Effect of Storage Temperature on the Nutritional Quality of Fresh Wolfberry

Xin Li[1, a], Sanmei Ma[1, b]

[1]Department of Bioengineering, Jinan University, Guangzhou 510632, China.

[a]2542861899@qq.com, [b]msmwdw@163.com

Abstract

To investigate the changes of fruit quality under different temperature, fresh harvested wolfberry fruit were stored in cold room at 4°C and -4°C respectively for 21 d, nutritional quality were analyzed during storage.The results showed that -4℃ storage can effectively delay the decline of themarketable fruit rate and firmness of wolfberry fruits,was beneficial to the accumulation of fruit TSS (14d), TA (7d and 14d), and was conducive to the maintenance of fruit flavor and free amino acids (7d), can increase the accumulation of polysaccharide content (14d).4°C storage was beneficial to the accumulation of total flavonoids of fruits.The content of mineral elements K and Ca have been at a relatively high level during lowtemperature storage, and the content of heavy metals Cd and Pb have been at a relatively low level.

Keywords

Storage temperature, wolfberryfruits, amino acid, nutritional quality.

1. Introduction

Wolfberry (Lyciumbarbarum L), belongs to the Solanaceous family, is an important traditional medicinal plant[1].It is widely grown throughout Ningxia, Xinjiang, Inner Mongolia, Hebei and some other provinces in northern China.Wolfberry is a traditional Chinese medicine which can be used both as food and medicine for its high nutritional value and outstanding pharmacological effects. Fresh wolfberry has rich nutritional value, which rich in wolfberry polysaccharides, flavonoids, carotenoids, amino acids, mineral elements, vitamins and other nutrients [2].

Currently, wolfberry fruit is mainly used as a dried product. However, the nutritional components in wolfberry could be seriously damaged and the fruit may be polluted during processing [3]. Its nutritive value can be preserved to the greatest extent by direct consumption of the fruit and meets the consumption concept ofgreen, natural and healthy. Nevertherless, wolfberry fruit is very perishable as its skin is thin and tender, the preservation and storage of wolfberry fruit is still a key problem limiting its transportation and circulation. The storage life of fresh wolfberry fruit under normal temperature conditions is only 2 to 3 days, which greatly limits their sales, and this has become a key factor affecting the development of fresh wolfberry industry.

The handling and storage conditions are the prime factors in the development of a successful postharvest protocol for any horticultural crop especially berry crops [4]. The perishable nature and the physiological changes are the most common reasons for postharvest decay in berries.Lowtemperature refrigeration can slow the respiration of berries through low temperature, reduce energy consumption, inhibit the reproduction of microorganisms, delaythe speed of berry corruption,so as to achieve preservation effect[5,6]. The fresh fruit is only available in the areas where it is cultivated, due to its highly perishable nature that limits its marketing and availability as the fresh fruit. Surprisingly, there is a lack of published data in the scientific literature about the postharvest behavior of wolfberry fruit.In this paper, “Ningqi No. 7” was used as the material,4 °C and -4 °C as the storage temperatures. The effects of different temperatures on the nutritional quality of fresh wolfberry were discussed in order to provide a theoretical basis for preservation and supply of freshwolfberry.

2. Materials and Methods

2.1 Fruit Source, Treatments, and Storage Conditions

Fresh wolfberryfruits （“Ningqi No.7”）were picked at the Zhongning, NinXia autonomous region. The fruits which were uniform size, no pests, no mechanical injury were selected.After fruits were randomly packed in plastic boxes, each temperature is 10 boxes,stored at -4 °C and 4 °C cold storage respectively. Three biological replicates were set up in the experiment and the results were averaged over three experimental data.

2.2 Measurements and Chemical Analysis

2.2.1 Mineral Element

The contents of eight kinds of mineral elements, including K,Ca, Mn, Fe, Se, Zn, Cd and Pb in fresh wolfberry fruits were detected using atomic absorption spectrometry (AA700, Jena, Germany) and inductively coupled plasma mass spectrometry (7700x, Agilent, USA).

2.2.2 Proportion of Ediblefruits

The fruitswere divided into five grades according to the appearance quality. Grade 0, no obvious shrinkage and saccharification and mildew spots;Grade1, visible shrinkage and saccharification, no obvious mildew spots, or mildew spotsdiameter $d \leq 1$mm;Grade2, mildewrangebetween$1<d<2$ mm, mild shrinkage and saccharification ;Grade 3, mildew range between $2<d<4$ mm, moderate shrinkage and saccharification;Grade 4, mildew in the range of $d \geq 4$ mm, severe shrinkage andsaccharification. Among them, grades 2 to 4 are considered to have no commodity value.

Product fruit rate (%) = (fruits ofgrade 0 + fruits ofgrade1) / (total number of fruits)×100%

2.2.3 Firmness

Reference blueberry measurement method[7](Blaker & Olmstead, 2014). Ten wolfberryfruits were picked random each time, and were measured by FT-7 Soft Fruit Hardness Detector.

2.2.4 Total Soluble Solids, Titratable Acidity and Their Ratio

TSS was tested with a hand-held refractometer (J1-3A, Guangzhou Scientific Instruments, Guangdong, China).The titratable acidity (TA) of wolfberryfruit was determined using a digital hand refractometer (PAL-1; Atago, Tokyo, Japan).The sugar-acid ratio was calculated by dividing the TSS by the TA, and is expressed as the TSS/TA ratio.

2.2.5 Free Amino Acidsandhydrolyzed Amino Acids

Free amino acid extraction: Free amino acids were extracted according to the method reported previously by Lin [8].

Determination of amino acids: The concentration of amino acids was determined following the method described by Xu [9] with some modifications. The amino acid extracts were filtered through a 0.45 μm of cellulose ester syringe filter (AMPEL Laboratory Technology, Shanghai, China) prior to analysis and analyzed by S-433D Amino Acid Analyzer (SYKAM, Germany).

2.2.6 Total Flavonoids Determination

The total flavonoids were determined as reported by Jatoi [10]. Rutinwas selected as the standard. A five-point standard calibration curve was plotted for 10 μg/mL to 50 μg/mL ($R2 = 0.993$).

2.2.7 Polysaccharides Determination

The amounts of polysaccharide was determined using the phenol-sulphuric acid method[11].The absorbance was measured at 490 nm and used to quantify polysaccharide, based on the standard curve of glucose, which was prepared by plotting five concentrations (10-100μg/mL) according to their absorbance ($R2=0.997$).The polysaccharides in each fraction were further diluted to adjust concentration within the linear range of the standard curve.

Polysaccharide were extracted according to the method reported previously by Wang [12].

2.3 Statistical Analysis

Using Excel 2010 for data statistics and use Sigmaplot 12.5 (USA) software for drawing figures.A completely randomized factorial design was used, with three replicates for all of the parameters, except for the firmness variables(10 replicates). Two-way ANOVA using a generalized linear model and LSD tests at the P≤0.05 level were performed using the SPSS Statistics 13.0 software (USA).

3. Results and Discussion

3.1 Marketable Fruit Rate and Firmness

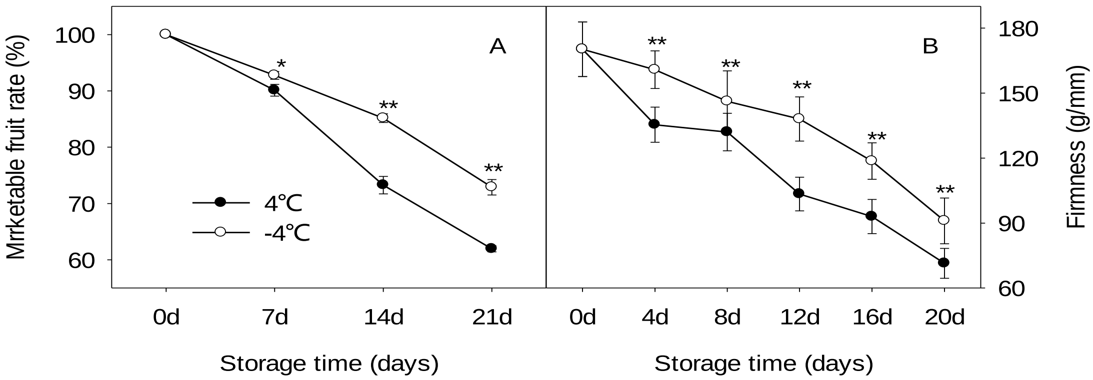

Fig 1. Effects of storage temperature on the marketable fruit rateandfirmness of wolfberry fruit

"*" and "**" indicate that there is a significant difference (p <0.05) and extremelysignificant difference (p <0.01) between different storage temperatures at the same time;same as below.

Postharvest wolfberry fruits are susceptible to saccharification, browning, and rot, resulting in loss of commodity value. Marketable fruit rate is an important indicator to measure the effect of preservation. During the entire storage period, the marketable fruit rate of wolfberryfruits stored at -4°C has been significantly higher than that at 4°C （Fig.3A）.After 21d of storage, themarketable fruit rate of wolfberry fruits stored at -4°C was still 72.89 %, which was 10.95% higher than that at 4 °C. It was shown that stored at -4°C can effectively maintain the marketable fruit rate of the “Ningqi No. 7”fruit during storage and effectively reduce the number of bad fruits, which was beneficial to the postharvest storage and transportation of wolfberry fruits and improve economic value.

The firmness of fruit affects the taste, and it is also an important factor in judging the quality of goods, resistance to storage and shelf life.The firmness of wolfberry fruit after harvest was 170.2 g/mm, which was lower than that of blueberry (204.98 g/mm) [7]. It indicated that the firmness of fresh wolfberry was extremely low, which was an important factor that caused it to be difficult to be stored.At the end of storage, the firmness of “Ningjing No.7” was only 71.4 g/mm at 4°C, while the firmness of the fruit at -4°C was 91 g/mm, they difference was extremely significant (Fig.3B).Those shows that -4 °C storage can significantly slow the decline of fruit firmness, maintain fruit quality.

3.2 Total Soluble Solids, Titratable Acidity and Their Ratio

As an important indicator of commodity quality, the internal quality of fruit can be significantly affected by its sugar and compositions and contents.The sugar contents of fruits can be influenced by ecological conditions and the sugar composition can also differ among varieties of a species [13].The organic acids of fruit have effect on enhancing appetite and helping digestion. The total sugar in the fruit constitute is themajor aspect of the medicinal quality of L. barbarum [14].The TSS content of wolfberry fruit showed a tendency of rising first and then decreasing during storage （Fig.3A）.The TSS content reached the highest at 4°C and -4°C storage after 7d and 14d respectively, reaching 15.6% and 16.75% respectively, both significantly higher than the pre-storage levels.The TA content of fruits also showed a tendency of rising first and then decreasing during stored at -4°C, reaching a maximum（1.6 mg/g）after 7 d（Fig.3B）.In the early of storage, the TA content of fruits stored at -4°C was significantly higher than at 4°C, indicating that -4°C storage was more conducive to the accumulation of TA in fruits.The TSS/TA of fruits was decreasing gradually during the two low temperatures storage periods, and the TSS/TA of fruits stored at 4°C was higher than at -4°C（Fig.3C）, indicating that 4°C storage is more conducive to the maintenance ofTSS/TA.

Mineral elements are essential for the human body to maintain normal life activities. In the human body, mineral elements combine with specific organic substances such as enzymes, hormones, and vitamins to complete a life activity together [15]. The mineral elements composition in wolfberryfruits has scarcely been investigated. Therefore, it is important to establish the composition of mineral elements in wolfberry fruit.The content of K and Cain the “Ningqi No. 7”fruits after harvest are much higher than other six kinds of trace elements. The order of the eight mineral elements content was K>Ca> Fe> Zn>Mn> Cd>Pb>Se (Table1).The content of K washighest ,approximately 3.631 mg/g, and the content of Cd and Pb were only about 0.013μg/g, which far below the national standard (GB 2762-2012) limit for Cd (0.05μg/g) and Pb (0.1μg/g) in fresh fruit, so it does not harm the human body. During the storage process of wolfberry fruit, the content of Se significantly increased at 4°C after 7 d, reaching 0.021μg/g, which was significantly different from that pre-storage (0.01μg/g)($p<0.05$).The content of Mn was highest (1.09μg/g) after stored at -4°C for 7 d. The content of Fe was decreasing during storage and decreased to the lowest level (6.143μg/g)when stored at -4°C for 14 d, which was significantly ($p<0.05$) lower than the level of pre-storage(7.5μg/g). The content of Ca during storage increased significantly ($p<0.05$) pre-storage, but it did not change significantly during the entire storage period.

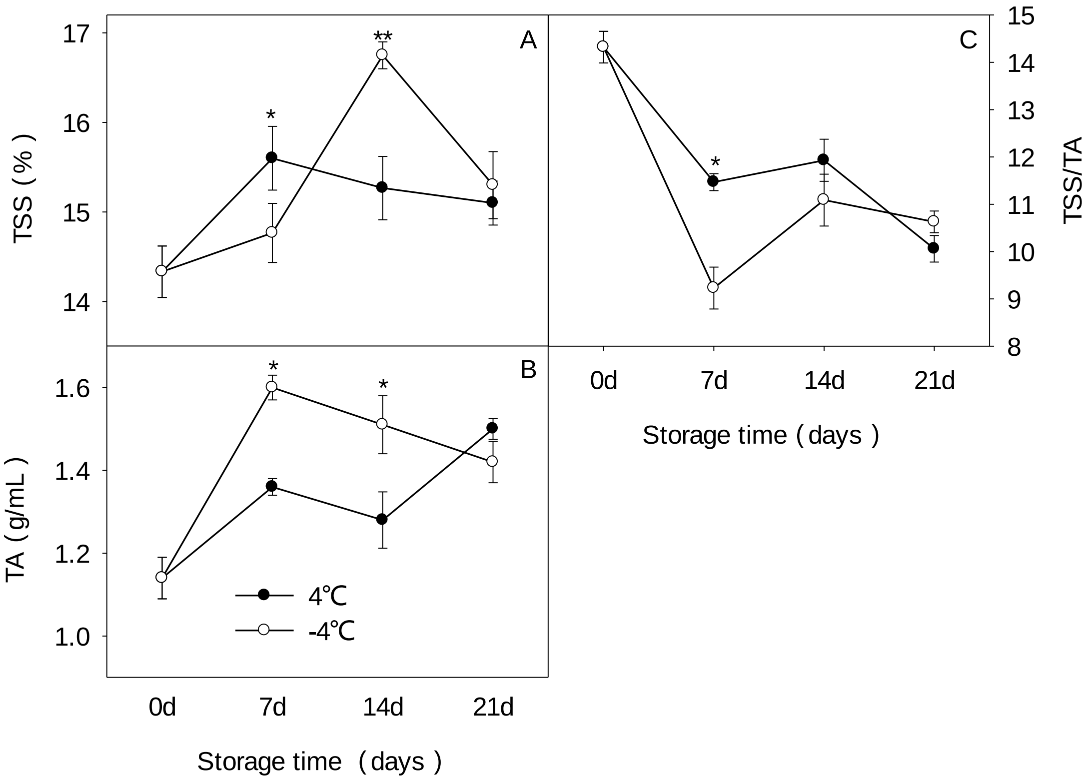

Fig 2. Effects of storage temperature on the contents of TSS and TA in wolfberry fruit

3.3 Mineral Elements Content

Table 1. Effects of storage temperature on the contents ofmineral elements inwolfberry fruit

Elements	0 d	7d		14d	
		-4℃	4℃	-4℃	4℃
Mn(μg/g)	1.704±0.262a	1.909±0.139a	1.807±0.062a	1.611±0.235a	1.873±0.240a
Fe(μg/g)	7.500±1.510a	7.009±0.237ab	6.824±0.788b	6.143±0.249b	6.592±0.386b
Zn(μg/g)	4.654±0.528a	4.761±0.368a	4.487±0.137a	4.447±0.345a	4.999±0.073a
Se(μg/g)	0.010±0.005b	0.008±0.002b	0.021±0.016a	0.009±0.001b	0.008±0.002b
Cd(μg/g)	0.013±0.002a	0.013±0.002a	0.012±0.001a	0.015±0.001a	0.018±0.001a
Pb(μg/g)	0.013±0.009a	0.007±0.002b	0.010±0.002ab	0.009±0.007ab	0.008±0.003b
K(mg/g)	3.931±0.172a	3.946±0.101a	4.081±0.173a	3.802±0.156a	3.911±0.214a
Ca(mg/g)	0.022±0.010b	0.036±0.004a	0.037±0.005a	0.038±0.005a	0.037±0.001a

Different letters represent significant differences (LSD tests; P≤0.05).

3.4 Free Amino Acids Content

Amino acid is the main nutrient component of wolfberryfruits, and it is also an important functional component,its species and content determine the nutritional quality and medicinal value of the fruit[16].Essential amino acids are easily absorbed and utilized,so evaluation of essential amino acids in wolfberry fruits is the most fundamental indicator to measure nutritional status of fruits.25 free amino acids were detected in postharvest “Ningqi No. 7” fruit, and the content ofasparagine was highest, approximately 3.791 mg/g, accounting for 43.6% of the total free amino acids（Table 2）.And 7 common essential amino acids were detected (tryptophan was not detected).The total free amino acid content ofwolfberry fruitsafter harvest reached 8.69mg/g, including the essential amino acid content of 0.61mg/g.The total free amino acid content reached the maximum (13.09 mg/g) after stored at -4°C for 7 d, which was significantly higher than the pre-storage (8.69 mg/g).Similarly, the total essential amino acids also reached their maximum (0.94mg/g) after stored at -4°C for 7 d.During the whole storage period, the total free amino acid content of fruits did not show a significant decrease, indicating that low temperature storage was beneficial to maintain the free amino acid content of wolfberry fruits.

Table 2. Effects of storage temperature on the contents of free amino acids inwolfberry fruit

	Amino acid content（mg/g）				
Amino acids	0 d	7d		14d	
		-4℃	4℃	-4℃	4℃
Asp	0.338±0.015	0.355±0.013	0.337±0.021	0.291±0.031	0.316±0.024
Thr*	0.381±0.016	0.556±0.034	0.513±0.061	0.284±0.024	0.386±0.029
Ser	0.821±0.031	1.306±0.064	1.063±0.093	0.926±0.084	0.865±0.062
Asn	3.791±0.193	6.258±0.534	5.44±0.22	4.528±0.39	4.078±0.268
Glu	0.027±0.002	0.165±0.019	0.002±0.001	0.253±0.016	0.04±0.003
Gly	0.023±0.001	0.043±0.005	0.033±0.009	0.62±0.042	0.432±0.027
Ala	1.026±0.053	1.385±0.116	1.239±0.21	0.235±0.088	0.262±0.018
Val*	0.057±0.002	0.094±0.004	0.078±0.003	—	—
Csy	0.01±0.001	0.004±0.001	0.003±0.001	0.02±0.002	0.019±0.004
Met*	0.014±0.001	0.02±0.001	0.015±0.002	0.041±0.005	0.054±0.013

Ile*	0.042±0.002	0.066±0.006	0.069±0.007	0.056±0.006	0.093±0.015
Leu*	0.083±0.005	0.132±0.006	0.159±0.015	0.011±0.001	0.014±0.003
Tyr	0.017±0.001	0.025±0.011	0.024±0.004	0.006±0.002	0.007±0.002
Phe*	0.004±0.002	0.008±0.003	0.007±0.001	0.012±0.002	0.011±0.002
His	0.123±0.007	0.155±0.007	0.121±0.012	0.119±0.011	0.111±0.009
Trp*	—	0.019±0.004	0.014±0.005	0.013±0.001	0.034±0.005
Lys*	0.038±0.003	0.055±0.002	0.04±0.001	0.044±0.005	0.042±0.005
Arg	0.861±0.048	1.163±0.048	0.909±0.025	0.917±0.083	0.879±0.062
Pro	0.324±0.043	0.682±0.057	0.982±0.035	0.289±0.057	0.527±0.075
Taurine	0.013±0.002	0.015±0.001	0.018±0.004	0.012±0.002	0.014±0.001
Citrulline	0.175±0.007	0.934±0.341	0.673±0.358	0.044±0.002	0.012±0.001
β-Ala	0.005±0.001	0.016±0.004	0.017±0.004	0.006±0.001	0.008±0.007
GABA	0.148±0.012	0.257±0.009	0.237±0.013	0.147±0.015	0.168±0.014
Ornithine	0.034±0.006	0.091±0.008	0.077±0.007	0.024±0.003	0.071±0.009
α-aminobutyric	0.005±0.001	—	—	0.04±0.003	0.054±0.002
Phosphatidylserine	0.03±0.006	0.034±0.004	0.032±0.002	0.025±0.005	0.029±0.002
β-AIBA	0.003±0.001	0.011±0.002	0.009±0.001	—	—
TFAA	8.69+0.211b	13.09+0.372a	12.11+1.082a	8.33+1.011b	8.47+0.580b
TEAA	0.61+0.032b	0.94+0.053a	0.89+0.085a	0.47+0.042c	0.64+0.011b

"*", "-"represent essential amino acids and not found respectively.Different letters represent significant differences (LSD tests; P≤0.05). TFAA, total free amino acids;TEAA, total essential amino acids.

3.5 Total Flavonoids Polysaccharides Content

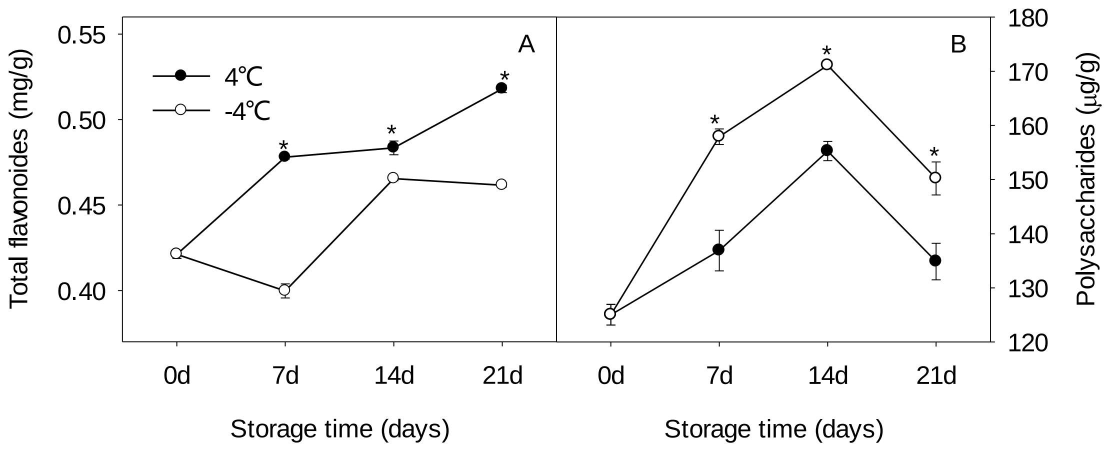

Fig 3. Effects of storage temperature on thecontents of total flavonoids and polysaccharides in wolfberry fruit

Flavonoids, a class of polyphenol compounds, are also widely distributed in plants, especially fruits and vegetables.The physiological activities of flavonoids, such as anti-cancer, anti- inflammation, and anti-atherosclerosis, have been well documented [12].The content of total flavonoids in the

"Ningqi No. 7" fruitsshowed a gradual increase at two storage temperatures. The contents of total flavonoids reached the maximum at 4 °C for 14 d, which was 0.64 mg/g, it significantly higher than at -4 °C storage level (0.50 mg/g) （Fig.3A）.At the end of storage, the total flavonoid content of fruits stored at 4°C decreased slightly, which was 0.62 mg/g. But at this time, the total flavonoid content of the fruits increased to a maximum value （0.57 mg/g） at -4°C. At the end of storage, the total flavonoid content of fruits stored at 4°C was still significantly higher than -4°C.The total flavonoid content of fruits at both storage temperatures were also significantly higher than that pre-storage (0.49mg/g) .The content of total flavonoids under storage conditions at 4°C was significantly higher than -4 °C during the whole storage period, indicating that stored at 4°C was more conducive to the accumulation of total flavonoids in wolfberry fruit.

Polysaccharides can be water-soluble or water-insoluble, with the former types being glucuronoβ-glucan and β-glucan, and they can enhance immunity through productionof interleukin and antibody [17].The content of polysaccharides in the "Ningqi No. 7" fruits after harvest was about125.04μg/g. The polysaccharide content of fruits also showed a tendency of rising first and then decreasing at both storage temperatures（Fig.3B）.After storage for 7 d, the polysaccharide content at -4°C has increased significantly to the level of 157.92 μg/g, which significantly higher than that at 4°C (136.89μg/ g).After storage for 14 d, the polysaccharide content of fruits at -4°C (171.11μg/ g) and 4°C (155.28μg /g) increased to the maximum, respectively.At the end of storage, the polysaccharide content of the fruits stored at -4°C was 150.22μg / g, while the storage conditions at 4°C decreased to 134.86μg / g. The polysaccharide content of wolfberry fruits under 4°Cstoragecondition was always higher than 4°C during storage. It was demonstrated that -4°C storage could effectively increase the polysaccharide content of wolfberry fruit and improve the nutritional value of the fruit.

4. Conclusions

The present study is an investigation into the possible prolongation of the storage life of wolfberry fruits through low temperature storage.The decrease of marketable fruit rate,fruit firmness, and the contents of TSS, TA, free amino acids was delayed at- 4℃ stored than that at4℃ stored in"Ningqi No. 7"wolfberryfruit. In addition, -4℃ storage also increased the accumulation of polysaccharides. However,the 4 °C storage was more conducive to the accumulation of polysaccharide content. The content of mineral elements in fruits was always at a relatively high level during low temperature storage, and the heavy metal content of Cr and Pbwere always at a low level.

In general, this study believes that fresh wolfberry fruit was more resistant to low temperatures.Under -4 °C storage conditions, the storage period of fresh wolfberry fruit could reach 21 d, and the contents of amino acids and other nutrients in fruits were well maintained.

References

[1] Yao X, Peng Y, Xu L, et al. ChemInform Abstract: Phytochemical and Biological Studies of Lycium Medicinal Plants[J]. Chemistry & Biodiversity, 2011, 42(38):976-109.

[2] Yang R M, Jing N H, Wang H L, et al. Analysis and Evaluation of Nutritional Composition and Bioactive Composition in Chinese Wolfberry (Lyciumbarbarum L.) Fruits from Different Regions of Qinghai Province[J]. Food Science, 2012, 33(20):265-269.

[3] Wen-Ping M A, Zhi-Jing N I, He L I, et al. Changes of the Main Carotenoid Pigment Contents During the Drying Processes of the Different Harvest Stage Fruits of LyciumbarbarumL[J]. Agricultural Sciences in China, 2008, 7(3):363-369.

[4] El-Ramady H R, ÉvaDomokos-Szabolcsy, Abdalla N A, et al. Postharvest Management of Fruits and Vegetables Storage[M] Sustainable Agriculture Reviews. Springer International Publishing, 2015:65-152.

[5] Paula C, Franciscom D A. Effects of postharvest treatments on fruit quality of sweet pepper at low temperature[J]. J Sci Food Agric, 2010, 90(15):2716-2722.

[6] Díaz-Pérez J C, Muy-Rangel M D, Mascorro A G. Fruit size and stage of ripeness affect postharvest water loss in bell pepper fruit (Capsicum annuum L.)[J]. Journal of the Science of Food & Agriculture, 2010, 87(1):68-73.
[7] Blaker KM, Olmstead JW. Effects of preharvest applications of 1-Methylcyclopropene on fruit firmness in southern highbushblueberry[J]. ActaHorticulturae, 2014, 1017:71-75.
[8] Lin L Y, Peng C C, Yang Y L, et al. Optimization of bioactive compounds in buckwheat sprouts and their effect on blood cholesterol in hamsters.[J]. J Agric Food Chem, 2008, 56(4):1216-1223.
[9] XuJianGuo, Hu QingPing, DuanJiangLian, et al. Dynamic changes in γ-aminobutyric acid and glutamate decarboxylase activity in oats (Avenanuda L.) during steeping and germination.[J]. Journal of Agricultural and Food Chemistry, 2010, 58(17):9759-63.
[10] Jatoi M A, Jurić S, Vidrih R, et al. The effects of postharvest application of lecithin to improve storage potential and quality of fresh goji (Lyciumbarbarum L.) berries [J]. Food Chemistry, 2017, 230:241-249.
[11] Dubois, M., Gilles, K. A., Hamilton, et al. Colorimetric method for determination of sugars and related substances.[J]. Analytical Chemistry, 1956, 28(3):350-356.
[12] C.C. Wang, S.C. Chang, B. Stephen Inbaraj, et al. Isolation of carotenoids, flavonoids and polysaccharides from Lyciumbarbarum, L. and evaluation of antioxidant activity[J]. Food Chemistry, 2010, 120(1):184-192.
[13] ZhengGuoQi, ZhengZiYan, Xu Xing, et al. Variation in fruit sugar composition of Lyciumbarbarum L. and Lyciumchinense Mill. of different regions and varieties.[J]. Biochemical Systematics and Ecology, 2010, 38(3):275-284.
[14] Tang W M, Chan E, Kwok C Y, et al. A review of the anticancer and immunomodulatory effects of Lyciumbarbarum fruit.[J]. Inflammopharmacology, 2012, 20(6):307-314.
[15] DemiRbas A. Oil, micronutrient and heavy metal contents of tomatoes.[J]. Food Chemistry, 2010, 118(3):504-507.
[16] İbrahim Kıvrak, ŞeydaKıvrak, Harmandar M. Free amino acid profiling in the giant puffball mushroom (Calvatiagigantea) using UPLC–MS/MS[J]. Food Chemistry, 2014, 158(158):88-92.
[17] Yuenshan H, Yu M S, Yang X F, et al. Neuroprotective effects of polysaccharides from wolfberry, the fruits of Lyciumbarbarum, against homocysteine-induced toxicity in rat cortical neurons[J]. Journal of Alzheimers Disease Jad, 2010, 19(3):813-827.