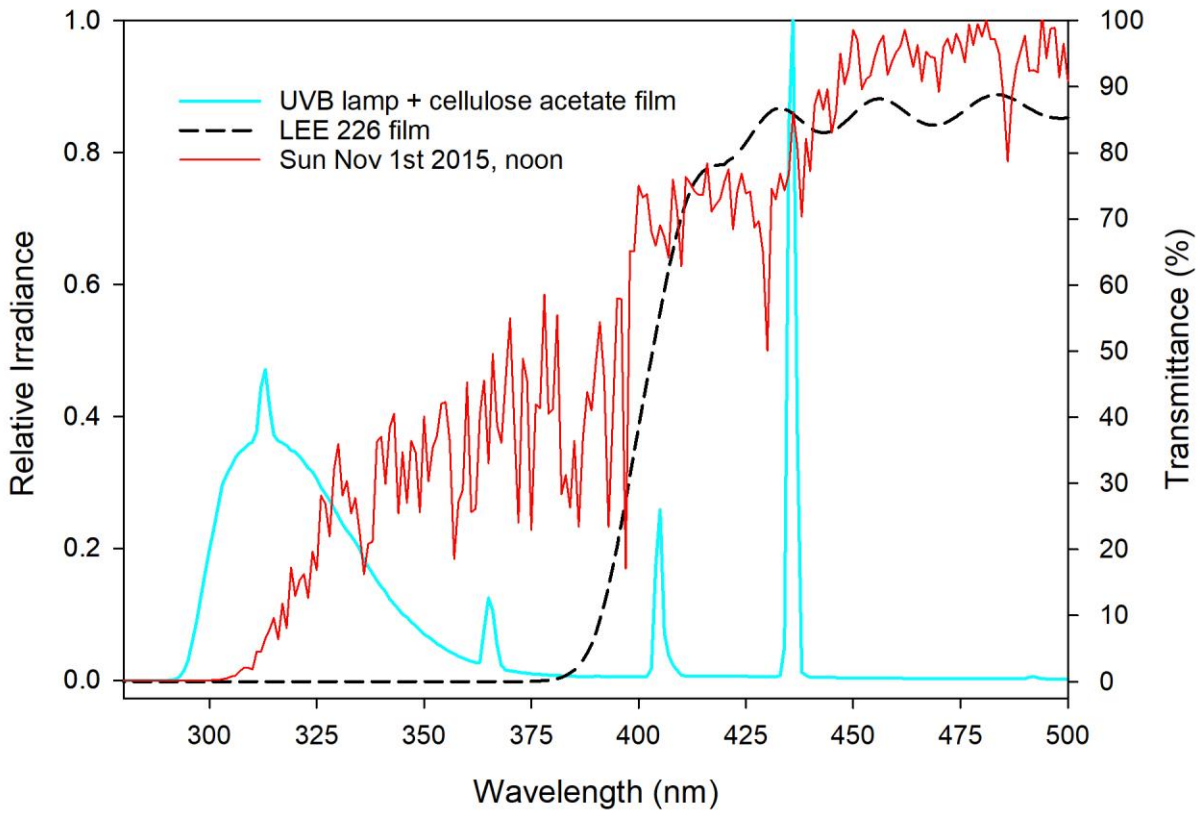
898 **Appendix A. Supplementary data**



899
900
901 Figure S1. Emission spectrum of a UVB lamp filtered by the cellulose acetate film and
902 transmittance spectrum of the LEE 226 film. The solar emission spectrum recorded on Nov 1st 2015
903 is also reported. Emission spectra are normalized to their maximum.
904

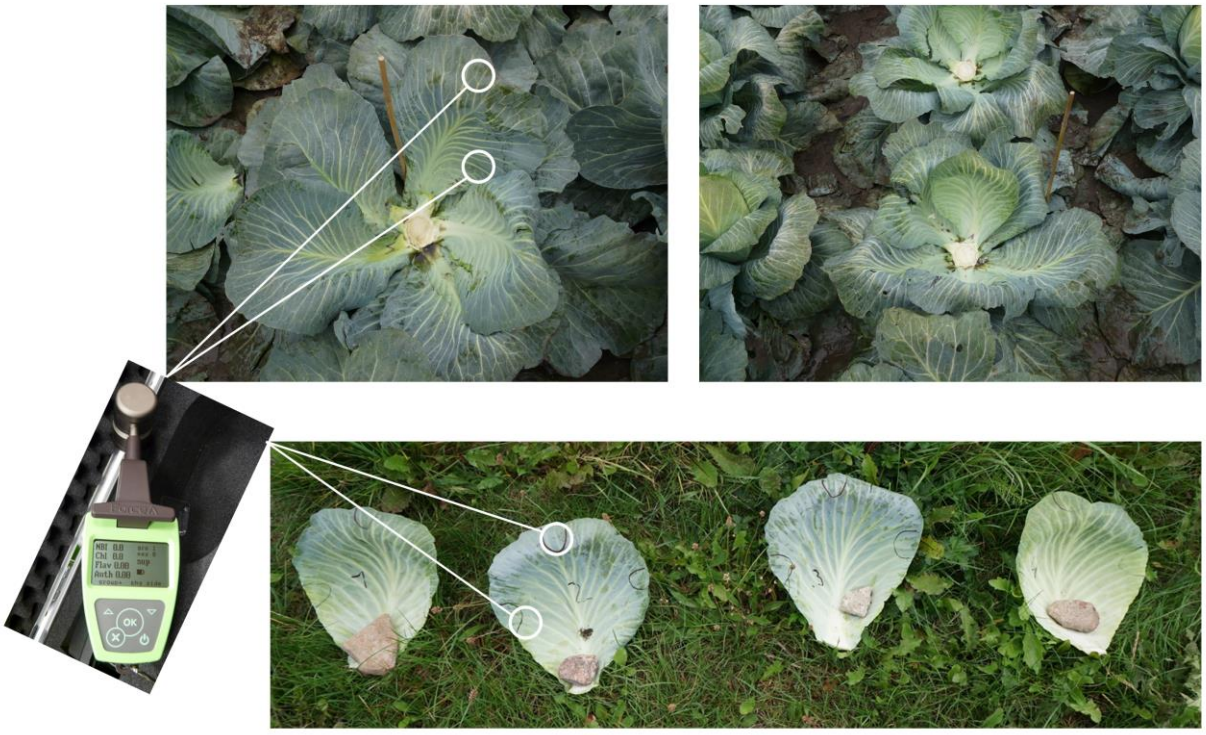


Figure S2. Postharvest sunlight exposure of cabbage leaves, attached to the stalk (top) or detached (bottom), and monitored by the Dualex sensor on the leaf apical and basal parts (Sep-Nov, 2015).

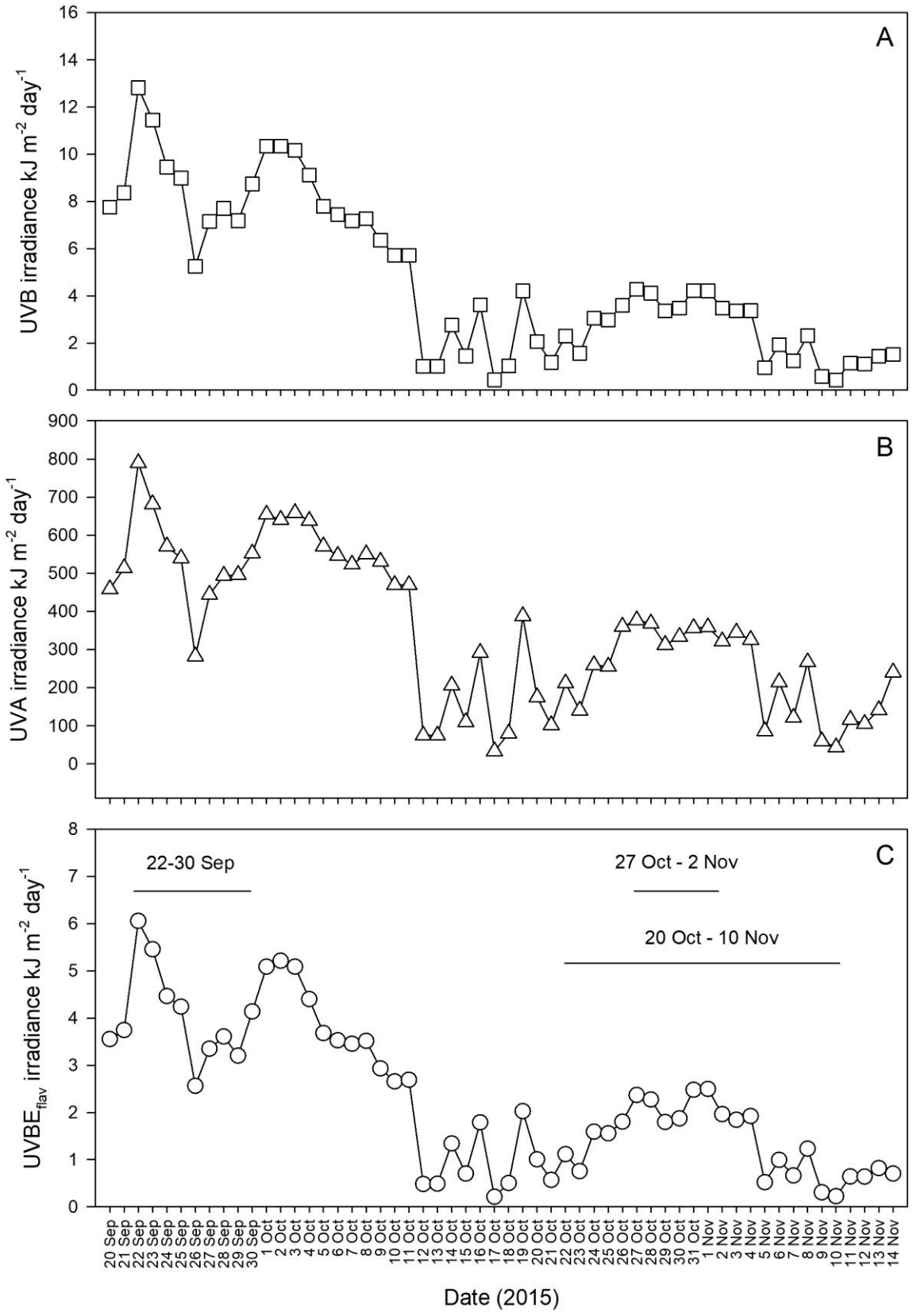


Figure S3. Average daily solar UVB (A) and UVA (B) irradiance and the biologically effective $UVBE_{flav}$ dose calculated using the flavonol induction in *M. crystallinum* weighting function during the Sep-Nov 2015 period.