238 2014, Vol.35, No.16 **食品科学** ※包裝贮运

Establishment of a Mathematical Model for Treatment of *Gynura bicolor* DC. by Nano-Packaging in Combination with Controlled Atmosphere

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Abstract: The effects of nano-packaging (NP) in combination with controlled atmosphere (CA) on the preservation and nutritional quality of fresh *Gynura bicolor* DC (*G. bicolor*). were investigated during storage at 0 °C. The nutrition and quality indices including decay index, respiration rate, as well as the contents of anthocyanin, chlorophyll, amino acid, protein, total carbohydrate and reducing sugar were tested every 5 days. The combined treatment (CA+NP) improved the nutritional quality of *G. bicolor* compared with polyethylene packaging in CA or NP in a modified atmosphere alone. After 20-day storage, CA + NP treatment significantly inhibited respiration intensity and maintained higher contents of total soluble solids, anthocyanin, chlorophyll, amino acids, protein, total sugar, and reducing sugar. The simplified regression model: Decay index/%=24.605 - 0.020 X_7 + 1.122 X_4 - 0.162 X_5 (where, X_4 : reducing sugar content, X_5 : anthocyanin content, X_7 : chlorophyll content) revealed satisfactory goodness of fit (X_7) on the content of the protein of the protein

Key words: Gynura bicolor DC.; phytic acid; preservation; quality; mathematical model

采后纳米材料包装结合气调处理对紫背天葵贮藏品质的影响及数学模型建立

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摘 要:以紫背天葵为试材,采用纳米材料自发气调、HDPE袋(15 μ m厚)人工气调(3% O_2)和纳米材料结合人工气调(3% O_2)包装处理,并置于0 C条件下贮藏20 d,每5 d测定一次生理指标,包括腐烂率、呼吸强度及叶绿素、花青素、还原糖、总糖、氨基酸、蛋白质含量。结果表明,纳米结合人工气调包装处理组可有效保持采后紫背天葵的叶绿素和花青素含量,保持良好的外观色泽,通过延缓花青素、还原糖、总糖、氨基酸和蛋白质含量的下降,有效保持品质,延缓衰老。建立的一元回归方程为:腐烂率/%=24.605 $-0.020X_7+1.122X_4-0.162X_5$ (式中: X_4 为还原糖含量; X_5 为花青素含量; X_7 为叶绿素含量),拟合度较好($R^2>0.9$),可应用于生产实践。

关键词: 紫背天葵; 纳米材料结合气调包装; 贮藏; 品质; 数学模型

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Gynura bicolor DC. (G. bicolor), a cultivated leafy vegetable, belongs to the genus Gynura Cass and is used as food throughout Asia for centuries^[1]. It is mostly produced in southern China and has once been used as a Chinese herbal medicine^[2]. Recently, many Chinese have a deep-rooted belief that 'even tastes bad, a well-selected Chinese herbal medicine uptake from daily diet provides health benefits and prevents diseases as a tonic' [3]. Thus, the production and consumption of G. bicolor have been expanding rapidly.

Since living organisms (water content >90%) with high metabolic activity after harvest are prone to losing nutrients and appearance during senescence, proper post-harvest treatments are in need for *G. bicolor* production. Heat treatment, edible coating, high-pressure processing, refrigerated storage and controlled atmosphere (CA) storage have been used for vegetables, but the protocols are not universal. Up to now, the studies on *G. bicolor* have focused on nutritional value, antioxidant activity^[4], as well as

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extracted anthocyanin^[5] and flavonoids^[6]. In contrast, eligible preservation technologies have only been reported by our group during the last 6 years. A previous research showed that package in combination with low temperature (0 °C) improved the nutritional quality of *G. bicolor* and decre ased the decay rate to 10% on Day $20^{[1]}$.

Low-level O₂ can delay senescence by significantly increasing antioxidant activity and total phenolic content, thus extending the storage life of fresh product^[7]. Nanopackaging (NP) materials, which have better barrier, mechanical and fresh-keeping properties than normal ones^[8-9], have been applied in a wide range of industries and markets. Huang Yuanyuan et al.^[9] reported that NP better retained VC, chlorophyll, polyphenol and amino acids in green tea than normal packaging did. Li Hongmei et al.^[8] also reported that the quality of *Ziziphus jujuba* Mill. was improved with NP preservation agents.

Although both CA storage^[10] and NP^[8-9,11] have been employed to extend the shelf-life of many vegetables, the two methods have never been combined. Our previous studies have shown that CA (3% O₂) could effectively delay the senescence of *G. bicolor* and maintain good quality. The objective of this work was to investigate the effect of combined NP and CA on the nutritional quality of *G. bicolor* during storage at 0 °C, and to establish a mathematical model describing the storage process.

1 Materials and Methods

1.1 Materials

G. bicolor samples were harvested in a commercial farmland in Shanghai, China. Then they were pre-cooled to 2-5 °C and transferred to the laboratory by a refrigerated vehicle immediately. The unified leaves were divided randomly into three groups and treated as follows: 1) Nanomaterial-packaging with modified atmosphere (NP, 30 bags × 600 g/bag); 2) normal polyethylene-packaging with a controlled atmosphere of 3% O2 and 97% N2 (controlled CO₂<1%) (CA, 30 bags×600 g/bag); 3) nano-materialpackaging combined with controlled atmosphere of 3% O₂ and 97% N₂ (controlled CO₂<1%) (CA + NP, 30 bags×600 g/bag). Then all bags were stored at 0 °C for 20 days. The nutritional qualities of three bags from each treatment were randomly analyzed on the 0, 5th, 10th and 20th days during storage. The gas compositions for CA and CA+NP were continuously controlled constant throughout the storage.

The nano-material bags, which contained 10.5% nano-Ag and 12% nano-TiO₂, were 40 μ m in thickness and 30 cm× 45 cm in size. Polyethylene bags with the same thickness and size without nano-powders were employed as controls.

1.2 Decay index

About 500 g leaves from each treatment were used, and decay degrees were visibly divided into four levels: 0, no decay; 1, decay < 1/3; 2, 1/3–2/3 decay; 3, decay > 2/3. Decay index was calculated according to the following formula:

Decay index/% = $[(1 \times N_1 + 2 \times N_2 + 3 \times N_3) \times 100 / (3 \times N)]$ where N is the total number of leaves showing different degrees of decay.

1.3 Respiratory rate

The respiratory rate of *G. bicolor* was determined with the small skep method based on CO₂ absorption and expressed as mg CO₂/(kg·h)^[1]. A glass plate containing 20 mL of 0.4 mol/L NaOH was added to absorb CO₂ produced by 300 g *G. bicolor* leaves during respiration. After 1 h, NaOH was transferred into a beaker and titrated with 0.2 mol/L oxalic acid.

1.4 Anthocyanin content

The total anthocyanin content was determined by a spectrophotometric method described by Dourtoglou et al. [10]. An aliquot of extract was diluted 1:10 with ethanolic HCl solution (0.25 mol/L). The solution was mixed thoroughly, and the absorbance at 520 nm ($A_{520 \text{ nm}}$) was read after 5 min, using the ethanolic HCl solution as blank. Cyanidin 3-glucoside (kuromanin chloride, Rotichrom®) was used as the standard substance.

1.5 Chlorophyll content

The chlorophyll content was measured by using acetone colorimetry method with slight modifications^[9]. Leaves (0.5 g) were fully ground, to which acetone ethanol solution (2:1, V/V) was then added until the constant volume of 25 mL. Then the absorbance at 652 nm ($A_{652 \text{ nm}}$) was detected. Total chlorophyll content was calculated using Lichtenthaler's equations.

1.6 Protein and reducing sugar contents

Leaves (0.5 g) were homogenized in distilled water (6 mL) and centrifuged at 10 000 $\times g$ for 10 min at 4 °C. Proteins in the supernatant were determined according to the method of Bradford^[12] with bovine serum albumin as standard. The amount of released reducing sugar was determined using the 3,5-dinitrosalicylate (DNS) reagent method with glucose as reference^[11].

1.7 Total carbohydrate and amino acid contents Total carbohydrate and amino acid in 1 g leaves were

extracted twice by hot distilled water (10 mL and 1 h each time) until the constant volume of 25 mL. The supernatant was used for test. Total carbohydrate content was detected according to the anthrone colorimetric method described by Pons et al.^[13]. Amino acid content was measured according to the ninhydrin method^[9].

The contents of anthocyanin, chlorophyll, protein, amino acid, total carbohydrate and reducing sugar were all determined as milligrams of equivalents per g of fresh weight (FW).

1.8 Statistical analysis

All the treatments and measurements were set up in a completely randomized factorial design with three replicates. All data were subjected to the analysis of variance (ANOVA) with SPSS 18.0 statistical software. A probability of <0.05 was considered as significant.

2 Results and Analysis

2.1 Respiratory rate and decay index

Respiration is an important metabolic process that provides energy for plants, during which various substrates for key synthetic metabolic pathways are produced. During storage at 0 °C, all three treatments increased the respiratory rate consistently, with the largest increase for CA (from 20.04 mg/(kg·h) to 56.61 mg/(kg·h), almost tripled as original) and the smallest for CA+NP (from 20.04 mg/(kg·h) to 41.27 mg/(kg·h), doubled as original) (Table 1). NP inhibited the respiration of *G. bicolor* more evidently than CA did, and CA+NP reduced the respiration most effectively and managed to maintain good quality as well. In contrast, the respiratory rate of green celery stored in CA decreased during storage^[14].

Vegetables decay following browning, yellowing and putrescence. Browning of fresh vegetables during storage often leads to quality loss, and thus has become one of the important factors responsible for short shelf-life and limited marketability^[15-16]. In this study, samples lost weight and browning was aggravated because the relative humidity of each treatment was more than 94%. The decay of *G. bicolor* leaves was only indicated by browning during low-temperature storage. As shown in Table 1, all treatments increase the decay indices of leaves during storage. The leaves stored in CA started decaying on Day 1 and peaked (9.64%) on Day 20. Meanwhile, NP and CA+NP groups were less prone to decay during storage, with the decay indices (9.23% and 8.97%) significantly lower than that of the CA group on Day 20 (P < 0.05). In contrast to NP treatments,

combining NP with CA could inhibit leaf decaying (8.97%) more effectively, which may be related to the better maintenance of cell membrane integrity that enhanced the resistance of leaves to infection and lesion. Similarly, different packages significantly changed the decay indices of *Flammulina velutipes*^[17]. Li Hongmei et al. [8] also found that nano-Ag, which was antibacterial, relieved the decay.

Table 1 Effects of NP in combination with CA on decay index, respiration rate, anthocyanin and chlorophyll contents of *G. bicolor*

Testing index	Treatment	Storage time /d					
		0	5	10	15	20	
	CA	0 ^{Ae}	2.96±0.11 ^{Ad}	3.78 ± 0.09^{Ac}	6.02 ± 0.09^{Ab}	$10.21\!\pm\!0.20^{Aa}$	
Decay index/%	NP	0^{Ae}	2.72 ± 0.09^{Bd}	$3.17\!\pm\!0.9^{Bc}$	5.22 ± 0.10^{Bb}	$9.95\!\pm\!0.12^{Ba}$	
	CA+NP	0^{Ae}	$2.13\!\pm\!0.05^{\text{Cd}}$	3.46 ± 0.07^{Cc}	5.37 ± 0.09^{Cb}	$8.87\!\pm\!0.10^{Ca}$	
Respiration	CA	$20.04\!\pm\!0.33^{\text{Ae}}$	$28.85 \!\pm\! 0.21^{Bd}$	47.42 ± 1.77^{Bb}	$32.27\!\pm\!1.69^{Bc}$	$56.61\!\pm\!0.69^{Aa}$	
rate/ (mg CO ₂ / (kg·h))	NP	$20.04\!\pm\!0.33^{Ad}$	$26.66 \!\pm\! 0.22^{\text{Cc}}$	$29.27\!\pm\!0.22^{Cb}$	$27.29\!\pm\!0.32^{\text{Cc}}$	$50.04\!\pm\!0.28^{Ba}$	
	CA+NP	$20.04\!\pm\!0.33^{\text{Ac}}$	$25.31\!\pm\!0.15^{Cb}$	$25.52\!\pm\!1.86^{Db}$	$24.02\!\pm\!0.27^{Db}$	$41.27\!\pm\!0.35^{Ca}$	
Anthocyanin content/ (mg/g)	CA	$6.82\!\pm\!0.02^{^{Ad}}$	$9.57 \pm 0.03^{\text{Cb}}$	$11.53\!\pm\!0.21^{Ca}$	8.69 ± 0.15^{Cc}	$6.42\!\pm\!0.01^{De}$	
	NP	$6.82 \pm 0.02^{\text{Ae}}$	10.78 ± 0.26^{Bb}	$12.16\!\pm\!0.17^{Ba}$	9.54 ± 0.06^{Bc}	7.61 ± 0.02^{Bd}	
	CA+NP	$6.82\!\pm\!0.02^{\text{Ac}}$	$11.09\!\pm\!0.17^{Ab}$	$13.35\!\pm\!0.15^{Aa}$	$10.25\!\pm\!0.14^{Ac}$	$8.17\!\pm\!0.04^{Ad}$	
Anthocyanin content/ (mg/g)	CA	$1.26\!\pm\!0.02^{^{Aa}}$	$1.22\!\pm\!0.02^{^{Ab}}$	$1.15\!\pm\!0.02^{Cc}$	1.10 ± 0.01^{Cd}	$0.97\!\pm\!0.01^{Be}$	
	NP	$1.26\!\pm\!0.02^{^{Aa}}$	$1.20\!\pm\!0.01^{Bb}$	1.13 ± 0.02^{Cc}	1.07 ± 0.02^{Cd}	$0.96\!\pm\!0.01^{Be}$	
	CA+NP	$1.26\!\pm\!0.02^{^{Aa}}$	$1.24 \pm 0.02^{\text{Aa}}$	$1.20\!\pm\!0.01^{Bb}$	$1.15\!\pm\!0.01^{Bc}$	$1.03 \pm 0.01^{\text{Ad}}$	

Note: Each value is the mean for three replicates and vertical bars indicate the standard deviation of each mean value (n=3). Mean \pm SD within the same column or row followed by the same uppercase or lowercase letter are not significantly different (LSD 0.05/0.01). Data are analyzed with SPSS. CA: controlled atmosphere packaging; NP: nano-packaging in combination with modified atmosphere; CA+NP: nano-packaging in combination with controlled atmosphere. Different letters in the same column or row indicate significant difference at the 0.05 level. Table 2 is same.

2.2 Anthocyanin and chlorophyll contents

As a main attribute of most vegetables, color plays a key role in food choice, preference and acceptability, and may even influence taste thresholds, sweetness perception and pleasantness^[18]. Since the back of *G. bicolor* leaves was purple and the other parts were green, color mainly indicated the contents of anthocyanin and chlorophyll.

The content of anthocyanin in the leaves of the CA+NP group increased, decreased, and reached maximum on Day 10. Individual CA and NP treatments exerted similar effects, but the contents were lower on the same day and reached significant difference (Table 1). These results suggested that combining NP with CA maintained stable anthocyanin content and the purple color more effectively.

All treatments decreased the chlorophyll content continuously but with slight differences (Table 1). The

chlorophyll contents resembled after CAP and NP treatments, but there were significant differences between CA+NP and NP or CAP treated leaves. Hence, CA+NP could keep the chlorophyll content at a higher level. Similarly, Huang Yuanyuan et al. [9] reported that the chlorophyll content in green tea with NP was 6.9% higher than that with normal packaging.

Many crops, such as *Brassica*, generate anthocyanin as a stress response indicating poor quality^[19]. Higher-level anthocyanin was induced in response to storage condition in all groups during the first ten days, and then anthocyanin degraded responding to senescence. Oxidation converted chlorophyll into pheophytin, so the chlorophyll content of *G. bicolor* leaves steadily decreased during storage. CA+NP maintained the color of *G. bicolor* better by inhibiting the decreases of anthocyanin and chlorophyll.

2.3 Amino acid and protein contents

Amino acid in *G. bicolor* treated with CA+NP during the 20-day storage increased from 7.64 mg/g on 0 day to 10.02 mg/g on the 10th day and thereafter decreased to 8.30 mg/g on the 20th day (Table 2). CA and NP treatments changed the contents of amino acids similarly and moderately, but the contents on the 20th day were significantly lower than that after CA+NP treatment, i.e. 9.08 mg/g on the 10th day and 6.64 mg/g on the 20th day for NP, and 7.09 mg/g on the 10th day and 6.39 mg/g on the 20th day for CA. Contrarily, Huang Yuanyuan et al. ^[9] reported that the amino acid content of green tea could be retained by using NP. Hence, CA+NP kept higher amino acid content of *G. bicolor* leaves. Table 2 presents that the anthocyanin and amino acid contents that are significantly positively correlated change similarly. However, the relationship between them remained unclear.

Moreover, the contents of protein in *G. bicolor* leaves kept decreasing to minimum on the 20th day of storage. The protein contents decreased from 9.79 mg/g to 8.41 mg/g for CA, to 8.94 mg/g for NP and to 9.26 mg/g for CA+NP on Days 20. CA+NP significantly delayed the decrease in protein content, which may be ascribed to NP material and combined CA treatment. Accordingly, nanoparticles could maintain the protein content at a higher level^[17].

The degradation rate of protein exceeded the synthetic rate, which thus decreased the protein content and probably increased the amino acid content. However, the amino acid contents of G. bicolor steadily decreased in all treatments owing to the enhanced respiration under low O_2 condition.

Table 2 Effects of NP in combination with CA on the postharvest contents of amino acid, protein, total carbohydrate and reducing sugar in G. bicolor

Testing index	Treatment	Storage time/d				
		0	5	10	15	20
Amino acid content/ (mg/g)	CK	7.64±0.03 ^{Ab}	6.46±0.09 ^{Cd}	8.13±0.12 ^{Ca}	7.17±0.13 ^{Cc}	5.99±0.03 ^{Ce}
	CA	$7.64\!\pm\!0.03^{Aa}$	$7.46\!\pm\!0.10^{^{Bab}}$	$7.09\!\pm\!0.11^{Dbc}$	$6.90\!\pm\!0.10^{Cc}$	$6.39\!\pm\!0.03^{Bd}$
	NP	7.64 ± 0.03^{Ac}	$6.77 \!\pm\! 0.13^{\text{Cd}}$	$9.08\!\pm\!0.10^{Ba}$	$8.92\!\pm\!0.10^{Bb}$	$6.64\!\pm\!0.27^{Bd}$
	CA+NP	7.64 ± 0.03^{Ae}	$8.78\!\pm\!0.13^{Ac}$	$10.02\!\pm\!0.14^{Aa}$	$9.43 \!\pm\! 0.07^{\text{Ab}}$	$8.30\!\pm\!0.11^{\text{Ad}}$
Protein content/ (mg/g)	CK	9.79 ± 0.03^{Aa}	$8.97\!\pm\!0.05^{Db}$	$8.02\!\pm\!0.03^{\text{Dc}}$	$7.82\!\pm\!0.05^{Dd}$	$7.53 \!\pm\! 0.03^{De}$
	CA	$9.79\!\pm\!0.03^{Aa}$	$9.50 \pm 0.06^{\text{Cb}}$	$8.95\!\pm\!0.04^{\text{Cc}}$	$8.67\!\pm\!0.02^{Cd}$	$8.41 \pm 0.07^{\text{Ce}}$
	NP	$9.79\!\pm\!0.03^{Aa}$	$9.58\!\pm\!0.01^{Bb}$	9.43 ± 0.08^{Bc}	$9.24\!\pm\!0.02^{Bd}$	$8.94\!\pm\!0.08^{Be}$
	CA+NP	9.79 ± 0.03^{Aa}	$9.76\!\pm\!0.03^{Aa}$	$9.65\!\pm\!0.04^{Ab}$	$9.48\!\pm\!0.02^{Ac}$	$9.26\!\pm\!0.04^{\text{Ad}}$
Total carbohydrate content/ (mg/g)	CK	$15.25\!\pm\!0.08^{Aa}$	$12.55\!\pm\!0.11^{Db}$	$12.68\!\pm\!0.09^{Bb}$	$11.54\!\pm\!0.07^{Dc}$	$9.87\!\pm\!0.08^{Bd}$
	CA	$15.25\!\pm\!0.08^{Aa}$	$14.05\!\pm\!0.10^{Bb}$	$12.78\!\pm\!0.07^{Bc}$	$11.89\!\pm\!0.07^{\text{Cd}}$	9.37 ± 0.11^{Ce}
	NP	$15.25\!\pm\!0.08^{Aa}$	$13.35\!\pm\!0.14^{Cb}$	$12.20\!\pm\!0.04^{\text{Cc}}$	$12.09\!\pm\!0.10^{Bd}$	$8.64\!\pm\!0.07^{De}$
	CA+NP	$15.25\!\pm\!0.08^{Aa}$	$14.67\!\pm\!0.15^{Ab}$	$13.49\!\pm\!0.13^{Ac}$	$12.68\!\pm\!0.05^{\text{Ad}}$	$10.34\!\pm\!0.10^{^{Ae}}$
Reducing sugar content/ (mg/g)	CK	$1.89\!\pm\!0.02^{Ad}$	$1.98\!\pm\!0.03^{Bc}$	$2.11\!\pm\!0.08^{Cc}$	$3.12\!\pm\!0.05^{Ca}$	$2.95\!\pm\!0.07^{Db}$
	CA	$1.89\!\pm\!0.02^{^{Ad}}$	2.85 ± 0.02^{Ac}	3.21 ± 0.08^{Bb}	$3.19 \pm 0.05^{\text{Cb}}$	$3.35\!\pm\!0.03^{\text{Ca}}$
	NP	$1.89\!\pm\!0.02^{^{Ae}}$	$2.85 \pm 0.03^{\text{Ad}}$	3.07 ± 0.04^{Bc}	$3.36\!\pm\!0.03^{Bb}$	$3.49\!\pm\!0.04^{Ba}$
	CA+NP	1.89 ± 0.02^{Ae}	2.86 ± 0.03^{Ad}	3.49 ± 0.04^{Ac}	3.80 ± 0.02^{Aa}	3.64 ± 0.02^{Ab}

2.4 Total carbohydrate and reducing sugar contents

Total carbohydrate in *G. bicolor* leaves decreased during storage and reached to 9.37 mg/g for CA, 8.64 mg/g for NP and 10.34 mg/g for CA+NP on Day 20 (Table 2). There were significant differences between the three treatments. Being consistent with the results of Yu Wenhua et al. (20), CA+NP remarkably retarded the dehydration and gave rise to quality loss.

The overall contents of reducing sugar in *G. bicolor* leaves of three treatments all increased during storage and reached maximum on Day 20, and CA+NP treatment led to the highest content compared with other two treatments did at each time point (Table 2). Thus, CA+NP treatment inhibited the metabolism of reducing sugar from respiratory processes and then kept quality better.

Moreover, total carbohydrate and reducing sugar contents were significantly negatively correlated. A part of reducing sugars originated from the soluble ones released during storage, probably due to the hydrolysis of total carbohydrate.

2.5 Establishment of mathematical model

The correlations between decay index (X_1) , protein content (X_2) , total carbohydrate content (X_3) , reducing sugar content (X_4) , anthocyanin content (X_5) , respiration rate (X_6) , chlorophyll content (X_7) and amino acid content (X_8) were analyzed. Most variables were highly correlated, so cluster and factor analyses were performed.

2.5.1 Hierarchical cluster and discriminant

Cluster and discriminant analyses are the fundamental

methods for categorization. All the treatment groups at different storage times (CA0, CA5, CA10, CA20, NP0, NP5, NP10, NP15, NP20, CA+NP0, CA+NP5, CA+NP10, CA+NP15 and CA+NP20) were divided into 3–5 clusters by using hierarchical clustering (Fig.1). The groups could be divided into three classes, *viz.*, CA0, NP0 and CA+NP0 in cluster 1, CA20, NP20 and CA+NP20 in cluster 3, and other groups in cluster 2. Accordingly, storage time mainly affected the quality of *G. bicolor*. When the groups were further divided into five classes, CA5, NP5, CA+NP5, NP10, CA+NP10 NP15 and CA+NP15 were all in cluster 2, indicating that NP and CA+NP treatments influenced preservation more obviously than CA treatment did and that packaging was the minor influencing factor.

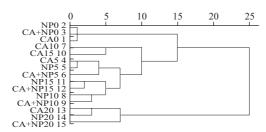


Fig.1 Dendrogram using average linkage (between groups) rescaled distance cluster

Since only storage period (prophase, metaphase and anaphase) needs to be predicted in practical production, a discriminant clustering equation was established herein based on cluster analysis. The coefficients of the equations are listed below:

This equation had good discrimination ability throughout *G. bicolor* storage and could be used to determine the storage period. The data were assigned to the cluster that scored highest respectively.

2.5.2 Factor analysis and regression

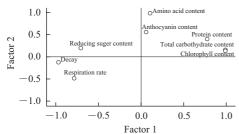


Fig.2 Factor plot in rotated factor space

The cumulative contribution rates of the first and second principal components are 62.210% and 23.805%, respectively. The first two principal component contribution rates, which exceeded 85%, were utilized to carry out subsequent analysis.

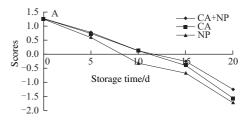
A rotated factor model was established by maximumvariance orthogonal rotation of the original component matrix. Then two common factors, factor 1 and factor 2, were extracted as the first and second principal factors respectively. The first principal factor was mainly determined by decay index (X_1) , total carbohydrate content (X_3) and chlorophyll content (X_7) , and their loads on the main factor were -0.969, 0.978 and 0.981, respectively (Fig.2). The second principal factor was mainly determined by amino acid content (X_8) , and its load on the main factor was 0.994. Therefore, factor 1 and factor 2 were referred to as quality factor and flavor factor, respectively. In addition, decay index, respiration rate and contents of reducing sugar, amino acid, anthocyanin, protein, chlorophyll and total carbohydrate might change similarly during G. bicolor senescence (Fig.2). Afterwards, the factor scores of observable variables were calculated by factor score coefficients with the equation bellow:

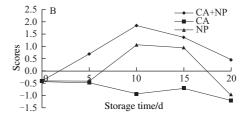
 $F_3 = 681.619X_1 + 3 187.018X_2 - 233.198X_3 - 1588.392X_4 - 339.378 8X_5 + 73.329X_6 + 9.271X_7 + 71.982X_8 - 18130.867$

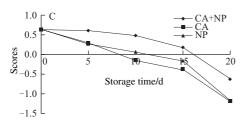
 $F_2 = 635.444X_1 + 2925.513X_2 - 197.672X_3 - 1457.447X_4 - 311.408X_5 + 66.425X_6 + 8.432X_7 + 56.799X_8 - 15402.681$

 $F_1 = 670.776X_1 + 3185.669X_2 - 238.858X_3 - 1686.828X_4 - 378.084X_5 + 72.984X_6 + 9.533X_7 + 94.686X_8 - 17989.190$

As suggested by the scores of all treatment groups at different storage time points (Fig.3), CA+NP and CA groups had higher antioxidant capacities, CA+NP and NP groups had better flavor, and CA+NP group had maximum storage capacity.







A. score of factor 1; B. score of factor 2; C. score of total.

Fig.3 Factor scores of all treatment groups

Table 3 Coefficientsa

Model -	Unstandardized coefficients		 Standardized coefficients 	+	Cia.
Wiodei	В	Std. Error	Standardized coefficients	ι	Sig.
(Constant)	32.219	2.105		15.303	0.000
Chlorophyll	-0.025	0.002	-0.968	-13.861	0.000
(Constant)	26.793	3.142		8.527	0.000
Chlorophyll	-0.022	0.002	-0.859	-10.749	0.000
Reducing sugar	-0.723	0.337	0.171	2.145	0.053
(Constant)	24.605	3.049		8.070	0.000
Chlorophyll	-0.020	0.002	-0.776	-9.253	0.000
Reducing sugar	1.122	0.367	0.266	3.057	0.011
Anthocyanin	-0.162	0.083	-0.133	-1.940	0.078

Note: a. Dependent variable: decay index.

The process was run in three steps (Table 3), and only X_4 , X_5 and X_7 remained in the model in the last step. The prediction equation was expressed below:

Decay index/%= $24.605-0.020X_7+1.122X_4-0.162X_5$

Decay index of *G. bicolor*, as suggested by the regression equation, was significantly negatively correlated with chlorophyll and anthocyanin contents, and was positively correlated with reducing sugar content. The content of oxygen free radicals increased with prolonged storage. In the meantime, chlorophyll, anthocyanin and reducing sugar were easily oxidized^[21], and the oxidation of them or the retention rate was able to reflect the senescence process. $R^2 > 0.9$, F statistic is 103.749, and the significance level of automatic system test is 0.000 1, suggesting that the equation allowed better fitting and gave very significant difference. Therefore, this regression equation can be applied to production practice.

3 Conclusions

In summary, NP in combination with CA exerted significant beneficial effect on the quality maintenance of *G. bicolor* than each applied separately did. The leaves of treated *G. bicolor* had higher antioxidant capacity, as evidenced by the high contents of chlorophyll, anthocyanin and reducing sugars. Hence, the senescence of *G. bicolor* leaves was delayed during storage. The mathematical models for predicting the storage duration and the decay of *G. bicolor* were established respectively, both being applicable to practice use.

References:

- JIANG Li, HOU Tianying, YUAN Xiaoyang, et al. Effect of storage temperature and packanging method on the deacay and physiology of fresh leaves of *Gynura bicolor* D.C[J]. Journal of Food Processing and Preservation, 2010, 34(5): 858-871.
- [2] SRITHI K, BALSLEV H, WANGPAKAPATTANAWONG P, et al. Medicinal plant knowledge and its erosion among the Mien (Yao) in northern Thailand[J]. Journal of Ethnopharmacology, 2009, 123(2): 335-342
- [3] CHAU C F, WU S H. The development of regulations of Chinese herbal medicines for both medicinal and food uses[J]. Trends in Food Science & Technology, 2006, 17(6): 313-323.
- [4] WANG Hongjiang, LIANG Chenyuan, ZHUO Min, et al. Comparison and evaluation of the nutrition of 3 wild vegetables from gynura[J]. Chinese Wild Plant Resources, 2004, 23: 48-49.
- [5] LI Hongying, YANG Haigui, ZHI Zhongqiang. Study on physicochemical property of natural red pig ment from begonia fimbristipula hence[J]. Journal of Zhao Qing University (in Chinese), 2002, 23(2): 77-78.
- [6] LU Xiaoxiang, TANG Jinzhong. Study on extraction conditions of flavonoids from gynura and its antioxidant effects[J]. Food Science (in Chinese), 2007, 28(4): 145-148.
- [7] SCHOTSMANS W, MOLAN A, MACKAY B. Controlled atmosphere storage of rabbiteye blueberries enhances postharvest quality aspects[J]. Postharvest Biology and Technology, 2007, 44(3): 277-285.
- [8] LI Hongmei, LI Feng, WANG Lin, et al. Effect of nano-packing on preservation quality of Chinese jujube (*Ziziphus jujuba* Mill. var. inermis (Bunge) Rehd)[J]. Food Chemistry, 2009, 114(2): 547-552.
- [9] HUANG Yuanyuan, HU Qiuhui. Effect of a new fashion nano-packing on preservation quality of green tea[J]. Food Science, 2006, 27(4): 244-246.
- [10] DOURTOGLOU V G, MAMALOS A, MAKRIS D P. Storage of olives (Olea europaea) under CO₂ atmosphere: effect on anthocyanins, phenolics, sensory attributes and in vitro antioxidant properties[J]. Food Chemistry, 2006, 99(2): 342-349.
- [11] HU Qiuhui, FANG Yong, YANG Yanting, et al. Effect of nanocomposite-based packaging on postharvest quality of ethylenetreated kiwifruit (*Actinidia deliciosa*) during cold storage[J]. Food Research International, 2011, 44(6): 1589-1596.
- [12] BRADFORD M M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of proteindye binding[J]. Analytical Biochemistry, 1976, 72(1/2): 248-254.
- [13] PONS A, ROCA P, AGUILÓ C, et al. A method for the simultaneous determinations of total carbohydrate and glycerol in biological samples with the anthrone reagent[J]. Journal of Biochemical and Biophysical Methods, 1981, 4(3/4): 227-231.
- [14] GÓMEZ P A, ARTÉS F. Controlled atmospheres enhance postharvest green celery quality[J]. Postharvest Biology and Technology, 2004, 34(2): 203-209.
- [15] MURATA M, TANAKA E, MINOURA E, et al. Quality of cut lettuce treated by heat shock: prevention of enzymatic browning, repression of phenylalanine ammonia-lyase activity, and improvement on sensory evaluation during storage[J]. Bioscience, Biotechnology and Biochemistry, 2004, 68(3): 501-507.
- [16] ZHENG Xiaolin, TIAN Shiping. Effect of oxalic acid on control of postharvest browning of litchi fruit[J]. Food Chemistry, 2006, 96(4): 519-523.
- [17] YANG Yanting, YANG Qin, YANG Fangmei, et al. Effect of nanopackaging material on quality of *Flammulina velutipes*[J]. Scientia Agricultura Sinica, 2009, 42(9): 3250-3258.
- [18] KAYS S J. Preharvest factors affecting appearance[J]. Postharvest Biology and Technology, 1999, 15(3): 233-247.
- [19] MOBIN M, KHAN N A. Photosynthetic activity, pigment composition and antioxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to cadmium stress[J]. Journal of Plant Physiology, 2007, 164(5): 601-610.
- [20] YU Wenhua, LI Jiezhi, CHEN Gong, et al. Research of nano fresh-keeping film and its application in the preservation of green pepper[J]. Sichuan Food and Fermentation (in Chinese), 2008, 44(5): 28-31.
- [21] KHANDAKER M M, BOYCE A N, OSMAN N. The influence of hydrogen peroxide on the growth, development and quality of wax apple (Syzygium samarangense, Blume Merrill & L.M. Perry var. jambu madu) fruits[J]. Plant Physiology and Biochemistry, 2012, 53: 101-110.