Research Article

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Effect of different light spectra on the pigmentation of stored elephant garlic

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Abstract

BACKGROUND: In the present study high-brightness light-emitting diodes were used to investigate the influence of different light spectra on garlic discoloration at different humidity levels and temperature. Many processes involved in the discoloration process of garlic/leek during storage under different conditions remain unanswered. For this reason in this study the ability of specific light spectra to enhance the production of desirable pigments has been evaluated in elephant garlic. It is well known that the pigments involved in the discoloration reaction are of great interest because of their potential ability to increase the nutritional value and health benefits of the food.

RESULTS: In the present study, we show how the chlorophyll content of the sprout increases directly proportionally to the wavelength of the light tested; green/blue light delays the greening process of garlic young shoots whilst red/infra-red light irradiance conditions increase the greening process at different storage temperatures and humidity. Moreover different lights in the visible spectrum have been observed to stimulate and enhance the outer layer purple coloration.

CONCLUSION: The use of different lights to modulate garlic pigmentation has been demonstrated and, in particular, the utilisation of red/green/blue lights and lower temperature resulted in higher red/pink pigments production supporting the hypothesis that this process involves more than one light to be fully performed and the low temperature is a condition that influences the formation of these products.

Keywords: *Allium ampeloprasum*; storage; LEDs light; pigmentation; temperature

INTRODUCTION

Bulbous garlic is a perennial plant of the genus *Allium* which also includes onions, shallots, leeks and chives from the Liliaceae family. The most common cultivated garlic species is *Allium sativum*, divided in two sub-species (var. *sativum* and var. *ophioscorodon*) while, recently, another type of garlic, called elephant garlic, has increased in popularity. It is closely related to wild leek and is a separate species, specifically *Allium ampeloprasum* or *scorodoprasum*, which produces a very big bulb. Elephant garlic has been used in this study and, even though it is not considered a true garlic, it will be referred to as 'garlic' in this work as its appearance, consumption, taste and use is more similar to that of the garlic species.

Garlic is present worldwide in the gastronomic traditions of many countries and its production is more than 20 million tons each year. China is by far the largest producer of garlic with more than 75% of the whole world production. The plants are all edible and the leaves and flowers are sometimes eaten but the bulb is the most important part for the market, sliced in parts called cloves. The absence of green shoots in the cloves, called sprouts, is considered an important quality descriptor because they provide an unpleasant taste that persists when garlic is consumed, derived from the presence of chlorophylls. The majority of growers sell their garlic as a fresh harvest at farmers' markets. Only very few companies trade over the winter because it is difficult to deal with sprout prevention and to maintain garlic quality during long-term storage.[1] For this reason it would be economically profitable for growers to be able to sell a consumable product during the season when fresh garlic is mostly unobtainable from the market.[2] Recommendations[2–5] for the long-term storage (over 6 months) of garlic suggest it should be kept from −5 to 0 °C and a relative humidity (RH) between 60% and 80%. However, the conditions used for the storage strongly affect garlic composition and quality (e.g. pigments production, organosulfur compounds and outer part damages or dehydration) and any rapid variations or fluctuations of the environmental parameters will initiate life processes by breaking the dormancy, such as sprouting or rooting.[6,7]

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Is well known that garlic possesses many beneficial properties for human health and has been widely studied for its antioxidant and antimicrobial bioactive effects for numerous treatments, e.g. to reduce the risk of cardiovascular problems and cancer or as an anti-ageing agent,[8–10] and storage temperature is able to condition the product by stimulating the phenolic metabolism-related genes.[11] The final products maintain their activity in several forms, such as dehydrated powder, essential or macerated oil or garlic extract.[12,13] Therefore, the possibility to modulate the organoleptic properties and to stimulate the production of specific compounds beneficial for the human health has attracted a wide range of studies. A recent example of the ability to transform and increase the compounds of garlic is represented by 'black garlic' which was introduced as a healthy food in the Korean market.[14] This product consists of aged garlic that by using specific conditions of high temperature and humidity becomes black because of browning compounds. During this ageing treatment, the garlic loses its pungent flavour, the acrid taste and converts its cloves to a black sweet gelatinous food similar to dry fruit. Black garlic does not cause unpleasant breath and body odour, in particular it exhibits a stronger antioxidant activity and higher phenolic content.[15] The colouring of black garlic is not a proper fermentation process because there are no microorganisms involved but it is dependent on the heating and relative humidity conditions that, between certain ranges, permit non-microbial chemical and biochemical transformations (mainly Maillard reactions) that occur also on dried garlic.[16] The production of reaction compounds can be modulated to the optimum desirable level by adjusting the temperature and the relative humidity levels.

It has been widely reported that during the processing of garlic, onion and leek several examples of discolouration occur, for example the generation of green or blue/green pigments in garlic or pink/red in onion and leek.[17,18] The discolouration phenomena during the manufacturing processes (e.g. powder, juice or puree) in general reduce the product quality and economic value[19] but in some cases, like for the black garlic, the 'colouring' is sought to obtain specific products. Another example of this phenomenon is the Chinese 'laba garlic',[20] a particular type of garlic typical of northern China that after an ageing process at low temperature and a subsequent treatment with vinegar becomes green.[21]

Several studies have shown isoalliin to be one of the primary compounds involved in the pinking of onion/leek and the greening of the outer part of garlic.[18,22–25] It has been hypothesised that hundreds of compounds may be involved in the reactions and pathways necessary for the formation of the pink/green colours, most of them still unknown,[26] and these compounds, despite sharing numerous equivalent characteristics, can vary among different species.[27]

Nevertheless, several studies have been performed regarding garlic/leek storage, although there is a lack of information about the influence of light during long-term preservation. In this context, the aim of this work was to test the influence of different lights on the storage of elephant garlic to investigate the possibility of modulating the production of garlic pigments and maintaining the quality of the product for a prolonged period of time by reducing the sprout greening process. For this purpose different conditions of temperature and humidity have been tested concomitantly with the use of light from high-brightness light-emitting diodes to discriminate the influence of irradiation from specific light regimes on garlic storage.

EXPERIMENTAL

Garlic storage and treatments

Garlic was obtained freshly immediately after the harvest from a grower in Mizumaki town, situated in Fukuoka prefecture, Japan. For the analysis of garlic storage, several intact garlic cloves were placed under different conditions.

Several garlic cloves were stored under different light conditions and examined after a long period of storage. All light intensities and spectra were measured by an illuminance spectrophotometer (CL-500A; Konica Minolta, Osaka, Japan) and regulated at an appropriate distance to furnish the same light irradiance of 15 ± 2.5 W m^{-2}.

Four different experiments were performed. In the first experiment (no. 1) about 15–20 garlic cloves, without the skin-tunic layer (the protective sheaths that wrap the clove), were used for each treatment. The cloves were maintained in a refrigerator at a temperature of 6 ± 1.4 °C with a RH of 53 ± 15% for about 15 weeks. Four different light regimes were tested: blue (peak at 450 nm), green (peak at 425 nm), red (peak at 660 nm) and RGB (red, blue and green light). As a dark control the cloves were covered by a black plastic box.

In the other three experiments (nos. 2, 3 and 4), five to eight garlic cloves, covered by the outer tunic layer, were used for each treatment. The cloves were maintained under three different temperature and humidity conditions and under specific light regimes for about 30 weeks. Blue light was not tested in these conditions because the outer tunic shielded the majority of the blue light furnished (Fig. 1).

In detail, the conditions were as follows. In experiment 2, cloves were kept at room temperature and humidity (RT) of 24.5 ± 2 °C with a RH of 65 ± 10% where several different light treatments were tested, in particular: under dark (black plastic box), room light (indirect fluorescent light), sunlight (on a window with direct sunlight exposition), UV, green (peak at 525 nm), short and long red (respectively with peaks at 625 and 660 nm), and infra-red (IR, peak at 735 nm). For the regulation of the irradiance of the UV light in which the spectra were outside the range of the illuminance spectrophotometer CL-500A, a photodiode connected to a multi-meter was used to calibrate an equivalent irradiance with respect to the other light treatments.

In experiment 3 a chilled refrigerator at 13.09 ± 0.5 °C with a RH of 83 ± 11.5% was used.

In experiment 4, a refrigerator at 10 ± 1.15 °C with a RH of 21 ± 11% was used, where the same four different light treatments

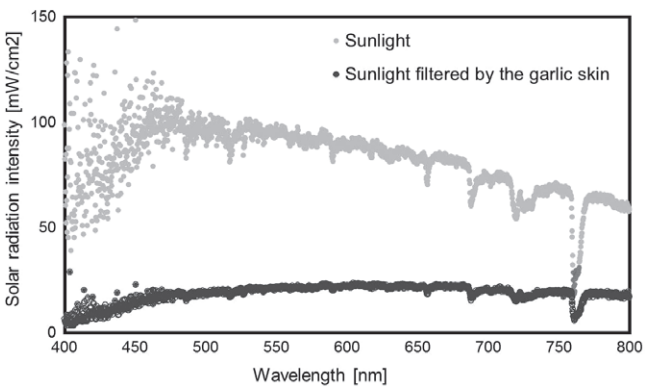

Figure 1. Two spectra, one of the direct sunlight and the second of the same light filtered by the garlic protective skin-tunic.

were tested, in particular: under dark, green (525 nm), long red (660 nm) and IR (735 nm).

Determination of pigments

The chlorophyll content was calculated in the sprout for all treatments and in the outer layer for only the garlic stored without the outer tunic protection.

The sprout was cut at the base, weighed (average weight 0.9 ± 0.78 g) and extracted in *N*,*N*-dimethyliminium chloride (DMF). Two subsequent extractions were performed after a period of 24–48 h at 4 °C in the dark (total volume from 2 to 5 mL depending on the fresh weight and the residual colouration of the sprout).

For extraction of the chlorophyll from the outer layer of the garlic a circle of 0.9 cm of diameter and approximately 2 mm in

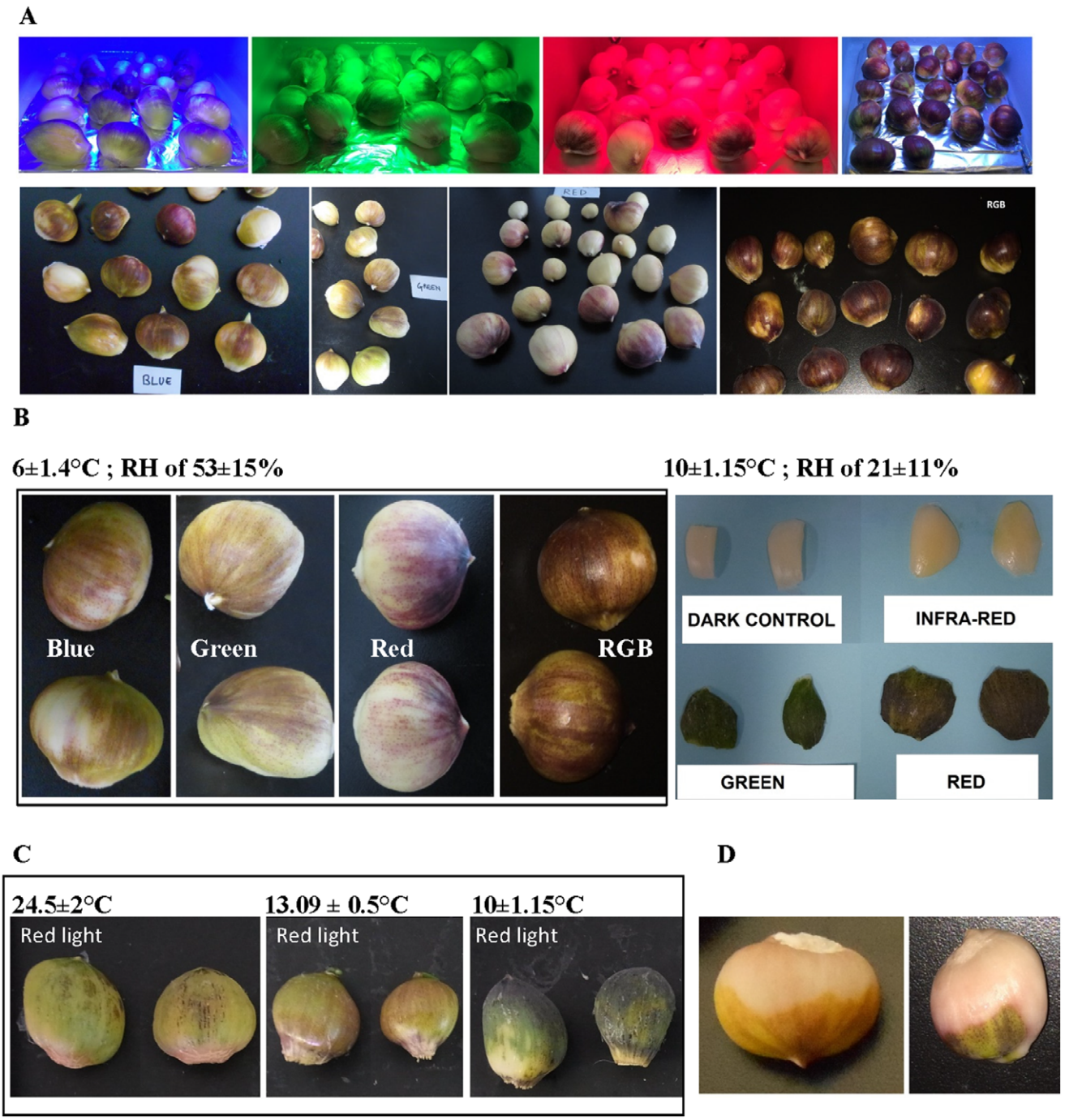

Figure 2. (A) The four groups of garlic subjected to different light treatments stored without the outer tunic at 6 ± 1.4 °C with a RH of 53 ± 15% for 15 weeks. Upper row: the garlic during the light storage conditions; lower row: some cloves samples after the light treatment on a black background. (B) Outer part pigmentation highlighted for the two low temperature treatments (left: 6 ± 1.4 °C and right: 10 ± 1.15 °C). (C) Examples of the outer pigmentation for garlic cloves kept under red light (660 nm) with the outer tunic for 30 weeks at different temperature/humidity conditions. (D) Effect of the presence of mildew and mould on clove pigmentation.

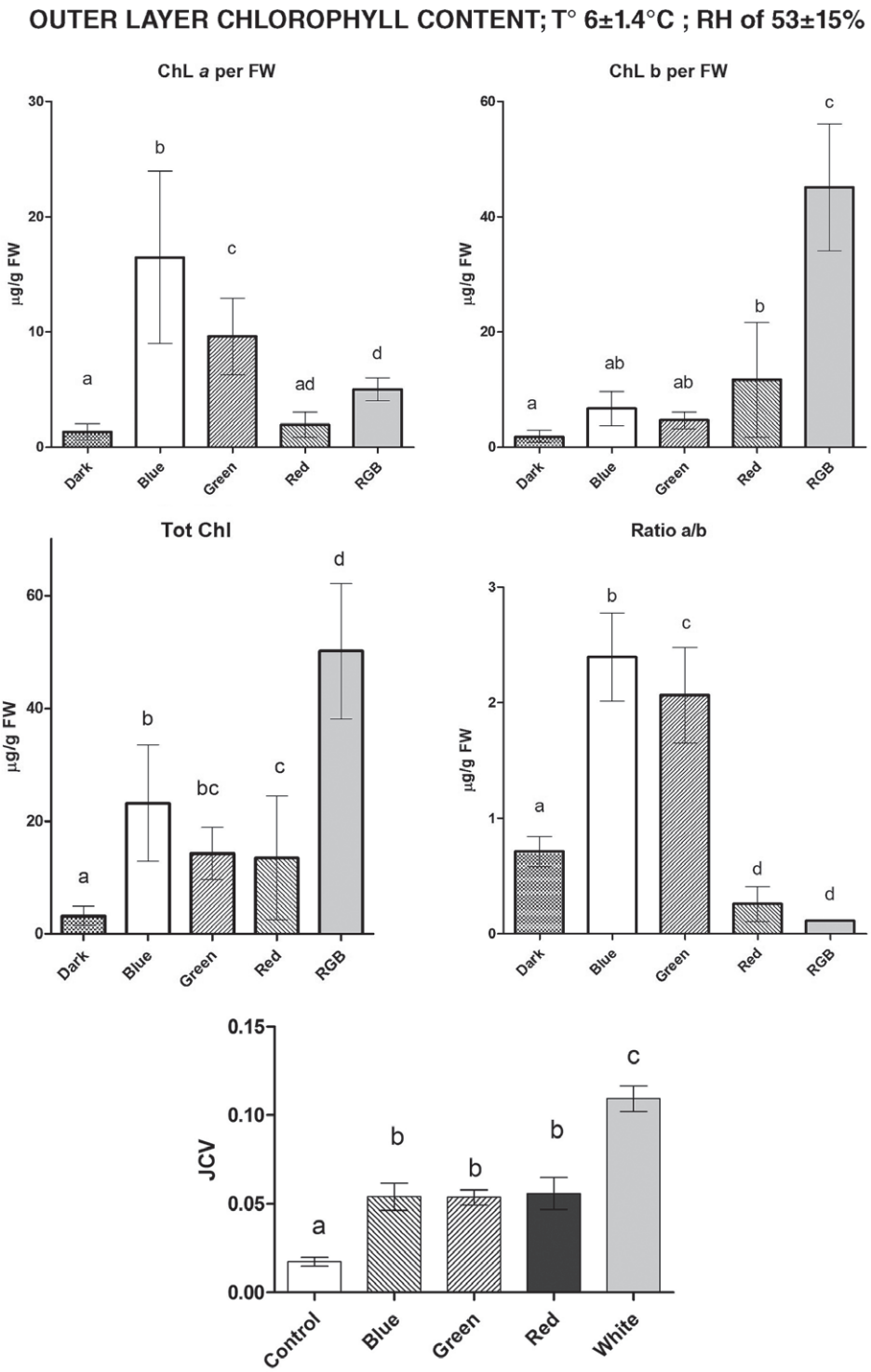

Figure 3. Calculation of the chlorophyll and the pink/red pigments (JCV) in the outer layer of the garlic content of four groups of garlic subjected at different light treatments without the outer tunic at 6 ± 1.4 °C with a RH of 53 ± 15% for 15 weeks ($n = 15–20$; error bars represents SD, different letters indicate statistical significance $P < 0.05$).

height (average weight 0.132 ± 0.014 g) was finely chopped and extracted in DMF. Two subsequent extractions were performed after a period of 24–48 h at 4 °C in the dark (total volume of 0.5–1 mL).

All samples were centrifuged at about 9000 RCF for 10 min and measured in a spectrophotometer 350–700 nm. The calculation was performed by considering the solvent dilution and the initial fresh weight of each sample and using the method described by Welburn[28]: (Chl$a = 11.65\text{Abs}_{664} - 2.69\text{Abs}_{647}$; Chl$b = 20.81\text{Abs}_{647} - 4.53\text{Abs}_{664}$).

Red/pink pigments were extracted by modifying the standard method of the Japan Food Additives Association.[29,30] The colour value of the pigment extract (JCV), which is a Japan commercial indicator of total anthocyanins-like compounds, was calculated

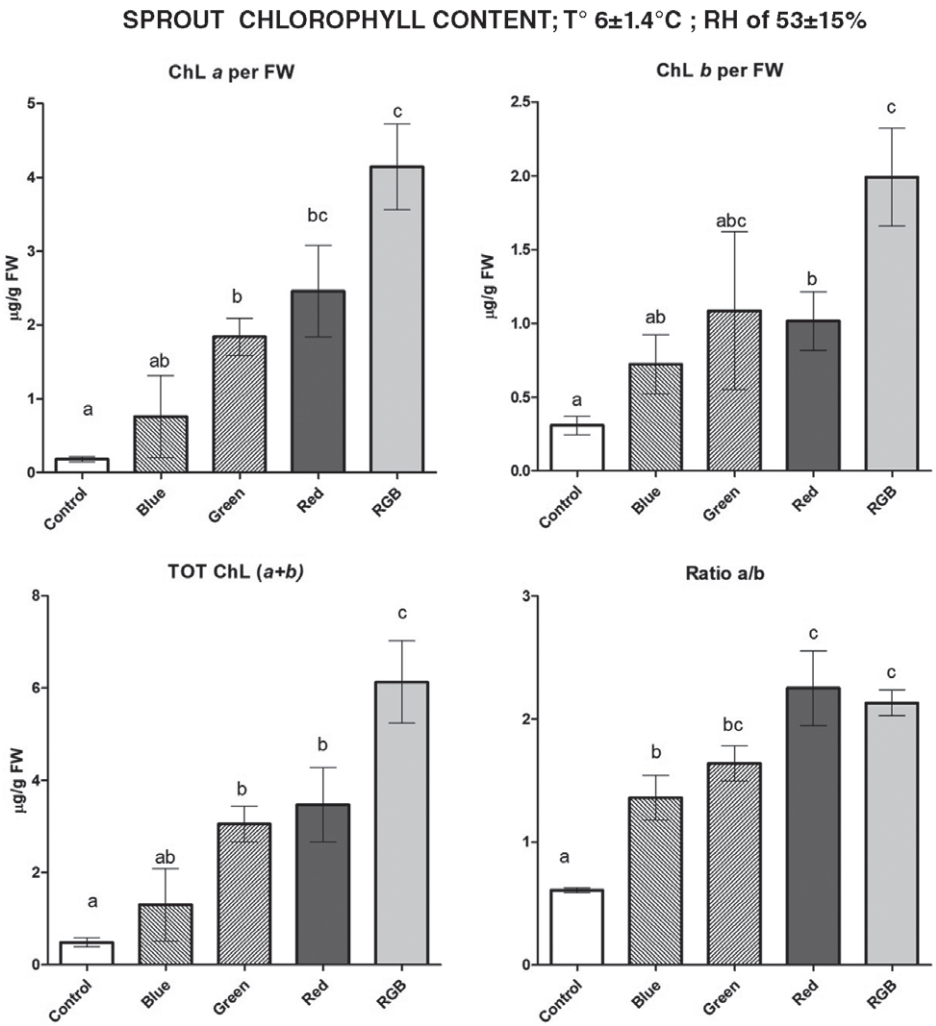

Figure 4. The sprout chlorophyll content of four groups of garlic subjected at different light treatments without the outer tunic at 6 ± 1.4 °C with a RH of 53 ± 15% for 15 weeks ($n = 5–8$; error bars represents SD, different letters indicate statistical significance $P < 0.05$).

using the following formula: $JCV = 0.1 \times OD_{520} \times D_1 \times D_2$, where D_1 and D_2 are the dilutions used for the extraction. In particular, one disc of 0.9 cm of diameter (0.64 cm^2) and 2 mm thick was ground in a mortar in 5–10 mL (D_1) of 50% acetic acid solution, and incubated for 24 h at 4 °C in the dark. The extract was centrifuged at about 9000 RCF for 10 min and the absorbance measured at 520 nm (OD_{520}) with a spectrophotometer with another dilution (D_2) using a McIlvaine buffer (pH = 3).

Data analysis

All results reported were calculated with Graphpad Prism software (San Diego, CA, USA) as result of the statistical analysis one-way ANOVA and a Tukey post-test ($P < 0.05$).

RESULTS AND DISCUSSION

The measure of sunlight filtered by the garlic protective skin-tunic underlined a natural mechanism of protection against light. In particular the blue–green spectra were the most affected by the garlic skin (Fig. 1). The overall results, described in detail below, showed that low light wavelength (i.e. blue/green) reduced the greening of the sprout, supporting the hypothesis that the function of the shielding sheath is to prevent the early sprouting of the garlic.

Regarding the sprout fresh weight, we did not observe any significant statistical difference between the temperature and humidity treatments. Although no substantial influence on sprouting and germination was noticed, new findings about the ability of different light to modulate pigmentation emerged. Different light storage conditions were able not only to influence the shoots' greening but also to stimulate the outside colouration of the cloves (Fig. 2). In our results, green pigmentation extracted with DMF produced a peak at 664 nm, thus putatively identified as chlorophyll, whilst a pink/red pigment resulted in a peak at 520 nm, putatively identified as flavonoids[31] or organosulfur compounds.[25]

These phenomena, in particular the formation of a purple colour, have been clearly observed in garlic subjected to light treatments stored at low temperature without the outer tunic (i.e. 6 ± 1.4 °C and 10 ± 1.15 °C). The effects were moderate with the outer tunic at a temperature of 13.09 ± 0.5 °C and minor for the groups of garlic stored at room temperature where only a green/yellow colour was present with a small purple spot (Fig. 2C). In fact the light was able to highly stimulate the outside purple pigmentation in cloves stored without the outer tunic at 6 ± 1.4 °C with a RH of 53 ± 15% for 15 weeks, in particular increasing the production of chlorophyll

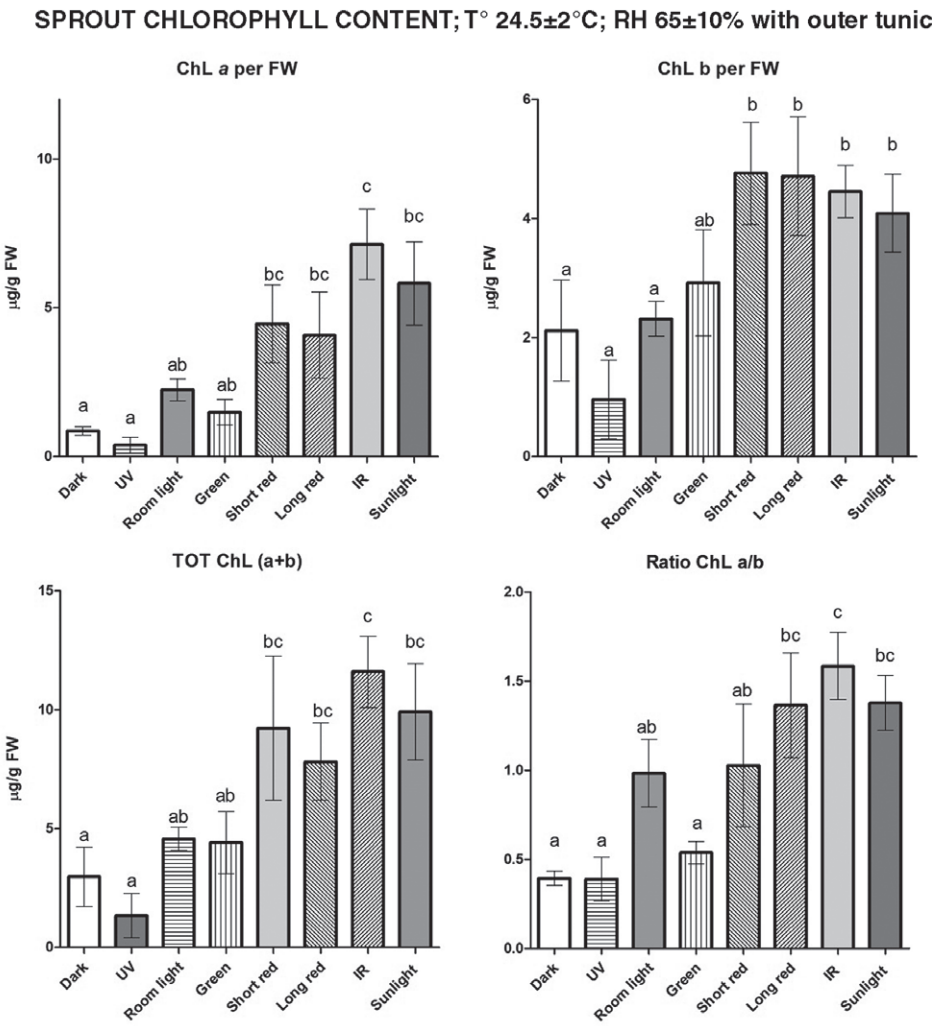

Figure 5. The sprout chlorophyll content of different groups of garlic subjected at different light treatments stored with the outer tunic at 24.5 ± 2 °C and with a RH 65 ± 10% for 30 weeks ($n = 5–8$; error bars represents SD, different letters indicate statistical significance $P < 0.05$).

and red/pink pigments in the outer layer part (Fig. 2 and Fig. 3). For this reason the low temperature played a key role in the formation of red/pink pigmentation in the outer layer. However, the production of pigments was almost absent in the outer part in all the dark treatments (both for chlorophyll and red/pink pigments) whilst the IR light treatment resulted in a pale yellowish skin as shown in Fig. 2B, where an outer part slice is shown for the treatment at 10 ± 1.15 °C. The purple colouration appeared to be distributed along the surface, mainly in the upper part of the cloves, and a cluster of more intense spots was present in most of the garlic (Fig. 2B). Our results are in accord with previous research demonstrating that storage at low temperature favoured garlic discolouration.[25] The pathway involves mainly two compounds, alliin and isoalliin, and other non-protein sulfur-containing amino acids.[7,32] In particular, isoalliin is essential for the discolouration to occur and if the temperature is higher than 23 °C an increase in cycloalliin rather than isoalliin is expressed. In fact, cycloalliin is not capable of influencing the colouring, and this explains why a low temperature is necessary for greening.[7] The dark conditions and IR light treatments did not produce any clear outer pigmentation, thus the light should be involved in inducing discolouration or is able to stimulate a precursor essential for this process.

Mildew and mould effects were very limited during all the experiments, especially when the tunic–layer protection was present and almost absent during the room temperature treatments. This was probably due to the fact that storage at room temperature caused some dehydration of the clove and resulted in a coarseness of the surface with respect to the other treatments. During the ongoing experiment all garlic cloves were periodically checked and if affected by mildew and/or mould the cloves were removed. The percentage of mouldy cloves was not correlated with any light treatment but it is noteworthy that when microbiological contamination appeared the pigmentation was inhibited by the formation of a clear zone neighbouring the affected part (Fig. 2D).

Among the light treatments in the groups of garlic stored for 15 weeks without the skin-tunic layer at a temperature of 6 ± 1.4 °C with a RH of 53 ± 15% for about 15 weeks (experiment 1), the RGB condition resulted in higher anthocyanins production in the outer layer (Fig. 3) and no statistical difference among blue, green and red light treatments were highlighted. The chlorophyll content in the outer layer was higher in the RGB light treatment and lower in the red light samples, but interestingly the Chl*a*/Chl*b* ratio was inversely proportional to the light wavelength meaning that the red light and RGB treatment mainly possess Chl*b* (Fig. 3) whilst

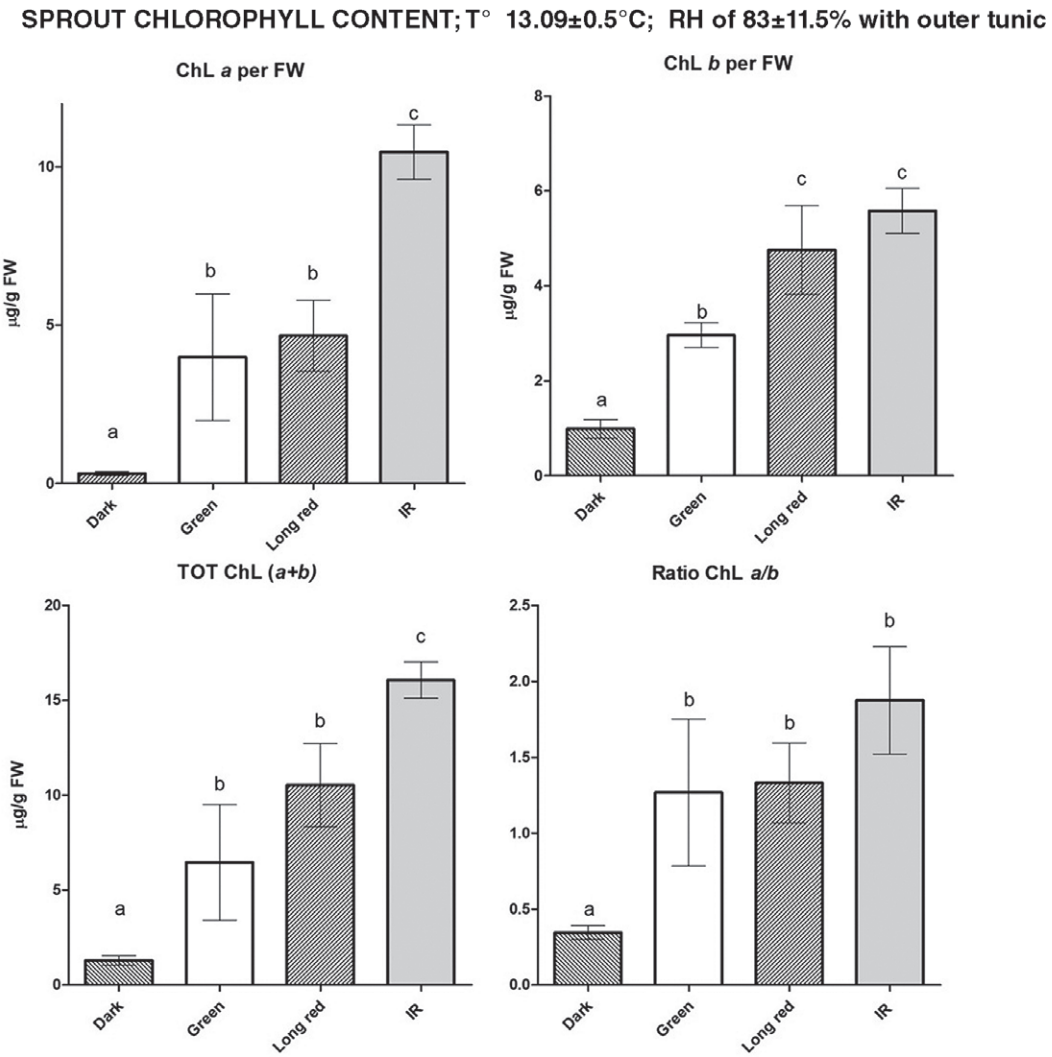

Figure 6. The sprout chlorophyll content of different groups of garlic subjected to different light treatments stored with the outer tunic at 13.09 ± 0.5 °C and with a 83 ± 11.5% for 30 weeks ($n = 5–8$; error bars represents SD, different letters indicate statistical significance $P < 0.05$).

green and blue mainly possess Chl*a*. The chlorophyll content of the sprout in these storage conditions was directly proportional to the increase of the light wavelength (Fig. 4) meaning that the greening process of the seedlings is mostly affected by high wavelength light.

The chlorophyll content of the sprout in experiments 2, 3 and 4 similarly always increased proportionally to the wavelength (Fig. 5, Fig. 6, Fig. 7) except for the treatment at 10 ± 1.15 °C with a RH of 21 ± 11% where the greening by the IR light was lower than by red light but these experiment presented a very high standard deviation, in particular for the Chl*b* content. In all the other conditions, IR light was the most effective light to enhance the sprout greening process, whilst completely dark conditions were the most effective way to prevent sprout greening. The UV light, the shaded garlics at indirect room light and the green light treatment, resulted in the lowest chlorophyll production. Among the experiments of 30 weeks of duration (experiments 2, 3 and 4) the treatment at a temperature 13.09 ± 0.5 °C with a RH of 83 ± 11.5% resulted in a higher production of chlorophyll when the same light conditions were compared, meaning that humidity played an important role in the greening process when the temperature was kept low. As expected, low humidity and temperature were the most effective treatments to prevent sprout greening.

CONCLUSIONS

Our study underlined that light should be considered as an additional natural stimulus that can modulate discolouration reactions in onion/leek and garlic. In particular, RGB lights can delay greening or enhance pigmentation. By using LED technology it is easy to investigate the influence of specific light spectra during different plant growth phases. In fact, all elements of the discolouration process have not yet identified and the mechanism requires a better understanding of all natural constituents involved. The use of RGB LEDs allows us to analyse and understand regulatory processes in a systematic manner. In almost all the storage conditions tested the chlorophyll content of the sprout was usually increased directly proportionally to the wavelength of the light tested. All light conditions stimulate the outside colouration of the cloves at low temperatures, in particular the mix of all three lights (RGB) enhanced the production of red/pink pigments, suggesting this process is enhanced by low temperature but involved more than one light to be entirely accomplished. The treatment with IR light

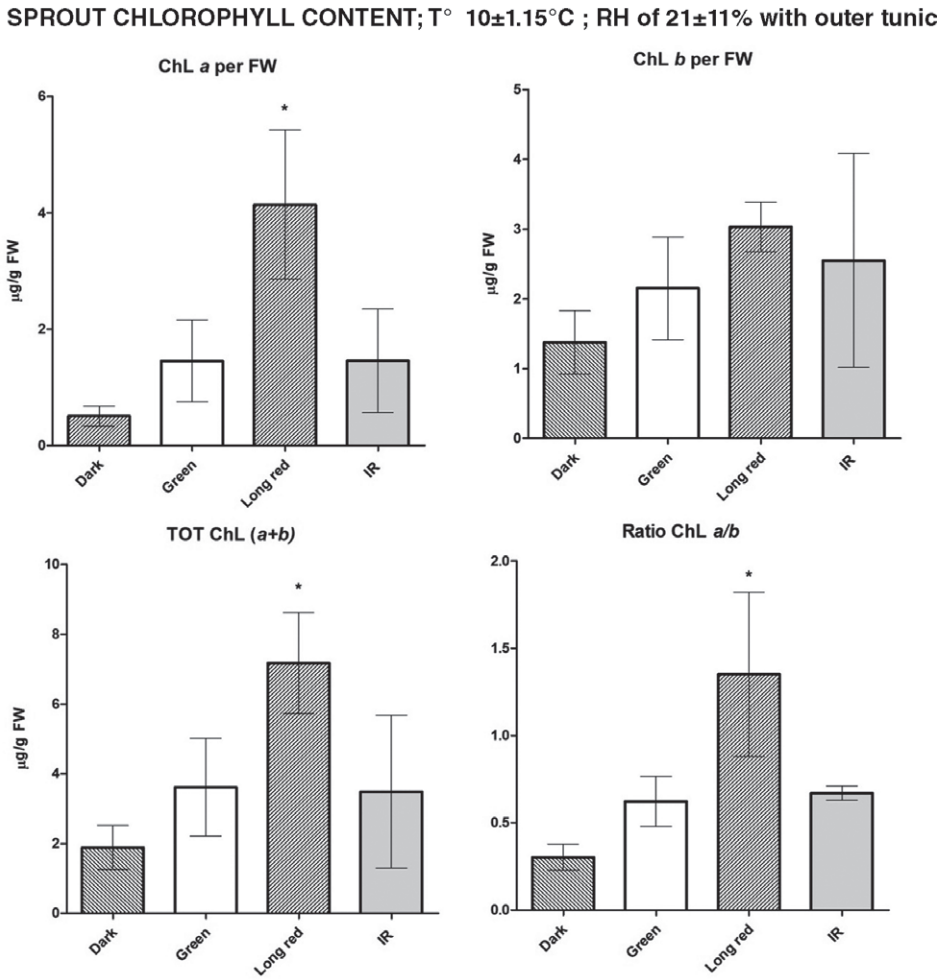

Figure 7. The sprout chlorophyll content different groups of garlic subjected to different light treatments stored with the outer tunic at 10 ± 1.15 °C and wit a RH 21 ± 11% for 30 weeks ($n = 5–8$; error bars represents SD, stars indicate statistical significance $P < 0.05$).

and dark conditions did not result in any green/purple pigmentation, and then the compounds responsible for the discolouration were produced in response to the light within the visible spectra. The pigments of many fruits and vegetables can act as powerful antioxidants or free-radical scavengers and have the potential to bring several health benefits. Their presence in the outer layer of garlic is a very attractive trait that can increase the market value of the product as fresh or dried powder. For this reason further study needs to be performed to elucidate the mechanism and identify the actors and the specific compounds involved in this process.

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