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Effects of preprocessing freeze-thaw cycles and thawing methods on nutrition quality of frozen *Toona sinensis* sprouts

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Abstract:
Aims and Objectives*: To study the effect of pretreatment and thawing methods on qualities of frozen Toona sinensis sprouts.*
Methodology: *Four different pretreatment (blanching and cooling) methods were conducted including WW, SW, WS and SS. Three thawing methods were included: 1) room temperature and air (Air); 2) 4°C fridge (Fridge); 3) tap water (Water). Weight loss, defoliation rate, vitamin C, chlorophyll content, total flavones, total phenolic content and sensory evaluation were determined during processing.*
Results: *Among four pretreatments, SS exhibited the best sensory properties, the lowest defoliation rate, the highest total flavones content after 7 freeze-thaw cycles. Samples of thawing at fridge possessed the highest vitamin C, reducing sugar, chlorophyll content, total flavones and phenolic content.*
Conclusion: *SS was the most effective pretreatment to reduce the nutritional quality of Toona sinensis sprouts. Thawing at fridge was chosen to be the best thawing method to maintain the quality of frozen Toona sinensis sprouts.*

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I. Introduction

Fresh *Toona sinensis* sprout (Chinese toona) is a seasonal vegetable available only in Spring, widely distributed in China[1, 2]. It has special and excellent flavor and is considered as a highly sought-after culinary item[3, 4]. It is also a rich source of anti-carcinogenic compounds and antioxidants, such as glucosinolates, phenolic compounds, vitamin C, and carotenoids[5-8]. As a perishable vegetable, *Toona sinensis* sprout usually needs suitable post-harvest techniques to extend its shelf life[9, 10], among which freezing is one of the most popular and effective processing approaches to maintaining post-harvest quality and nutritional properties of *Toona sinensis* sprouts for extensive periods of time[11]. Quick-freezing could be a good choice to prolong its shelf-life to meet ever-increasing demands of consumers for high quality, fresh, nutritious, and conveniently prepared vegetable. Up to now, little is known about the changes of main bioactive compounds in *Toona sinensis* sprouts during pre-freezing processing, washing, blanching, cooling and thawing. Therefore, the objective of this study was to evaluate the effect of pretreatment and thawing methods on qualities of frozen *Toona sinensis* sprouts.

II. Material And Methods

Sample preparation and pretreatment

Toona sinensis sprouts (TSS) were purchased from a local market (Zibo, Shandong, China) on the day of harvest and transported to the laboratory without delay. Fresh TSS without visual defects and with a length ranging from 11 to 12 cm were chosen. TSS is subjected to processing including sorting, blanching, cooling and draining prior to freezing. In this study, four different pretreatment (blanching and cooling) methods were conducted as follows: 1) blanching with boiling water and cooling with 4 ℃ cold water, denoted as WW; 2) blanching with boiling saline (1%, w/w) and cooling with 4 ℃ cold water, denoted as SW; 3) blanching with boiling water and cooling with 1% (w/w) saline (4 ℃), denoted as WS; 4) blanching with hot saline (1%, w/w) and cooling with 1% (w/w) saline (4 ℃), denoted as SS. Samples without blanching or cooling were taken as control (CK). All pretreatments were blanched for 30 s and immediately cooled as above, then drained and packaged with polyethylene bag prior to freezing. The freeze-thaw cycle test was carried out to observe the effect of different pretreatments on qualities of TSS during storage at -18 ℃ (DW-FW351 cryogenic

refrigerator, Zhongke Meiling Cryogenics Co., Ltd, Hefei, China). All frozen samples were taken to thaw at 4 ℃ refrigerators (Haier Pharmacy Refrigerators, Qingdao, China) at fixed time for further analysis.

Preparation for different thawing methods

Samples pretreated by the selected groups were conducted to freeze under -18 ℃, followed by different thawing methods. Three thawing methods were included: 1) room temperature and air (denoted as Air); 2) 4°C fridge (denoted as Fridge); 3) tap water (Water). Each sample (100 g) was wrapped in polyethylene film, the melting of ice coat on the exterior surface was observed. A probe thermometer was used to determine the central temperature of Chinese toon to help determine the end point of thawing (about 20°C in Water and Air, and more than 4°C in refrigerator). Different physical and chemical properties were measured before and after thawing.

Weight loss and defoliation rate

Weight loss and defoliation rate were calculated as following equations:

$$\text{Weight loss} = (M_1 / M_2) / M_1 \times 100\% \quad (1)$$

$$\text{Defoliation rate} = (N_1 / N_2) / N_1 \times 100\% \quad (2)$$

where, M_1 is Pre-storage mass, M_2 is Post storage mass, N_1 is total number of leaves shed, N_2 is the total number of leaves.

Total flavones and total phenolic content assays

Total flavones content was determined by colorimetric analysis[9]. The standard calibration curve was established using the absorbance of gradient solutions of rutin at 510 nm, which could be used to determination the content of total flavones quantitatively. Methanol at 80% was used as control. As a result, equation of the standard curve was Y=4.053X-0.02851 (R^2=0.9964), showing that excellent linearity relationship was obtained. Generally, 80% methanol and method of $NaNO_2$-Al $(NO_3)_3$-NaOH was used to extract and determine total flavones in samples. The flavones amount could be determined according to the above equation of standard curve, and finally the total flavonoids content in the sample was denoted as mg RE/100 g FW equivalent of rutin in 100g sample. Folin method was used to measure total phenolic content[12], equation of the standard curve was Y=0.00528X+0.03714 (R^2=0.9912), showing that excellent linearity relationship was obtained.

Vitamin C assay

The content of vitamin C was determined by iodine titration, after extracting with 2 mol/L acetic acid[13]. Vitamin C was calculated as following equation:

$$\text{Vitamin C content} = C \times V \times M / v \quad (3)$$

where, C is the concentration of iodine solution, V is the volume of iodine solution used in titration, M is the molar mass of vitamin C, v is the volume of sample.

Reducing sugar assay

Reducing sugar assay was conducted according to DNS method as described by Zhao et al.[1]

Chlorophyll content assay

Chlorophyll content was determined by spectrophotometer after extracting with ethanol[10]. The absorbance of chlorophyll extracting solution was measured at wavelengths of 663nm and 645nm. Ethanol at 95% was used as the control. Finally, Chlorophyll content was calculated as following equations:

$$C_a = 12.71 \times A_{663} — 2.59 \times A_{645} \quad (4)$$

$$C_b = 22.88 \times A_{645} — 4.67 \times A_{663} \quad (5)$$

$$C = C_a + C_b = 8.04 \times A_{663} + 20.29 \times A_{645} \quad (6)$$

$$\text{Chlorophyll content (mg/g dry sample)} = C \times V_{total} / m \quad (7)$$

where, C_a, C_b and C (mg/L) are contents of chlorophyll a, chlorophyll b and total chlorophyll, respectively; A_{663} and A_{645} are absorbance of sample, V_{total} is the total volume of chloropgyll extraction, m is the mass of sample.

Water content assay

Water content was measured by weighing after heating at 60 ° C until constant weight. It was calculated as following equation:

$$W = (m_2 - m_1) / m_1 \times 100\% \quad (8)$$

where, W (%) is water content, m_1 is the mass of wet sample, m_2 is the mass of dry sample.

Sensory evaluation

Five-point system was used to evaluate sensory properties, including color, odor, flavor, tenderness, and overall acceptability. The scale was categorized as: five=like very much, four=like slightly, three=neither

like nor dislike, two=dislike slightly, and one=dislike very much. Samples with mean scores of more than 3 were considered acceptable.

Statistical analysis

All experiments were performed in triplicate on different treatments. SAS software (SAS 9.0 for windows, SAS (Shanghai) Software Co., Ltd, Shanghai, China) was used for data analysis. Significant differences between treatments were analyzed by least significant difference (LSD) at a significance level of $P < 0.05$. The mean values as shown in this study were calculated as the mean ± SD (n=3).

III. Result and discussion

Fig. 1 showed the sensory evaluation of frozen TSS with different pretreatments during freeze-thaw cycles. Sensory properties of all samples tended to decease with the increase of the number of freeze-thaw cycles. Among them, the control group (CK) deteriorated fastest, almost lost most of the sensory properties and had been rotten after 7 freeze-thaw cycles. Samples of WW maintained the best sensory properties during freeze-thaw cycles, followed by those of SS. Samples of SW and WS were in the middle, which were better than the control group.

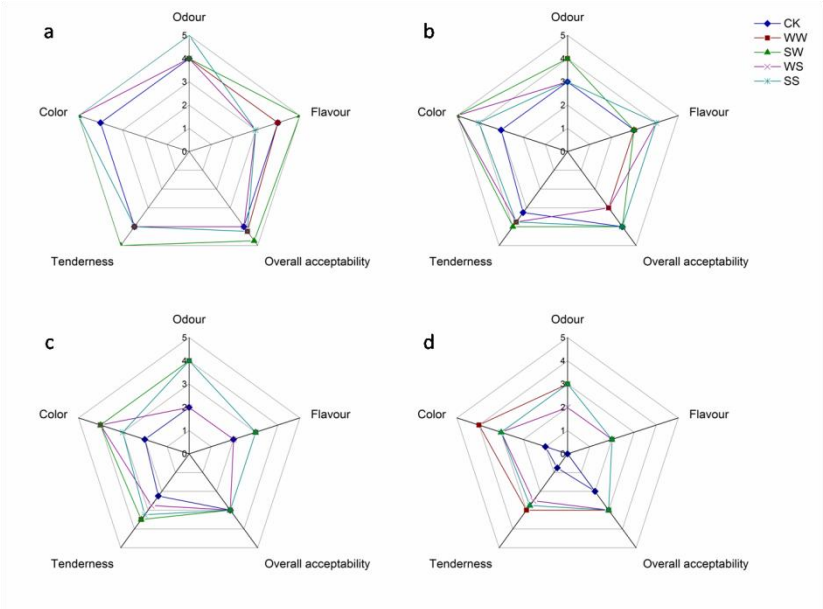

Fig. 1 Sensory evaluation of frozen *Toona sinensis* sprouts with different pretreatments during freeze-thaw cycles: a-1 freeze-thaw cycle, b-3 freeze-thaw cycles, c-5 freeze-thaw cycles, d-7 freeze-thaw cycles.

Defoliation rate of frozen TSS with different pretreatments were determined during the freeze-thaw cycles. As shown in Fig.2, defoliation rate of frozen T. sinensis sprouts increased with increasing freeze-thaw cycles, and control group (CK) always got the highest value in each freeze-thaw cycle, it was as high as 20% after 7 freeze-thaw cycles. There was no significant difference between each treatments within 4 freeze-thaw cycles ($p>0.05$). It was showed that SW, WS and SS exhibited lower defoliation rate than those of WW and CK, indicating that defoliation rate could be reduced either blanching with salt or cooling with salt.

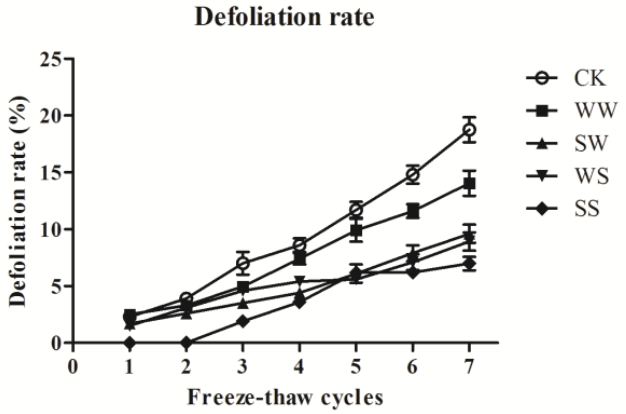

Fig. 2 Defoliation rate of frozen *Toona sinensis* sprouts with different pretreatments during freeze-thaw cycles

Water in TSS was condensed into ice during freezing, while it evaporated at higher temperatures when thawing[11]. Also, its juice was lost at the same time, leading to the weight loss of frozen TSS. As shown in Fig.3, samples of WW exhibited the highest weight loss, which were significantly higher than those of other pretreatment groups after 3 freeze-thaw cycles, suggesting that pretreatments with salt (SW, WS and SS) were beneficial to reduce weight loss rate. Weight loss of the control (CK) was the lowest since no plant cell damage without blanching pretreatment.

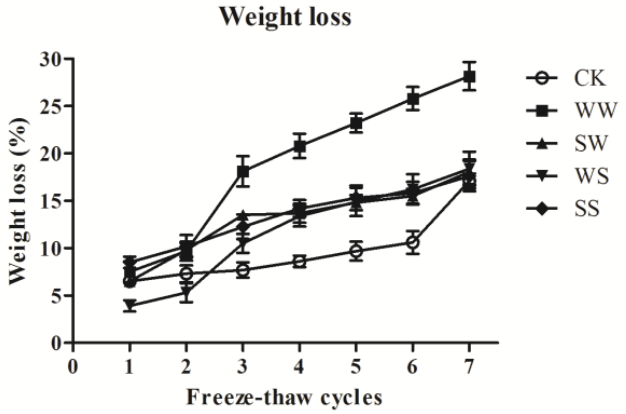

Fig. 3 Weight loss of frozen *Toona sinensis* sprouts with different pretreatments during freeze-thaw cycles

Flavonoids are important medicinal compositions in TSS, with a strong ability of antibacterial and anti-inflammatory[14, 15]. They are easily soluble in water, ethanol, methanol and other polar solvents, which are relatively stable under neutral conditions. Fig.4 showed total flavones content of frozen TSS with different pretreatments during freeze-thaw cycles. Total flavones had the tendency to decrease during freeze-thaw cycles. Among them, SS exhibited the highest total flavones contents at the end of 7 freeze-thaw cycles with the lowest reducing rate, while WW showed the lowest level with significant differences ($p < 0.05$). SW and WS groups were similar with that of CK after 7 freeze-thaw cycles with no significant difference ($p < 0.05$). In short, results indicated that SS exhibited the best protective effect to decrease the reducing rate of total flavones in frozen TSS.

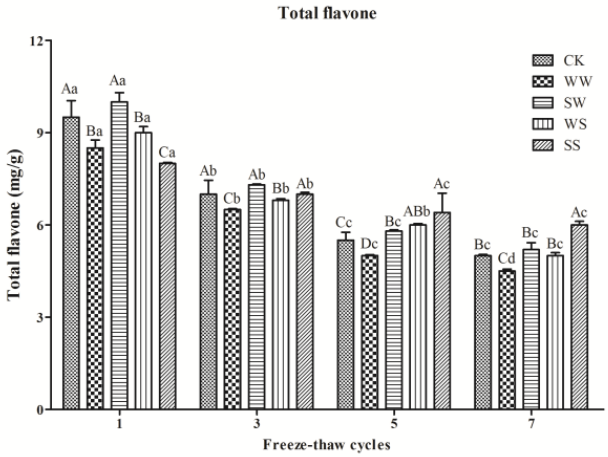

Fig. 4 Total flavones content of frozen *Toona sinensis* sprouts with different pretreatments during freeze-thaw cycles.

Effect of different thawing methods on frozen TSS was evaluated by total flavones, total phonelic, vitamin C, reducing sugar, chlorophyll and water content, since those were the main quality indicators during storage[9, 10, 12]. As shown in Fig.5, almost six parameters of WW and SS of samples were lower than those of CK. However, some quality indicators of SS were better than those of WW, like vitamin C and reducing sugar, indicating that SS was beneficial to maintain the quality of TSS during freezing and thawing. It could further confirm the above results on pretreatments. Three different thawing methods had great influence on the qualities of frozen TSS, among which thawing at Fridge possessed the best protective effect followed by Air group and Water thawing treatment was the worst. It was found that chlorophyll contents of samples in Air and Water thawing groups reduced more than 40% when compared to those of Fridge group for both WW and SS pretreatments. Similar significant differences were found in other parameters ($p<0.05$). In a word, Fridge was chosen to be the best thawing method to maintain the quality of frozen TSS.

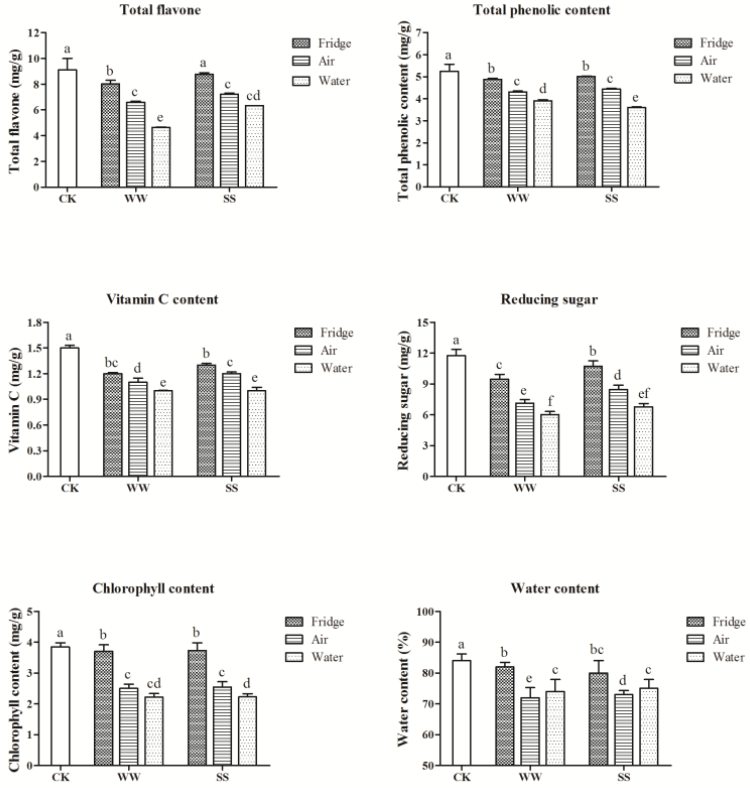

Fig. 5 Effect of different thawing methods on frozen *Toona sinensis* sprouts.

IV. Conclusion

Results indicated that SS was the most effective pretreatment to reduce the nutritional quality of frozen Toona sinensis. Also, fridge was chosen to be the best thawing method to maintain the quality of frozen Toona sinensis sprouts.

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