Modeling ammonia accumulation and color changes of arugula (*Diplotaxis tenuifolia*) leaves in relation to temperature, storage time and cultivar

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Abstract

Leafy greens produce ammonia under stressful conditions during postharvest handling. The objective of this study was to understand the relationship between ammonia accumulation and color variation of arugula leaves in relation to cultivar and storage temperature and time. Leaves of five cultivars ('Bellezia', 'Grazia', 'Letizia', 'Tricia', and 'Wild Thing') were washed, centrifuged, placed in unsealed plastic bags, and stored at 0, 5 or 10°C for 28, 20 and 12 days, respectively. Visual quality score, color (hue angle) and ammonia accumulation were monitored every 4 or 8 days, depending on temperature. Ammonia increased from 11-15 µg g^{-1} fresh weight (FW) to 150-220 at 0°C, 340-450 at 5°C and >590 µg g^{-1} FW at 10°C by the end of storage-life. While there were some differences among cultivars, temperature played the major role in ammonia accumulation. Color varied due to leaf yellowing, which increased with increasing temperature. Both hue angle and ammonia changes were fitted with traditional (first- and zero-order kinetics) and Weibullian models, with the latter explaining a higher percentage of experimental data variance. Moreover, considering that time was the common variable between the two kinetics, a new mathematical equation describing ammonia versus hue angle for each cultivar/temperature was obtained. In all cases, there was a good correlation between ammonia content and color changes, with more accurate results at 5 and 10°C (r>0.98), since color at 0°C was quite stable (r>0.72). These results demonstrate that ammonia may be a good indicator of senescence in arugula, since it correlated well to color change with storage temperature and time.

Keywords: leafy greens, zero-order kinetics, first-order kinetics, Weibull model

INTRODUCTION

Arugula, or rocket, is a leafy green from the family *Brassicaceae* that is usually eaten alone or in a mixed leafy salad, as it adds flavor and nutritional components (Martínez-Sánchez et al., 2006; Bennett et al., 2006). The two species commonly sold commercially are known as salad rocket (*Eruca sativa*) and wild rocket (*Diplotaxis tenuifolia*); the latter is known to have longer storage-life. Low temperature is very important to achieve maximum storage-life or to maintain the quality of arugula (Koukounaras et al., 2007; Watada et al., 1996). A temperature of 0°C with >95% relative humidity (RH) is required to optimize the storage-life of many specialty leafy greens (Cantwell et al., 1998), though commercial handling temperatures through distribution and retail may range from 2 to 10°C. Koukounaras et al. (2007) reported a storage-life of 16 days for arugula stored at 0°C, which decreased to 13 days at 5°C and 8 days at 10°C. Color loss and yellowing, resulting from chlorophyll degradation, have been reported to be the most critical postharvest alterations in arugula leaves. Fresh arugula should be dark or bright green in color, but, during senescence, color changes occur due to chlorophyll loss, which lead to yellowing and a general loss of visual quality. This condition also affects the overall quality evaluation by the

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consumer, who judges the product as not fresh. Storage at low temperature should minimize this effect, as it delays metabolic activity, thereby retarding chlorophyll degradation.

Another aspect associated with leaf senescence is ammonia accumulation in plant tissues as a consequence of protein catabolism (Toivonen, 1997). Ammonia is produced in appreciable quantities in some plant tissues (Treshow, 1970; King et al., 1990), and high concentrations were reported during early stages of senescence in various leafy and floral vegetables such as broccoli, cauliflower, romaine lettuce, spinach, and sugar-snap peas (Cantwell et al., 2010). Chibnall (1939) reported that ammonia accumulation was the cause of the darkening process associated with the senescence of leaves. Cantwell et al. (2010) also reported darkening of spinach leaves coincident with ammonia accumulation during storage. Leafy greens accumulate ammonia under stressful postharvest conditions because of a decrease in the activity of glutamine synthetase, the enzyme responsible for ammonia reincorporation during protein turnover. Ammonia is toxic to cells, and accumulation in large amounts may cause tissue damage (Toivonen, 1997).

Based on these antecedents, it is of interest to understand whether ammonia can be considered a senescence indicator and if it is well correlated with other quality changes. Quality loss may be analyzed using mathematical models as a function of the time and temperature of storage. Degradation reactions are generally described through the conventional zero-, first- or second-order kinetics (Labuza, 1982; Tauokis et al., 1997; Giannakourou and Taoukis, 2003; Nisha Rekha et al., 2004; Nisha et al., 2005; Zanoni et al., 2005; Rodrigo et al., 2007; Sothornvit and Kiatchanapaibul, 2009). After the estimation of a kinetic constant, the Arrhenius equation is traditionally used to evaluate the rate constant at each temperature to predict the shelf-life of the food, but this methodology is not able to describe quality changes that depend on time. The Weibull model takes into account time as a variable and has been used to describe changes in chemical and sensory attributes of fresh-cut produce (Iqbal et al., 2005; Oms-Oliu et al., 2009; Odriozola-Serrano et al., 2009). Amodio et al. (2015) used the Weibull model to describe several degradation reactions of quality attributes of packaged arugula leaves, obtaining good data fitting and estimation of product storage-life at different temperatures. The objective of this study was to understand whether a mathematical relationship between ammonia accumulation and color change could be found and, to this aim, whether the Weibullian or another conventional kinetic model could be used to fit quality changes over time.

MATERIALS AND METHODS

Raw materials and experiment setup

Five cultivars of arugula ('Bellezia', 'Grazia', 'Letizia', 'Tricia', 'Wild Thing') were obtained from an Enza Zaden variety trial in a grower's field near San Juan Bautista, CA, USA, on 29 September 2014. Product was manually cut using sharp knives, placed in unsealed plastic bags and transported to the laboratory in coolers with ice and held overnight at 0°C. About 1500 g batches were washed and sanitized (5:1 ratio of wash solution to product weight) using swirling chlorinated water for 20 sec (50 ppm NaOCl at 20°C, pH 7.0) and then centrifuged using a Dynamic spinner (model 06140). Quantities of 65-70 g were placed in small unsealed plastic bags on trays, which were enclosed in larger unsealed bags to prevent water loss. Samples were stored at 0, 5 and 10°C and overall visual quality, objective color values and ammonia concentrations were evaluated periodically. Based on previous tests, sampling days for the three temperatures were 0, 8, 16, 20, 24 and 28 days for leaves stored at 0°C, 0, 8, 12, 16 and 20 days for samples at 5°C, and 0, 4, 8 and 12 days for leaves stored at 10°C. Three replicates were taken for quality evaluations and composition on each sampling day.

Quality evaluation and composition

The overall visual quality was scored on a 9 to 1 scale, where 9 = excellent, fresh appearance, 7 = good, 5 = fair (limit of marketability), 3 = fair (usable, but not salable), and 1 = unusable. Color evaluation was based on L*a* and b* color values using a Minolta color meter (Minolta, CR-300); hue angle (H) and Chroma (C) values were calculated as H=arctan(b*/a*) and C=√(a*^{2}+b*^{2}).

Ammonia was determined by a colorimetric method (Weatherburn, 1967; Beecher and Whitten, 1970) using a Shimadzu UV-1700 PharmaSpec spectrophotometer.

Four grams of arugula leaves was cut and frozen at -80°C until analysis. Tissue was homogenized with 20 mL distilled water for 1 min, and a 1.5 mL aliquot was centrifuged for 10 min at 14,000 g at 5°C. A 0.5 mL aliquot was added to a solution of nitroprusside with phenol and alkaline hypochlorite in a reaction mixture, which was incubated at 37°C for 15 min, and color development was measured at 635 nm. Ammonium sulfate was used as a standard.

Mathematical modeling

Zero- and first-order kinetics are traditionally used to describe degradation reactions in foods and may be generically written (Giannakourou and Taoukis, 2003; Polydera et al., 2005; Zanoni et al., 2005; Nisha et al., 2005) as:

$$\frac{dC_t}{dt} = -kC^m \quad (1)$$

where C_t is the concentration of the quality index at time t, k is the rate constant and m is the kinetic order of the equation. The equation may be integrated easily, obtaining the well-known decay functions. In particular the zero-order kinetic model (m=0) is written as $C_t=C_0$-kt, whereas first-order kinetics (m=1) are written as $C_t=C_0e^{-kt}$.

As an alternative, according to Corradini and Peleg (2004), the degradation reactions can be analyzed using the cumulative form of the Weibull distribution (Equation 2):

$$C_t/C_0=\exp[-btn] \quad (2)$$

where b and n are temperature-dependent coefficients and C_t/C_0 is the fraction of "molecules" that retain their "intact" form over storage, which is referred to as a conserved initial quality, with C_t and C_0 being the numbers of "intact" molecules at times t and t=0, respectively. This equation can be applied to any attribute that decreases over time, and was used for H changes whereas, for ammonia accumulation, Equation 3 was used:

$$C_t/C_0 =1-\exp[-btn] \quad (3)$$

Since the only variable in common between Equations 2 and 3 is the storage time t, the following mathematical relation Equation 4 between changes in ammonia and hue angle was obtained:

$$C_A(t) = 1 - \exp\left[-b_A\left(\frac{\log(H)}{b_H}\right)^{\frac{1}{n_H}}\right]^{n_A} \quad (4)$$

After a one-way ANOVA to test the effect of time of storage for each quality parameter, curve fitting was obtained using the non-linear model option in STATISTICA 7 (Stat Soft. Inc., Tulsa, OK, USA). The goodness of fit was evaluated by the correlation coefficient (r), the sum of squares error (SSE) and the root mean square error (RMSE). Moreover, the kinetic parameters were compared by the confidence interval (CI) (95% probability).

RESULTS AND DISCUSSION

Temperature was clearly the most important factor affecting quality changes of arugula leaves; shelf-life of arugula leaves based on visual quality assessments was much longer at lower temperature, averaging across cultivars 22, 11 and 6 days at 0, 5 and 10°C, respectively. Hue angle was used to describe changes in visual quality, with these measurements being more objective than sensory evaluation.

Ammonia (A) increased from an initial 11-15 µg g^{-1} FW to 180-270 at 0°C, 440-570 at 5°C and >570 µg g^{-1} FW at 10°C at the end of storage-life (Figure 1). While there were some differences among cultivars, temperature played the major role in ammonia accumulation. Also, for hue angle, temperature was the most important factor. Hue angle decreased over time from 124 to 118 (5°C) and 115 (10°C), while remaining almost unchanged at 0°C (Figure 1). Both ammonia and hue angle were fitted by zero- and first-order kinetic models as well as the Weibull model. Among conventional kinetics, A was better described by first-order kinetics, whereas H showed a linear variation. The results of the fitting are reported in Tables 1 and 2, respectively. For ammonia (Table 1), all models were highly accurate (r>0.96), whereas, for hue angle, the fitting at 0°C gave low regression coefficients, particularly when zero-order kinetics were used. This was due to the little color variation measured at this temperature, particularly for cultivars 'Bellezia' and 'Tricia'.

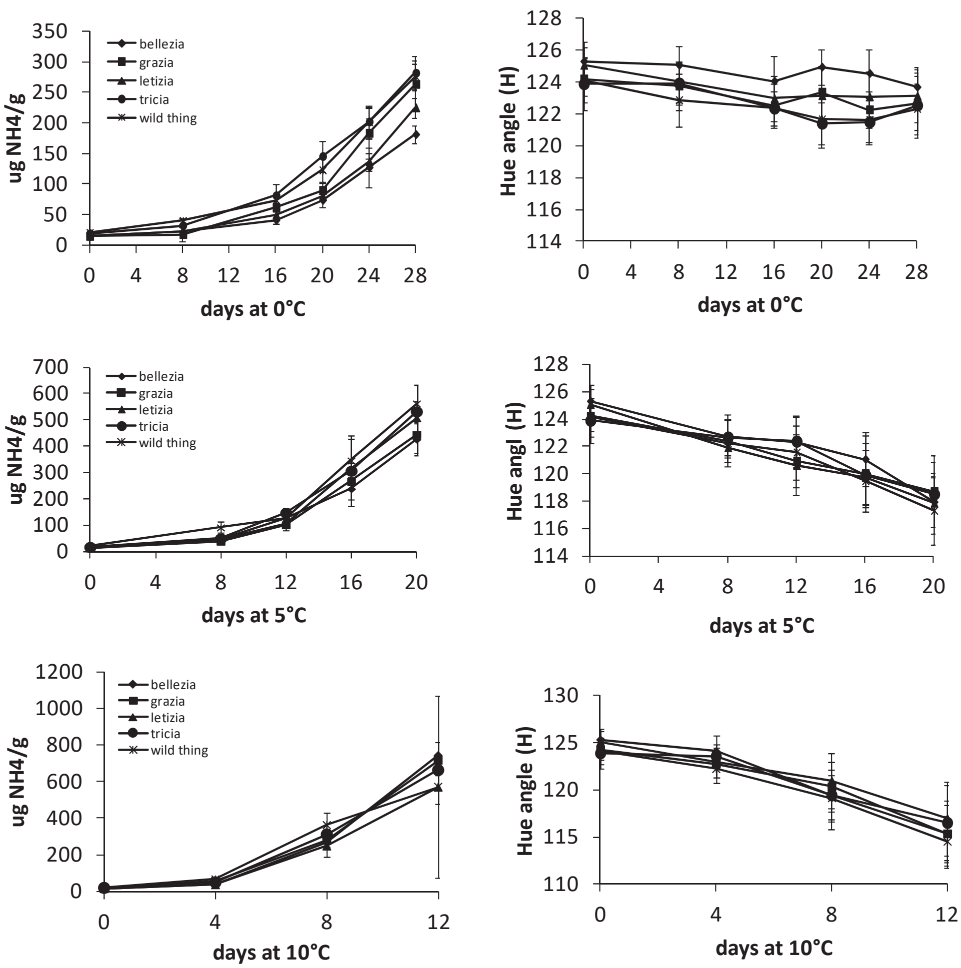

Figure 1. Ammonia accumulation and hue angle changes for five arugula cultivars stored at three temperatures. Data are means ± standard deviation.

Table 1. Weibull parameters for the fitting of ammonia changes over time of arugula leaves stored at different temperatures.

Cultivar	Temp. (°C)	b (d-1)	Confidence interval	*n*	Confidence interval	*r*	*r**1
Bellezia	0	2.85E-08	1.331E-08-2.852E-08	5.531	3.764-7.297	0.997	0.969
	5	4.03E-06	4.032E-06-4.032E-06	4.460	3.251-7.336	0.980	0.994
	10	1.50E-06	1.505E-06-1.505E-06	6.036	1.008-11.064	0.999	0.970
Grazia	0	1.33E-08	1.320E-08-1.340E-08	5.761	3.454-8.069	0.993	0.957
	5	4.02E-07	4.018E-07-4.031E-07	5.294	4.02E-07	0.996	0.994
	10	6.85E-06	-0.00007-0.00009	5.367	-0.186-10.920	0.998	0.990
Letizia	0	1.62 E-08	1.510E-09-1.719E-06	5.656	2.915-8.397	0.989	0.976
	5	1.81E-07	1.808E-07-1.820E-07	5.589	3.669-7.511	0.998	0.983
	10	1.15E-06	-0.000069-0.000092	5.181	1.836-8.525	0.999	0.972
Tricia	0	2.18E-06	2.180E-06-2.182E-06	4.205	2.696-5.7133	0.994	0.989
	5	2.01E-06	1.808E-07-1.809E-07	4.714	2.288-7.142	0.998	0.984
	10	3.12E-05	-0.000163-0.000226	4.754	1.771-7.737	0.994	0.974
Wild Thing	0	2.18E-06	2.941E-07-2.941E-06	5.713	2.927-6.719	0.993	0.993
	5	8.44E-07	8.416E-07-8.537E-07	4.714	1.642-8.432	0.989	0.987
	10	0.0005	0.0004-0.0014	3.639	2.767-4.512	0.979	0.959

[1]*r** is the regression coefficient for conventional first-order kinetics.

Table 2. Weibull parameters for the fitting of hue angle changes over time of arugula leaves stored at different temperatures.

Cultivar	Temp. (°C)	b (d-1)	Confidence interval	*n*	Confidence interval	*r*	*r**1
Bellezia	0	0.00017	0.00125-0.00159	1.232	-1.393-3.857	0.760	0.586
	5	0.00067	-0.00134-0.00268	1.483	0.419-2.546	0.974	0.922
	10	0.00150	-0.00159-0.00458	1.621	0.755-2.487	0.996	0.955
Grazia	0	0.00114	-0.00375-0.00602	0.757	-0.632-2.146	0.852	0.712
	5	0.00141	0.000478-0.00234	1.159	0.924-1.394	0.999	0.993
	10	0.00064	-0.00042-0.00170	1.908	1.217-2.599	0.999	0.929
Letizia	0	0.00449	-0.00194-0.01092	0.402	-0.073-0.876	0.962	0.796
	5	0.00358	0.00061-0.00655	0.926	0.627-1.225	0.996	0.991
	10	0.00197	-0.00198-0.00593	1.411	0.562-2.259	0.995	0.969
Tricia	0	0.00098	-0.00517-0.00713	0.873	-1.150-2.896	0.791	0.920
	5	0.00013	-0.00026-0.00053	1.944	0.922-2.965	0.986	0.621
	10	0.00080	-0.00279-0.00438	1.761	-0.119-3.640	0.994	0.890
Wild Thing	0	0.00527	-0.00518-0.01572	0.381	-0.276-1.038	0.929	0.731
	5	0.00038	-0.00020-0.00096	1.666	1.129-2.202	0.995	0.941
	10	0.0016	0.0006-0.0026	1.574	1.309-1.839	0.999	0.965

[1]*r** is the regression coefficient for conventional zero-order kinetics.

Regression coefficients for these cultivars increased from 0.59 to 0.76 and from 0.71 to 0.85 when passing from conventional to Weibull models. For all the other cultivar-temperature combinations, fitting with the Weibull equation always gave very accurate results (r>0.96), better than zero-order kinetics, except for 'Tricia' at 0°C (r=0.79 for Weibull and 0.92 for zero-order kinetics). These results confirm the idea that conventional zero- and first-order kinetics have low flexibility to obtain good estimation under different conditions, and the results agree with those of several other authors (Manso et al., 2001; Nisha Rekha et al., 2004, Rodrigo et al. 2007, Sothornvit and Kiatchanapaibul, 2009; Oms-Oliu et al., 2009;

Odriozola-Serrano et al., 2009). Oms-Oliu et al. (2009) obtained correlation coefficients always greater than 0.976, fitting the changes of vitamin C content and phenolic content of fresh-cut melon samples stored between 5 and 20°C with the Weibull model. Amodio et al. (2015) found that the Weibullian model fitted several quality attributes of arugula leaves (including sensorial score for appearance, color, off-odor and texture, vitamin C, and CO_2 and O_2 changes in the package) with higher accuracy than first-order kinetics.

The shape of the curve changed with the storage temperature (Figure 2). As reported by van Boekel (2002), values <1, observed at 0°C for all cultivars except 'Bellezia' and for 'Grazia' also at 5°C, lead to an upward concavity, whereas values >1, leading to a downward concavity, are generally observed at higher temperatures.

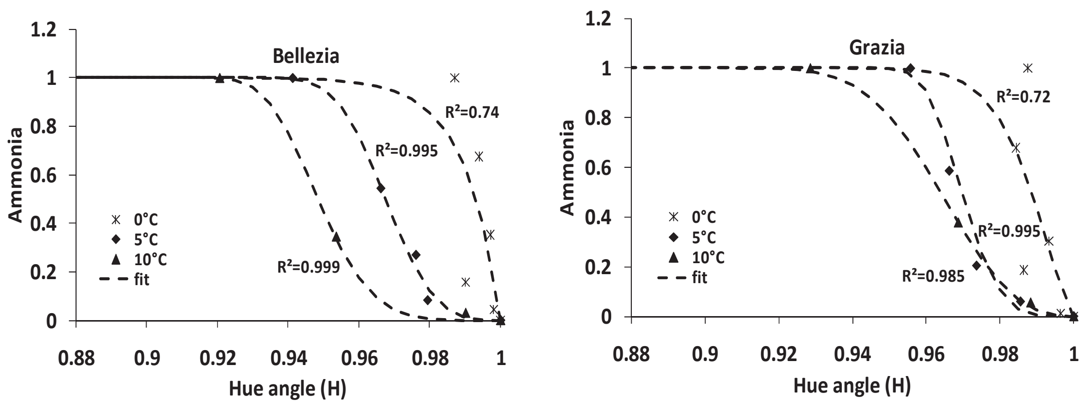

Figure 2. Fitting of the changes of ammonia vs. hue angle for two of the five arugula cultivars for each storage temperature.

Moreover, considering that time was the common variable between the two kinetics describing ammonia (A) and hue (H) changes over time, a new mathematical equation describing a relation between A and H for each cultivar/temperature of storage was obtained. The new mathematical equation gave a good correlation, with more accurate results at 5 and 10°C (r>0.98), since color at 0°C was quite stable (r>0.72). In Table 2, the fitting result of this equation is shown for cultivars 'Bellezia' and 'Grazia'. These results indicate a faster ammonia accumulation at the higher temperature, which can be related to the color changes of arugula leaves.

CONCLUSIONS

Changes in ammonia concentrations were highly correlated to changes in color of arugula leaves stored at 5 or 10°C (r>0.98), while a lower correlation (r>0.72) was obtained at 0°C due to color stability at this temperature. These results indicate that ammonia accumulation is related to leafy green senescence, encouraging further studies to validate these results and to better understand the factors affecting the postharvest accumulation of ammonia.

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