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Comparison of Hurdle Treatment and Thermal Treatment on Ascorbic Acid and Total Phenolic Content of Winter Melon Puree Stored at Different Temperature

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Abstract. Winter Melon (WM) is an important fruit in China, India, Philippines and elsewhere in Asia and due to its perishable characteristic it is a necessity to process it into a puree form. Hence in this study, WM fruit was processed into puree and hurdle technology (HT) treatment was compared with traditional thermal treatment (TT) to see the effect on Ascorbic acid (AAC) and Total Phenolic (TPC) content stored at different temperatures for 6 months of storage. HT WMP was adjusted to pH 3 using citric acid while for TT WMP was prepared by heating at 84.5°C for 94 seconds. Both treated WMP were stored at 25°C, 5°C and -20°C and were analyzed at 1 months storage intervals till 6 months. AAC test was conducted using 2, 6-dichlorophenol-indophenol titration method and TPC was determine by using Folin-Ciocalteu. A gradual decrease in AAC and TPC were observed during storage for both treatments. These changes were more pronounced at 25°C storage for both treatments ($P < 0.05$), however, TT shows high reduction of AAC and TPC ($P < 0.05$) at 0 months throughout 6 months of storage as compared to HT. Overall, WMP for HT stored at 5°C and -20°C shows lowest reduction for AAC and TPC for the last 6 months of storage. By processing WM into puree, it will be more convenient to use especially by the food manufacturer. In contrast to traditional TT for common fruits puree preparation, HT could imply new opportunities for WMP in developing value-added product with reasonable shelf life.

Keywords: Hurdle technology, Thermal treatment, Winter melon puree, Total phenolic content, Ascorbic acid content

1. Introduction

Winter melon (WM) or its scientific name *Benincasa hispida* belongs to a family of *Curcubitaceae* and its origin is from South East Asia and also can be found in area outside Asia [1]. Some scientific studies carried out reveal the potential of WM as anti-inflammatory [2], anti-diarrheal [3], anti-ulcer [4], antioxidant [5] and antimicrobial agent [5]. In Malaysia, WM is popular fruit among Chinese and Indian communities and it has potential for the food industry. It is prized for its pleasant smell and juicy flesh. However, it softens rapidly during ripening and becomes mushy and difficult to consume in fresh. Moreover due to these reasons, it will be rejected by the consumers if these fruits are bruised. Therefore, there is a need to process WM into puree form. Winter melon puree (WMP) needs a considerable treatment before canning and before storage. Therefore in this study, two treatments were proposed which are thermal treatment (TT) and hurdle technology (HT). Thermal treatment (TT) is a combination of the temp-time profile to ensure microbial destruction and to stabilise the WMP by removing microorganisms and enzyme inactivation that could produce fermentation or spoilage. Study by Sanchez et al. (1990)[6]; Umme et al. (2001)[7] and Bartolome et al. (1995)[8] reported that fruit puree processed below 93°C, canned and stored frozen showed no much

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changes in texture and organoleptic properties. On the other hand, HT used a combination of milder preservation techniques to achieve product safety and stability and the concept of combining several factors have been developed by Leistner [9]. Ohlsson & Bergstsson, (2002)[10] reported that application of combined factors such as reduced water activity and high acidity in fruits jam increased the product quality and achieved microbial stability. Although recently there have been many studies that investigated the effect of combining hurdle technology in food laboratory, the interactions of many hurdles are still not clear. A better understanding of the interacting of different preservation hurdle in foods can form a logical basis for improvements in food preservation. This study has been designated to evaluate the effect of HT in comparison with traditional TT on TPC and AAC stored at different temperatures for 6 months.

2. Materials and Methods

2.1. Preparation of Winter Melon Puree

Winter melon was obtained from a local market in Selayang, Selangor, Malaysia. Sample was blended in a mechanical food processor (Panasonic, MK-5080M). Puree was packed in polyethylene plastics and sealed using impulse foot sealer (SINGAR SEGA CO.LTD) and stored at -21°C prior to use.

2.2. Thermal Treatment and Hurdle Technology

For TT, the WMP was heated at optimum condition of 84.5°C for 94 seconds [11]. In HT, sample puree (300g) was placed in a container and after then stabilizer, Fructose was added to WMP. pH of the purees were adjusted by using citric acid to pH 3.0 and incorporated with potassium sorbate.

2.3. Storage Test

TT and HT WMP were packed in cans and stored at 25°C, 5°C and -20°C for a period of 6 months. AOE and AOA analysis of WMP were evaluated at 0, 1, 2, 3, 4, 5 and 6 months. Triplicates samples from 3 batches at each of the three storage temperatures were randomly selected for each analysis.

2.4. Ascorbic Acid Content Analysis

Ascorbic acid content was determined using the 2,6-dichlorophenol-indophenol titration with meta-phosphoric acid (MPA) solution. Ascorbic acid standard was used to prepare a standard solution (1 mg/mL).

2.5. Total Phenolic Content Analysis

Total phenolic content was determined by Folin-Ciocalteau method, which was adapted from Singleton and Rossi (1965)[12]. 0.1 ml of an aliquot of the extract was added to 0.5 ml Folin-Ciocalteau reagent in a test tube and then mixed well using a vortex. The mixture was allowed to react for 3 minutes then 1.5 ml of 7.5 % w/v Na_2CO_3 and 7.9 ml distilled water was added and mixed well. The solution was incubated at room temperature (23°C) in the dark for 2 hours. After 2 hours incubation at room temperature, the absorbance was read at 765nm.

2.6. Statistical Analysis

All data was carried out in triplicates and expressed as mean ± standard deviation. Data were analysed using SAS (9.1) year 2008). P-values < 0.05 were considered statistically significant. Data were analysed as a factorial with seven storage times, one type of packaging material, three storage temperatures and two treatments (Thermal and Hurdle).

3. Results and Discussion

3.1. Ascorbic Acid Content (AAC)

The AAC of thermal treatment (TT) WMP showed significant low AAC as compared to the hurdle treatment (HT) WMP (Table 1). TT WMP stored at -20 ℃ started to decrease significantly ($P<0.05$) from the 1st month until 6th month of storage. Generally, at -20 ℃ and 5 ℃, no significant decrement in AAC was observed between 0th month until 4th month, however at 5th month of storage TT WMP showed slightly declined ($P<0.05$) in AAC. On the other hand, HT WMP, frozen storage showed no significant ($P>0.05$) decrease in AAC with time from 0th month until 2nd month but started to decrease significantly ($P<0.05$) at 3rd month until 6th month of storage. In contrast to TT, HT WMP showed a significant ($P<0.05$) AAC

between storage temperature (-20 ℃, 5 ℃ and 25 ℃) from the 1st until 6th month of storage. However, both processing (TT WMP and HT WMP) showed similarity at -20 ℃ storage where WMP stored at this temperature retained highest AAC compared to 5 ℃ and both treatments also revealed that the lowest AAC in WMP were encountered in samples stored at 25 ℃. Comparison between treatments (TT and HT WMP) on AAC significantly showed that AAC in HT WMP were much higher throughout 6th month of storage period. Even though treatment methods utilizing heat can irreversibly inactivate ascorbate oxidase, thus counteracting enzymatic decomposition, AA is not stable at high temperature [13] and this might resulted in AAC reduction in TT WMP. This is supported by study by Somsub *et al*., (2008)[14] who reported that conventional heat treatment led to excessive loss of AAC in most Thai vegetables and fruits. For HT WMP, additional citric acid to reduce the pH of puree helps to stabilize AA in WMP. Study done by Wang *et al*., (2007)[15] found that the organic acids and low pH in most fruits are critical for the preservation of derivatives products.

3.2. Total Phenolic Content (TPC)

The substances classified as phenolics contain a phenol group –a hydroxyl group (-OH) attached to an aromatic ring and although phenolic compounds represent secondary metabolites but due to its chemical diversity they play an important roles in plants [16]. Table 2 showed TPC for TT WMP and HT WMP stored at different temperature for 6th months of storage period. In TT WMP, storage time and storage temperature were significantly effect ($P<0.05$) the TPC in TT WMP during 6th months of storage. The ranking in descending order: frozen (-20 ℃)> chill (5 ℃)> room (25 ℃) and all samples stored at different temperatures gradually decreased significantly ($P<0.05$) from 0th month until 6th month of storage. In contrast, HT WMP at -20 ℃ and 5 ℃ showed slightly declined in TPC at 2nd month of storage until 5th month of storage. Both frozen and chill HT WMP showed no significant difference in TPC between the storage temperatures until the 4th month of storage. However, a gradual decrease of TPC was observed for HT WMP stored at 25 ℃ from 0th until 6th month of storage. As comparison between treatments, HT WMP showed highest TPC compared to TT WMP at different storage time and storage temperature. Study by Ranilla *et al*., (2010)[17] reported that heat treatment had a decomposition effect on phenolic compound. Furthermore, storage and processing also reduced the content of phenolic compounds as some of them are easily oxidized. In previous study on strawberry, corn and peaches revealed that these fruits showed no significant decreased in total phenolics stored at frozen (-12 ℃) for up to 6th month compared to fruits that were stored at ambient temperature. Study on white saffron by Pujimulyani *et al*., (2010)[18], showed an increment in TPC when the extracts contained 0.05% citric acid and this supported the high TPC in HT WMP compared to TT WM.

Table 1: Ascorbic acid content (mg AA/100g Fresh Weight) for thermal treatment and Hurdle technology of WMP during 6 months of storage

Storage Temperature (^{0}C)	Ascorbic Acid content (mg AA/100g Fresh Weight)													
	Storage time (months)													
	Thermal							Hurdle						
	0	1	2	3	4	5	6	0	1	2	3	4	5	6
-20	31.84 ±1.38A Da	28.79 ±0.64A FHb	28.55 ±0.59A Ga	26.73 ±1.05A Jb	22.19 ±1.65 AMc	18.51 ±1.44 APRc	17.29 ±0.72 AQd	35.36 ±2.78 ADa	31.43 ±0.21 ADa	30.20 ±1.22A DEFa	30.06 ±0.38 AEa	28.13 ±0.32 AFa	29.00 ±0.40 AFa	25.00 ±0.10 AIa
5	31.32 ±0.42A Da	28.58 ±0.90A Eb	27.60 ±0.72A Ic	25.64 ±1.64A lb	21.31 ±0.94 ANd	16.00 ±0.49 BRd	15.06 ±0.47 BSe	35.20 ±0.40 ADa	28.36 ±0.85 BFb	28.30 ±0.50B Fb	26.80 ±0.50 BGb	24.93 ±0.42 BIb	21.96 ±0.50 BJb	20.63 ±0.35 BLb
25	29.44 ±0.81B Eb	27.77 ±0.68A Hb	25.99 ±0.51B Kb	20.33 ±1.34B Od	17.15 ±0.82 BRe	13.59 ±0.81 CTe	10.95 ±1.12 CUf	34.60 ±3.90 ADa	25.76 ±0.15 CHc	26.03 ±0.25C Hd	23.66 ±0.76 CJc	23.16 ±0.78 CJc	20.33 ±0.68 CKc	19.26 ±0.25 CMc

[a] Values was carried out in triplicates and expressed as mean ± standard deviation

[b] A…C = means within a column (by temperature) with different letters are significantly different ($P<0.05$). a…f = means within a row (by processing) with different letters are significantly different ($P<0.05$). D…M = means within a row by month different letters are significantly different ($P<0.05$).

Table 2: Total Phenolic Content (mg GAE/g Fresh Weight) for thermal treatment and Hurdle technology of WMP during 6 months of storage

Storage Temperature (^{0}C)	Total Phenolic Content (mg GAE/g Fresh Weight)													
	Storage time (months)													
	Thermal							Hurdle						
	0	1	2	3	4	5	6	0	1	2	3	4	5	6
-20	21.13 ±0.03 ADb	19.63 ±0.10 AEc	18.61 ±0.08 AFb	17.55 ±0.05 AIc	16.16 ±0.10 AKd	14.65 ±0.05 AMd	13.08 ±0.06 APc	28.59 ±1.67 ADa	27.90 ±3.20 ADa	26.62 ±0.61 AEb	25.34 ±0.79 AEa	24.93 ±0.21 AEa	21.64 ±0.75 AEFa	20.30 ±0.92 Aga
5	21.08 ±0.16 ADb	19.10 ±0.10 BFd	18.18 ±0.08 BGc	17.03 ±0.08 BJd	15.46 ±0.37 BLe	14.06 ±0.08 BNe	12.31 ±0.23 BQd	28.51 ±1.29 ADa	27.69 ±8.01 ADa	24.90 ±1.61 AEa	24.64 ±1.34 AEa	23.41 ±1.11 BEb	20.31 ±0.12 BHb	19.51 ±0.51 AIa
25	20.65 ±0.30 ADb	18.85 ±0.56 BFd	17.68 ±0.10 CHd	16.36 ±0.33 CKe	14.56 ±0.08 CMf	13.41 ±0.23 COf	12.18 ±0.50 BQd	28.50 ±0.59 ADa	24.50 ±0.06 BEb	26.30 ±0.12 AEa	21.80 ±0.40 BFb	22.35 ±0.36 BEFc	18.78 ±0.31 CIc	15.55 ±0.12 BJb

[a] Values was carried out in triplicates and expressed as mean ± standard deviation

[b] A…C = means within a column (by temperature) with different letters are significantly different (P<0.05). a…f = means within a row (by processing) with different letters are significantly different (P<0.05). D…Q = means within a row by month different letters are significantly different (P<0.05).

4. Conclusion

By implying hurdle technology with combine preservation method for WMP, it is able to maintain functional properties of the purees. This study revealed that HT WMP able to retain the AAC and TPC throughout 6th month of storage period. The AAC was found to be relatively high at storage temperature -20 ℃ and TPC was found to be no significant decrease at -20 ℃ and 5 ℃ for HT WMP, thus, this study was found that HT to be the most suitable treatment to maintain the functional properties of WMP during 6th month of storage and commercial short term storage at temperature above -20 ℃ therefore also appeared to be quite feasible.

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