Food and Applied Bioscience Journal

FABJ

ISSN: 2286-8615
Volume 9 Issue 1
(January-April 2021)

FABJ

Food and Applied Bioscience Journal

TABLE OF CONTENTS

PAGE

ISSN: 2286-8615
Volume 9 Issue 1
(January-April 2021)

Food and Applied Bioscience Journal

Effect of pre–treatments and freezing methods on frozen mulberry qualities for fresh fruit consumption

Pichan Powpongchan and Somchai Jomduang*

Division of Food Science and Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50100, Thailand
**Corresponding author. E–mail: somchai.j@cmu.ac.th*
Received: 26 February 2020, Accepted: 24 September 2020

Abstract

After short annually harvesting period, fresh mulberry fruits are perishable easily because of their soft texture. This study was to investigate the effect of pre–treatment and freezing methods on frozen mulberry fruits for off–season fresh fruit consumption. There were two methods including pre–treatments for firmness and sanitizing qualities. Soaking in 2% (w/w) calcium chloride for 10 min at room temperature was the most appropriate treatment providing fruit firmness (41.58 ± 10.50 N of firmness), meanwhile adding of 0.3% (w/w) calcium hypochlorite into 2% (w/w) calcium chloride solution provided the greatest inhibitory effect (less than 1×10^5 CFU/g of microbial load). Among three freezing methods (household freezing at −18°C, air–blast freezing at −25°C and cryogenic freezing with liquid nitrogen), cryogenic freezing provided the shortest freezing time (only 0.47 min). There were textures cracking occurred in some cryogenic frozen samples due to extreme low temperature. Air–blast freezing was considered as the most suitable for mulberry fruit freezing due to its lower drip loss and higher firmness after thawing with reasonable freezing time (26 min). In addition, among frozen mulberry fruits, air-blasted samples gave the highest Hedonic scores in terms of texture and overall acceptance qualities (5.94 ± 1.58 and 6.16 ± 1.49, respectively). Therefore, air–blast freezing had the most potential freezing method suggesting for fresh fruit consumption.

Keywords: Mulberry fruits, pre–treatment, firmness, air–blast freezing, fresh fruit consumption

1. Introduction

Ripe mulberry fruits have high anthocyanin content which are known to be effective in human healthy such as protection against various diseases, against oxidative damage to the cell, minimization of tissue damage, improving of cell survival, anti−cancer, improving of immune function, and increasing of healthy longevity (Azevedo *et al.,* 2003; Lee *et al.,* 2009; Tan *et al.,* 2018; Thanthanasupawat, Y. 2018). There are also have 1−deoxynojirimycin (DNJ) which has high potential to control blood glucose (Pham, *et al.,* 2017). In addition, resveratrol in mulberry fruits displays cardioprotective (Voloshyna *et al.,* 2012), anti−inflammatory (De Sa Coutinho *et al.,* 2018) and anticancer agent (Cai *et al.,* 2015).

In Thailand, there are some varieties of mulberry plant. Chiang Mai variety is popular for mulberry fruit production. Maturity index for mulberry fruit harvesting is the color of fruit which can be classified into three levels; unripe, ripe and over ripe. Ripe mulberry fruits have about 70% of black color on surface which are suitable for fresh fruit consumption (Wasan, 2003). During mulberry fruit harvesting period (January−April), there are over supplied of mulberry fruits. Ripe mulberry fruits have soft texture which are quick perishable after harvesting. Some mulberry fruits are processed to some products such as mulberry jam, mulberry wine, mulberry candy, dried mulberry and mulberry juice. Frozen mulberry fruit is one of processed products that can be kept more than one year. The frozen products can be utilized as fresh consumption or raw material for other products. Normally, household freezer (still air freezer) which is slow freezing method can keep mulberry fruits for other purposed, not for fresh consumption because of the soft texture of mulberry fruits (Nopparat *et al.,* 2017). Slow freezing has the problem of liquid leaking from frozen products (drip loss) during thawing before consumption. The problems caused by formation of large ice crystals in cells during freezing processes which large ice crystals denatured or damaged to cells. This problem can be solved by firmness pre−treatment and quick freezing method. Soaking in some commercial calcium salts (calcium chloride and calcium lactate) can raise up the firmness texture of raw material (Chen *et al.,* 2011; Tangtua *et al.,* 2014). Quick freezing method creates small ice crystals to reduce damage in cells more than slow freezing (Estrada, 2002). Currently, air−blast freezing was suitable for vegetable and fruits. Cryogenic freezing was suitable for meat, seafood, poultry, fruits, vegetables, bakery products, and specialty items which high value product. Utilization of quick freezing in mulberry fruit for fresh consumption has not reported yet.

In this study, suitable pre−treatments of mulberry fruits in order to increase the fruit firmness and reduce the microbial loads before freezing were investigated. In addition, two quick freezing methods (air−blast and cryogenic freezing) were applied for mulberry fruits. Qualities of thawed fruits after freezing were compared to a traditional household freezing. The high potential of quick freezing method was selected in order to utilize as fresh fruit consumption.

2. Materials and Methods

2.1 Effect of pre−treatment of mulberry fruits

In this study, ripe (about 70% black in color of surface) mulberry *(Morus alba)* fruits, Chiangmai variety were collected from mulberry orchard in Chiang Mai province, Thailand. Each batch of mulberry fruit harvesting was collected for each experiment.

2.1.1 Effect of calcium salts and concentrations on firmness of fresh mulberry fruits

There were two types of calcium salt solutions (calcium chloride and calcium lactate) which were prepared four difference concentrations; 0, 1, 2 and 2.5% (w/w). Each 1 kilogram of ripe mulberry fruits was soaked in 2 litter of calcium salt solutions for 10 min at room temperature. The experiment design was 2 × 4 factorial in completely randomized design with three replications. After soaking, each replication was firmness determined using maximum compression force by TA.XT Plus Texture Analyzer with cylinder probe number P/50 (modified method from Han *et al.*, 2017) from randomized fifty fruits. All data were statistical analyzed using analysis of variance (ANOVA) and mean compared using Duncan's new multiple range test ($P \leq 0.05$). Statistical analysis was performed using statistical software SPSS Ver.17 (Chicago, Illinois, USA). The suitable calcium salt and concentration that could provide high firmness texture was selected for further study.

2.1.2 Optimal calcium salt soaking time on firmness of fresh mulberry fruits

Ripe mulberry fruit were soaked in selected calcium salt solutions at room temperature with the ratio of 1:2 (w/w). Four soaking times were studied; 0, 10, 20 and 30 min. Completely randomized design (CRD) with three replications was employed in this study. After soaking, mulberry fruits were determined the maximum firmness as same as the previous study. All data were statistical analyzed using analysis of variance (ANOVA) and mean compared using Duncan's new multiple range test ($P \leq 0.05$). The suitable soaking time that could provide high firmness texture was selected for further study.

2.1.3 Effect of sanitizers on microbial load reduction in fresh mulberry fruits

Ripe mulberry fruits were soaked with selected calcium salt combined with sanitizer solution. There were two types of food sanitizer; calcium hypochlorite and peracetic acid. Four levels of sanitizer concentrations were studied; 0, 0.1, 0.2 and 0.3% (w/w). After soaking at room temperature for 10 min, each treatment was determined total microbial load following the Bacteriological Analytical Manual (BAM) method (BAM, 2001). The suitable sanitizer that could control safety microbial load according to Thai National Bureau of Agricultural Commodity and Food Standards, Ministry of Agriculture and Cooperatives (ACFS, 2013) was selected for further study.

2.2 Optimal freezing method of mulberry fruits

Ripe mulberry fruits were pre−treated with selected method. Three freezing methods were studied; household freezing using freezer (SF−PC900, Panasonic) at -18°C, air−blast freezing using air−blast freezer (March cool, Thailand) at -25°C and cryogenic freezing with liquid nitrogen. Freezing time was determined from the beginning of freezing until complete freezing (-18°C at center of fruit). All frozen mulberry fruits were stored at -18°C for one week before quality checking.

Completely randomized design (CRD) was employed in this study. Frozen fruits were thaw at room temperature before quality checking. Drip loss was determined following Liang *et al.* (2015) method. Fruit firmness was determined as same as previous experiment. All data from three replications were statistical analyzed using analysis of variance (ANOVA) and mean compared using Duncan's new multiple range test ($P \leq 0.05$).

In addition, sensory evaluation was performed using randomized complete block design (RCBD). Fifty test panelists evaluated sensory quality using 9−point Hedonic scale. All data were statistical analyzed using analysis of variance (ANOVA) and mean compared using Duncan's new multiple range test ($P \leq 0.05$). The suitable freezing method that provided low drip loss, high firmness and high sensory evaluation was selected.

3. Results and Discussion

3.1 Effect of pre−treatment

3.1.1 Effect of calcium salts and concentrations on firmness of fresh mulberry fruits

Ripe mulberry fruits without soaking had 40.84 ± 10.91 Newton (N) of firmness. After 10 min soaking with difference calcium salts, it was found that both calcium salts (calcium chloride and calcium lactate) could raise up the similar firmness of fruits (46.94 ± 17.21 and 46.99 ± 16.20 N, respectively), not statistical significant difference ($P > 0.05$) (Table 1). It was also found that at 2% (w/w) concentration could provide the high firmness (49.88 ± 16.42 N) similar to 2.5% (w/w), so 2% (w/w) calcium salt solution should be selected because of the low cost. Calcium chloride price was about 2 times cheaper than calcium lactate. For further study, soaking in 2% (w/w) calcium chloride solution was selected for firmness pre−treatment.

Table 1 Effect of concentration for soaking with calcium salt solutions on firmness of mulberry fruits

Factor	Firmness (N)*
Type of calcium salt ns	
Calcium chloride	46.94 ± 17.21
Calcium lactate	46.99 ± 16.20
Calcium salt concentration (% w/w)	
0	40.84^{c} ± 10.91
1	45.28^{bc} ± 17.67
2	49.88^{ab} ± 16.42
2.5	51.87^{a} ± 18.75
Calcium salt x concentration	
Calcium chloride, 0% (w/w)	40.84^{b} ± 11.00
Calcium chloride, 1% (w/w)	46.53^{ab} ± 20.52
Calcium chloride, 2% (w/w)	49.26^{ab} ± 14.76
Calcium chloride, 2.5% (w/w)	51.15^{a} ± 19.80
Calcium lactate, 0% (w/w)	40.84^{b} ± 11.00
Calcium lactate, 1% (w/w)	44.03^{ab} ± 14.52
Calcium lactate, 2% (w/w)	50.51^{a} ± 18.16
Calcium lactate, 2.5% (w/w)	52.58^{a} ± 17.95

*Means followed by different letters in the same column were significantly different ($P \leq 0.05$), ns: no significant difference ($P > 0.05$)

Table 2 Effect of soaking time with 2% (w/w) calcium chloride solution on firmness of mulberry fruits

Soaking time (min)	Firmness (N) *
0	36.35^{b} ± 10.29
10	41.58^{a} ± 10.50
20	43.36^{a} ± 13.36
30	47.45^{a} ± 12.61
40	41.25^{a} ± 15.73

*Means followed by different letters in the same column were significantly different ($P \leq 0.05$).

3.1.3 Effect of sanitizers on microbial load reduction in fresh mulberry fruits

Ripe mulberry fruits from the new batch were soaked in 2% (w/w) calcium chloride combined with different sanitizer solutions and concentrations for 10 min. It was found that 2% (w/w) calcium chloride combined with 0.3% (w/w) calcium hypochlorite solution could reduce the microbial load from 8.5 x 10^{6} to 9.7 x 10^{4} CFU/g (Table 3). That level was in the safety microbial load following Thai

National Bureau of Agricultural Commodity and Food Standards, Ministry of Agriculture and Cooperatives (less than 1x10^5 CFU/g). Thus, pre−treatment before freezing of ripe mulberry fruits was soaking in 2% (w/w) calcium chloride combined with 0.3% (w/w) calcium hypochlorite solution for 10 min with 1:2 (w/w) ratio of fruit to soaking solution.

Table 3 Effect of calcium chloride combination with sanitizer solution soaking for 10 minutes on microbial load of fresh mulberry fruits

Methods	Microbial load (CFU/g)
No soaking (fresh mulberry fruits)	8.5 x 10^6
Soaking in calcium chloride, 2% (w/w) + calcium hypochlorite, 0.1% (w/w)	4.1 x 10^6
Soaking in calcium chloride, 2% (w/w) + calcium hypochlorite, 0.2% (w/w)	1.4 x 10^5
Soaking in calcium chloride, 2% (w/w) + calcium hypochlorite, 0.3% (w/w)	9.7 x 10^4
Soaking in calcium chloride, 2% (w/w) + peracetic acid solution, 0.1% (w/w)	2.8 x 10^6
Soaking in calcium chloride, 2% (w/w) + peracetic acid solution, 0.2% (w/w)	1.5 x 10^5
Soaking in calcium chloride, 2% (w/w) + peracetic acid solution, 0.3% (w/w)	3.0 x 10^5

3.2 Optimal freezing method for mulberry fruits

After pre−treated mulberry fruits were frozen, household freezing took very long time for freezing (380 min) (Table 4). Air−blast freezing could provide 26 min for freezing time without fruit cracking. During not very fast freezing by air−blast freezing, water at outer and inner of fruit was uniform changed into ice crystals. After thawing, mulberry fruits from this method could provide low drip loss (14.57 ± 1.83%) and high firmness (51.40 ± 7.67 N). Cryogenic freezing provided very short freezing time (0.47 min) Although short freezing time but mulberry fruits were cracked after freezing because of extremely low temperature of liquid nitrogen treatment (Cao *et al.*, 2018). At the first period of cryogenic freezing, water at outer layer of mulberry fruit was fast changed to ice crystals without flexible texture. When the water inside the fruit changed to ice crystals and expanded, so the outside solid layer was cracked. From the observation during freezing, cryogenic freezing used a large amount of liquid nitrogen per kilogram of mulberry fruits which costed very high value for production. This method was not suitable for mulberry fruit freezing.

Frozen mulberry fruits (after one week storage) were thawed then compared the sensory evaluation to fresh mulberry fruits. It was found that fresh mulberry fruits had high sensory evaluation score for all qualities (6.42 ± 1.58 to 7.58 ± 1.14) which was at the level of like slightly to like moderately (Table 5). Consideration to texture sensory evaluation score of thawed air−blast freezing frozen fruits (5.94 ± 1.58), it was significantly lower than fresh mulberry fruits score (7.48 ± 1.30). It was indicated that firmness pre−treated fruits before freezing, the hard texture was not firmness similar to the fresh fruits. From this study, the freezing temperature of air−blast freezing was -25°C which may be not low enough for small size crystal forming in mulberry fruits, so the drip loss after thawing was still high because of some collapsed cells. If the lower freezing temperature was applied, it should be affected to shorten freezing time, low drip loss and high texture firmness (Estrada, 2002). Other sensory evaluation scores of air−blast frozen fruits were also lower than fresh fruits which was at the level of like slightly (5.14 ± 1.59 to 6.16 ± 1.49). Thawed frozen mulberry fruits from other freezing methods (household freezing and cryogenic freezing) had very low sensory evaluation scores (5.04 ± 1.44 to 6.08 ± 1.55). From this study, it was indicated that air−blast freezing had the highest potential to apply for mulberry fruit freezing in order to fresh consume.

Table 4 Effect of freezing methods on quality of frozen mulberry after thawing

Freezing methods	Freezing time (min)	Qualities after thawing*	
		Drip loss (%)	Firmness (N)
Household freezing	380	$17.49^{b} \pm 3.42$	$26.12^{b} \pm 1.16$
Air-blast freezing	26	$14.57^{ab} \pm 1.83$	$51.40^{a} \pm 7.67$
Cryogenic freezing	0.47	$8.28^{a} \pm 1.41$	$35.00^{b} \pm 1.35$

*Means followed by different letters in the same column were significantly different ($P \leq 0.05$)

Table 5 Hedonic scores of frozen mulberry from fresh mulberry fruits and difference freezing methods

Sensory qualities*	Fresh mulberry fruits (control)	After thawing of treated fruits		
		Household freezing	Air−blast freezing	Cryogenic freezing
Appearance	$6.76^{a} \pm 1.99$	$5.56^{b} \pm 1.70$	$6.16^{b} \pm 1.49$	$6.00^{b} \pm 1.59$
Color	$6.84^{a} \pm 1.39$	$5.80^{b} \pm 1.75$	$5.14^{b} \pm 1.59$	$6.08^{b} \pm 1.55$
Odor	$6.42^{a} \pm 1.58$	$5.04^{b} \pm 1.44$	$5.70^{b} \pm 1.91$	$4.80^{b} \pm 1.50$
Flavor	$7.56^{a} \pm 1.23$	$5.34^{b} \pm 1.81$	$5.90^{b} \pm 1.85$	$4.84^{c} \pm 1.80$
Texture	$7.48^{a} \pm 1.30$	$5.16^{c} \pm 1.81$	$5.94^{b} \pm 1.58$	$5.48^{c} \pm 1.83$
Overall acceptance	$7.58^{a} \pm 1.14$	$5.32^{c} \pm 1.70$	$6.16^{b} \pm 1.49$	$5.52^{c} \pm 1.49$

*Means followed by different letters in the same row were significantly different ($P \leq 0.05$).

4. Conclusion

The pre-treatment method for ripe mulberry fruits was soaking in the solution at the ratio of 1:2 (w/w) for 10 min at room temperature. The soaking solution consisted of 2% (w/w) calcium chloride and 0.3% (w/w) calcium hypochlorite solution. This pre−treatment could provide high firmness value and safety microbial load of mulberry fruits for fresh consumption. Air-blast freezing at -25°C had provided lower drip loss and high firmness of thawed mulberry fruits than household freezing and cryogenic freezing. It took 26 min for freezing time of ripe mulberry fruits using air−blast freezing. After thawing of frozen mulberry fruits, mulberry fruits from air−blast freezing had a little bit lower sensory evaluation score than fresh ones. It was indicated that air−blast freezing had high potential to apply in mulberry fruits for fresh consumption. The lower temperature of air−blast freezing than -25°C should be further studied in order to shorten freezing time, drip loss reduction and firmness texture improvement.

Acknowledgements

This research was supported by The Queen Sirikit Department of Sericulture. The authors would like to express our deep gratitude to from Queen Sirikit Sericulture Centre, Chiang Mai, Thailand and Baan Suan Punya Farm for the raw material supply, and the Faculty of Agro−industry, Chiang Mai University, Thailand for providing the instruments and facilities.

References

Azevedo, L. A. J. C., Gomes, J. C., Stringheta, P. C., Gontijo, Á. M., Padovani, C. R., Ribeiro, L. R., and Salvadori, D. M. F. 2003. Black bean (*Phaseolus vulgaris* L.) as a protective agent against DNA damage in mice. Food and Chemical Toxicology. 41(12): 1671-1676.

Bacteriological Analytical Manual (BAM). 2001. US food and drug administration. Chapter 3, Aerobic Plate Count.

Cai, H., Scott, E., Kholghi, A., Andreadi, C., Rufini, A., Karmokar, A., Rufini, A., Karmokar. A,, Britton, RG., Horner−Glister, E., Greaves, P., Jawad, D., James, M., Howells, L., Ognibene, T., Malfatti, M., Goldring, C., Kitteringham, N., Walsh, J., Viskaduraki, M., West, K., Miller, A., Hemingway, D., Steward, WP., Gescher, AJ., Brown, K., and James, M. 2015. Cancer chemoprevention: Evidence of a nonlinear dose response for the protective effects of resveratrol in humans and mice. Science Translational Medicine. 7(298): 117.

Cao, X., Zhang, F., Zhao, D., Zhu, D., and Li, J. 2018. Effects of freezing conditions on quality changes in blueberries. Journal of the Science of Food and Agriculture. 98(12): 4673−4679.

Chen, F., Liu, H., Yang, H., Lai, S., Cheng, X., Xin, Y., and Bu, G. 2011. Quality attributes and cell wall properties of strawberries (*Fragaria annanassa* Duch) under calcium chloride treatment. Food Chemistry. 126(2): 450−459.

De Sá Coutinho, D., Pacheco, M. T., Frozza, R. L., and Bernardi, A. 2018. Anti−inflammatory effects of resveratrol: Mechanistic insights. International Journal of Molecular Sciences, 19(6): 1812.

Estrada−Flores, S. 2002. Novel cryogenic technologies for the freezing of food products. The Official Journal of Airah. 16: 21.

Han, Q., Gao, H., Chen, H., Fang, X., and Wu, W. 2017. Precooling and ozone treatments affects postharvest quality of black mulberry (*Morus nigra*) fruits. Food Chemistry. 221: 1947−1953.

Lee, J. H., Kang, N. S., Shin, S. O., Shin, S. H., Lim, S. G., Suh, D. Y., ... & Ha, T. J. 2009. Characterisation of anthocyanins in the black soybean (*Glycine max* L.) by HPLC-DAD-ESI/MS analysis. Food Chemistry, 112(1), 226-231.

Liang, D., Lin, F., Yang, G., Yue, X., Zhang, Q., Zhang, Z., and Chen, H. 2015. Advantages of immersion freezing for quality preservation of litchi fruit during frozen storage. LWT−Food Science and Technology. 60(2): 948−956.

National Bureau of Agricultural Commodity and Food Standards (ACFS). 2013. Code of practice for the manufacturing of frozen agricultural commodities.

Nopparat, T., & Wasusri, T. 2017. The logistics cost analysis for mulberry fruit supply chains in Nan province. Thai Agricultural Research Journal.

Pham, P. P., Morales, N. P., Pitaksuteepong, T., & Hemstapat, W. 2017. Antioxidant activity of mulberry stem extract: A potential used as supplement for oxidative stress-related diseases. Songklanakarin Journal of Science & Technology, 93(3).

Tan, B. L., Norhaizan, M. E., and Winnie−Pui−Pui Liew, H. S. 2018. Antioxidant and oxidative stress: A mutual interplay in age-related diseases. Frontiers in Pharmacology. 9: 1162.

Tangtua, J., Leksawasdi, N., & Rattanapanone, N. 2014. Quality Changes in Ripened Mango and Litchi Flesh After Cryogenic Freezing and During Storage. Chiang Mai University Journal of Natural Sciences, 13(3), 281-296.

Thanthanasupawat, Y. 2018. Effect of Microwave-Vacuum Drying on Drying Characteristics and Quality of Mulberry. Thai Society of Agricultural Engineering Journal, 24(2).

Vichasilp, C., Nakagawa, K., Sookwong, P., Higuchi, O., Luemunkong, S., and Miyazawa, T. 2012. Development of high 1-deoxynojirimycin (DNJ) content mulberry tea and use of response surface methodology to optimize tea−making conditions for highest DNJ extraction. LWT−Food Science and Technology. 45(2): 226−232.

Voloshyna, I., Hussaini, S. M., and Reiss, A. B. 2012. Resveratrol in cholesterol metabolism and atherosclerosis. Journal of Medicinal Food. 15(9): 763−773.

Wasan Nuipirom. 2003. Mulberry and Processing. Bangkok. Department of Agriculture Ministry of Agriculture and Cooperation.