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Effect of storage on physicochemical properties and microbiological stability of osmodehydrated pineapple (*Ananas comosus)* treated with sucrose-sorbitol mixtures

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ABSTRACT

Osmotic dehydration (OD) is an effective fruits preservation technique with minimal nutrients loss. The study aimed to determine physicochemical properties and microbial stability of osmodehydrated pineapple. The experiment were conducted using 65 °Brix sucrose-sorbitol mixtures (100:0, 0:100 and 25:75) at 40 °C for 240 minutes OD and continued for hot air drying to 20% moisture content at 50 °C. Samples were vacuum-packed and stored at 4 °C. Textural, physicochemical properties (moisture content, aw), flavonoids, vitamin C and microbial stability were determined. Results showed that samples treated with sucrose-sorbitol mixtures (0:100 and 25:75) able to extend the shelf life of pineapple up to 35 days and 49 days at bacteria count ≤106 CFU/g and yeast and mould ≤104 CFU/g, respectively. While, sucrose (100:0) treated pineapples only able extend the shelf life up to 14 days. In conclusion, reducing water activity of sorbitol able to extend the shelf life of osmodehydrated pineapple.
Keywords: Osmotic dehydration, sorbitol, vitamin C, bromelain, shelf life

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INTRODUCTION

Pineapple (*Ananas comosus*) fruits are widely grown in Johor state of peninsular Malaysia and known as one of the largest producers in the world (MPIB, 2012c). Based on the nutritional values of pineapple from Malaysia Pineapple Industrial Board, pineapple is rich with vitamin C, vitamin B (B1 and B3), potassium, sodium and contain beneficial enzyme bromelain (Corzo, Waliszewski, & Welti-Chanes, 2012; MPIB, 2012a). Vitamin C content for pineapple is 47.8mg/100gram and it is known as a good source of vitamin C because the content of vitamin C is comparable to the vitamin C content in orange fruit, 48.5% in 100gram (U.S. Department of Agriculture, 2012a; U.S. Department of Agriculture, 2012b). According to Hajare *et al.* (2006), ripen pineapple have short and limited shelf life up to 4 - 6 days only. Therefore, there were several types of processed pineapple products that able to extend the shelf life of pineapple such as pineapple jam, candied pineapple and canned pineapple (MPIB, 2012b). Although these products have a longer shelf life, there are not preferred by certain consumers because of high sugar content and loss of nutritional values due to high thermal processing (Saxena, Mishra, Chander, & Sharma, 2009).

Osmotic dehydration (OD) is a process of removing water from water-containing solids by osmotic pressure in the osmotic solution (Rastogi & Raghavarao, 2004). Osmotic pressure is the driving force for OD and this driving force will cause the natural osmosis of water from the fruits into the osmotic solution through cell membrane (Rastogi, Raghavarao, & Niranjan, 2005). OD is becoming more popular in recent years as an effective method in preserving fruits due to the ability to preserve the natural characteristics of the fruits and produced superior quality of dried fruit products at low temperature treatment (Chavan & Amarowicz, 2012; Igual, Contreras, & Martínez-Navarrete, 2010; Lombard, Oliveira, Fito, & Andrés, 2008). Moreover, Rastogi *et al.* (2005) reported that OD combined with other drying methods able to produce high quality fruit products such as osmodehydrated pineapple, apple, guava, strawberry, grapefruits and kiwi (García-Martínez et al., 2002; Lombard et al., 2008; Nuñez-Mancilla, Pérez-Won, Uribe, Vega-Gálvez, & Di Scala, 2013; Panadés-Ambrosio, Treto-Cárdenas, Fernández-Torres, Castro, & M, 1996; Saputra, 2001; Silveira, Rahman, & Buckle, 1996). Besides, osmodehydrated pineapple are becoming more popular among consumers mainly due to the extended shelf life, retention of nutritional value and storage cost compared to fresh pineapple (Leistner, 1992; Thakur & Singh, 1995).

Enhancement of shelf life of pineapple fruits is very crucial for food industries because even though the shelf life could be extended for a few days, it will give a significant economic advantage for them (Sipahi, Castell-Perez, Moreira, Gomes, & Castillo, 2013).

Flavonoids are one of the polyphenols and mainly present as coloring pigments in plants also function as potent antioxidants at various levels (Hossain & Rahman, 2011; Islek, Nilufer-Erdil, & Knuthsen, 2014). Studies showed that flavonoids have powerful antioxidant properties in protecting membrane lipids from oxidation (Islek et al., 2014; Terao, Piskula, & Yao, 1994), several possible health benefits on anti-tumor properties and protect against coronary heart disease (Kesarkar, Bhandage, Deshmukh, Shevkar, & Abhyankar, 2009). Vitamin C is an essential nutrient in human diets and is the safest, most effective nutrient which able to help in preventing more serious complications for common cold and it is necessary in maintaining connective tissues and bones (NCBI, 2014; WebMD, 2014a). Bromelain is an enzyme that found in fresh pineapple mainly in juice and stems (WorldHealth, 2005). Bromelain have its beneficial functional properties which are used as anti-inflammatory agent, used for ulcerative colitis, removing dead tissue after burn, anti-cancer properties and so on (WebMD, 2014b).

The objective of this study are to determine physicochemical properties, microbial stability and kinetic of osmodehydrated pineapple during storage studies.

MATERIALS AND METHODS

Pineapple preparation

Queen cultivar pineapples were obtained from Kompleks Pasar Borong in Seri Kembangan, Selangor, Malaysia. The stalk and the top of pineapple fruits were cut off and wash thoroughly to remove any contaminants. The pineapples were peeled, cored and cut into small triangle shaped using Pineapple Multipeeler Plus (MY-151701-A) constructed at Food Process Engineering Laboratory, Faculty of Engineering, Universiti Putra Malaysia. The fruits were then blanched at 80°C for 45 seconds (Phisut, 2012) before OD process.

Osmotic solution formulation

Sucrose was purchased from local hypermarket while sorbitol was purchased from Euro Chemo Pharma Sdn Bhd as osmotic solution for OD process. The solutions used were at 65% (w/w) sucrose level and there are three different formulations. The formulations consist of 100% sucrose and partial substitution of sucrose with sorbitol at 75% and 100%.

Osmotic dehydration process

The OD method was as described by Lombard *et al.* (2008) with some modifications. All pineapple fruits were marked wrapped in a net and were immersed into osmotic solution. The OD was processed using Ezy Cooker (MY-142132) constructed at Food Process Engineering Laboratory, Faculty of Engineering, Universiti Putra Malaysia. These processes were conducted at 40°C and atmospheric pressure for 240 minutes. The ratio of osmotic solution to sample is 10:1 as recommended by (Khoyi & Hesari, 2007; Silva, Silva, Mariani, & Darche, 2012). After 240 minutes of OD process, samples for all three formulations were further dried to about 20% moisture content in cabinet dryer in Food Engineering Laboratory, Faculty Food Science and Technology, Universiti Putra Malaysia. Samples collected at the end of the process were vacuum packed in aluminized polyethylene vacuum bags and kept in the cold room at 4°C until further analysis for up to eleven weeks of storage studies.

Physicochemical properties

The samples were grinded using grinder prior to physicochemical analysis. The moisture content of the samples were measured using standard AOAC oven drying method AOAC (2000) and the water activity of the samples were measured using water activity meter (TE8309, AQUALAB, UK).

Textural properties analysis

The textures properties of samples of each formulations were analyzed using Texture Analyzer (TA-XR2i, Stable Micro Systems, UK) (Lombard *et al.*, 2008) with some parameters modification. The firmness of the samples were determined using puncture test method using 5 kg load cell at 20% strain, 1.5 mm/sec pretest speed, 1.5 mm/sec test speed, 10 mm/sec posttest speed and trigger force of 25 g. The probe used was P2 probe (2mm cylinder probe). The samples were loaded one at a time and the distance of penetration was 50% of the samples' height. Ten different samples were randomly selected and analyzed using texture analyzer for all osmotic dehydration treated pineapple.

Ascorbic acid analysis

Ascorbic acid analysis was carried out using standard AOAC method 967.21 for vitamin C (ascorbic acid) in vitamin preparations and juices with some modifications (AOAC, 1990). In determination of vitamin C, 10.0 g of pineapple were weighed and homogenized in 50 mL oxalic acid for 3 × 10 seconds. The homogenized samples were filtered through cotton wool with vacuum pump and washed with some oxalic acid. 10.0 mL of the filtrated solution were titrated with 2, 6-dichoroindophenol standard solution which was standardized with standard vitamin C solution. The titration volume was recorded and the amount of vitamin C content present in the samples was calculated in mg/100g sample.

Total Flavonoid content

Total flavonoid contents of OD pineapple were determined using aluminium colorimetric method as described by Islek *et al.* (2014) and Sulaiman, Sajak, Ooi, Supriatno, and Seow (2011), with some modifications. Samples were extracted using 50.0 mL 95% ethanol. Extract were filtered using vacuum pump. 2.5 mL of extract, 0.5 mL of 10% aluminium chloride, 0.5 mL of 1M sodium acetate solution and 1.5 mL of distilled water mixed well with vortex. Blank is the same mixture quantity but with pure ethanol. Mixtures were incubated for

30 minutes at room temperature (27 ± 1 °C) and absorbance of the samples were measured at wavelength 415nm using spectrophotometer (YO-02654-22, Thermo Scientific Genesys 10S UV-Vis, USA). The total flavonoid contents of samples were calculated as quecertin equivalent milligram per milliliter (mg QE/ml) using regression equation obtained from the calibration curve.

Bromelain activity

Bromelain activity in OD pineapple was determined by casein digestion unit (CDU) method using Hammersten grade casein as substrate in the presence of cysteine and EDTA (Dapeou, 1976; Kumar, Hemavathi, & Hebbar, 2011; Umesh Hebbar, Sumana, & Raghavarao, 2008). 5 mL casein substrate solution in 3 labelled screw cap test tubes was placed in 37 °C water bath for 10 minutes. At zero time, 1.0 mL of sample was added into each of the tubes, vortex and returned to the water bath immediately. The tubes were incubates in 37 °C water bath for exactly 10 minutes. 5mL protein precipitant (TCA) stock solution was added into the tubes after 10 minutes and continues to incubate in 37 °C water bath for an additional 30 minutes. The content of each tube was filtered twice through Whatman #1 filter paper and absorbance of clear filtrate was measured at 275 nm using spectrophotometer (YO-02654-22, Thermo Scientific Genesys 10S UV-Vis, USA). Bromelain activity was calculated using equation below.

$$\text{Activity (CDU/mL)} = ((E_t - E_b)/E_s) \times \text{Concentration of standard l-tyrosine} \times (V_r/t_r) \times D_f$$

Where E_t, E_b and E_s are absorbance of enzyme sample, enzyme blank and standard L-tyrosine, respectively. Df is the dilution factor, V_r is the reaction volume and t_r is the reaction time.

Microbiological analysis

Microbiological analysis for all samples were conducted based on total plate count (TPC), yeast and mould count and *Escherichia coli* with some modifications (ICMSF, 2002). OD pineapples (10g) were aseptically homogenized for 2 minutes in sterile stomacher bags with 90 ml peptone water using Stomacher Blender. Media used for analyses were tryptic soy agar (TSA) (Difco), potato dextrose agar (PDA) (Difco) and Eosin Methylene Blue (EMB) (Difco) agar. The analyses were conducted on weekly basis.

Kinetic of quality change

Quality changes of osmodehydrated pineapple with storage is expressed in terms of rate constant (k) and dependence of rate constant on storage duration (t), The changes of quality index A with time (dA/dt) is represented by the following kinetic equation:

$$dA/dt = \pm k (A)^n$$

Where k is the rate constant; n is the order of reaction; A is the concentration of quality characteristics.

For zero and first order rate, the kinetic equation on the quality changes with storage can be representing by the equations below,

$$A = \pm kt + A_o$$
$$\ln A = \pm kt + \ln A_o$$

Where A_o is the initial value at time 0; A is value at time, t, and k is the rate constant. The positive or negative sign indicates the quality characteristics can be either increases or decreases with storage (Maftoonazad and Ramaswamy 2008).

Statistical analysis

All analyses were conducted in triplicates and the data obtained were analyzed using analysis of variance (One-way ANOVA, Tukey’s Method) by Minitab (Version 16).

RESULTS AND DISCUSSION

Throughout the storage period of 11th week, the moisture content for 100:0 (% sucrose : % sorbitol) samples were ranging from 49.29% to 57.00%, for 0:100 (% sucrose : % sorbitol) were ranging from 37.24% to 44.69%, and for 25:75 (% sucrose : % sorbitol) the values were ranging from 43.06% to 49.57% as shown in Table 1. The moisture content of the samples increased gradually from the beginning of storage to the 11th week of storage. Moisture content for three of the formulations showed significant different ($p \leq 0.05$) by comparing the initial and the end of storage. Moreover, samples for 100:0 showed statistically different ($p \leq 0.05$) for the 0:100 and 25:75 samples.

Table 1: Effect of different osmotic solution formulations (100:0, 0:100 and 25:75) and duration of storage on moisture content of osmodehydrated pineapple.

Week of Storage	Formulations (% Sucrose:% Sorbitol)		
	100 : 0	**0 : 100**	**25 : 75**
0	50.61 $^{b, x}$ ± 1.47	37.24 $^{b, y}$ ± 4.62	43.06 $^{b, y}$ ± 6.43
1st	49.29 $^{b, x}$ ± 2.08	43.09 $^{ab, y}$ ± 4.64	45.98 $^{ab, xy}$ ± 0.37
3rd	53.76 $^{ab, x}$ ± 2.81	43.72 $^{ab, y}$ ± 4.46	46.07 $^{ab, y}$ ± 0.32
5th	53.71 ab, ^{x} ± 0.19	43.50 $^{ab, z}$ ± 1.63	48.44 $^{a, y}$ ± 1.21
7th	56.22 $^{a, x}$ ± 4.29	43.99 $^{ab, y}$ ± 3.48	47.88 $^{a, y}$ ± 1.95
9th	56.26 $^{a, x}$ ± 3.04	44.00 $^{ab, z}$ ± 3.92	49.57 $^{a, y}$ ± 0.28
11th	57.00 $^{a, x}$ ± 4.47	44.69 $^{a, z}$ ± 2.62	49.43 $^{a, y}$ ± 0.40

***** mean values which do not share the same superscript letter are significantly different at 5%), where a and b represent the comparison for different storage duration while x, y and z represent the comparison among different formulations

From the results, it showed that the moisture content for 100:0 samples increased after 2 weeks of storage and samples for 100:0 and 25:75 increased after 1st week of storage and remained almost constant until the end of storage. These results come in an agreement with the research finding of Saxena *et al.* (2009) intermediate moisture pineapple slices. The increased of moisture content at the beginning state of storage most probably because of the improper drying of pineapple cubes as reported in Saxena *et al.*, (2009) are due to the thickness of pineapple cubes which is greater than 1 cm will caused uneven drying of pineapple. However, the moisture content remained almost constant and showed no significant different after 1st or 2nd week of storage because the aluminized polyethylene vacuum bags is known as a good moisture barrier properties (Basantia, Arora, Seth, & Singh, 2000).

Table 2: Effect of different osmotic solution formulations (100:0, 0:100 and 25:75) and duration of storage on water activity (aw) of osmodehydrated pineapple.

Week of Storage	Formulations (% Sucrose:% Sorbitol)		
	100 : 0	**0 : 100**	**25 : 75**
0	0.92 $^{cd, x}$ ± 0.01	0.86 $^{a, y}$ ± 0.02	0.87 $^{a, y}$ ± 0.02
1st	0.92 $^{bcd, x}$ ± 0.01	0.87 $^{a, y}$ ± 0.02	0.89 $^{a, y}$ ± 0.01
3rd	0.92 $^{d, x}$ ± 0.01	0.86 $^{a, y}$ ± 0.02	0.88 $^{a, y}$ ± 0.02
5th	0.93 $^{abc, x}$ ± 0.02	0.88 $^{a, y}$ ± 0.01	0.90 $^{a, z}$ ± 0.01
7th	0.94 $^{a, x}$ ± 0.01	0.88 $^{a, y}$ ± 0.02	0.89 $^{a, y}$ ± 0.01
9th	0.93 $^{abcd, x}$ ± 0.01	0.88 $^{a, y}$ ± 0.02	0.89 $^{a, y}$ ± 0.004
11th	0.94 $^{ab, x}$ ± 0.01	0.87 $^{a, y}$ ± 0.02	0.89 $^{a, y}$ ± 0.01

* mean values which do not share the same superscript letter are significantly different at 5%), where a, b, c and d represent the comparison for different storage duration while x and y represent the comparison among different formulations

The changes of a_w for the storage period from 1st week to 11th week were shown in Table 2. The a_w for 100:0 samples have higher a_w values of about 0.93 as compared to the other two formulations which was statistically different from 0:100 and 25:75 samples at about 0.87 and 0.89,respectively. For formulations treated with sorbitol and mixture of sucrose-sorbitol, both of them having no significant difference ($p>0.05$) throughout the storage period. Water activity is important in food industry as a determinant for the stability of food products and microorganism growth (Maltini *et al.*, 2003). According to Barbosa-Cánovas *et al.* (2003), the intermediate moisture food (IMF) having water activity ranging from 0.65-0.90. Samples of 0:100 and 25:75 having a lower water activity which are theoretically microbial safe. The water activity for 100:0 samples have a higher water activity which is correlate to the higher moisture content of samples compared to other samples. This may lead to the deterioration of samples as microorganisms will grow at this range of water activity (Barbosa-Cánovas *et al.*, 2003). There were several indicators to determine the shelf life of fruit products such as the moisture content, water activity and microbial load on the fruits itself. Water activity of the fruits will affect the microbial load in the fruits as reported by Maltini, Torreggiani, Venir, & Bertolo (2003). This showed that the measurement of water activity of fruits was important as it is a determinant for stability of food products and microbial loads.

Table 3: Effect of different osmotic solution formulations (100:0, 0:100 and 25:75) and duration of storage on firmness of osmodehydrated pineapple.

Week of Storage	Formulations (% Sucrose:% Sorbitol)		
	100 : 0	0 : 100	25 : 75
0	68.84 $^{a, x}$ ± 19.66	72.79 $^{a, x}$ ± 9.01	70.91 $^{a, x}$ ± 14.02
1st	66.88 $^{a, x}$ ± 9.09	69.14 $^{ab, x}$ ± 11.78	69.78 $^{a, x}$ ± 15.42
3rd	65.90 $^{ab, x}$ ± 16.15	69.57 $^{a, x}$ ± 14.09	68.97 $^{a, x}$ ± 13.27
5th	57.03 $^{ab, x}$ ± 12.82	60.36 $^{abc, x}$ ± 14.68	59.12 $^{ab, x}$ ± 15.23
7th	53.22 $^{ab, x}$ ± 12.02	54.52 $^{bc, x}$ ± 14.25	58.56 $^{ab, x}$ ± 15.66
9th	53.85 $^{ab, x}$ ± 18.64	59.90 $^{abc, x}$ ± 13.84	56.19 $^{ab, x}$ ± 14.77
11th	50.65 $^{b, x}$ ± 12.10	50.02 $^{c, x}$ ± 12.11	52.96 $^{b, x}$ ± 11.88

* mean values which do not share the same superscript letter are significantly different at 5%), where a, b and c represent the comparison for different storage duration while x represent the comparison among different formulations

Besides, sensory attributes for food products is also an important aspect to be taken care by food industries. Sensory attributes are including physical appearance such as the texture properties of the products (Osorio *et al.*, 2007). Myung, Hamilton-Kemp Tr Fau - Archbold, and Archbold (2006) reported that firmness of food products could be reduced after cutting process and during storage of fruits. This is mainly due to the disruption of tissues and enzyme degradation by the fruits itself. Firmness of samples throughout the storage period was reported in Table 3. The results showed that the firmness of the samples decreased with time increased. There were significant ($p \leq 0.05$) decreased of firmness by comparing the samples at the end of storage to the beginning of storage. The decreased of firmness through time mainly due to hydrolytic enzymes released during cutting process that caused disruption of tissues and breakdown of membrane. Enzymatic degradation of cellulose, hemicellulose and pectin during storage led to the loss of firmness (Myung *et al.*, 2006). However, there were no significant different ($p > 0.05$) among samples for three formulations which showed that the different percentage of glucose and sorbitol used have no significant effect on the firmness of samples. From the results, it showed that firmness of pineapple treated with sorbitol reduced greater ($p \leq 0.05$) compared to the other two samples through storage. It is because that sucrose with greater molecular size will tend to form sugar crystal on the surface that contribute to the firmness of the pineapple (Naknean *et al.* 2013), while sorbitol may soften the firmness as sorbitol act as humectant that will bind water to its water molecules, thus reduced the water activity while samples will remained moist. Ronda, Gómez, Blanco, and Caballero (2005) reported that sorbitol could reduce the hardness of sponge cake which the same phenomenon can be seen when the application of sorbitol on pineapples.

Table 4: Effect of different osmotic solution formulations (100:0, 0:100 and 25:75) and duration of storage on vitamin C of osmodehydrated pineapple.

Week of Storage	Formulations (% Sucrose:% Sorbitol)		
	100 : 0	0 : 100	25 : 75
0	11.67 $^{b, x}$ ± 0.09	20.00 $^{a, y}$ ± 9.18	15.75 $^{a, xy}$ ± 1.93
1st	14.53 $^{a, x}$ ± 3.66	16.30 $^{a, x}$ ± 1.58	13.84 $^{a, x}$ ± 0.78
3rd	6.29 $^{c, x}$ ± 0.30	6.56 $^{b, x}$ ± 0.71	6.05 $^{b, x}$ ± 2.61
5th	5.11 $^{c, x}$ ± 0.24	5.44 $^{b, x}$ ± 1.37	5.62 $^{b, x}$ ± 0.06
7th	2.19 $^{d, z}$ ± 0.74	6.08 $^{b, x}$ ± 1.74	4.27 $^{bc, y}$ ± 0.06
9th	2.01 $^{d, x}$ ± 0.29	1.74 $^{b, x}$ ± 0.42	2.25 $^{cd, x}$ ± 0.92
11th	1.41 $^{d, x}$ ± 0.28	1.21 $^{b, xy}$ ± 0.36	0.94 $^{d, y}$ ± 0.19

* mean values which do not share the same superscript letter are significantly different at 5%), where a, b, c and d represent the comparison for different storage duration while x, y and z represent the comparison among different formulations

Vitamin C content of samples showed significant loss throughout storage period. From Table 4, vitamin C content of 100:0 samples reduced from 11.67 ± 0.09 mg/100g to 1.41 ± 0.28 mg/100g. While, 0:100 and 25:75 were reduced from 20.00 ± 9.18 mg/100g to 1.21 ± 0.36 mg/100g and 15.75 ± 1.93 mg/100g to 0.94 ± 0.19 mg/100g respectively. There were significant ($p \leq 0.05$) reduced from the initial to week 11th storage. The final vitamin C content is 1.41 ± 0.28 mg/100g for 100:0 samples which was higher compare to the other two samples and the lowest is 25:75 samples. Although there were significant ($p \leq 0.05$) differences between 100:0 and 25:75, these results showed vitamin C cannot be retained much throughout the storage even though different osmotic solutions used. This finding was comparable with the findings by Bierhals, Chiumarelli, and Hubinger (2011) and Mantilla, Castell-Perez, Gomes, and Moreira (2013) in which vitamin C content of pineapple reduced significantly during storage.

Table 5: Effect of different osmotic solution formulations (100:0, 0:100 and 25:75) and duration of storage on total flavonoid content of osmodehydrated pineapple.

Week of Storage	Formulations (%Sucrose:% Sorbitol)		
	100 : 0	0 : 100	25 : 75
0	$0.022^{a,x} \pm 0.002$	$0.017^{a,z} \pm 0.003$	$0.019^{a,y} \pm 0.001$
1st	$0.020^{ab,x} \pm 0.003$	$0.012^{bc,y} \pm 0.002$	$0.012^{c,y} \pm 0.002$
3rd	$0.020^{ab,x} \pm 0.002$	$0.013^{bc,y} \pm 0.004$	$0.012^{c,y} \pm 0.002$
5th	$0.019^{ab,x} \pm 0.002$	$0.013^{bc,y} \pm 0.002$	$0.013^{c,y} \pm 0.002$
7th	$0.018^{b,x} \pm 0.001$	$0.015^{ab,z} \pm 0.001$	$0.016^{b,y} \pm 0.001$
9th	$0.016^{c,x} \pm 0.002$	$0.011^{c,y} \pm 0.002$	$0.012^{c,y} \pm 0.001$
11th	$0.009^{d,x} \pm 0.004$	$0.007^{d,xy} \pm 0.003$	$0.005^{d,y} \pm 0.001$

* mean values which do not share the same superscript letter are significantly different at 5%), where a, b, c and d represent the comparison for different storage duration while x, y and z represent the comparison among different formulations

The changes of total flavonoid content through storage period were reported in Table 5. The results showed that the total flavonoid content had a decreasing trend with time. The total flavonoid content for all three formulations showed significant ($p \leq 0.05$) reduced through storage. There was 59.09% reduction of flavonoid content from 0.022 ± 0.002 mg QE/100g to 0.009 ± 0.004 mg QE/100g for 100:0 samples. While, for 0:100 samples, there was reduction of 58.82% flavonoid content from 0.017 ± 0.003 mg QE/100g to 0.007 ± 0.003 mg QE/100g and from 0.019 ± 1.926 mg QE/100g to 0.005 ± 0.190 mg QE/100g flavonoid content for 25:75 samples, which is reduction about 73.38%. The degradation of total flavonoid content in osmotic dehydrated pineapple may due to the enzymatic activities by the naturally synthesized enzymes like poliphenoloxidase (PPO) and peroxidase (POD) by fruits themselves (Baltacio, Velio, & Karacabey, 2011). PPO and POD were reported as enzyme that responsible for degradation of phenolic compounds in plants (Tomás-Barberán & Espín, 2001).

Table 6: Effect of different osmotic solution formulations (100:0, 0:100 and 25:75) and duration of storage on bromelain activity of osmodehydrated pineapple.

Week of Storage	Formulations (% Sucrose:% Sorbitol)		
	100 : 0	0 : 100	25 : 75
0	$2484.6^{a,x} \pm 232.4$	$2633.1^{a,x} \pm 115.0$	$2040.0^{bc,x} \pm 89.2$
1st	$2196.3^{b,x} \pm 68.5$	$1991.0^{b,xy} \pm 99.8$	$2327.9^{a,x} \pm 85.2$
3rd	$1463.9^{c,y} \pm 330.7$	$1243.5^{d,y} \pm 94.5$	$2206.9^{ab,x} \pm 123.2$
5th	$707.2^{d,z} \pm 64.1$	$1591.8^{c,y} \pm 43.3$	$2230.7^{a,x} \pm 171.6$
7th	$584.8^{d,x} \pm 34.2$	$1470.8^{cd,x} \pm 380.6$	$1981.8^{c,x} \pm 110.1$
9th	$537.2^{de,x} \pm 41.2$	$879.5^{e,x} \pm 253.3$	$1539.1^{d,x} \pm 59.1$
11th	$278.2^{e,y} \pm 27.5$	$699.4^{e,x} \pm 98.4$	$699.4^{e,x} \pm 46.5$

* mean values which do not share the same superscript letter are significantly different at 5%), where a, b, c, d and e represent the comparison for different storage duration while x, y and z represent the comparison among different formulations

Bromelain content for all the formulations were showed in Table 6. The initial Bromelain content for 100:0, 0:100 and 25:75 were 2484.6 ± 232.4 CDU/ml, 2633.1 ± 115.0 CDU/ml and 2040.0 ± 89.2 CDU/ml respectively. While, the Bromelain content at the end of storage were 278.2 ± 27.5 CDU/ml, 699.4 ± 98.4 CDU/ml and 699.4 ± 46.5 CDU/ml. These showed significant ($p \leq 0.05$) reduction of Bromelain content during storage. Besides that, both 0:100 and 25:75 samples have a higher Bromelain content ($p \leq 0.05$) compared to samples treated with 100 % glucose as osmotic solution. The decreasing trend of Bromelain content in three of the samples come with an agreement with the research done by Truc, Thanh, and Muoi (2011) which showed that the Bromelain activity decreased with time for cool storage at 4°C.

For microbial stability, TPC was conducted on the samples on a weekly basis. Fig. 1 showed that the microbial load for 0:100 and 25:75 samples increased with storage time, while, microbial loads for 100:0 was maintained between 4 to 7 log CFU g^{-1} from the beginning until the end of storage. The microbial loads for 100:0 samples were ranging from 4.33 to 6.26 log CFU g^{-1}. For 0:100 and 25:75, both formulations were ranging from 1.85 to 6.55 log CFU g^{-1} and 1.85 to 5.31 log CFU g^{-1} respectively. There were significant ($p \leq 0.05$) increased in microbial loads for samples treated with 100 % sorbitol osmotic solution. However, there were no coliform detected during the storage studies.

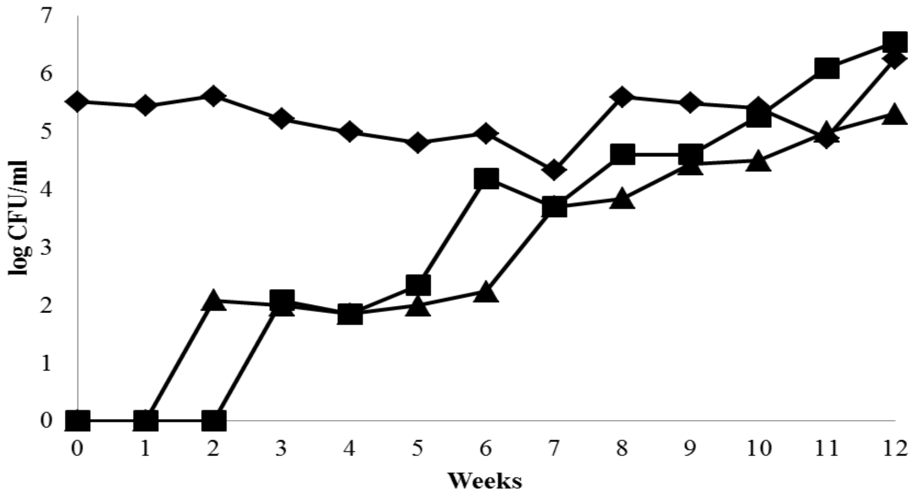

Fig. 1. Changes of total plate count (TPC) of 100:0 (♦), 25:75 (▲) and 0:100 (■) (%Sucrose:%Sorbitol) for 11th week of storage.

According to Food Standard Australia New Zealand (2001), the satisfactory and marginal microbial load for intermediate moisture products was $\leq 10^6$ CFU g^{-1} and $\leq 10^7$ CFU g^{-1}. By comparing the TPC results to the standards stated, 100:0 samples were satisfactory from the first week until 11th week but the microbial load at the end of storage exceed the satisfactory limit. While, for 0:100 samples, the microbial load exceed the satisfactory limits on 11th week. However, sample treated with mixture of sorbitol and sucrose (25:75), having microbial load at the satisfactory limit throughout the storage studies.

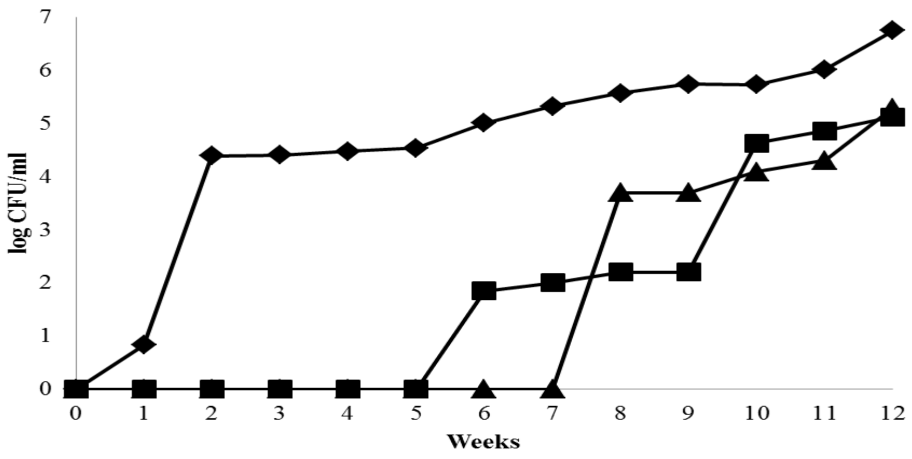

Fig. 2. Changes of yeast and mould of 100:0 (♦), 25:75 (▲) and 0:100 (■) (%Sucrose : %Sorbitol) for 11th week of storage.

Other than TPC, detection of yeast and mould were also conducted on the samples. Fig. 2 reported that yeast and mould loads for all three formulations increased with time. From the figure, it showed that 100:0 samples started to show growth of yeast and mould starting from the second week of storage. On the contrary, growth of yeast and mould started to be detected on week 6 and week 8 of storage for 0:100 and 25:75 samples respectively. There were significant ($p \leq 0.05$) increased of yeast and mould loads in both 100:0 and 25:75 samples by comparing between the initial and the end of storage. Yeast and mould loads for 100:0 were ranging from 0.83 to 6.75 log CFU g^{-1}. For 0:100, samples' loads were ranging from 1.85 to 5.12 log CFU g^{-1} and for 25:75 were ranging from 3.70 to 5.29 log CFU g^{-1}.

The maximum tolerance limit for yeast and mold were ≤ 10^4 CFU g^{-1} (Woolworths Quality Assurance, 2012). The results showed that 0:100 and 25:75 treated with sorbitol have an extended shelf life up to five weeks and seven weeks of storage respectively. While, samples treated with only sucrose, the extended shelf life only up to two weeks of storage. These results were due to the higher water activity of 100:0 samples which had been reported in Table 2. While, for samples treated with sorbitol with lower water activity have a longer extended shelf life. This is mainly due to the characteristics of sorbitol which act as humectant that could reduce water activity in the product by forming of hydrogen bonding with water that lead to the increment of bound water in the samples (Naknean *et al.*, 2013).

Kinetic changes of quality during storage for vitamin C content and bromelain activity were characterized using zero and first-order rate model. Table 7 summarized the changes of vitamin C content and bromelain activity which was modeled based on first-order rate model. For vitamin C for all three formulations best fitted to the first-order rate model at R squared values ranging from 0.8577-0.9549. These modeling showed a comparable results as reported by Vikram, Ramesh & Prapulla (2005) and Chakraborty, Rao and Mishra (2016), showing that vitamin losses are generally considered to follow the first-order kinetic model. The degradation of bromelain during the storage of osmodehydrated pineapple were modeled with best fitted to first-order rate model at R squared 0.9632 for sample treated with 100% sucrose and R squared of 0.8302 for sample treated with 100% sorbitol. While, for the mixture of 25 : 75 (% sucrose : % sorbitol), the best fitted model is also first-order rate model at R squared 0.6021.

Table 7: Kinetic parameters for changes in vitamin C content and bromelain activity during storage duration.

Formulation (% Sucrose: % Sorbitol)	Parameter	First-order model	
		k	***R^2***
100 : 0	Vitamin C content	0.8577	0.8577
	Bromelain activity	-0.1943	0.9632
25 : 75	Vitamin C content	0.9549	0.9549
	Bromelain activity	-0.0807	0.6021
0 : 100	Vitamin C content	0.9212	0.9212
	Bromelain activity	-0.1015	0.8302

CONCLUSIONS

Osmodehydrated pineapples undergo further drying and cool storage at 4 ºC were found to be effective to extend the shelf life of pineapple up to 14 days, 35 days and 49 days for 100 : 0, 25 : 75 and 0 : 100 (% sucrose :% sorbitol), which were treated with sorbitol as osmotic solutions.. All three formulations showed a good microbial stability throughout the extended storage which the total plate count for bacteria, yeast and mould were within under the limits of bacteria at less than 10^6 CFU/g and yeast and mould at less than 10^4 CFU/g. However, through storage studies, the results shows that all three formulations with different osmotic solution having insignificant effect on the retention of vitamin C, total flavonoid content and bromelain activity in which all these three analyses shows significant degradation during storage. Besides, the firmness of all three formulations also shows decreasing trend through storage. First-order rate reaction was best fitted for both vitamin C and bromelain activity which the kinetic changes of these quality attributes can be predicted. The specific objective of this research was fulfilled because results shows that sorbitol treated osmodehydrated pineapple have a better extended shelf life although the retention of vitamin C, total flavonoid content and bromelain activity did not shows significant effect.

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