A New Role for UV?
Shelf Life Extension of Plant Foods by UV-induced Effects.

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ABSTRACT

In its traditional disinfective role, UV is used to bring about the more or less direct inactivation of micro-organisms by a number of different mechanisms, chief amongst these being damage to microbial DNA. The time scale for such events is typically of the order of seconds. However, a new way of exploiting UV is beginning to emerge which has particular relevance to plant foods such as fruits and vegetables. In this relatively new role, exposure to UV triggers a series of biochemical events within the plant tissue. A number of quite distinct responses have been identified; some involve the synthesis of enzymes that have activity against molds, whilst others result in the generation of a host of so-called 'phytoalexins' which are inhibitory to micro-organisms.

Crucially, these effects are produced by the use of very low UV doses, and the time scale for the induction of such events is measured over hours or even days and the term 'hormesis' has been applied to this type of treatment. Equally intriguing is the fact that some of the compounds induced by UV are known to have beneficial health effects – the most well studied of these being resveratrol synthesis in grapes.

The claim that has been made for this type of low UV dose treatment is that it can result in the extension of shelf life of a number of perishable commodities. However, to date few efforts have been made to 'translate' these laboratory observed phenomena into the field. A number of key questions remain unanswered, for example, it has to date only been applied as a post-harvest treatment, and whilst this may be suitable for some types of produce, it excludes others; can protective effects be obtained by pre-harvest exposures to UV? This review surveys the evidence for such UV-induced effects and discusses the problems that will have to be overcome if this technology is to be applied commercially.

Key words: Ultraviolet treatment; Fruits and vegetables; Hormesis; Phytoalexins; Health benefits.

INTRODUCTION

Post harvest losses of perishable commodities such as fruit and vegetables occur at every stage in their growth, processing, distribution and retail. Disposal by consumers, or rather, would be consumers, is arguably less controllable and much discarded produce ends up in landfill.

Whilst pesticides and fungicides have hitherto been relied upon to reduce such losses, a trend is developing towards minimising the use of chemical agents. This is occurring partly in response to regulatory demands but also as a result of pressure by consumer groups on the basis that chemical residues are associated with threats to human health (Wilson et al. 1991). If, as seems likely, this trend continues and losses are not to increase still further as a consequence, alternative methods of preserving perishable produce must be found. One possibility is the use of UV. The application of UV in the food industry is by no means novel. However, research conducted in the 1990s has revealed a radically different approach to UV treatment in relation to fresh produce. This research is described here as well as the difficulties that would have to be faced in translating these laboratory findings to the commercial sector.

Conventional UV Treatment

UV treatment applied to a solid object with the express purpose of inactivating microorganisms on the object in question scarcely needs any elaboration. However, in order to compare with what follows below it will be useful to provide the essential elements of this type of treatment. Firstly, for maximum lethal effect UV sources which emit primarily shortwave UV should be used. The most useful wavelengths are in the so called UV-C region of the spectrum (100 to 280 nm) and they are sometimes referred to as "germicidal." More specifically, wavelengths in the region of 250 nm have been shown to be most efficient at inactivating microorganisms (Harm 1980). Fortuitously, the principal emissivity of the low pressure mercury burner happens to be at 253.7 nm making them ideal sources for this purpose. Treatment requires that every surface of the object is exposed to UV light for a time sufficient for any microorganisms present to accumulate a lethal dose.

This type of UV treatment may also be characterised by its relatively short time scales. This is essentially the length of time to which the microorganisms present on a solid object will be exposed to UV, and is generally of the order of seconds, although times as high as minutes are not unknown. However, the defining characteristic of this form of UV treatment is that it is direct – only whilst organisms are exposed to UV will they accumulate damage to cellular targets. In other words, once they are outside the UV field no further damage is sustained. Many microbial components are susceptible to, and ultimately damaged by, UV but the most labile of these is the cell's DNA (Harm 1980).

At the length scale of microbial cells (i.e. dimensions of the order of 1µm), surfaces can display wide varieties of topographies. For instance, some surfaces may be almost perfectly smooth so that any microorganisms present on them will be fully exposed to incident UV light. Other surfaces may offer niches in which microorganisms may obtain protection or shielding from incident UV. The effects of shielding were demonstrated by Gardner and Shama (1998) who deposited spores of *Bacillus subtilis* on various grades of filters and membranes and showed that spores were activated at lower UV doses on filters in which the spores remained closer to the surface and therefore more relatively exposed to incident UV.

Microbial cells that are completely shielded by surface features may escape UV treatment unscathed and remain fully viable. The only way of inactivating microorganisms that are partially shielded at the surface of an object is to increase the dose of UV that is applied. However, where foods are involved there are limitations to the UV doses that may be applied without bringing about deleterious or even harmful effects. To a large extent considerations such as these have tended to limit the application of UV for food treatment.

The Concept of Hormesis and the "Responsive Target"

The first hormetic phenomena were apparently recorded in the 1880s and since then hormesis has attracted a more or less steady stream of attention (Calabrese, 2002). Hormesis involves the use of small doses of potentially harmful agents directed against a living organism or living tissue in order to elicit a beneficial or protective response. The agent used to bring about such effects may be either physical or chemical, and of particular relevance here is the fact that UV constitutes one such agent. The most studied UV induced hormetic phenomena have been in fruit and vegetables.

It is essential to distinguish hormetic UV treatment from conventional UV treatment described above. In conventional treatment the UV is directed at microorganisms present on the surfaces of an object, whereas in hormetic UV treatment it is the object itself that is the target of the incident UV. Where fruits or vegetables are concerned these should not therefore be seen simply as passive carriers of microorganisms. The objective of the treatment is to elicit an anti-microbial response in the fruit and vegetable tissue. Both types of UV treatment employ the same wavelengths but for hormetic treatments only low UV doses are required. These were recently reviewed for fruit by Shama and Alderson (2005), and vary according to the type of fruit but the doses range from 0.1 to 9.0 kJ/m^2.

The essential feature of hormetic phenomena is that they are induced: the UV in effect is perceived as an external stress and the tissue responds by activating certain metabolic pathways. It should be stated here that other physical treatments such as heat or gamma irradiation are also capable of eliciting such stress responses (Schreiner and Huyskens-Keil 2006). Induction implies an elapse of time between the application of treatment and the maximal protective response. This period of time can vary from hours to days in some cases.

The induction of metabolic pathways means that the response is generated throughout the entire tissue rather than at its surface. This is in stark contrast to conventional UV treatment where direct inactivation effects are confined to the surfaces of solid objects. In fruit, for example, it has been shown that UV is rapidly adsorbed by the "exocarp", the most outer layer of the fruit wall (Blanke 1996) and therefore would not be able reach microorganisms just a few microns from the surface. The literature contains numerous reports of hormetic UV treatments resulting in protective effects against microorganisms throughout the entire tissue rather than at its surface only. For example, Stevens et al. (1999) showed that sweet potatoes inoculated with spores of *Fusarium solani* as deep as 12 mm below the surface could successfully be protected from infection following hormetic UV treatment.

The concept of the 'responsive target' is further reinforced by work described below. The term 'epiphyte' is used in the plant sciences to describe a microorganism that either grows on or is otherwise associated with plants. The epiphytic microflora may have an important role as natural antagonists of pathogens (Wilson et al., 1991) and therefore it is important that any applied treatment should not negatively impact on the epiphytic population. Nigro et al. (1998) observed increases in epiphytic yeast and bacteria following low dose UV treatment. They speculated that the growth of these organisms may have been stimulated by the UV-induced permeabilization leading to the leakage of nutrients. Presumably conventional UV treatment directed at surface phytopathogens, and requiring higher UV doses, would have a greater probability of disrupting the epiphytic microflora,

When treating peaches with low doses of UV Stevens et al. (1998a), found that the population of epiphytic yeasts belonging to the species *Debaromyces hansenii* actually increased with applied doses of up to 7.5 kJ/m^2. Most intriguing was the fact that doses of this magnitude had severely depleted populations of the same yeast on the surface of paper disks. This led the authors to conclude that the yeast were under the genetic control of the host i.e. the peach. A similar explanation might account for the results obtained by Lopez-Rubin et al. (2005) who treated pomegranate arils with UV and found that whilst bacterial counts were reduced by certain UV doses, yeast counts remained essentially constant.

Hormetic Mechanisms

The accumulated literature on UV hormesis in fresh produce reveals two distinct effects. The first is protection against attack by phytopathogens – mainly fungi - and the second is delaying the onset of ripening. Both effects are obviously desirable and would contribute to reducing losses. In some cases the identities of some or all of the elicited chemical species is known, whilst in others it remains to be discovered.

Perhaps the greatest attention has focussed on citrus fruit and in such fruits; enhancement of resistance to phytopathogens such as *Penicillium digitatum* has been attributed to accumulation of the phytoalexins scoparone (Kim et al. 1991; Rodov et al. 1992; D'hallewin et al. 1999) and scopoletin (D'hallewin et al. 2000). Phytoalexins have been described as low molecular weight compounds that accumulate in plants as a result of infection or stress (Kuc 1995). In carrots, UV treatment has been shown to result in accumulation of 6-methoxymellein (Mercier et al. 1993). Nigro et al. (1998) treated grapes with low doses of UV and attributed increased resistance in the treated grapes to resveratrol.

In addition to phytoalexins a number of workers have reported the induction of phenylalanine ammonia lyase (PAL) as well as in a host of so-called pathogenesis-related (PR) proteins (chitinases and β-1,3-endoglucanases) in both citrus fruit and peaches (Droby et al. 1993; Porat et al. 1999). Of the PR-proteins produced by plants, chitinases, glucanases and lysozymes have the ability to hydrolyse insoluble polysaccharides from the cell walls of fungi and bacteria. Glucanases and chitinases inhibit fungi by hydrolysing β-1,3-ether linkages in β-1,3-glucans and β-1,3-1,6-glucans and hydrolysing β-1,4-ether linkages in chitin. Lysozymes and some chitinases also inhibit bacteria, with the lysozymes hydrolysing the carbohydrate component of bacterial peptidoglycan (Shama and Alderson 2005).

Ripening in fruit and vegetables is a complex process in which the growth regulator ethylene plays a key role. It is not the intention here to enter into a description of this process but rather to focus on those aspects of it that are affected by low doses of UV. Polyamines are known to play an important role in ripening and Maharaj et al. (1999) showed that low UV doses led to increased levels of the polyamine putrescine which inhibits ripening. Similarly, Stevens et al. (1998b) attributed delayed ripening of UV-treated tomatoes to high levels of induced putrescine and spermine. Barka et al. (2000b) noted less activity of cell wall-degrading enzymes, i.e. polygalacturonase, pectin methyl esterase, cellulase, xylanase, β-D-galactosidase and protease, in tomato fruit treated with UV irradiation and in later work observed an increase in lipoxygenase and PAL activities (Barka et al. 2000a).

New Functional Foods?

Rising rates of cancers and heart disease have become virtually synonymous with a western industrialized lifestyle. Compelling evidence exists that the incidence of such diseases can be reduced by increased consumption of fruit and vegetables, and national governments and a diversity of other agencies have for some time been promoting changes to dietary habits (Schreiner and Huyskens-Keil 2006). One such campaign is the consumption of 5 pieces of fruit and vegetables per day and only time will determine to what extent such promotions are successful. Recent studies suggest that it may be possible to increase the protective effects that fruit and vegetables can confer by treatment of such produce with low doses of UV. The chemical species at the heart of these effects are identical to those giving protection against phytopathogens and in delaying ripening – i.e. the phytoalexins. Perhaps the well-studied of these is resveratrol.

Resveratrol has been claimed to have cardioprotective, anti-inflammatory and anti-tumorogenic properties, and perhaps most intriguingly, that it extends the lifespan of lower organisms. With the exception of the latter, the other effects have only been demonstrated *in-vitro* but as Baur and Sinclair (2006) point out in their review of the therapeutic potential of resveratrol, it will be necessary to validate the claims *in-vivo*, and these authors give details of ongoing large-scale clinical trials.

Assuming that future studies confirm the desirability of increasing the dietary intake of resveratrol, strategies for doing so may already be in place. Cantos et al. (2001) showed that delivering low doses of UV to grapes caused resveratrol levels to increase significantly and they went so far as to describe UV-treated grapes as a "functional food". In addition, levels of resveratrol increased by UV treatment persist in wines produced from the grapes (Cantos et al. 2003).

Resveratrol is by no means the only health protective phytoalexin whose concentration in fresh produce may be increased by low dose UV treatment. For example, tomatoes contain a number of potentially very interesting species. These include tomatine and tomatidine and a host of related glycoalkaloids. In a recent review Friedman (2002) discussed the roles of this family of compounds in relation to human health.

An issue that has largely been unaddressed to date is how will consumers respond to foods treated with UV? This will to some extent be influenced by marketing and how the beneficial effects of UV-induced phytoalexins are promoted. In connection with consumer acceptance it is possibly advisable that terms such as "irradiation" be avoided in describing the treatment of fresh produce with UV as the term has become associated in the popular mind with the use of ionising radiation.

Adverse Effects of UV Treatment

Although hormetic effects are elicited by low UV doses, damage to fresh produce even at low doses has been reported. It should be borne in mind that such effects are, after all, brought about by agents (in this case UV) which are harmful at high doses. These undesirable effects include skin discoloration in tomatoes (Maharaj et al. 1999), browning and drying of calyxes in strawberries (Marquenie et al. 2002), increasing susceptibility to brown rot in peaches (Stevens et al. 1998a) and premature ripening in mangoes (Gonzalez-Aguilar et al. 2001). Prolonged exposure of tomato fruits to UV has actually been found to accelerate ripening and senescence of tomatoes (Liu et al. 1993). All of these adverse effects would contribute to postharvest losses and any proposed treatment methods should have in place measures for carefully controlling the maximum UV dose that can be delivered to individual fruits.

Process Requirements

A potentially very interesting finding was made by Stevens et al. (2005) who reported that for apples, peaches and tangerines it was not necessary to expose the entire surface of the fruits to UV to elicit the desired hormetic response. These workers found that the UV dose resulting in optimal protective effects could be delivered solely at the stem end of the fruit. They suggested that the vascular tissue in these fruits might play a role in signal transduction from the receptor tissue at the stem end. If this were confirmed to hold for other types of fresh produce, it would have significant consequences for commercial treatment as produce could be packed in a certain way as to enable their stem ends to be exposed to UV. Clearly, this is one area where further investigations are required.

An equally important finding to that described above is that many of the effects induced in living systems by UV –

including hormetic effects - have been shown to be partly, or in some cases wholly, reversible by subsequent exposure to light of a longer wavelength, typically either UVA or visible light. Stevens et al. (1998a) exposed UV-treated peaches to ordinary fluorescent white light sources at high light intensity continuously for 48 hrs and found that the beneficial effects of the UV-C in reducing brown rot disease caused by *Monilinia fructicola* were completely eliminated. By contrast, storage in the dark did not result in a reversal of beneficial effects of UV treatment.

The possibility that post treatment exposure to visible light would reverse hormetic effects would require careful control of the post treatment environment. There appears to be currently no information in the literature concerning the length of time after irradiation that produce should be protected from exposure to photoreversing wavelengths. It would be crucial therefore to establish after what period of time after treatment does UV-induced damage become irreparable. Presumably after the elapse of time it would be safe to permit exposure of the treated produce to visible light. Further work needs to be conducted to obtain the answers to these questions.

Another factor in the UV treatment of fresh produce is the time of harvesting. D'hallewin et al. (2000) found that UV induced higher levels of phytoalexins in grapefruit harvested late in the season (February and May) than those harvested early in November. They also noted that decay control for fruit harvested late in the season was generally more difficult. Droby et al. (1993) had earlier shown that grapefruit harvested in February required approximately double the UV dose to confer resistance against *Penicillium digitatum*.

Another finding with implications for commercial applications is the physiological status of the produce at time of treatment. de Capdeville et al. (2002) found that fresh apples were more responsive to treatment than were fruit stored for 3 months in a controlled atmosphere environment, relatively little is currently known about how fruit with different postharvest 'ages' respond to UV treatment. This is clearly one area in which more work is needed.

All previously published work on the application of low doses of UV to fresh produce has been restricted to the treatment of produce after it has been harvested i.e. post-harvest. Is pre-harvest treatment a possibility and might it offer advantages? Certain soft fruits such as strawberries are traditionally harvested directly into punnets (a small light basket used as a measure for fruits) without any form of treatment simply because the fruit are delicate and almost any form of mechanical handling results in damage and subsequent losses.

There is currently very little information to assess whether pre-harvest treatment could result in the induction of hormetic effects or indeed whether it would be practical and ultimately economically viable. Specific questions to be answered include at what point in the growth cycle of the fruit should the UV be applied? Would a single dose or multiple doses be required? What effects would the UV have on the growing plant?

Should future studies validate pre-harvest treatment, the practicalities of applying UV doses to fruit on the vine, as it were, could presumably be addressed. For instance, returning to strawberries, the current methods of cultivation in glass house with the plants arranged in rows with the fruit overhanging for ease of picking coincidentally offers possibilities for potential UV treatment. In this context a patent filed by Michaloski (1991) may be of potential interest. This patent describes a bank of UV sources designed to be pulled by a tractor between rows of vines for the purposes of eradicating powdery mildew from the vines. Such a device could readily be adapted for the treatment of fruit such as strawberries.

Conclusions

The concept of employing low doses of UV to elicit hormetic responses in fresh produce has been firmly established by a host of laboratory-based studies. However, more work remains to be undertaken to enable this method of treatment to be applied commercially. One promising new area of investigation might be whether pre-harvest treatment could ever be a practicality. It must also be shown that key quality attributes of the produce are not significantly compromised by UV treatment. In addition, methods of treating delicate produce that minimises mechanical handling must be devised. Finally, the attitude of consumers must be taken into account in marketing produce treated with UV and the benefits of treatment clearly explained.

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