

## Research Article

### Process and Storage Quality of Quick Freezing Blueberries in LN<sub>2</sub>-spraying Fluidized Bed

<sup>1,2</sup>Zhang Qinggang, <sup>1</sup>Tao Leren and <sup>1</sup>Cai Meiyang

<sup>1</sup>Institute of Cryogenics and Food, University of Shanghai for Science and Technology, Shanghai 200093, China

<sup>2</sup>Institute of Refrigeration and Air Conditioning, Harbin University of Commerce, Harbin 150028, China

**Abstract:** Liquid nitrogen spraying fluidized bed was used for Individual Quick Freezing (IQF) of blue berries; processing technology and quality change of frozen samples during storage stage were analyzed in this study. Regular and mixed experimental design were used to confirm the quick freeze process of blueberries by means of setting different freezing temperature, wind velocity and the height of bed-layer. Multiple linear regression analysis indicates that the suitable process conditions for quick-freezing blueberries in LN<sub>2</sub>-spraying fluidized bed were -40°C freezing temperature, 4.5 m/sec wind speed and 3.6 cm bed height. Environmental Scanning Electron Microscopy (ESEM) results show that the inner microstructure of the fluidized quick-frozen blueberries has better compactness, uniformity and destructiveness than that of the slow-frozen blueberries in -18°C refrigerator. Texture Analysis (TPA) and the technology of image processing were used to analyze the different of blueberries in firmness and internal color between the fluidized quick-frozen and slow-frozen in -18°C refrigerator. The results show that the quality of the fluidized quick-frozen blueberries has better than that of the slow-frozen blueberries in -18°C refrigerator. The storage life of blueberry by LN<sub>2</sub> spraying fluidized quick-frozen was confirmed that is around 150 days which is much better than that of the slow-frozen sample with the storage life of 100 days.

**Keywords:** Blueberry, fluidized, image processing, process, quick-frozen

## INTRODUCTION

Blueberries (*Vaccinium corymbosum* L.), called as “Vaccinium fruit”, have special flavor and rich nutrition (Stojanovic and Silva, 2007). Blueberries are likely to promote the generation of retinal “rod cells” and relieve eye muscle fatigue (Sun *et al.*, 2007). They can prevent the oxidation of free radical with powerful antioxidant function and anti-allergic activities which protect cranial nerve from oxidation and stabilize the brain functions (Skrede *et al.*, 2000; Youdim *et al.*, 2000). Blueberry is juicy berry and its fruit maturation period ranges from June to August which is a hot and rainy season (Sun, 2002). So the fruit is apt to be rotten and difficult for storage. How to preserve blueberries after harvesting for long term has been a key technology in the development of blueberries industry. Presently, the methods of storing blueberries include hyperoxia (Zheng *et al.*, 2008), controlled atmosphere, ultraviolet irradiation and chitosan coating (Zhu and Jiangang, 2011), etc.

With the characteristics such as quick freezing and high quality of food after thawing, fluidization quick freezing has gradually been one of important industrial freezing methods of IQF products (Khairullah and Singh, 1991). However, the technology of quick

freezing blueberries with liquid nitrogen fluidized bed has been rarely reported domestically. This study aims to freeze quickly blueberries using liquid nitrogen fluidized bed and determine the technological parameters; By combining with Environmental Scanning Electron Microscopy (ESEM), Texture Analyzer (TPA) and image processing, the synthetic method is used to analyze the different change laws of blueberries quality in storage life on the conditions of quick freezing and slow freezing; Meanwhile the feasibility and storage life of quick freezing blueberries with liquid nitrogen is determined. The study provides a theoretical basis for the further optimization of fluidization quick freezing technology of blueberries.

## MATERIALS AND METHODS

**Materials:** Blueberries of the same maturity and growing conditions were collected from Qingpu modern agriculture park in Shanghai. Sorting was conducted in the finishing room of the cold storage. Blueberries (average diameter of 12±2.5 mm) with no pest and mechanical damages were selected. In experiment, the required 2, 6 dichlorophenol indophenol, Cellulase, Xylene, sodium phosphate, disodium hydrogen phosphate, potassium ferricyanide

**Corresponding Author:** Zhang Qinggang, Institute of Cryogenics and Food, University of Shanghai for Science and Technology, Shanghai 200093, China

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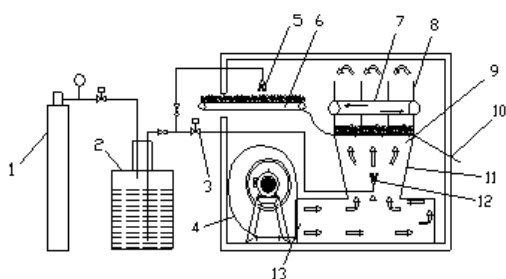


Fig. 1: The structure of liquid nitrogen fluidized bed  
 1: Nitrogen bottle; 2: Liquid nitrogen container; 3: Cryogenic solenoid valves; 4: Centrifugal blower; 5: Liquid nitrogen spray nozzle; 6: Pre-freezing conveyor belt; 7: Freezing conveyor; 8: Scraper; 9: Wind outlet; 10: Discharge port; 11: Airduct; 12: Liquid nitrogen spray nozzle; 13: Inlet duct

and DPPH (2, 2-diphenyl-1-picrylhydrazyl) are chemically pure.

**Experimental methods:**

**Experimental device:** The structure of the liquid nitrogen fluidized bed used in this study is shown in Fig. 1. The device is composed mainly of a pre-frozen part of liquid nitrogen spray and a fluidized frozen part. The freezing process is as follows. First, the food was delivered through the conveyor belt into the pre-frozen segment in which the liquid nitrogen was sprayed from spray nozzle 5. Subsequently, the pre-frozen food was delivered into the fluidized phase. Food finally frozen was discharged from the outlet port. High-pressure nitrogen bottle 1 pressurized liquid nitrogen container 2 to 0.2 to 0.3 MPa and the mist nitrogen sprayed from nozzle 12 evaporated rapidly into air duct 11 as a result of heat transferring with the supplied air while cooling the air in duct 11. Cooling air flowed up through the materials, freezing them rapidly in a fluidized state.

**Technology parameters of quick freezing:** The blueberries are cleaned firstly and conducted air-drying. According to different quick freezing conditions, the samples are divided into ten groups (Table 1) to perform quick freezing on liquid nitrogen fluidized bed. In each experiment, the temperature sensors of three thermocouples are used to test central temperatures of different blueberries respectively and the temperatures are displayed by prova 800 temperature sensors (TES, Taiwan). The experiment is not finished until the central temperature is up to -15°C. On the basis of the time recorded and the decreased quality of liquid nitrogen containers displayed on electronic scale, the freezing time and consumption of liquid nitrogen of samples in each group are measured; In addition, the indexes of samples after conducting quick freezing are tested to determine the optimum technological parameters.

Table 1: U<sub>10</sub> (10<sup>4</sup>) uniform experimental design

Factors			
Level	Freezing temperature	Wind velocity	Height of bed-layer
1	-30	3.5	2.4
2	-35	4.0	1.2
3	-40	4.5	3.6
4	-45	5.5	2.4
5	-50	5.0	1.2
6	-30	4.5	3.6
7	-35	5.5	4.8
8	-40	4.0	6.0
9	-45	5.0	6.0
10	-50	3.5	4.8

**Detection of chemical indexes and methods:** The 2, 6-dichloroindophenol titration method is used to test the Vc content (Chen *et al.*, 2010); and the weighing method is applied to measuring the water loss ratio; while the soluble solids is tested with GB12295-90 method (China Standard, 1990), expressed by relative content; the content of Anthocyanins is tested by referring (Li, 2008) and (Penelope Perkins *et al.*, 2008) using differential method (Huo *et al.*, 2005) to prepare buffer solution; the method of testing the free radical clearance rate of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) are applied (Burda and Oleszek, 2001; Gao *et al.*, 2010); the absorption value AA of the solution and absorptions value AB of DPPH ethanol solution are tested at 517 nm. The samples' capacity of eliminating free radical is expressed by clearance rate. And the formula is obtained: clearance rate = [(AB - AA) / AB] × 100%; Reducing power is tested based on the method in Halici *et al.* (2005) and the absorption value is tested at 700 nm. The larger the absorption value, the more powerful the reducing power is (the above clear liquid is blank). All the measurements are repeated three times and then the average values are obtained.

**Microstructure measurement:** ESEM (Quanta FEG, FEI) is used to observe the microstructures of blueberries which are frozen and refrigerated for 24 h with different methods. The samples are divided into 4°C cold storage, slow freezing at -18°C, fluidized bed quick freezing (stored in refrigerator at -25°C). And three samples in each group are measured, while the group with the highest comprehensive evaluation in ten groups is selected as the samples of fluidized bed quick freezing. Before testing, all the samples are thawed for half an hour at 4°C. In order to improve observation effect, the samples before observing are performed pretreatment processes such as dehydration, freeze-drying, spray-gold and so on (Allan-wojtas *et al.*, 2001). The ESEM is used to observe with accelerating voltage of 20 kV and amplification of 500 times.

**Firmness testing:** The firmness not only presents effects on the appearance of fruits, but the taste. And it is an essential index to judge the storage quality of fruit.

TA.XT plus Texture Analyzer is applied to the firmness frozen and cold stored for 24 h with different methods. The samples are divided into 4°C cold storage, slow freezing at -18°C, fluidized bed quick freezing (stored by refrigerator at -25°C) and 13 samples in each group are tested. The group with the highest comprehensive evaluation in ten groups is selected as the samples of fluidized bed quick freezing. Before testing, all the samples are thawed for half an hour in incubator at 4°C. The testing parameters are set: probe is P/0.5; and the speed before testing is 2.0 mm/sec; testing speed is 1.0 mm/sec.

#### **Internal color change and rotten rate of blueberry:**

Because the internal color change of blueberries is one index of measuring quality change and unlikely to be distinguished in a short term, the image processing method is used to distinguish accurately the color change of blueberries with different processing ways in different storage lives. The method is described in details as follows: a digital camera (Canon EOS 550D) is used to take photos for the cut blueberries under same light environment, with same photo parameters (distance, aperture, exposure time, etc.); three blueberries are selected in each group and each one is taken 3 times; and then the computer software is applied to conduct image processing to get the gray histogram of each photograph. The cold stored samples at 4°C are taken photos in every 5 days; but the slow freezing samples at -18°C and the samples of fluidized bed quick freezing (the group with the highest comprehensive evaluation is stored in refrigerator at -25°C) are taken photos at the 20, 40, 60, 90, 120, 150 and 180<sup>th</sup> day, respectively.

At the same time, the rotten rate is recorded, rotten rate = (rotted fruit number/total fruit number) × 100%. The rotted fruit refers to the one that has at least on juice leakage or presents putrefactive phenomenon. The effective storage lives are recorded in accordance with the rotten rate. The storage days with rotten rate less than 10% are defined as the effective storage life. In each group, 100 fruits are recorded randomly in each group.

**Statistics analysis:** The MATLAB 7.0 software is applied to conduct regression analysis of data and image processing.

## **RESULTS AND ANALYSIS**

#### **Quick freezing time of blueberries on different conditions and consumption of liquid nitrogen:**

Table 2 shows the experiment results of quick freezing time of liquid nitrogen fluidization quick freezing and consumption of liquid nitrogen on different technological conditions.

From Table 2, it can be found that when the freezing temperature ranges from -30 to -50°C, with the decrease of temperature, the quick freezing time of blueberries is shortened. However, the relationships between quick freezing time and wind velocity and height of bed-layer are not apparent. But with the decrease of freezing temperature, the consumption of liquid nitrogen per unit time increases.

#### **Effects of different quick freezing technological parameters on blueberries quality and functional activity:**

Table 3 indicates the experimental testing results of liquid nitrogen fluidized bed quick freezing of blueberries. Seen from the table, all the water loss rates are less than 3% in the ten groups of experiments, but the difference is large in the range of 0.41~2.71%; the reducing power is almost around 2, showing less effects from different freezing conditions; but the stability of Anthocyanins is good during -30 and 50°C, with little difference. The standard deviation of each index can reflect the degree of influence of different freezing conditions on the index. Therefore, the degree of influence of different freezing conditions on different indexes can be compared: water loss rate (0.562>Vc (0.164>soluble solids (0.143> Anthocyanins (0.115) ≈ reducing power (0.118) ≈ DPPH clearance (0.112).

**Weight arrangement and regression analysis:** The important degree of six experimental indexes in actual production is different. For most of refrigerated and cold stored blueberries, as ordinary processing material, it has ensured basically the quality of blueberries to guarantee excellent indexes such as Vc, soluble solids, water loss rate and so on. According to actual production requirement, the subjective weighting method is adopted to perform different weight arrangement and then the regression analysis based on the calculated comprehensive values to determine the suitable liquid nitrogen fluidized bed quick freezing technology of blueberries.

Grade the Vc, Anthocyanins, reducing power, soluble solids, water loss rate and DPPH in U U<sub>10</sub> (10<sup>4</sup>) uniform experiment. And then the above six indexes are performed value weight based on the degree of influence of different freezing conditions on different indexes. And Vc, Anthocyanins, reducing power, soluble solids, water loss rate and DPPH are weighted with 2.5, 1, 1, 2, 2.5 and 1, respectively (water loss rate is as important as Vc). After weight, the comprehensive value is got, shown as in Table 4.

Regression result is:

$$Y = -35.6182 + 1.7206X_1 + 6.6531X_2 + 46.8967X_3 - 6.4885X_2^2 - 5.9925X_3^2 - 0.8064X_1X_2 + 0.338X_1X_3 + 4.073X_2X_3$$

Table 2: Quick-frozen time and liquid nitrogen consumption for quick-freezing blueberries in LN<sub>2</sub>-spraying fluidized freezer

Number	1	2	3	4	5	6	7	8	9	10
Freezing time (min)	19.60	17.20	14.50	12.00	7.90	18.50	15.70	15.80	9.40	10.10
Consumption of LN <sub>2</sub> (kg)	4.03	3.64	4.47	3.94	2.89	5.11	5.46	6.07	5.93	5.25

Table 3: Experimental indexes test results of blueberries' quick frozen in liquid nitrogen fluidized bed

Number	Vc mg/100 g	Anthocyanins mg/100 g	Reducing power	Soluble solids (%)	Moisture (%)	DPPH (%)
1	11.95	39.54	2.20	11.68	0.68	26.48
2	10.33	34.22	1.98	8.93	1.85	25.67
3	16.90	29.12	2.69	12.74	0.75	25.99
4	12.40	35.26	2.21	13.26	2.22	32.29
5	11.56	34.32	2.12	10.45	2.71	33.05
6	16.20	28.31	2.60	12.36	0.88	27.02
7	15.99	28.17	2.24	13.44	0.97	26.11
8	14.72	31.70	1.90	14.01	1.21	25.00
9	15.50	33.45	2.01	14.89	1.53	26.26
10	13.29	37.22	2.05	13.88	0.41	23.77

Table 4: Evaluation values of experimental results

Number	Vc	Anthocyanins	Reducing power	Soluble solids	Moisture	DPPH	Total
1	24.70	100	38.0	46.1	11.7	29.2	29.19
2	0.00	53.2	10.1	0.0	62.6	20.5	-7.27
3	100	8.4	100	64.0	14.8	24.0	47.34
4	31.50	62.4	39.2	72.7	78.7	91.8	22.08
5	18.70	54.1	27.8	25.5	100	100	2.97
6	89.30	1.2	88.6	57.6	20.4	35.0	41.23
7	86.10	0.0	43.0	75.7	24.3	25.2	37.41
8	66.80	31.0	0.0	85.2	34.8	13.3	29.47
9	78.70	46.4	14.0	100	48.7	26.8	36.22
10	45.10	79.6	19.0	83.1	0.0	0.0	37.76

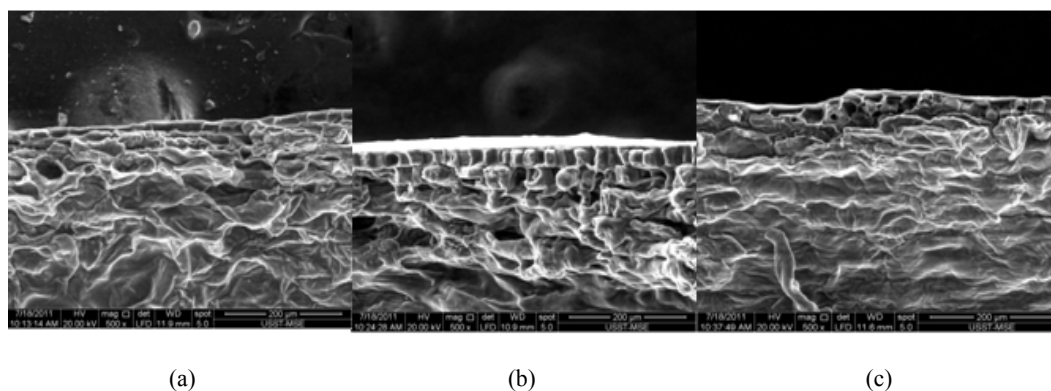


Fig. 2: The picture of Environmental Scanning Electron Microscope (ESEM) of blueberries' section parts near the skin, (a) fresh (cooling at 4°C), (b) fluidized bed quick freezing and (c) frozen at -18°C

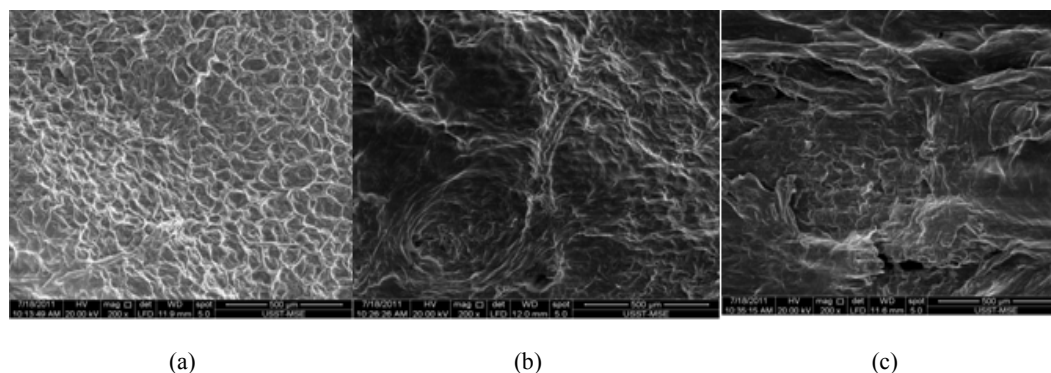


Fig. 3: The picture of Environmental Scanning Electron Microscope (ESEM) of blueberries' internal organization, (a) fresh (cooling at 4°C), (b) fluidized bed quick freezing and (c) frozen at -18°C

Multiple correlation coefficients R and F are 0.9992 and 731.3467 respectively; Significant level  $p = 0.0286 < 0.05$ , therefore, regression equation is significant. The regression result shows that Y is not relevant to  $X_1^2$ . By calculating by software, when  $X_1 = -40$ ,  $X_2 = 4.5$  and  $X_3 = 3.6$ , Y is maximum. This is consistent with conditions in 3<sup>rd</sup> group (10 groups of experiments in total). The maximum of  $Y = 47.733$  which is 0.83% less than of score values measured. The optimum parameters combination including freezing temperature at  $-40^\circ\text{C}$ , fan wind velocity of 4.5 m/sec and 3.6 cm in height of bed-layer is obtained.

**Study on the microstructure of blueberries:** Figure 2 shows the microstructure of the part near the epidermis of the blueberries under conditions of fresh, quick-freezing on a fluidized bed and average frozen. When fresh (cooling at  $4^\circ\text{C}$ ), the skin of the blueberry was smooth and did not have an obvious boundary with internal organization. When frozen in the fluidized bed, the skin of the blueberry was smoother and had a very obvious boundary with internal organization. When frozen at  $-18^\circ\text{C}$ , the skin of the blueberry was uneven and slightly disconnected from the internal organization, forming a clear separating line. In addition, a small amount of cells ruptured. When the blueberries were frozen in liquid nitrogen fluidized bed, a thin ice shell membrane quickly formed on the surface of the food at certain strength, which can improve the mechanical strength of fragile foods. Food adhesions were also less likely to occur.

Figure 3 shows the microstructure of the internal part of the blueberries under conditions of fresh, quick-freezing and average frozen on a fluidized bed. When fresh (cooling at  $4^\circ\text{C}$ ), the internal organization of the blueberries was homogeneous, dense, non-deformed and non-fractured. When frozen in fluidized bed, the internal organization of the blueberries was also homogeneous and dense, but a slight local deformation and fracture phenomenon were observed. When frozen at  $-18^\circ\text{C}$ , the internal organization of the blueberries was uneven and very loose. In addition, local tissues were collapsed. Thus, when the blueberries were frozen in liquid nitrogen fluidized bed, food can have complete contact with the cooling medium, faster freezing rate, smaller ice crystals and smaller damage to the internal structure of the food. As a result, the loss of food nutrients can be effectively reduced. Freezing in nitrogen fluidized bed complies with moisture loss rate and other testing results.

**The firmness comparison of blueberries in different processing conditions:** The firmness decrease of fruit is due to the changes of pectin and moisture content in the tissues of the fruits. On the one hand, protopectin is

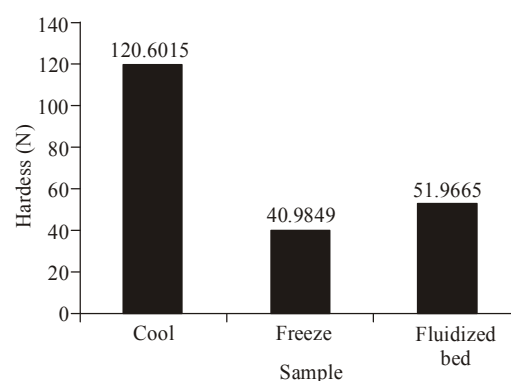
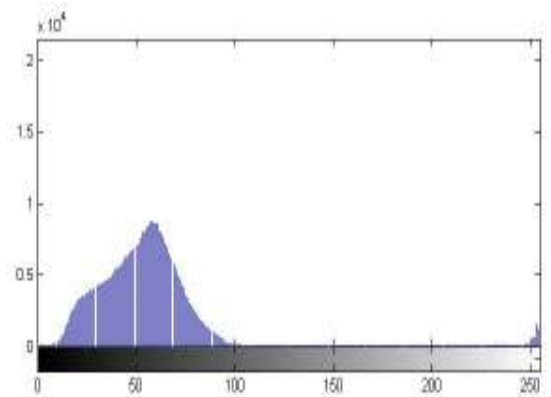
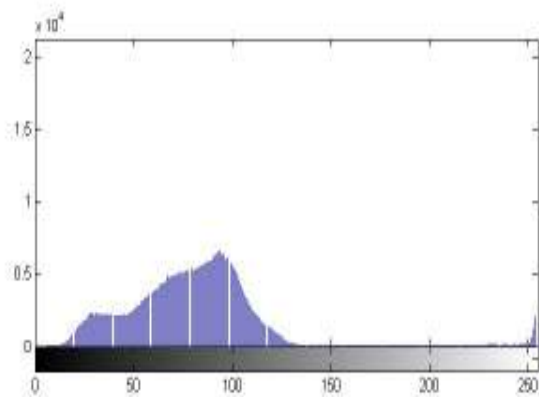
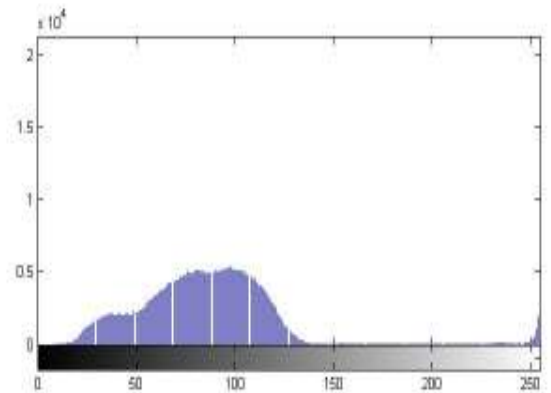
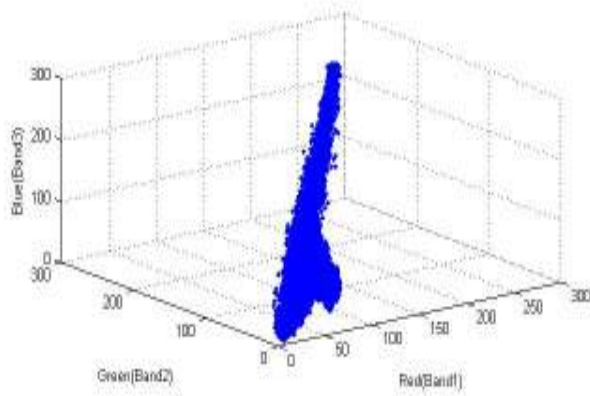
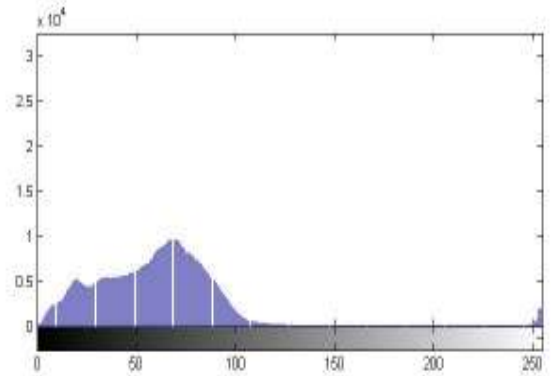
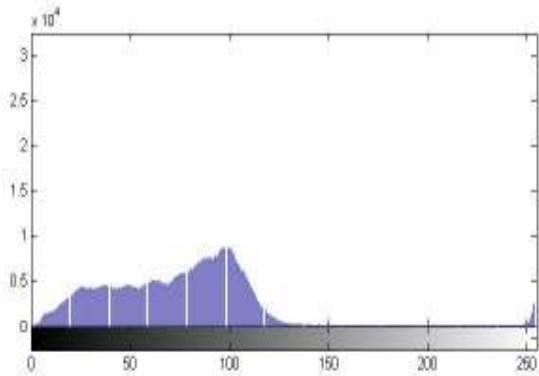
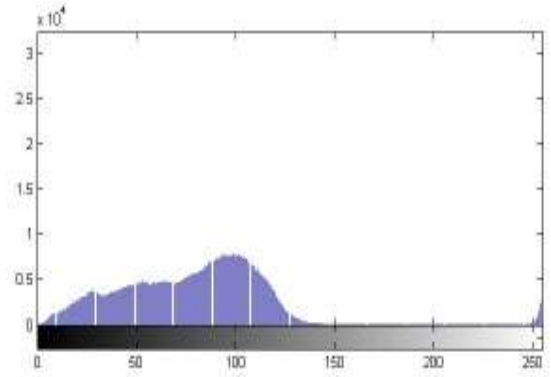
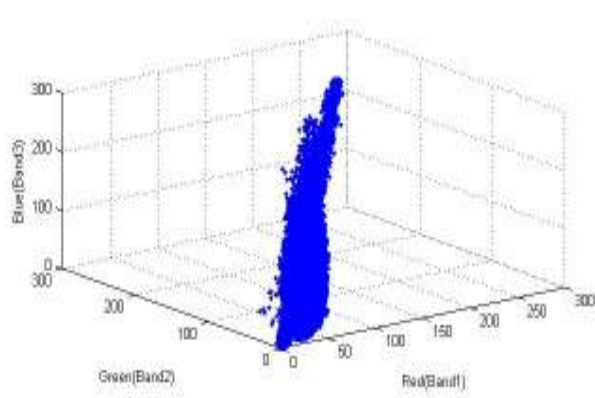


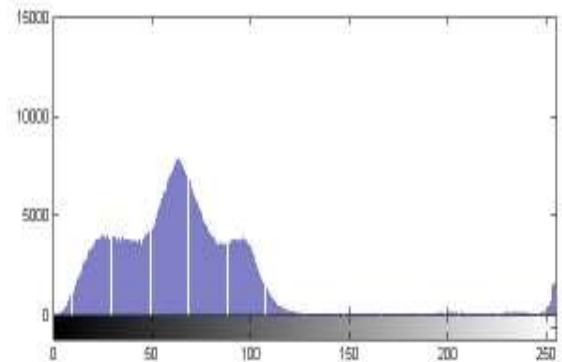
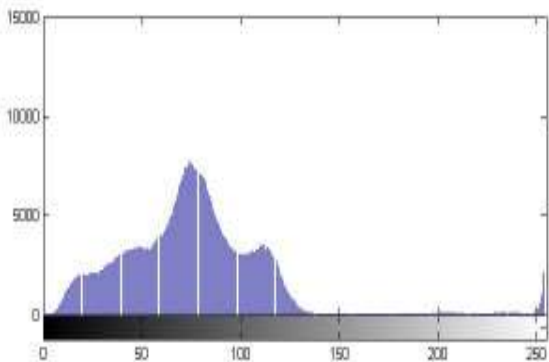
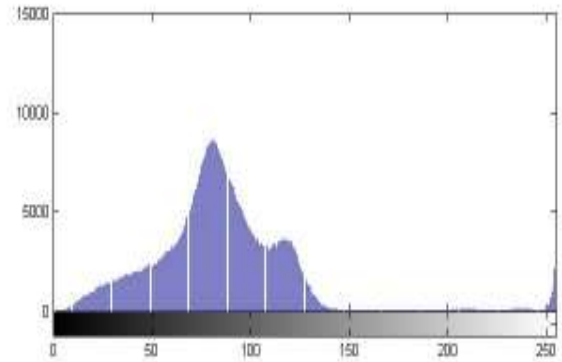
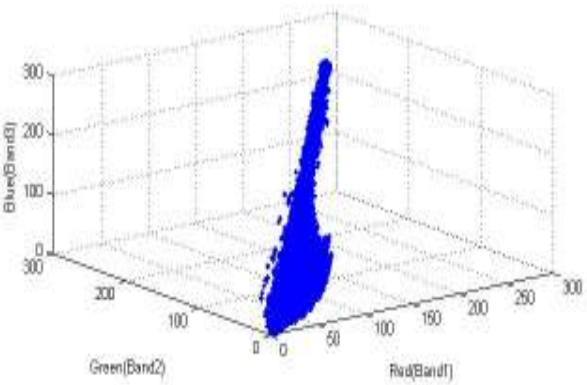
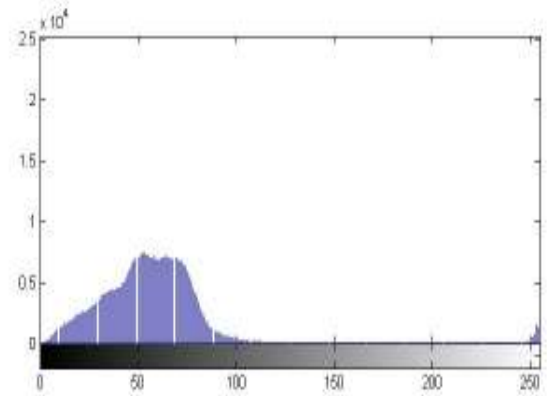
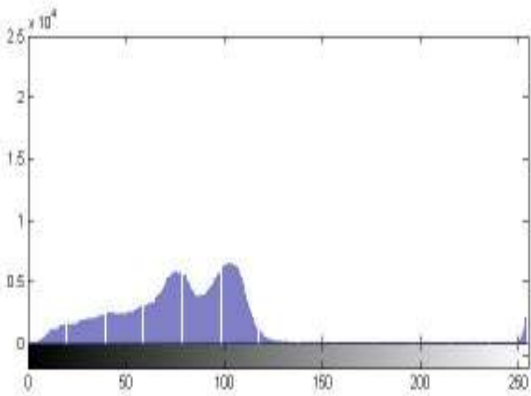
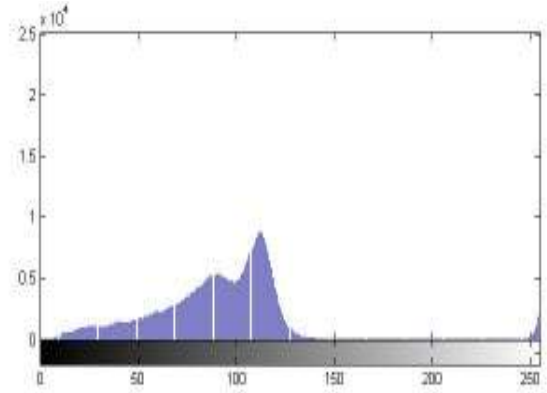
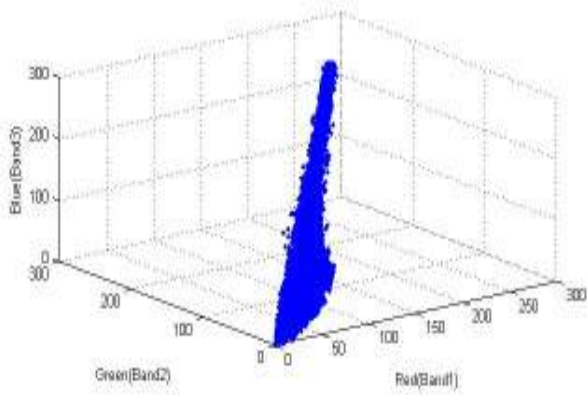
Fig. 4: The firmness comparison of blueberries in different processing conditions

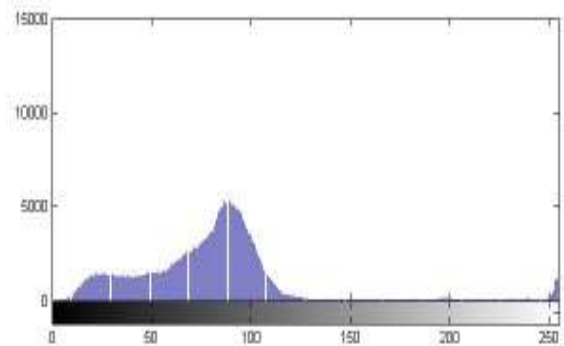
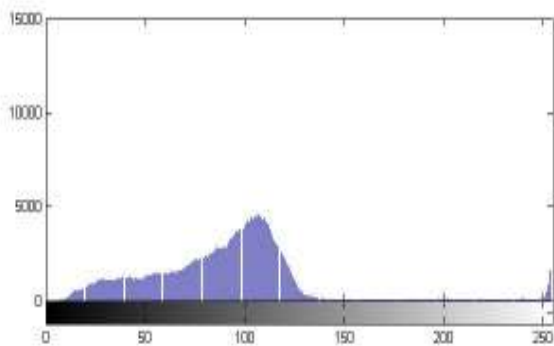
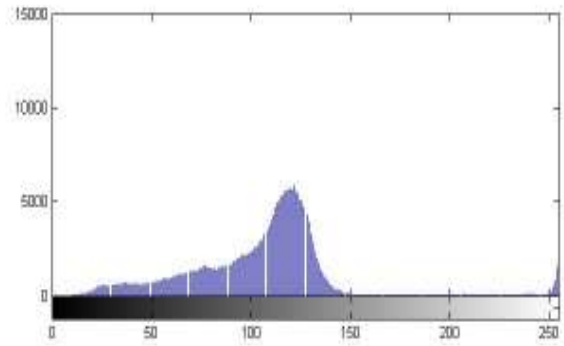
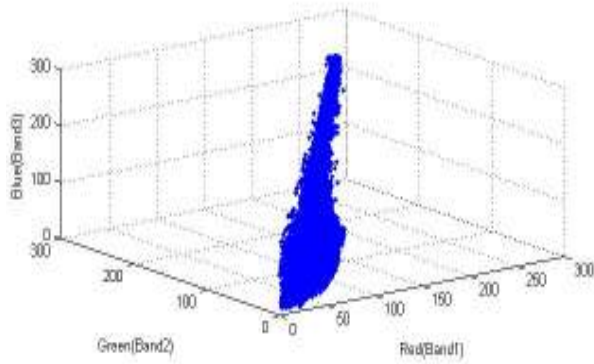
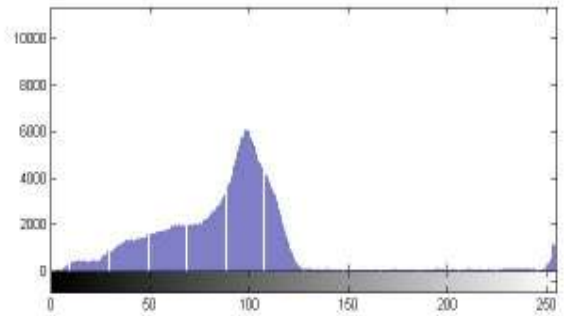
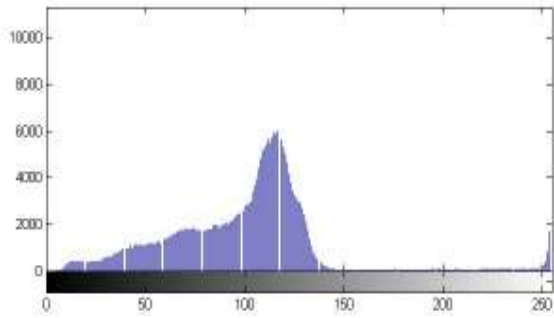
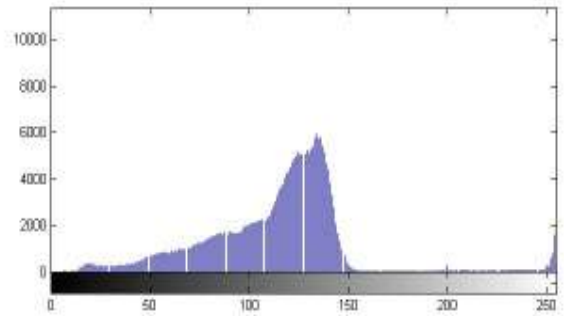
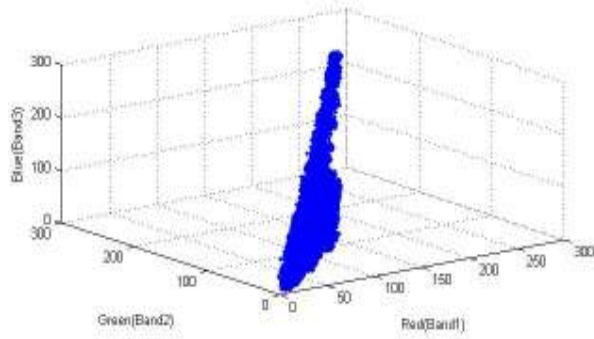
transferred into pectin gradually with the influence of pectinase. The fruits become softening and further transform into pectic acid; On the other hand, the water loss leads to the decrease of cell swelling and the fruits soften. Figure 4 indicates the firmness of blueberries at cold storage of  $4^\circ\text{C}$ , slow freezing at  $-18^\circ\text{C}$  and fluidized bed freezing respectively. The figure shows that the firmness of blueberries after freezing and thawing is lower than those blueberries without freezing. Besides, in the same freezing condition, the firmness of blueberries by fluidized bed freezing is stronger than of blueberries with slow freezing.

**Sensory test results of the samples in storage life:**

Figure 5 and 6 show the internal color and gray histogram of corresponding pictures when blueberries are frozen by fluidized bed freezing and slow freezing at  $-18^\circ\text{C}$  in different storage life. Gray histogram is described as gray scale function. It presents the number of each pixel with gray scale and reflects gray scale's turn-up-frequency in digital image. The abscissa of gray histogram is gray scale; while ordinate indicates the turn-up frequency of the gray scale. Each gray histogram consists of four parts: the image in upper left is color histogram, the three-dimensional coordinates show red, green and blue respectively; The rest of three images present red, green and blue monochrome histograms respectively; For monochrome histogram, the more the peak is closer to zero in abscissa, the darker the pictures and vice versa. The more apparent the peak, the more monotonous the color shows. The clearer the boundary is. On the contrary, the color is equilibrium and presents variation with blur boundary. Figure 5 and 6 indicate that the peak of histogram is obvious after 120 days by quick freezing of fluidized bed. However, blueberries by slow freezing at  $-18^\circ\text{C}$  show multi-peaks and tend to be moderate. The relative positions of green peak and red peak in Fig. 6 are larger, while their relative positions with blue peak









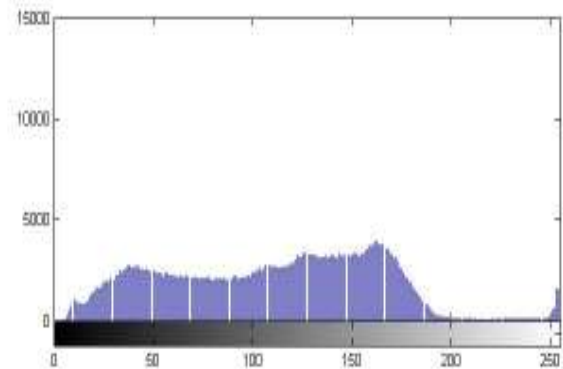
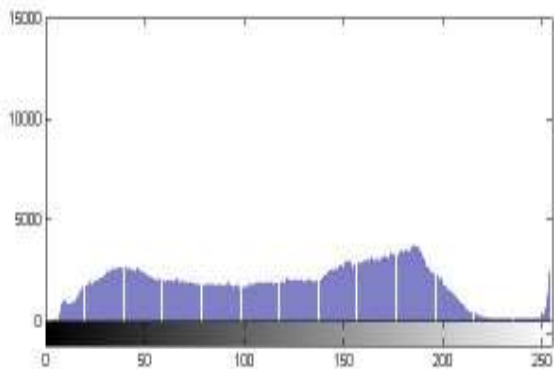
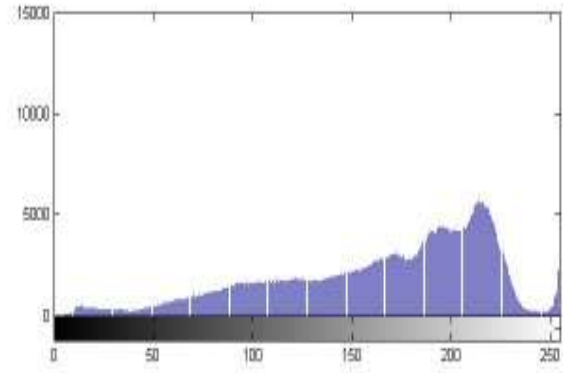
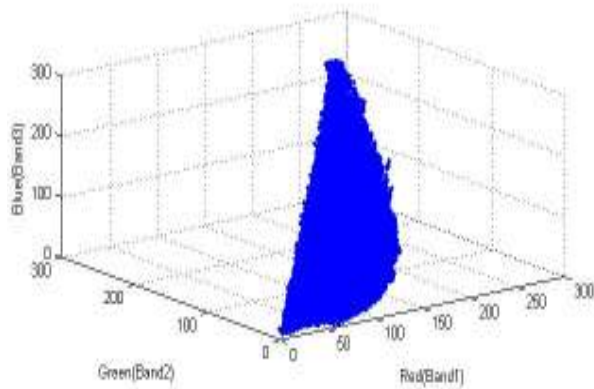
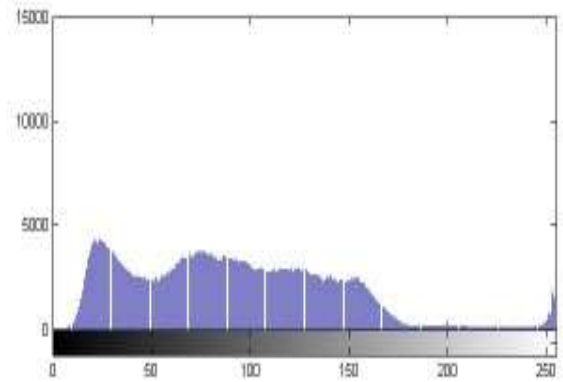
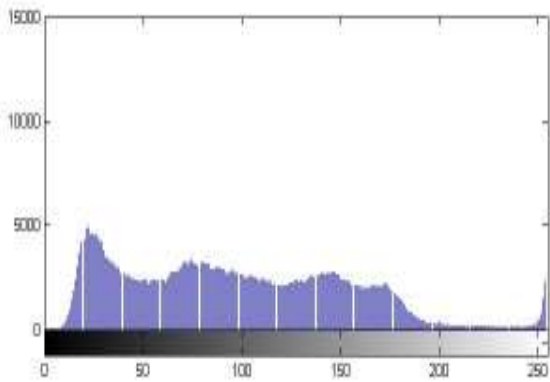
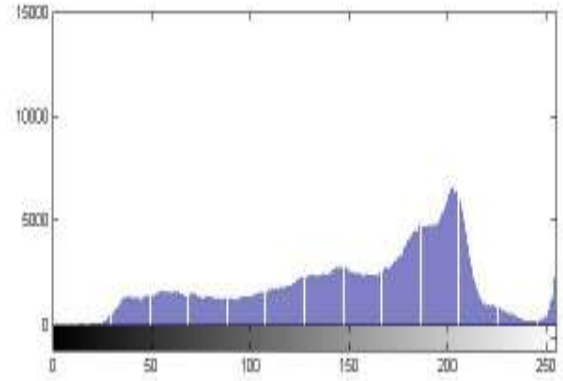
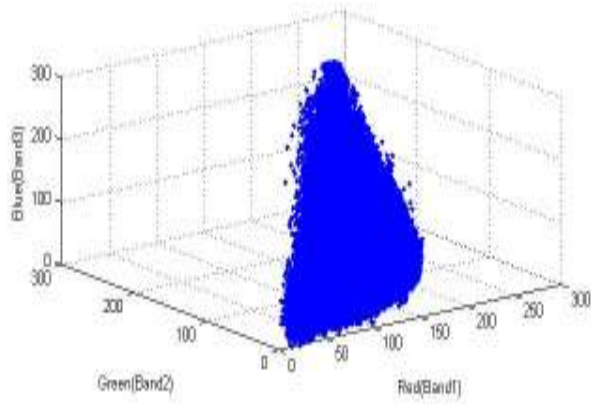
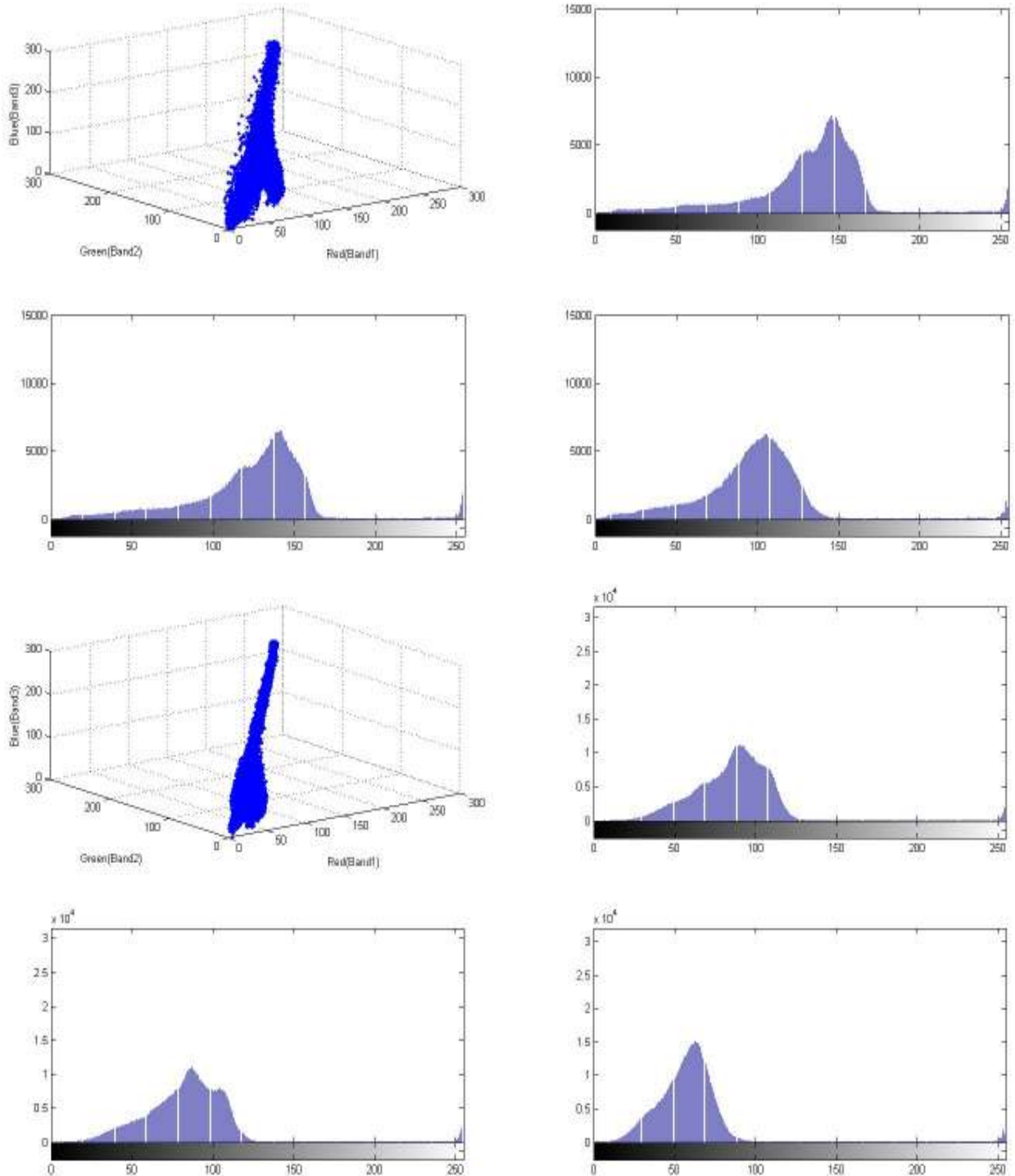
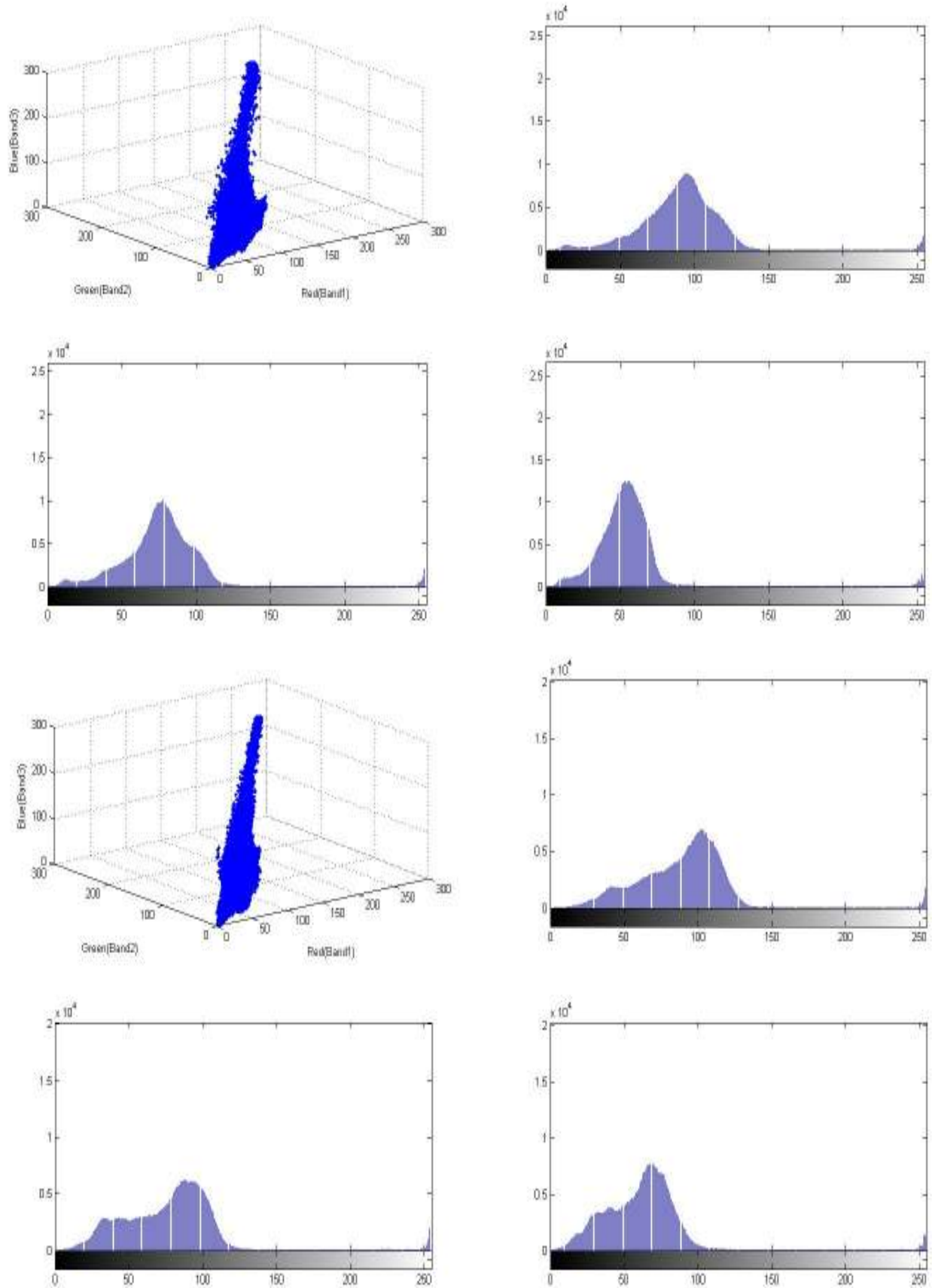
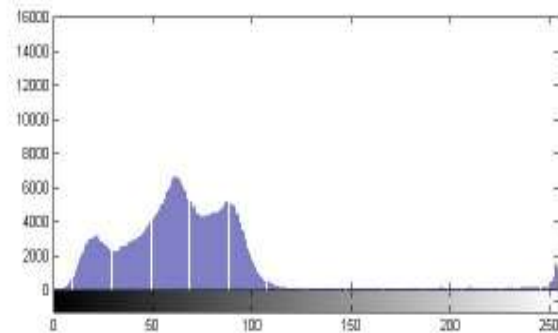
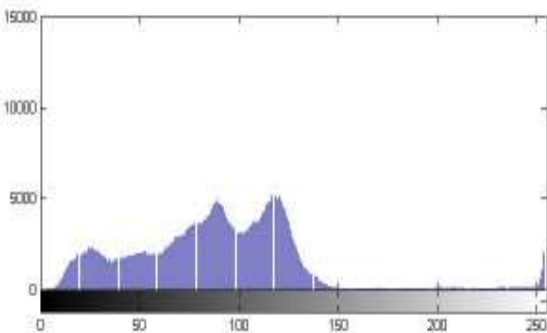
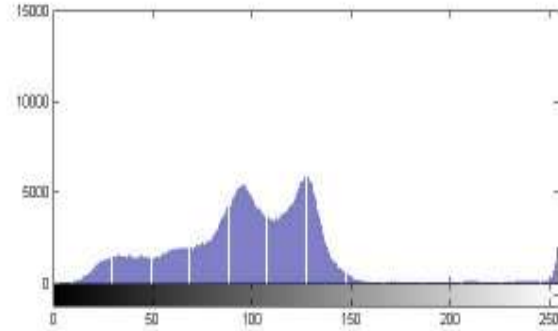
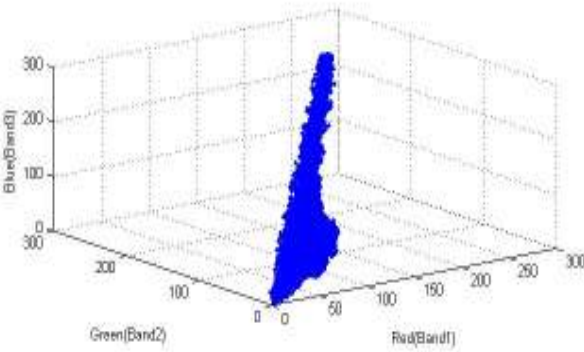
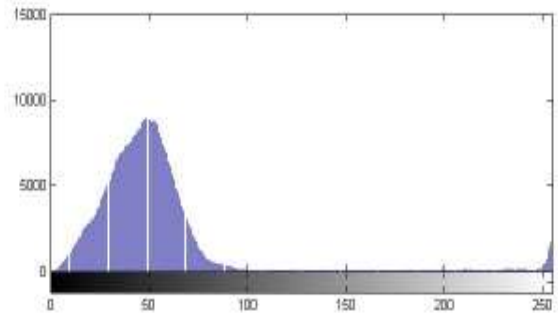
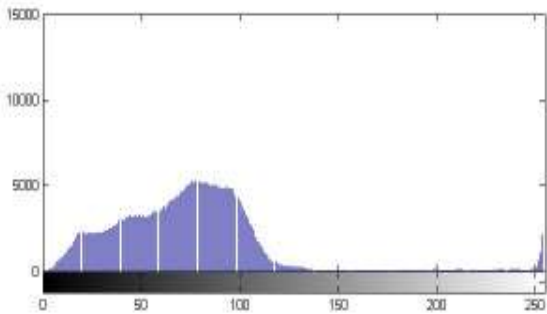
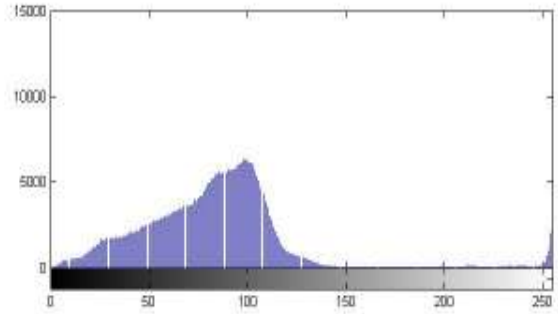
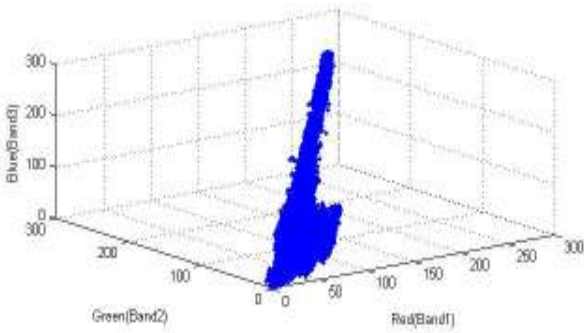




Fig. 5: Internal color and gray histogram of blueberries after quick freezing by fluidized bed in different storage life







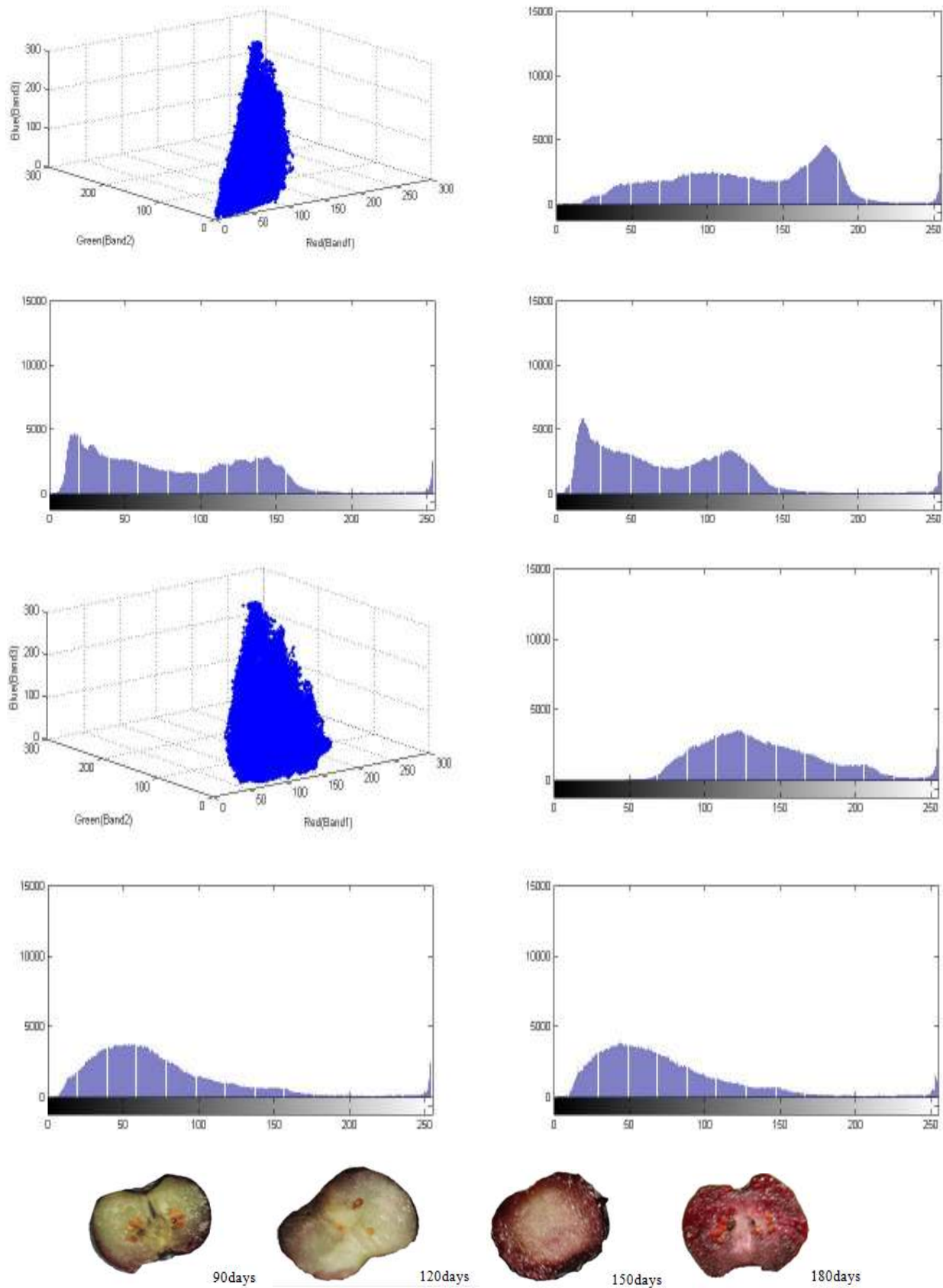


Fig. 6: Internal color and gray histogram of blueberries after slow freezing at  $-18^{\circ}\text{C}$  in different storage life

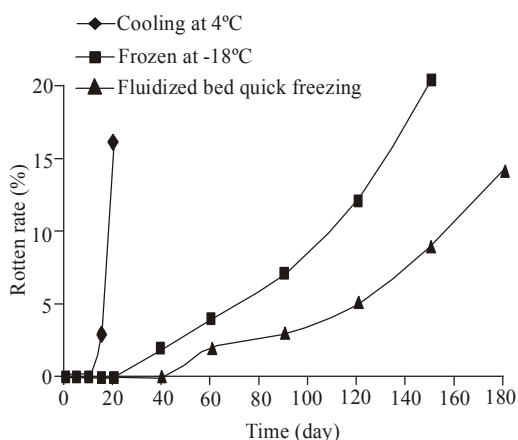


Fig. 7: The influence of different processing methods on the rotten rates of blueberries

decreases. This phenomenon shows that internal color of blueberries tends to change from distinct black and white to yellow, then blue-green and finally dark. The results show that fluidized bed freezing performs better than that of slow freezing at  $-18^{\circ}\text{C}$  in terms of color variation.

**The fruit rotten rate changes in storage life:** As shown in Fig. 7, rotten rates of three groups of samples at cold storage of  $4^{\circ}\text{C}$ , slow freezing at  $-18^{\circ}\text{C}$  and fluidized bed freezing are conducted statistically. The results indicate that: the rotten rate of blueberries at cold storage of  $4^{\circ}\text{C}$  about 18 days is more than 10%; the rotten rate of blueberries by slow freezing at  $-18^{\circ}\text{C}$  is more than 10% in 105 days; the rotten rate of blueberries after quick freezing by fluidized bed reaches to 10% in 150 days. The storage lives of blueberries with different processing methods are determined to be 18, 105 and 150 days respectively.

### CONCLUSION

The optimum processing conditions for blueberries quick freezing of liquid nitrogen fluidized bed consists of freezing temperature at  $-40^{\circ}\text{C}$ , fan wind velocity of 4.5 m/sec and bed-layer height of 3.6 cm.

ESEM shows the blueberries after quick freezing by liquid nitrogen fluidized bed is more superior to that of slow freezing at  $-18^{\circ}\text{C}$  in terms of compactness, uniformity and destructiveness of internal microstructure. The feasibility and superiority of blueberries quick freezing by liquid nitrogen fluidized bed are verified.

By conducting sensory tests on blueberries in storage life, the results show that the frozen samples by fluidized quick freezing is more superior to that of the samples by slow freezing at same stages in terms of indexes including firmness, internal color and appearance. By conducting statistics on blueberries

rotten rate, the storage life of blueberries after quick freezing by liquid nitrogen fluidized bed is approximately 150 days. It is more superior to that of slow freezing in 105 days.

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