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Fate of Flavonoids in the Outer Skins of Onion (*Allium cepa* L.) Throughout Curing

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Reducing the current U.K. curing temperature from 28 to 20 °C would help to reduce energy costs; however, onion skin appearance and consumer acceptability may be detrimentally affected. The aim of this study was to elucidate the compounds responsible for the difference in color between brown and red onions cured at 20 and 28 °C by monitoring dynamic biochemical changes in the skin at set intervals during curing and after storage from two years' data. Sugar concentrations appeared to play no role in the difference in onion skin appearance when cured at different temperatures. Using regression, principal component, and partial least-squares discriminant analyses, the decrease in skin H° after the curing of brown onion cultivars at 28 °C was linked to a decrease in individual flavonol concentrations, possibly due to their oxidation at higher temperatures into brown pigmented compounds. Red onion cultivars cured at lower temperatures and for a shorter curing period had higher concentrations of individual anthocyanins as well as a darker skin color. Skin water content was reduced significantly in only the first 6 days of curing. Taken together, this suggests that current U.K. curing practice could be carried out at a lower temperature (20 °C) and/or for a shorter duration, resulting in reduced curing costs and possibly improved skin appearance.

KEYWORDS: ***Allium cepa*** **L.; anthocyanins; flavonols; fructose; glucose; objective color**

INTRODUCTION

Due to the maritime climate of the United Kingdom, onions are not usually cured outside in the field as in many other parts of the world, but are cured artificially, in closed environments. The purpose of curing is to remove excess water from the outer skins and seal the neck of the bulb, which acts as a barrier against pathogens (e.g., *Botrytis allii*) and minimizes weight loss from the flesh (*1*). Various curing regimes have been proposed (*2*−*4*); nonetheless, the consensus is that relative humidity (RH) should be controlled at ca. 75% as excessive water loss tends to occur below 60% RH, which can result in skin splitting (*5*). Current practice involves curing onions at 28 °C for 3−6 weeks; however, reducing this temperature to 20 °C would reduce energy use and, therefore, costs by 35% (D. O'Connor, personal communication). Apart from the aforementioned reasons, curing also creates a darker brown skin appearance, which is appreciated by the consumer. Therefore, changing the curing temperature and hence the skin appearance may also influence the appeal of the product to consumers. Recent work by Downes et al. (*6*) showed that the skins of red onions cured at 28 °C were less red than those cured at 20 °C and also contained lower concentrations of certain anthocyanins, possibly caused by an increased rate of degradation of these compounds at the higher temperature. Additionally, brown onions were found to be darker when cured at 28 °C compared to 20 °C; however, the reason for this was not elucidated as no interaction with flavonols, sugars, or dry weight was identified by the authors.

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The pigment of red onions results from anthocyanins, which are mainly composed of cyanidin and peonidin derivatives; however, the pigment of brown onions has yet to be conclusively identified. It has been postulated that the pigmentation of brown onions is derived from flavonols, specifically quercetin and its derivatives, although the maximum absorbance of these compounds is at 370 nm, whereas that of dried outer skin of brown onions is around 450 nm (*7*). Ito et al. (*7*) identified a novel yellow pigment as a xanthylium, specifically 9-carboxy-1,3,6,8-tetrahydroxyxanthylium, which they named cepaic acid. A mechanism was hypothesized whereby cepaic acid is synthesized from quercetin via a multilevel pathway that involves oxidation. Quercetin can be oxidized via peroxidase (POX), resulting in the synthesis of many antifungal phenolic compounds such as 3,4-dihydrobenzoic acid, the concentration of which increases during the skin browning process (*8*). Onions that do not synthesize flavonoids are prone to pathogenic infection, suggesting the browning process is linked to the production of some preformed antifungal compounds (*9*, *10*).

The major sugars found in onion skin are fructose and glucose (*6*) (see the Supporting Information). It has been suggested that sugars may play a regulatory role in the production of flavonoids because both anthocyanindins and flavonols can exist as sugar conjugates (*11*). In addition, sugars can react with amines to produce brown products via the Maillard reaction, which is common in foodstuffs occurring at elevated temperatures or after prolonged periods of storage (*12*).

The aim of this study was to elucidate the compounds responsible for the difference in color between onions cured at 20 and 28 °C by monitoring dynamic biochemical changes in the skin at

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set intervals during curing. Two years of data were investigated (2008 and 2009), and using parametric and nonparametric multivariate analyses, the relationship between biochemical and physiological data was identified.

MATERIALS AND METHODS

Plant Material. In 2008, one red and two brown onion cultivars were selected, namely, 'Red Baron', 'Sherpa', and 'Wellington', respectively, and grown on sandy clay loam (Alastair Findlay's, Cardington, Beds., U.K.; 1.2 × 0.3 ha). Onions were grown from seeds drilled on March 5, 2008, at a rate of 57 seeds m^{-2} with pesticides applied as per commercial practice. Plants were hand-harvested at 100% fall-down on September 17, 2008. In 2009, onion cultivars 'Red Baron', 'Sherpa', and 'Wellington' and an additional red cultivar, 'Kamal', were grown on the same site as in 2008. Onions were grown from seeds drilled on March 16, 2009, at a rate of 54 seeds m^{-2} with pesticides applied as per commercial practice. Onions were hand-harvested on September 7, 2009, at 100% fall-down.

Experimental Design. Two growing seasons were used, each focusing on different aspects of the curing process. In 2008, onion skins were studied at three time points, before curing, after curing, and after long-term cold storage at 1 ± 0.5 °C for 35 weeks, to investigate the effects of curing and storage on skin appearance and to identify the principal compounds responsible for these changes. In 2009, skin was sampled throughout curing at weekly intervals to identify the temporal profile of the changes recorded in 2008.

Both experiments were completely randomized with three replicates harvested from three equal plots of the field. Bulbs were harvested into nets and buried among other loose bulbs in 1 tonne capacity wooden crates for curing. In 2008, three crates of onions, one per replicate, were each cured at two curing temperatures, 20 or 28 °C, for 6 weeks (65−75% RH), at Sutton Bridge Crop Storage Research (Lincs., U.K.). After curing, all netted onions were transported to Cranfield University within 2.5 h. Diseased or damaged bulbs were removed and the remaining bulbs transferred to individual stackable plastic trays and stored for 35 weeks at 1 ± 0.5 °C. Five bulbs per cultivar, curing temperature, and replicate were taken for analysis immediately before (n = 45) and after (n = 90) curing and after 35 weeks of cold storage (n = 90). In 2009, nets were divided between two crates, one at 20 °C and the other at 28 °C, to facilitate frequent sample collection during curing. Skin from three bulbs per cultivar, curing temperature, and each of the three replicates was analyzed before curing (n = 36) and after 6, 13, 20, and 41 days of curing (n = 72).

Color Measurement and Sample Preparation. Loose outer skins and dry roots were removed as per commercial practice. Objective color (chroma (C^*), hue angle ($H°$), and lightness (L^*)) was assessed using a Minolta CR-400 colorimeter and a DP-400 data processor (Minolta Co. Ltd., Japan) as the mean of three measurements taken from around the equator of the onion bulb (*6*). The first layer of intact dry outer skin from each bulb was removed, and a sample (250 mg) was retained and stored at −40 °C. All biochemical analysis was conducted on fresh skins; however, a portion was lyophilized for analysis of dry weight using an Alpha 1-4 Christ LDC-1 freeze-dryer and pump (Edwards Super Modulo, Sussex, U.K.).

Biochemical Analysis. *Quantification of Sugars.* Sugars were extracted and measured as described by Chope et al. (*13*) with slight modifications. The sugars were extracted from 150 mg of onion skin (torn into pieces of approximately 1 cm^2) using 3 mL of 62.5:37.5 HPLC grade methanol/water (v/v). The skin samples were incubated for 1 h at room temperature before being placed in a shaking water bath at 55 °C for 15 min and agitated every 5 min to prevent layering. Filtered extracts were not diluted prior to HPLC analysis due to the low concentrations found in the skin versus inner scales (*14*). Extracts were analyzed using a HPLC system with a P580 pump and a GINA 50 autosampler (Dionex, Sunnyvale, CA). The extract (20 μL) was injected into a Rezex RCM monosaccharide Ca^+ size exclusion column of 300 mm × 7.8 mm diameter, 8 μm particle size (Phenomenex, Torrance, CA; part 00H-0130-K0) with a Carbo-Ca^{2+} guard cartridge of 4 mm × 3 mm diameter (Phenomenex; part AJ0-4493). The mobile phase was HPLC grade water at a flow rate of 0.6 mL min^{-1} held at 75 °C. An evaporative light scattering detector (ELSD 2420, Waters, Milford, MA) connected to the system via a UCI-50 universal chromatography interface detected the eluted carbohydrates. Sugar concentrations were calculated against authentic calibration standards of fructose, glucose, and sucrose ranging from 0.05 to 2.5 mg mL^{-1} (Sigma, Dorset, U.K.).

Quantification of Flavonols. Individual flavonols were extracted and quantified according to the method of Vågen and Slimestad (*15*), with slight modifications (*6*). Onion skin (150 mg) was mixed with 3 mL of 70:29.5:0.5 methanol/water/hydrochloric acid (v/v/v) and vortexed to mix thoroughly. The vials were held at room temperature for 1 h before being incubated at 37 °C for 1.5 h in a shaking water bath and agitated every 15 min. Cooled samples were filtered through a 0.2 μm Millex-GV filter unit and stored at −40 °C.

Flavonols were separated and quantified as described by Vågen and Slimestad (*15*) with slight modifications. Extracts were diluted 1 in 5 and analyzed using an Agilent 1200 series HPLC system (Agilent, Berks., U.K.). The extracts (10 μL) were injected into an Agilent Zorbax Eclipse XDB-C18 column, 4.6 mm × 150 mm, 5 μm particle size (part 993967-902), with an Agilent Zorbax Eclipse XDB guard column, 1.0 mm × 17 mm (part 5185-5921). The mobile phase consisted of HPLC grade water with 0.5 g L^{-1} trifluoroacetic acid (TFA) (A) and acetonitrile with 0.5 g L^{-1} TFA (B). The gradient involved a linear increase/decrease of solvent B (5−10%, 5 min; 10−25%, 5 min; 25−85%, 6 min; 85−5%, 4 min; 5%, 5 min) at a flow rate of 0.8 mL min^{-1} and a column temperature of 30 °C. An Agilent 1200 DA G1315B/G1365B photodiode array detected eluted flavonols at a wavelength of 370 nm. The data were presented in Agilent ChemStation Rev. B.02.01 software, and flavonol concentration was calculated against authentic calibration standards (quercetin 3′-quercetin, quercetin 4′-glucoside, and quercetin 3,4′-diglucoside; PlantChem, Sandnes, Norway).

Quantification of Anthocyanins. Anthocyanins were separated and quantified according to the method of Giné Bordonaba and Terry (*16*) with modifications (*6*). The same extract and HPLC system were used to measure anthocyanins and flavonols. The undiluted extracts (10 μL) were injected into an Agilent Zorbax Eclipse XDB-C18 column, 4.6 mm × 250 mm, 5 μm particle size (part 990967-902). The mobile phase consisted of HPLC grade water with 2.5% acetonitrile and 5% formic acid (A) and acetonitrile (B). The program involved a linear increase/decrease of solvent B (5−10%, 9.5 min; 10−18%, 9.2 min; 18−65%, 3.3 min; 65%, 3 min) at a flow rate of 1 mL min^{-1} and a column temperature of 35 °C. Anthocyanins were detected at a wavelength of 520 nm. Each peak was assigned an identity according to the guidelines of Downes et al. (*6*). Anthocyanin concentrations were expressed as milligrams of cyanidin 3′-glucoside (Extrasynthese, Lyon, France) equivalent per gram of fresh weight (FW) (mg C3GE g^{-1} FW).

Statistical Analysis. All statistical analyses were conducted using Genstat for Windows version 10.1.0.147 (VSN International Ltd., Herts., U.K.). Analysis of variance (ANOVA) was performed on the data specifying a nested treatment structure of a common baseline (observation before curing at day 0 was the starting point for both curing temperature treatments), plus a factorial combination of time and curing temperature for each year separately. Least significant differences (LSD; P = 0.05) were calculated for each analysis. Significant differences are quoted at $P < 0.05$ for all physiological and biochemical parameters for both 2008 and 2009. Correlation coefficients (Pearson's) were calculated between all biochemical and physiological data sets. To further identify relationships between the biochemical and physiological data sets, principal component analysis (PCA; an unsupervised multivariate technique) and partial least-squares discriminant analysis (PLSDA; a supervised multivariate technique) were adopted. As anthocyanins were detected only in red onions ('Red Baron' in 2008 and 'Kamal' and 'Red Baron' in 2009), data sets for red onions ('Kamal' and 'Red Baron') and brown onions ('Sherpa' and 'Wellington') were analyzed separately for correlations and multivariate analysis. For the brown onion cultivars, nine variables (C^*, dry weight, fructose, glucose, $H°$, L^*, quercetin, quercetin glucoside, and quercetin 3,4′-diglucoside) were considered as analytical data for PCA, whereas the addition of nine individual anthocyanins took the number of variables for the red onion cultivars to 18.

RESULTS AND DISCUSSION

In the U.K., onions are currently cured at 28 °C, although reducing the curing temperature to 20 °C could reduce energy

Table 1. Quercetin, Quercetin 4′-Glucoside, and Quercetin 3,4′-Diglucoside Concentrations in Onion Skins of 'Red Baron', 'Sherpa', and 'Wellington' Cured for 6 Weeks at 20 or 28 °C before Being Transferred to Cold Storage (1 ± 0.5 °C) for 7 Months in 2008 (n = 15)[a]

	curing temp (°C)	quercetin (mg g^{-1} FW (DW))			quercetin 4′-glucoside (mg g^{-1} FW (DW))			quercetin 3,4′-diglucoside (mg g^{-1} FW (DW))		
		Red Baron	Sherpa	Wellington	Red Baron	Sherpa	Wellington	Red Baron	Sherpa	Wellington
before curing	n/a	11.5a (14.1)	35.2b (43.8)	16.5a (19.6)	12.0a (14.7)	9.7a (12.1)	7.7a (9.1)	2.72a (3.30)	0.60a (0.75)	0.30a (0.35)
after curing	20	7.6a (8.9)	16.5a (18.5)	14.8a (17.4)	7.9a (9.2)	9.6a (10.7)	4.8a (5.7)	0.37a (0.43)	0.61a (0.68)	0.35a (0.41)
	28	8.9a (10.1)	9.6a (10.8)	8.0a (9.1)	20.3a (23.0)	5.5a (6.2)	5.0a (5.8)	2.48a (2.83)	0.23a (0.26)	0.43a (0.50)
after cold storage	20	9.3a (11.6)	7.6a (10.2)	8.6a (10.7)	20.4a (25.4)	3.7a (4.6)	13.7a (17.1)	5.04b (6.27)	0.13a (0.16)	1.48a (1.84)
	28	11.0a (14.0)	5.6a (6.3)	7.3a (9.1)	12.9a (16.2)	3.3a (3.7)	3.5a (7.0)	0.93a (1.17)	0.14a (0.16)	0.64a (0.80)

[a] LSD (P = 0.05); quercetin = 11.51 (13.88), quercetin 4′-glucoside = 17.25 (20.79), quercetin 3,4′-diglucoside = 3.669 (4.541). Values followed by the same letter are not significantly different from each other calculated using the LSD.

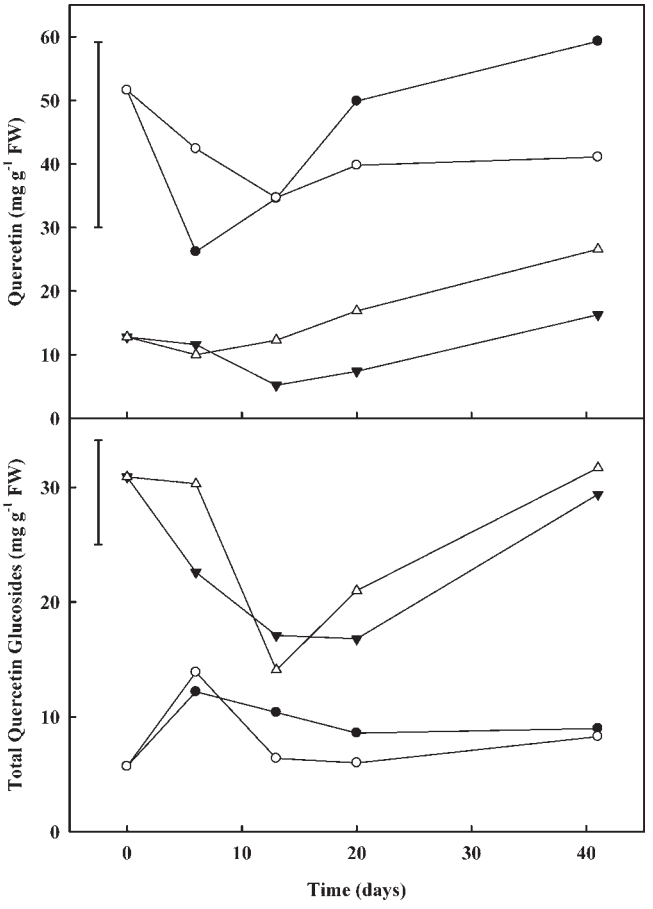

Figure 1. Flavonol concentrations in the skins of brown (mean of 'Sherpa' and 'Wellington'; circles) and red (mean of 'Kamal' and 'Red Baron'; triangles) onion in 2009 during 42 days of curing at 20 (solid symbols) or 28 °C (open symbols) (n = 18).

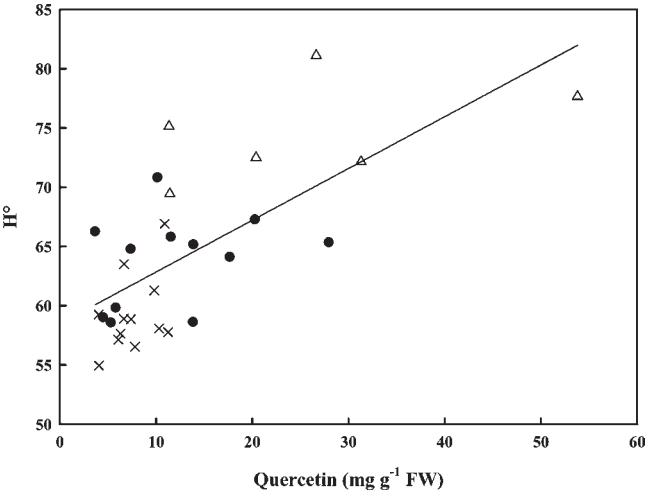

Figure 2. Correlation between $H°$ and quercetin content of onion skin 'Sherpa' and 'Wellington' before curing (open triangles) and after curing at 20 °C (solid circles) or 28 °C (crosses) in 2008 (r = 0.68; p < 0.001).

inputs and, therefore, costs. Previous works by Chope et al. (unpublished) found curing onion cv. 'Red Baron', 'Sherpa', and 'Wellington' at the lower temperature of 20 °C compared with 28 °C had no significant effect on sprout growth, sprout incidence, bulb dry weight, pungency, or disease incidence. In fact, onions cured at 20 °C had lower root incidence than those cured at 28 °C. The aim of this study was to investigate the effect of different curing temperatures on skin composition and, to the best of our knowledge, for the first time measure skin biochemistry and color throughout the curing process.

There was no main effect of curing on quercetin, quercetin 3,4′-diglucoside, or quercetin 4′-glucoside concentrations in 2008 and 2009 (**Table 1** and **Figure 1**). Nevertheless, in 2009, the mean quercetin 3,4′-diglucoside concentration for all cultivars throughout curing at 28 °C was significantly lower (0.72 mg g^{-1} FW) than throughout curing at 20 °C (1.21 mg g^{-1} FW). Freshly harvested brown onions 'Sherpa' and 'Wellington' contained higher concentrations of quercetin and a wider range (from ca. 10 to 55 mg g^{-1} FW) of values compared with cured onions. Thus, skin samples from brown onions cured at 20 °C contained quercetin concentrations below ca. 30 mg g^{-1} FW, and those cured at 28 °C were even lower at ca. 4−10 mg g^{-1} FW. This trend (quercetin before curing > 20 °C > 28 °C) was also observed for $H°$ with the skins of freshly cured brown onions having the highest $H°$ (ca. 70−80 indicating a yellow color) followed by those cured at 20 °C (ca. 60−70) and those cured at 28 °C (ca. 55−65) becoming successively browner and resulting in a positive correlation between quercetin and $H°$ (r = 0.69; n = 30). These data are presented in **Figure 2** and include samples taken before curing and from onions cured at 20 or 28 °C both immediately after curing and after 7 months cold storage. It could be assumed that the range of quercetin concentrations or $H°$ values would be greater in cured and stored onion skin than those before curing, yet this was not the case. This suggests that curing results in a more uniform skin color and quercetin content, which persists throughout cold storage as highlighted in **Tables 1** and **2**. Commercially, uniformity of color is a desirable characteristic, and reducing curing temperatures to 20 °C seems to create as uniform a color as 28 °C. Although there was no significant correlation between $H°$ and quercetin in the 2009 data, the PCA of brown onion cultivars (**Figure 3**) highlights the relationship between $H°$ and flavonol content.

Table 2. Chroma (C^*), Hue Angle ($H°$), and Lightness (L^*) of Skins of Onion 'Red Baron', 'Sherpa' and 'Wellington' before Curing, after 6 Weeks of Curing (at 20 or 28 °C), and after Cold Storage (1 ± 0.5 °C) for 7 Months in 2008 (n = 15)[a]

		chroma (C^*)			hue angle ($H°$)			lightness (L^*)		
	curing temp (°C)	Red Baron	Sherpa	Wellington	Red Baron	Sherpa	Wellington	Red Baron	Sherpa	Wellington
before curing	n/a	16.34a	27.11ef	27.66efg	14.15a	74.10f	75.23f	32.54a	61.14d	59.62cd
after curing	20	21.25b	27.71efg	28.10fghi	12.23a	65.20de	67.72e	32.31a	60.06cd	59.14cd
	28	22.62bc	28.01efgh	29.74ghij	14.48a	58.53bc	62.69cde	33.24a	56.41bc	56.62bc
after cold storage	20	25.47de	30.42hij	30.65ij	12.26a	61.37bcd	60.77bcd	34.67a	61.64d	58.75bcd
	28	24.55cd	31.25j	31.94j	15.55a	58.43bc	57.30b	34.45a	58.78bcd	55.19b

[a] LSD (P = 0.05); C^* = 2.542, $H°$ = 5.046, L^* = 3.920. Values followed by the same letter are not significantly different from each other calculated using the LSD.

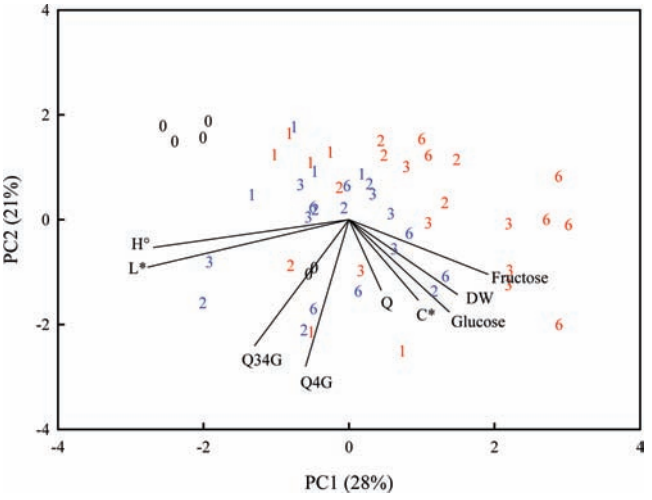

Figure 3. Principal component analysis biplot of all brown onion cultivars in 2009, labeled by number of weeks of curing (0, 1, 2, 3, and 6 weeks) and by curing temperature: before curing (black), after curing at 20 °C (blue) or 28 °C (red).

Although both 2008 and 2009 data were analyzed using PCA, only 2009 data have been presented because the same trends were found for both years. The skins of brown onion cultivars showed a clear separation of samples on PC1 (captured 28% of the variance) and PC2 (captured 21% of the variance) accounting for, in total, almost 50% of the variance. The inclusion of a third principal component did not improve sample clustering. Samples were mainly separated according to time and curing temperature (**Figure 3**). According to the biplots, the most important variables for differentiating between samples from different time points throughout curing were fructose, glucose, dry weight, and C^*, which all increased with time. In contrast, the most important variables for distinguishing between curing temperatures were $H°$, L^*, and both quercetin glucosides, which were all higher in skins cured at 20 °C. Takahama and Hirota (*8*) suggested that quercetin is formed from the deglucosylation of quercetin glucosides on the border between the dry brown skin and the drying section, because deglucosylation requires water. Since the 2009 data were recorded at weekly intervals mainly throughout the early stages of curing (weeks 0, 1, 2, 3, and 6), the quercetin glucosides may not yet have been deglucosylated into quercetin. In 2008, correlations between $H°$ and individual quercetin glucosides (quercetin 4′-glucoside and quercetin 3,4′-diglucoside) were very low (r = 0.43 and 0.14, respectively); however, the correlation between total flavonols and $H°$ was reasonable (r = 0.66), thus suggesting that both quercetin aglycon and quercetin glucoside concentrations may contribute to brown onion skin color. The 2008 data were recorded before curing, after curing, and after cold storage; 80% of the samples were from the two latter time points, by which time the quercetin glucosides may have already been converted to quercetin. Gökçe et al. (*19*) correlated onion scale color with total phenolics and total antioxidant capacity; this said, although significant, the correlations did not rise above r = 0.42. The lack of strong correlations may have been due to the use of total phenolics which, apart from quercetin, takes account of isorhamnetin, kaempferol, and other phenolic acids found in red onion skin such as gallic acid, ferulic acid, and protocatechuic acid as well as vitamin C and reducing sugars (including fructose and glucose), which may not all contribute to skin color changes (*20*, *21*).

Onion skin L^* was affected by curing temperature, where onions cured at 28 °C became significantly darker than those cured at 20 °C in both years (**Table 2** and **Figure 4**). The $H°$ of brown skin significantly decreased throughout curing at 20 °C (from 73.79 to 68.34) in 2009 and between the end of curing at 20 °C and the end of cold storage (from 74.6 to 63.77) in 2008, turning from yellow to orange. Those cured at 28 °C showed a greater change, becoming more orange/brown throughout curing in 2009 (from 73.79 to 62.93) and after curing at 28 °C and after cold storage (from 74.67 to 59.24) in 2008. During curing, C^* and L^* decreased steadily week by week, whereas the largest change in red and brown skin $H°$ was in the first 6 days of curing. In 2009, the PCA of the brown onion cultivars confirmed that at 20 °C onion skins were paler (higher L^*) and more yellow (higher $H°$) and contained higher concentrations of quercetin glucosides than the onions cured at 28 °C. It has previously been hypothesized (*22*) that the formation of brown pigmentation during onion skin drying may be due to the conversion of quercetin glucosides into the aglycon form because this conversion occurs during dry skin formation. On the other hand, Ito et al. (*7*) found quercetin and its glucosides are unlikely to contribute themselves to skin color because they are too pale and have a maximum wavelength of 370 nm, whereas the pigmented compound in the dry scales of yellow onions had a maximum wavelength of 450 nm. Ly et al. (*23*) identified many pigmented compounds in the dry outer scales of onion bulbs (cultivars not described). Along with several yellow pigmented compounds including quercetin and quercetin glucosides, the authors also identified several brown oxidative products of quercetin using NMR. The brown pigmented compounds identified were protocatechuic acid, a benzofuranone, and quercetin dimers. Under oxidative conditions, Makris and Rossiter (*24*) found the major degradative product of quercetin was protocatechuic acid, which agrees with Ly et al. (*23*). The concentration of protocatechuic acid was double that of each quercetin dimer and 5-fold greater than the benzofuranone (*23*). This suggests that there may be multiple oxidative products of quercetin apart from the dominant protocatechuic acid that contribute to the brown pigmentation of onion skin when cured at a higher temperature, which may include not only the smaller oxidative products of quercetin but also the larger dimers.

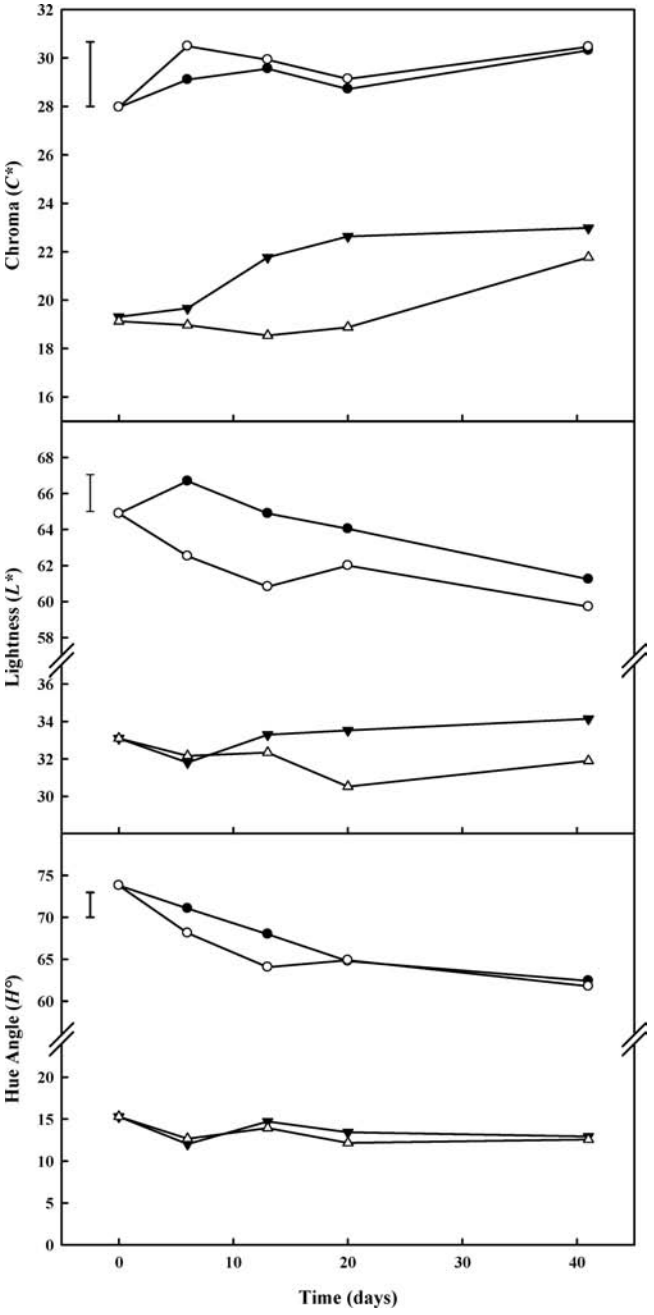

Figure 4. Chroma (C^*), hue angle ($H°$), and lightness (L^*) of skins from brown (mean of 'Sherpa' and 'Wellington'; circles) and red (mean of 'Kamal' and 'Red Baron'; triangles) onion in 2009 during 42 days of curing at 20 (solid symbols) or 28 °C (open symbols) (n = 18).

A relationship between color and quercetin glucoside content was also found in the skins of the red onions using multivariate analysis. The PCA on the red onion physiological and biochemical data showed a separation of samples according to time in PC1 (capturing 28% of the variance; data not shown); on the other hand, PC2 captured only a further 16% of the variance. Again, the inclusion of a third principal component did not improve sample clustering. When samples were labeled according to curing temperature, no clear separation was observed. Therefore, PLSDA (a supervised multivariate technique) was employed with clustering biased toward curing temperature (**Figure 5**). The most important variables contributing to clustering according to curing temperature were C^*, L^*, quercetin, and cyanidin 3′-glucoside. The profiles of anthocyanin content in the flesh

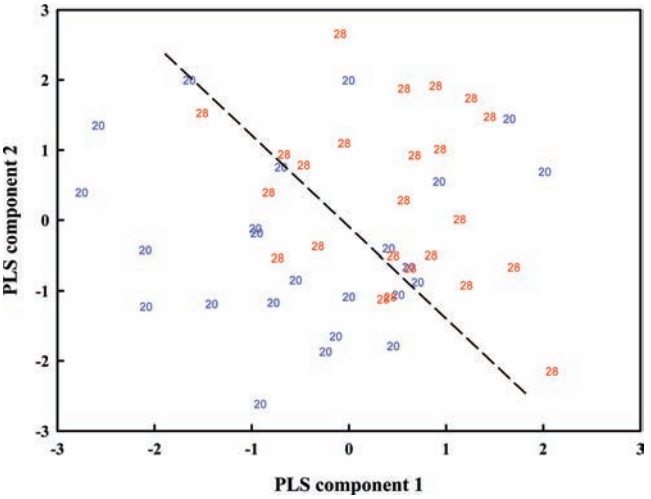

Figure 5. Partial least-squares discriminant analysis of all red onion cultivars in 2009 discriminated according to curing temperature, cured at 20 °C or 28 °C.

Table 3. Anthocyanin Concentrations in Onion Skins of 'Red Baron' Cured for 6 Weeks at 20 or 28 °C before Being Transferred to Cold Storage (1 ± 0.5 °C) for 7 Months in 2008 (n = 15)[a]

		anthocyanin (mg C3GE g^{-1} FW (DW))		
	curing temp (°C)	cyanidin 3-(6″-malonoyl-laminariboside)	cyanidin 3-(6″-malonoyl-glucoside)	cyanidin 3′-glucoside
before curing	n/a	0.37b (0.45)	2.37b (2.88)	0.52a (0.63)
after curing	20	0.38b (0.44)	2.10b (2.46)	0.93ab (1.09)
	28	0.45b (0.51)	3.07b (3.50)	1.17b (1.33)
after cold storage	20	0.36b (0.45)	1.98b (2.44)	0.87ab (1.08)
	28	0.10a (0.13)	0.66a (0.85)	0.48a (0.61)
LSD (P = 0.05)		0.199 (0.243)	1.358 (1.639)	0.524 (0.636)

[a] Values followed by the same letter are not significantly different from each other calculated using the LSD.

and skins of red onion (**Table 3**) are comparable, unlike those of flavonols as discussed earlier (*6*, *18*). The PLSDA revealed that red onions cured at 20 °C contained a higher concentration of quercetin in the skins and had a lower C^* value corresponding to a less saturated appearance compared with those cured at 28 °C. Like the relationship between quercetin/quercetin glucoside concentrations and color in brown onions, the same explanation can be applied to the red onion skins, where oxidation of quercetin into highly pigmented oxidative products may increase the strength of color in the red onion skins and therefore result in a higher C^* value. The difference in skin color of the 2008 brown onion cultivars cured at different temperatures was qualified by differences in $H°$, although the high red pigmentation of the red onions due to the anthocyanin content may have masked the effect of changes in flavonol content on $H°$, and therefore only changes in color saturation (C^*) between curing temperatures were recorded. However, differences in L^* were also found in the PLSDA of the 2009 data, where onion skins cured at 28 °C were lighter than those cured at 20 °C, although this is more likely to be due to the degradation of cyanidin 3-(6″ malonoyl-laminariboside)) and cyanidin 3-(6″-malonoylglucoside) at the higher temperature also found in 2008 (**Figure 3**), both of which have previously been shown to be sensitive to curing temperature (*6*). This concludes

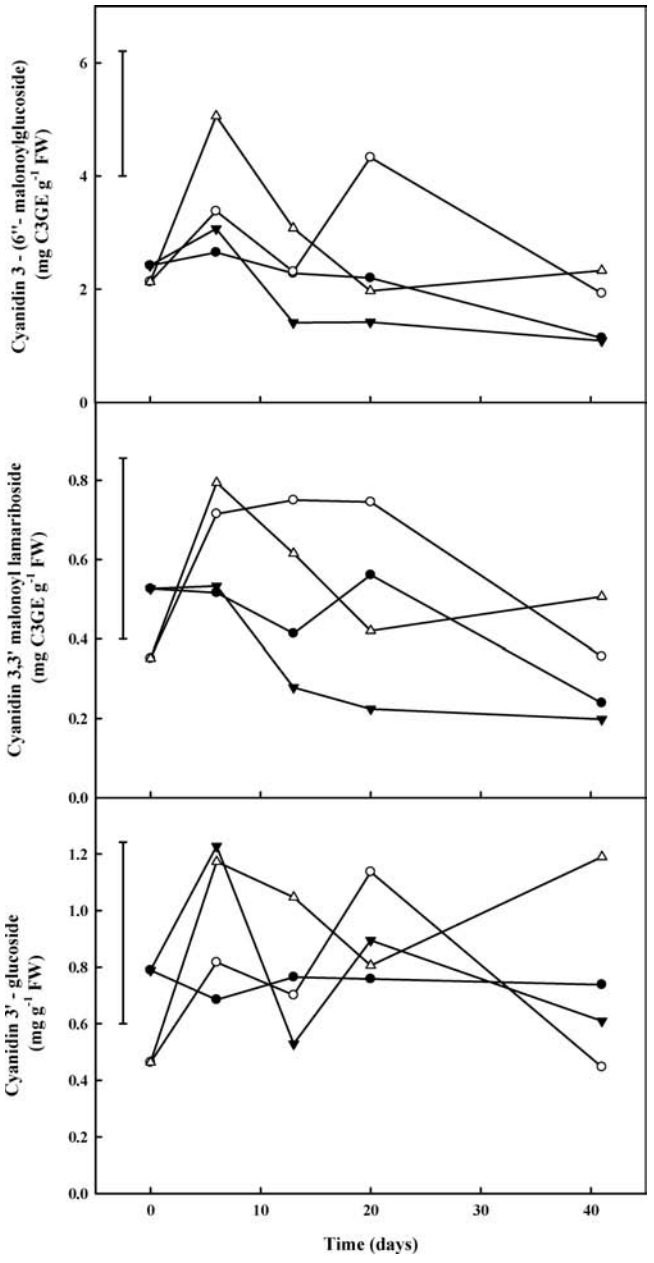

Figure 6. Anthocyanin concentrations in skins from red onion 'Kamal' (solid symbols) and 'Red Baron' (open symbols) in 2009 during 42 days of curing at 20 (circles) or 28 °C (triangles) (n = 9).

that curing at 20 °C reduces the degradation of skin anthocyanin content, preserving a redder appearance. Reducing the curing temperature to 20 °C would not only save energy costs but also potentially create an improved red onion appearance.

In 2009, cyanidin 3-(6″-malonoylglucoside) concentrations in 'Red Baron' onions peaked after 6 days in those cured at 28 °C and after 20 days in those cured at 20 °C (**Figure 6**). This variation in anthocyanin production between curing temperatures could not be due to differences in the rate of skin water loss because there were no significant differences in skin dry weight between onions cured at different temperatures. In 2009, monitoring dry weight throughout curing showed that the largest decrease in skin water content occurred within the first 6 days (712.3−793.8 mg g^{-1} FW). Therefore, the standard U.K. practice of curing for up to 6 weeks may not indeed be necessary. To the best of our knowledge this is the first time dynamic changes in skin water content have been measured during curing.

Sugars were measured in the skins of red and brown onions as some biproducts of the Maillard reaction are pigmented, and the regulatory role sugars possibly play in the production of anthocyanins and flavonol glucosides was examined (*11*, *12*). Fructose and glucose concentrations in onion skins were not affected by cultivar or curing temperature in either 2008 or 2009. Gennaro et al. (*11*) identified a positive relationship between anthocyanin concentration and free sugar concentrations in the edible portion of red onion cv. 'Tropea'. Sugar concentrations were not correlated with anthocyanin or flavonol concentrations nor was a relationship with color or dry weight identified in either 2008 or 2009 in the skins of any onion cultivars, agreeing with Downes et al. (*6*). Gökçe et al. (*19*) found that soluble solids in onion scales were positively and significantly correlated with total phenolics and total antioxidant capacity, but the correlations were weak (0.41 and 0.43, respectively).

In conclusion, fructose and glucose concentrations appeared to play no role in explaining the difference in onion skin appearance when bulbs were cured at different temperatures. Quercetin and its glucosides appear to be linked to the intensities of brown pigmentation in onions cured at different temperatures. Skins cured at 28 °C became darker and browner possibly due to the conversion of quercetin and its glucosides into brown oxidative products. This same relationship between skin flavonol content and color intensity was also observed in the skins of red onion cultivars. Additionally, curing at 28 °C resulted in greater reduction in anthocyanin content therefore reducing the curing temperature to 20 °C may not only save costs but also improve red onion appearance. Finally, dynamic changes in skin biochemistry and color throughout curing appeared to occur mainly in the first few weeks. Because the only significant change in skin dry weight also occurred in the first week of curing, the standard U.K. practice of up to 6 weeks could be reduced, resulting in additional cost reductions.

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Supporting Information Available: Fructose and glucose concentrations in the skins of brown and red onions before curing, after curing at 20 or 28 °C, and after long-term cold storage in 2008 (S1) and during 42 days of curing at 20 or 28 °C in 2009 (S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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