

Effect of Vacuum Impregnation and High Pressure in
Osmotic Dehydration and Air Drying on Physicochemical
Properties of Mango (*Mangifera indica* L.) Cubes –
Maturity Stage 1

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Abstract

Conventional drying method can decrease the quality of dried fruit products due to the heat sensitivity of the nutrients. Osmotic dehydration (OD) as a food preservation method, which has better retention of colour, flavour and nutrition, is getting more attention. Different pre-treatments can be applied before OD to increase mass transfer rate and may improve the overall quality of product. This study was conducted to determine the effect of different pre-treatments (Vacuum impregnation and High pressure processing) prior to OD on physicochemical properties of mango. The effect of pectin methylesterase (PME) addition in the osmotic solution is also investigated. Mango cubes (cv. Kent) at maturity stage 1 were osmotically dehydrated at 50°C in 60° Brix sucrose solution with 2% Calcium lactate and with or without 0.48% PME. Three treatment times were conducted, which are 0.5h, 2h and 4h. The physicochemical properties of mango were characterised by analysing water loss, soluble solid gain, OD performance index, water activity, colour (L^* , a^* , b^*) and texture attributes of mango cubes. Samples without pre-treatments showed the highest water loss (14%, 32.7%, 48% at 0.5h, 2h, 4h, respectively) and lowest soluble solid gain (6.5%, 11.3%, 11.8% at 0.5h, 2h, 4h, respectively) ($P \leq 0.05$), given an overall highest OD performance index, followed by sample applied with high pressure and vacuum ($P \leq 0.05$). Application of pre-treatments (VI and HPP) resulted in higher soluble solid gain and lower water loss. Vacuum treated sample showed higher rate of solid gain (26.4%) after 4h treatment compared to high pressure treated sample (18.75%) and sample without pre-treatment (11.8%), indicating a higher degree of structural change in mango tissue by vacuum. The application of vacuum caused the most decreased (31%) in sample lightness ($P \leq 0.05$), while applying high pressure can lead to better retention of colour in both L^* and b^* values of mango. Water activity of mango did not show significant difference by applying different pre-treatments ($P > 0.05$), a 2% decrease in average was observed after 4h treatment. Textural analysis of mango did not give clear results due to large variance, work of shear and adhesiveness was reduced by OD treatments. Subsequent hot air drying led to the reduction in water content and firmness but higher adhesiveness. Osmotically dehydrated samples required longer hot air drying time (11h to 13h) than fresh samples (6h) despite their lower water content. The effect of PME addition was not significant in most measured parameters, and no distinctive pattern was observed.

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1. Introduction

Mango (*Mangifera indica* L.) is the second most important tropical fruit, widely consumed worldwide and especially in Asian countries (Singh & Singh, 2012). Mangos have high nutritional value, good sources of vitamin A and vitamin C. Tommy Atkins, Kent, Keitt and Haden are the most popular varieties of mango (Medina & Garcia, 2002). Since mango is an easily perishable fruit with a short shelf life, drying can be used to provide microbiological stability and increase shelf life. However, due to the heat sensitivity of the nutrients, the quality of dried products decreases. Therefore, new preservation techniques such as osmotic dehydration, is getting more attention, since it can extend the shelf-life of fruit, meanwhile preserving its safety and quality (Chandra & Kumari, 2015).

Osmotic dehydration (OD) is a process that removes water from lower concentration of solute to higher concentration by immersing the product in concentrated aqueous solutions (Van Buggenhout *et al*, 2008; Yadav & Singh, 2014). The interest on the application of OD in the preservation of food is increasing due to it lower the water activity of fruit, low temperature and energy requirement, and better colour, nutrition and flavour compound retention value (Torres *et al*, 2006; Yadav & Singh, 2014). In this research, the texture-enhancing substances, Pectinmethylesterase (PME) and Ca^{2+} are added into the osmotic solution as both substances can promote the bond forming between Ca^{2+} and pectin in plants, thus modify its structural response. The fortification of fruit using Ca combined with OD can improve the mechanical properties of fruit tissue (Van Buggenhout *et al*, 2008; Torres *et al*, 2006).

Since the reduction of water activity by OD is not sufficient enough to obstruct the growth of microorganisms, the application of high pressure processing (HPP) as a pre-treatment before OD is used to enhance the drying rates and improve the overall quality of processed products (Perez-Won *et al*, 2016). According to Torres *et al*, applying vacuum for a short time before OD can have a beneficial effect on process kinetics and quality of product, the process is called vacuum impregnation (VI). A subsequent treatment like hot air drying should be applied as well for food safety and better preservation (Van Buggenhout *et al*, 2008).

The maturity stage of mango is classified into 5 stages, the changes associated with the ripening of mango including colour, textural and compositional changes (Brecht, 2013). A research on the effect of different pre-treatments of mango dehydration on the physiochemical quality of mango has been studied, which targeted on mango variety Kent, maturity stage 4 (Alarcón, 2016). Since the maturity stage of mango has a great influence on the properties of fresh mango, it is assumed that the quality and properties of osmotic dehydrated mango will also be affected by different maturity stage. Therefore, to investigate this assumption and gather a better understanding of the quality of osmotic dehydrated mango under different processing condition as well as maturity stage, this research will focus on the same variety of mango but in maturity stage 1.

2. Background information

For this research, Vacuum Impregnation (VI) and High Pressure Processing (HPP) are considered as pre-treatments prior to drying methods which are Osmotic Dehydration (OD) and Hot Air Drying (HAD).

2.1 Kent mango and maturity stage

Kent mango, as one of many varieties of mango, is originating from Florida, US. It has a sweet and rich flavour, juicy and tender flesh with limited fibres, which is ideal for juicing and drying (NationalMangoBoard, 2016).

The increase of mango maturity stage has an influence on the skin and flesh colour, decrease in firmness and increase in soluble solid content due to the conversion of starch into sugar. These indicators mentioned also vary along with mango variety. Table 1 shows the firmness and soluble solid content (°Brix) in different maturity stages of Kent mango (Brecht, 2013).

Table 1 Firmness and °Brix in different maturity stages of Kent mango

Maturity	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Firmness (lbs)	19-22	14-18	11-13	5-8	2-4
°Brix	8-10	9-11	12-13	12-14	14-15

2.2 Pre-treatments

The pre-treatments are applied during OD in order to enhance the drying rate and improve the overall quality of final product.

2.2.1 Vacuum Impregnation (VI)

Vacuum Impregnation (VI) is the application of low pressure to a solid-liquid system, and then followed by the restoring to atmospheric pressure. The VI of a product involves the exchange of internal gas through open pores of the sample for an external liquid phase (Moreno *et al*, 2012; Torres *et al.*, 2006). A phenomenon called hydrodynamic mechanism. After vacuum period, a relaxation period is applied by keeping immersing the product in atmospheric pressure. Both periods affect the reaching of an equilibrium state of the food structure (Derossi *et al.*, 2012). The application of VI before OD can improve mass transfer kinetics, increase the rate of water loss and solid gain, also leads to better retention of nutrition and sensory quality of products (Moreno *et al*, 2012) (Lin, Luo, & Chen, 2016)

2.2.2 High Pressure Processing (HPP)

The treatment of high pressure on the food product can inactivate enzymes and microorganisms (Igual *et al*, 2013), also enhance the drying rate and improve the overall quality of product before applying OD (Perez-Won *et al*, 2016). In addition, HPP does not destroy the nutritional and sensory components of food product due to the use of lower temperature than conventional thermal processing (Barba, Esteve, & Frigola, 2012).

2.3 Drying methods

Osmotic dehydration is selected as the drying method of mango in this research as it can obtain a higher overall quality of the final product than other common drying methods.

2.3.1 Osmotic Dehydration (OD)

The application of OD can remove water from lower concentration of solute to higher concentration by immersing the product in concentrated aqueous solutions (Yadav & Singh, 2014; Van Buggenhout *et al*, 2008), a schematic demonstration of OD is shown in figure 1. As a treatment before air drying, OD in sugar solution can be employed to fresh-cut fruits, with the impregnation of substances: PME and Calcium ion, in order to obtain the desired product (Nagai, *et al*, 2015). Since OD require low temperature and energy, also results in better appearance, colour, texture characteristics, nutrition and flavour compound retention value of the fruit (Yadav & Singh, 2014; Torres *et al*, 2006).

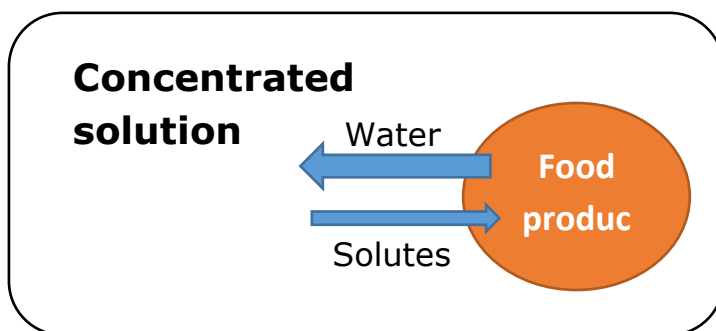


Figure 1 Schematic demonstration of osmotic dehydration process

However, the main disadvantage of OD is that it increases the sweetness of product and may reduce its characteristic taste. This problem can be avoided by controlling the factors that can affect the solute diffusion, such as time of OD treatment and pre-treatments. (Chandra & Kumari, 2015).

2.3.2 Hot air drying (HAD)

Fruit drying is the removal of water in different forms and different amount, it decreases the water activity of fruit (Barta, Balla, & Vatai, 2006). Apart from that, drying process also causes destruction of ascorbic acid and loss of the volatiles account for the flavour of fresh fruits (Shakuntala & Manay, 2001). Hot air drying is one of the convective and most effective way of drying fruits, the temperature and velocity of drying air as well as the thickness of the mango slices can have a great effect on mango drying (Mercer, 2012). Drying step should be carried on until a 0.65 water activity is reached which corresponded with the microbiological stability of the fruit (Korbel *et al*, 2013).

2.4 Addition of Pectin methylesterase (PME) and Ca^{2+} in OD solution

Textures of fruits are related to the structural integrity of the cell wall which is mainly constituted by pectin. Processes such as dehydration cause irreversible damages on the tissues thus texture of food. The use of PME can overcome the negative effects on texture during processing. The demethylation of pectin in plants occurs with the presence of PME. After the addition of Ca^{2+} , the free carboxyl groups generated in pectin molecules crosslink with Ca^{2+} and leads to the formation of networks among pectin molecules. As a result, the pectin is stabilised and tissue firmness is increased (Kohli, Kalia, & Gupta, 2015). Several studies on the firming of fruit using PME have been done which support this statement (Degraeve, Saurel, & Contel, 2003) (Suutarinen *et al*, 2000).

3. Research Objectives and Questions

3.1 Research Objectives

- Determine the effect of PME in the presence of Calcium without a pre-treatment, in combination with VI, or in combination with HP on the physicochemical properties of mango (maturity stage 1) by OD and HAD
- Determine the effect of different pre-treatments (VI, HPP) prior to OD and HAD on physicochemical properties of mango and the degree of methylation of pectin

3.2 Research Questions

- What are the effects on physicochemical properties of mango cubes by adding PME in the presence of Calcium to the osmotic solution?
- What are the different effects on physicochemical properties of mango cubes by applying VI and HPP during OD?
- What is the influence of maturity stage on physicochemical properties of mango on the OD and HAD of mango cubes?

4. Materials and Methods

4.1 Materials

Mangos (Variety: Kent) were provided by Nature's Pride, maturity stage 1 of mangos were peeled then selected manually based on firmness according to Table 1 (National Mango Board, 2010). The firmness was measured twice on each side of mango flesh using an 8mm penetrometer ensure the ripeness of mango is consistent. Then the mango flesh was cut into cubes with a size of 1.2 x 1.2 x 1.2 cm. For each treatment, 150g of mango cubes were used, an additional 150g was needed for samples that treated by hot air drying after OD. PME was purchased from NovoShape by Novozymes A/S, Copenhagen, Denmark and stored at 5°C. Calcium L-lactate pentahydrate purchased from Fluka analytical (BCBN1455V) was used as the source of Ca^{2+} in this research.

4.2 Methodology

The experimental design including the number of replicate of each variable measured is shown in the flow diagram in figure 2. The experiment was performed in duplicate.

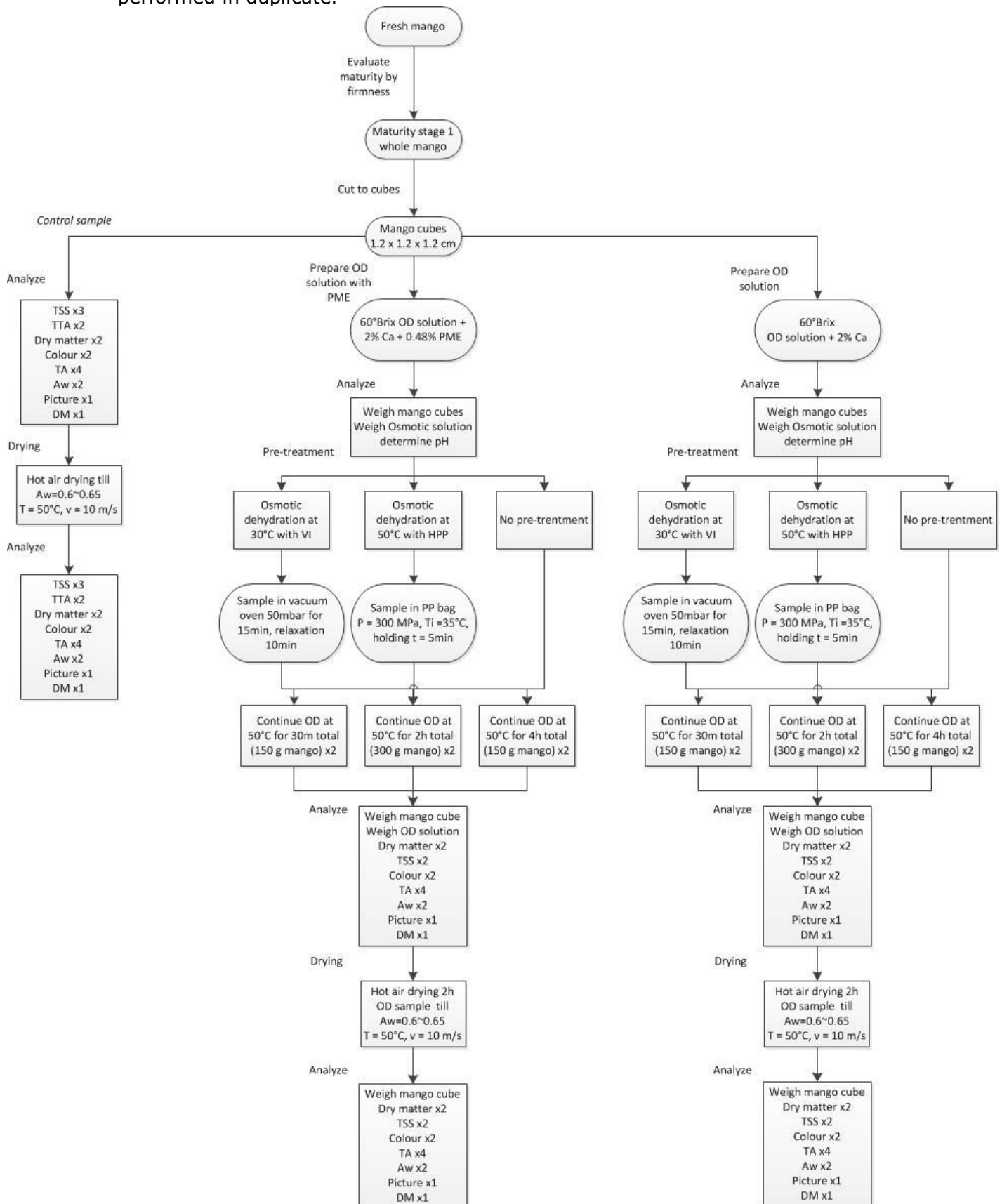


Figure 2 Flow diagram of experiment

4.2.1 Pre-treatments

4.2.1.1 Vacuum Impregnation (VI)

The pre-treatment of vacuum impregnation was performed using a vacuum oven, based on Laboratory Protocol 66 from Food Quality & Design Department, Wageningen University. The oven was pre-heated for approximately 30 minutes at 30 °C; the pump was set at 50 mbar. The beaker with mango cubes in OD solution was vacuumed for 15 minutes, including the 10 minutes to reach 50 mbar, followed by a relaxation time of 10 minutes with the restoration of atmospheric pressure. Despite the experimental design, the pump only reached 60mbar due to technical problems of the equipment. Therefore, the results obtained are treated at 60mbar. After VI, the samples were moved to continue OD on a hot plate at 50°C, which took approximately 30 minutes to reach the set temperature.

4.2.1.2 High Pressure Processing (HPP)

High pressure was applied on mango cubes in OD solution inside a polypropylene bag using High Pressure equipment in Food & Bio-based Research laboratory. Initial temperature was set at 35 °C and increases to 50 °C as this is the optimal temperature of PME (Ni, Lin, & Barrett, 2004). The working pressure was set at 300 MPa with 5 minutes holding time. The temperature increased along with the increase of pressure, which is approximately 3-5 °C per 100 MPa (Alarcón, 2016).

4.2.1.3 No Pre-treatment

Fresh mangos undergo OD without pre-treatment were obtained for the purpose of comparison of the results.

4.2.2 Drying methods

4.2.2.1 Osmotic Dehydration (OD)

Osmotic solution was prepared by adding 60% w/w sugar, 0.48% v/v PME and 2% w/w calcium lactate in demi-water. The solution was mixed well with a stirring bar at 50 °C. Then mango cubes were immersed into the OD solution in a 2L beaker, with a metal plate inside to keep them from floating.

The OD condition of mango cubes was following the settings by (Alarcón, 2016), for comparing the effect of different maturity stage on the same process condition.

Table 2 Osmotic dehydration condition and solution

	Description	Value
Fixed	Sample size	1.2 x 1.2 x 1.2 cm
	Solute solution	60 °Brix sucrose solution
	Ratio solution to fruit (w/w)	4:1
	Temperature	50 °C
	Calcium concentration (w/w)	2%
Variable	PME added (v/v)	0, 0.48%
	Pre-treatment	None, VI, HPP
	OD treatment time	0.5h, 2h, 4h

4.2.2.2 Hot air drying (HAD)

After OD, part of mango cubes was dried further using Quick Drying Machine TG200 by Retsch at 50 °C with 10m/s air velocity, samples were dried until the a_w reaches 0.6-0.65. The drying time differed depending on different pre-treatment and the addition of PME in the OD solution.

4.2.3 Analysis

4.2.3.1 Firmness of the whole mango

The firmness of mango is a key indicator of maturity of mango. It was measured twice or thrice on both side of mango flesh using a penetrometer with an 8mm tip.

4.2.3.2 Total Soluble Solids (TSS)

Total soluble solids were measured for mango cubes using a HANNA refractometer with juice pressed from mango cubes using a cheese cloth. Sugar was considered as the only TSS in this case. TSS measured represented the sucrose content in the sample. Since hot air dried mango did not have enough juice to be extracted, samples were cut and added with demi-water to make a 50%w/w mixture, stirred well and pressed again for measurement. The TSS value was then calculated by multiply with the dilution factor 2.

4.2.3.3 Total Titratable Acidity (TTA)

Total titratable acidity was determined using 10 mL of mango juice obtained by blending the cubes and filter with cheese cloth. The juice was titrated with 0.1N NaOH until pH reaches 8.1. The percentage of acid and sugar acid ratio can be calculated with equation 1 and 2.

$$\% \text{ of acid} = \frac{\text{mL of NaOH} * 0.0064 * 100}{10 \text{ mL mango juice}} \quad \text{Eq1}$$

$$\text{Sugar acid ratio} = \frac{^{\circ}\text{Brix}}{\% \text{ of acid}} \quad \text{Eq2}$$

4.2.3.4 Water loss, soluble solid gain, weight reduction and OD performance index

Water content is determined by using Protocol 1e, in which the sample was dried in an incubator overnight at 105°C, the difference in weight was the amount of water in the sample. The water loss was the difference of weight of mango cubes before and after OD and HAD (Eq3). Soluble solid gain was calculated with the water content of the samples (Eq4). Weight reduction was the ratio of weight change due to processing (Eq5). OD performance index was an indication of the efficiency of process and estimated by the ratio between water loss and soluble solid gain (Eq6).

$$\text{Water Loss} = \frac{(M_t)(x_{w,t}) - (M_o)(x_{w,o})}{M_o} \quad \text{Eq3}$$

$$\text{Soluble Solid Gain} = \frac{(M_t)(x_{s,t}) - (M_o)(x_{s,o})}{M_o} \quad \text{Eq4}$$

$$\text{Weight Reduction} = \frac{M_t - M_o}{M_o} \quad \text{Eq5}$$

$$\text{OD Performance Index} = \frac{\text{Water Loss}}{\text{Soluble Solid Gain}} \quad \text{Eq6}$$

M_o : initial weight of sample (g)

M_t : weight of sample at time t (g)

$x_{w,0}$: mass fraction of water initial content

$x_{w,t}$: mass fraction of water content at time t

$x_{s,0}$: mass fraction of solids initial content

$x_{s,t}$: mass fraction of solids content at each sampling times

4.2.3.5 Water activity

Water activity of mangos was measured using Novasine Labmaster-Aw according to Protocol 32. Samples were prepared by cutting the cubes into smaller pieces. The measurement was set with 2 minutes stability time for a_w measurement and 3 minutes for temperature stabilisation.

4.2.3.6 Texture analysis

A Texture analyser (TX2, Stable Micro System) was used for determination of firmness, adhesiveness, work of shear of mango cubes before and after OD and HAD. The analysis was performed according to Protocol 68. The measurement was performed by bulk shearing using a Kramer Shear cell and 50 kg load, for each measurement 4 cubes were placed in the cell to decrease variance. The compression force was measured at 1.5 mm/s test speed, the probe was set to return to start position when it has reached the bottom of the cell.

4.2.3.7 Colour

The colour of fresh mangos, before and after OD and HAD was measured in Hunter L^* , a^* , b^* scale based on Protocol 36 using Hunterlab Color Flex. The samples were cut into small pieces, then put into a glass cuvette. The colour is measured under mode 45/0 and each sample was measured 3 times with the cuvette being turned to avoid deviation.

4.2.3.8 Preparation of Alcohol insoluble residue and Degree of methylation analysis

In fruit cell walls, the most abundant component is polysaccharides (Ting, 1970). These wall polysaccharides, for example pectin and cellulose, can be isolated by alcohol extraction (Brito & Vaillant, 2012). The isolated residues are called Alcohol insoluble residue (AIR). The degree of methylation (DM) of pectin was calculated by the determination of methyl esters proportion in pectin. Mango cubes was frozen by liquid nitrogen and

grounded using IKA A11 basic analytical mill. AIR was obtained by filtration of sample with 95% ethanol, dried overnight in incubator at 40°C. The content of methanol and anhydrous galacturonic acid can be determined with AIR, and then the ratio between them was used to estimate DM. This analysis was performed in Food Chemistry laboratory according to Protocol 67.

4.2.3.9 Statistical analysis

All results were subjected to statistical analysis in order to obtain information on the significant difference among variables, including the time and PME within the same pre-treatment, and pre-treatment in the same processing condition (same time and both with/without PME). Samples treated with OD and HAD were analysed separately. Analysis of variance (ANOVA) was performed using SPSS Statistics 23 software (IBM), the significance was defined at $P \leq 0.05$.

5. Results and discussion

Osmotic dehydration of mango with different pre-treatments (HPP, VI and no pre-treatment) and conditions (treatment time and with or without PME addition) was conducted in this study, to understand its influence on physicochemical properties of mango.

5.1 Physicochemical properties of fresh sample

Three OD treatments were conducted on separate days since the preparation of mango cubes and treatment itself is time-consuming. To ensure the properties of raw material were consistent and the results obtained were comparable, the physicochemical properties of fresh mango were measured and presented in Table 3.

Table 3 Physicochemical properties of mango used in this study (maturity stage 1, Variety Kent)

	Water content(%)	TSS (°Brix)	TTA (%)	Firmness(lbs) ^a	Colour		
					L*	a*	b*
Day 1	83.2±0.4	13.2±0.1	0.76±0.05	20.22±1.60	68.36±0.48	10.86±0.97	58.79±1.29
Day 2	84.5±2.5	13.0±0.1	0.83±0.09	20.25±1.41	68.70±0.68	7.34±0.58	54.84±1.14
Day 3	85.3±1.7	13.3±0.1	0.83±0.02	20.66±1.28	68.87±0.42	7.04±0.39	53.04±0.47
Average	84.3±1.5	13.2±0.1	0.80±0.04	20.4±1.45	68.64±0.53	8.41±0.65	55.56±0.97

^a Firmness of whole mango flesh

From Table 3, it can be seen that except firmness, the standard deviation of average results is small ($SD < 5\%$), results from fresh samples subjected to different treatments were believed as valid data. Firmness had a higher standard deviation but was within the targeted range (19-22 lbs) in Table 1. TSS of fresh mango did not fall in the TSS range (8-10°Brix). However, previous research for Kent mango with different maturity stage also had higher TSS than reference (Alarcón, 2016). Therefore only firmness was used as the basis for screening mango samples for the experiment.

5.2 Water loss, soluble solid gain and OD performance index

The analysis of water loss and soluble solid gain are important indications to evaluate the performance of osmotic dehydration, which accounts for the total mass change and affecting sample shrinkage (Giraldo, Talens, Fito, & Chiralt, 2003).

From Figure 3 (a) and (b), the water loss and soluble solid gain increased along with OD treatment time was expected since the main mechanism of OD is the removal of water and, in the meantime, the diffusion of solute into tissues. This agreed with the general principles of OD of fruit, that is a larger amount of solute penetration and water loss would take place with longer treatment time (Chandra & Kumari, 2015).

Water loss of sample without pre-treatment was the highest among all treatments which corresponded to its lowest soluble solid gain. This is because that the water coming out of the sample surface through cell membrane restricted the solute penetration into the cellular material (Marcotte & Le Maguer, 1992; Sablani & Rahman, 2003). The higher soluble solid gain and the lower water loss tendency from sample treated with VI is in line with previous studies (Torres *et al.*, 2006; Moreno *et al.*, 2004), as VI favours the hydrodynamic gain of the osmotic solution in the tissue pores, therefore the application of VI resulted in more solid gain and decreased the drying rate of the product (Fito & Chiralt, 2001). According to Chewastek (2014), the relatively high solid gain from HPP treatment is due to the cell disruption caused by high pressure, which increased permeability of the structure, the mass transfer rate is then enhanced. In Figure 4, a the solid gain of sample with different pre-treatment is shown. A higher rate of solid gain can be seen in VI treated sample after 4h treatment. Since solid gain is mostly dependent on the microstructure of food tissue (An, Zhao, Ding, Tao, & Wang, 2013), this result has indicated that vacuum led to more structural change in the mango tissue than other two pre-treatments.

The effect of PME addition on water loss of sample did not shown significant difference on most samples, except 4h sample without pre-treatment, 30m and 4h sample treated with HPP. Solid gain also had no significant effect by the addition of PME except 30m and 4h sample without pre-treatment, and 4h sample treated with VI. A possible reason for this result is the initial activity of PME in mango itself. During the ripening of mango, the activity of PME decreases (Yashoda, Prabha, & Tharanathan, 2007). Therefore, the mango used in this study (maturity stage 1) had a relatively high PME activity, and the addition of small amount of PME (0.48%) did not shown significant effect. However, similar findings were reported by (Alarcón, 2016), who study the effect of different pre-treatments and PME addition on OD of Kent mango in maturity stage 4. The effect of PME was found also not significant on water loss and soluble solid gain in most samples. Maxime, Marcotte, & Taherian (2004) studied the firmness and water loss of raspberry affected by PME and OD, reported different results. The gel network obtained from pectin and Ca hindered water transfer between fruit and osmotic solution, which can reduce the water loss for the same treatment time. Germer *et al.*, (2014) conducted the OD of papaya with different additives in syrup, results shown that addition of PME and Calcium chloride increased both water loss and solid incorporation. A speculation of reason is that the fruit materials had large differences in their structural and physicochemical properties, which can greatly influence the effect of PME during processing.

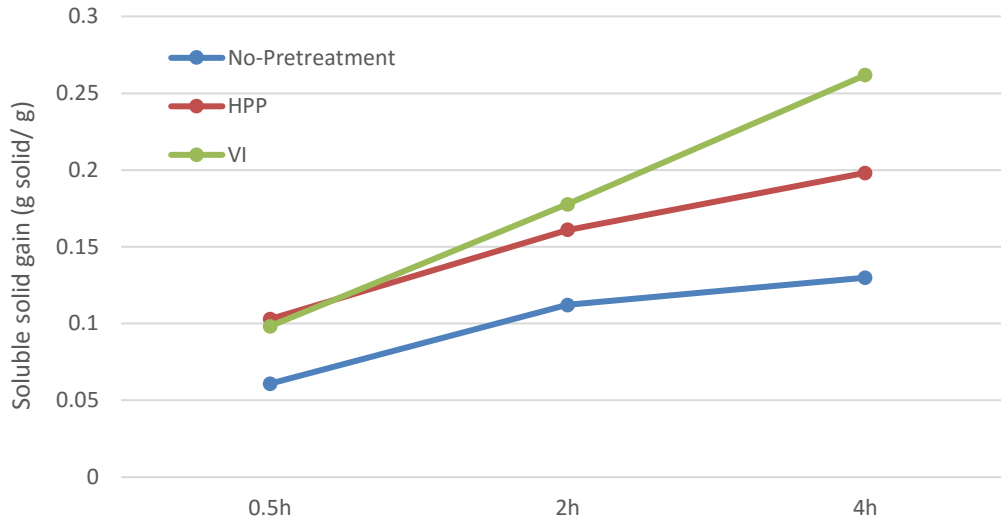


Figure 4 Soluble solid gain (g solid/g) of OD mangos with different pre-treatment

The soluble solid gain after 4h treatment is lower than the result of 2h in the sample without pre-treatment. This result was not expected, as the increase of TSS between these two samples from no pre-treatment is within the normal range comparing to other samples applying VI and HPP (Figure 7(a)). On the other hand, the weight change due to treatment for 2h no PME (-22.9%) and 4h no PME (-42.47%) has a significant increase. In Figure 5(a) and (b) shows the shrinkage and size decrease from 2h to 4h sample. Since the solid gain can reinforce the solid matrix strength in the tissue, which decrease porosity and oppose structural collapse (Nahimana *et al.*, 2011). In this case, the low solid gain obtained from 4h treatment is in line with high degree of shrinkage, but the solid gain at 4h should not be lower than at 2h. A justified explanation cannot be given other than data variance.

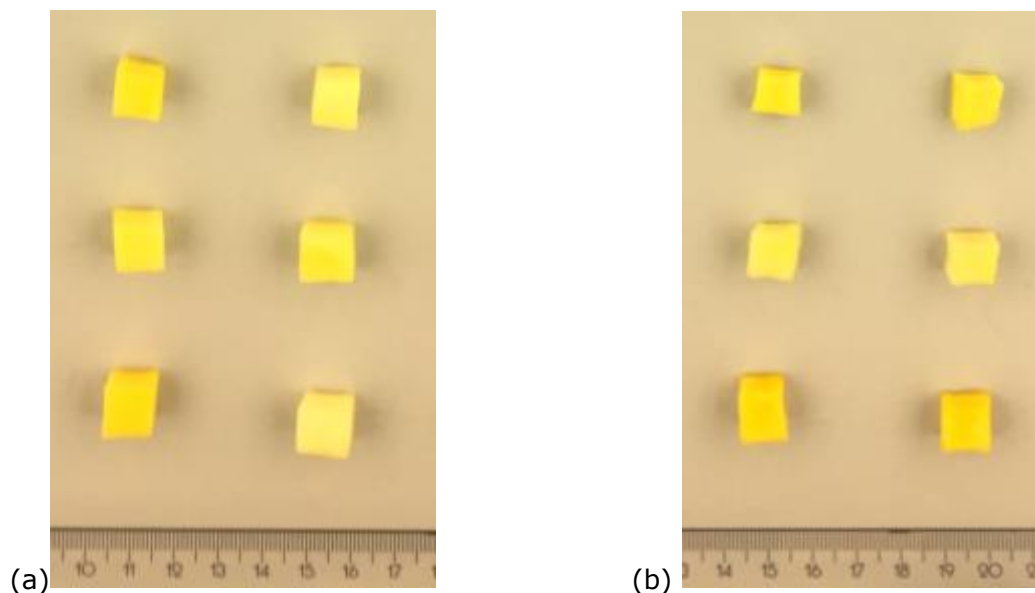


Figure 5 Pictures of mango cubes treated without pre-treatment and no PME at different OD time (a) 2 hours (b) 4 hours.

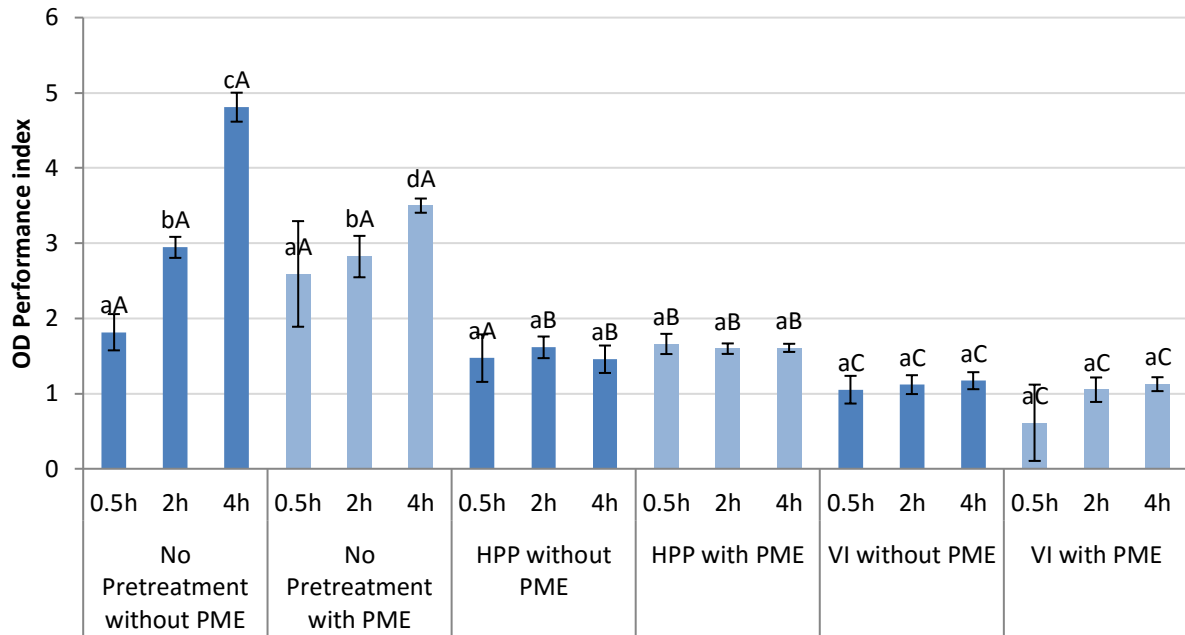


Figure 6 Osmotic dehydration performance index of OD mangos with different pre-treatments and time. Mean values with different small letters are significantly different ($P \leq 0.05$) among different processing condition for the same pre-treatment. Mean values with different capital letters are significantly different ($P \leq 0.05$) among pre-treatments at the same processing condition.

OD performance index is the ratio between water loss and soluble solid gain, it can indicate the efficiency of the OD treatment. According to Figure 6, it can be seen that sample without pre-treatment has the highest OD performance index, owing to its highest water loss and lowest soluble solid gain. For sample without pre-treatment, the effect of PME is only significant at 4h treatment ($P \leq 0.05$), which is the same case for water loss and solid gain. Meanwhile, VI is the least efficient treatment in this study. As discussed before, it should be noted that even though the sample without pre-treatment has higher OD performance index, it owns higher degree of shrinkage as well.

5.3 Total soluble solid, water content and water activity

Total soluble solids (TSS), represents the sugar content of the sample, is shown in figure 7(a) for all treatments. The significant increase of TSS along with the osmotic dehydration process taken place is because of the loss of water and intake of sugar from osmotic solution. Hot air drying caused a much higher TSS compared to OD due to the rapid decrease of water content. There are significant differences of TSS between no pre-treatment and HPP samples ($P \leq 0.05$) with at the same OD time. Sample treated with HPP had the lowest TSS after 4h treatment, while VI treated sample had the highest TSS. This indicating the different effect of high pressure and vacuum on the mango structure, which can affect the sugar intake in further OD treatment. The effect of PME is significant on 30m and 4h pre-treated sample using VI or HPP. Due to fruit materials have large variances themselves, this result cannot be concluded whether PME has an significant effect on TSS.

While TSS increased, the water content of the sample reduced from 84.3% to 60.1% as the osmotic dehydration treatment progress with time, indicating the removal of water and incorporation of solutes by OD. The fresh and 2h OD sample are then hot air dried to

remove more water to 77.74% and 59.35%, respectively. Similar to the results of TSS, the sample treated with HPP had the highest decline rate of water content at the beginning of OD, then gradually dropped.

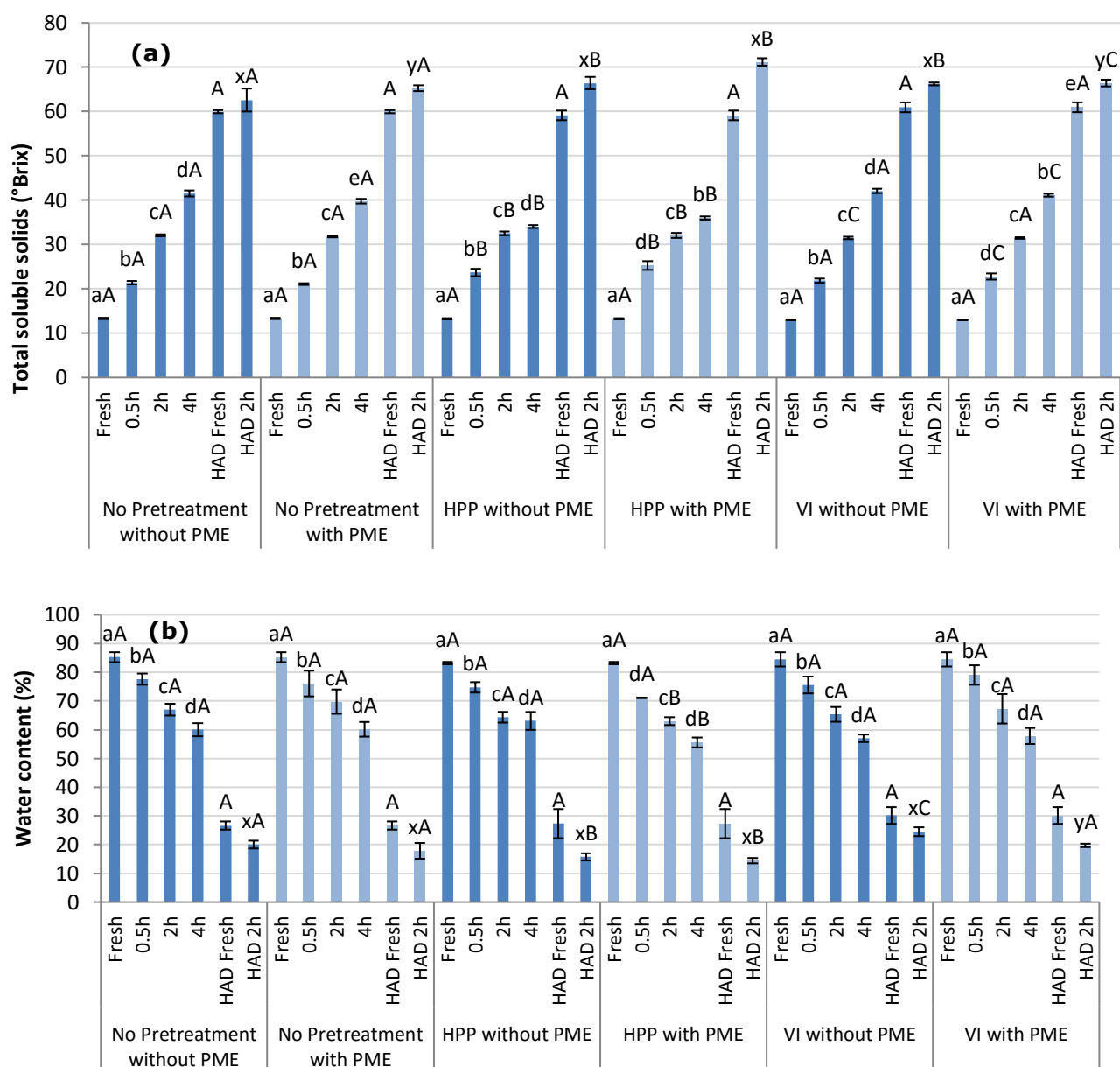


Figure 7 (a) Total soluble solids (°Brix) and (b) Water content (%) of Fresh, OD and HAD mangos with different pre-treatments and time.

Mean values with different small letters (a to e for OD, x and y for HAD) are significantly different ($P \leq 0.05$) among different processing condition for the same pre-treatment. Mean values with different capital letters are significantly different ($P \leq 0.05$) among pre-treatments at the same processing condition.

The water activity of food product refers to the unbond water which can support the growth of micro-organism therefore important for food safety (Nielsen, Bilde, & Frosch, 2012). As can be seen from figure 8, the a_w of samples had a reduction of nearly 2% by OD, from 0.979 to 0.960. Samples with no pre-treatment did not have significant differences ($P > 0.05$) with different treatment time and PME addition. However, for samples treated with HPP and VI, treatment time had a significant effect ($P \leq 0.05$) on

lowering a_w value but not for the addition of PME. Though the treatment lowers the a_w , the values are still in the range that allows the growth of spoilage microorganisms (FAO, 2003), proper preservation method and/or further treatment such as hot air is required.

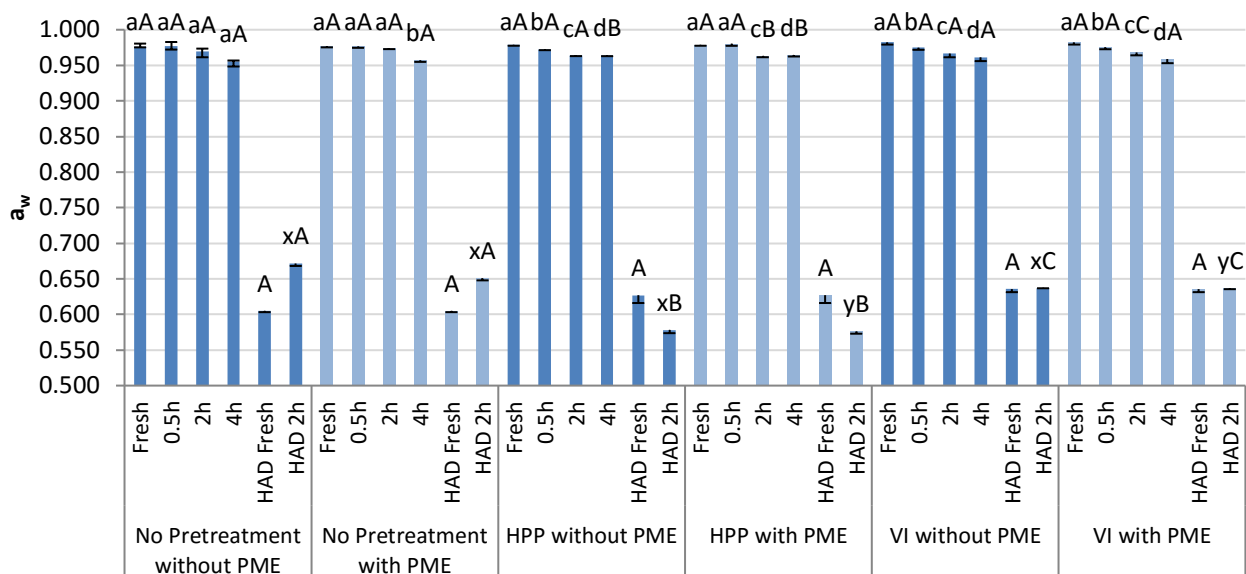


Figure 8 water activity of Fresh, OD and HAD mangos with different pre-treatments and time.

Mean values with different small letters (a to d for OD, x and y for HAD) are significantly different ($P \leq 0.05$) among different processing condition for the same pre-treatment. Mean values with different capital letters are significantly different ($P \leq 0.05$) among pre-treatments at the same processing condition.

Table 4 shows the drying time for samples underwent different treatments to reach an a_w of 0.6 to 0.65. The untreated fresh sample had a much lower drying time comparing to the treated ones. Since the samples were immersed in sucrose solution, the sucrose also acted as a stabiliser during dehydration (Patist & Zoerb, 2005, Crowe *et al.*, 1996). As tissues are dried, hydrogen bonds replaced the water of hydration at the membrane-fluid interface, preventing phase transition and consequent leakage (Neito *et al.*, 2013). Therefore though the water content is lower in treated sample, there is less unbound water in the tissue which required more energy to achieve the same a_w . The addition of PME did not show effect on drying time, samples without pre-treatment had shorter drying time but the difference is not evident as the range of targeted a_w is too broad.

Table 4 Hot air drying time of fresh and OD mangos with different pre-treatments

		Drying time (h)	Water activity
No PME	Fresh Fruit	6	0.623
	No Pre-treatment	11	0.672
	Vacuum impregnation	11.5	0.637
	High pressure	13	0.578
PME	No Pre-treatment	11	0.651
	Vacuum impregnation	11.5	0.637
	High pressure	13	0.576

5.4 Colour change

Figure 9 has given the L^* , a^* , b^* value of the samples. As L^* represents the lightness as higher value for higher lightness, red-green for a^* as green at negative and red at

positive a^* values, yellow and blue for positive and negative b^* values, respectively. In Appendices, the pictures of mango cubes from all treatments are also shown to collaborate the colour measurement.

Mango flesh has a bright colour, which is reflected in the high L^* value. Fresh sample had a mean value of 68.64 in lightness, while darker colour is measured in treated samples. The most remarkable decrease in lightness was observed in sample treated with VI, a 31% decrease from 68.70 to 47.23, while HPP has the least reduction from 68.52 to 64.51. The strong influence on lightness change induced by VI is due to the effect produced for total or partial substitution of the air present in the pores by the impregnation solution, leading to air loss which associated with transparency gain (Moreno *et al.*, 2004).

The a^* and b^* values are associated with chlorophyll and carotenoid contents in mango (Corzo & Alvarez, 2014). According to (Ornelas-Paz, E, & Gardea, 2008), correlation is found between the concentration of carotenoids and a^* values in Manila and Ataulfo mangos. The high concentration of carotenoids in mango flesh causing the intense yellow to orange (b^* value) colour (Brecht & Yahia, 2009).

Figure 9 (b) and (c) shows the change of colour after treatment in terms of a^* and b^* values. In samples without pre-treatment, an interesting pattern of both a^* and b^* value change was observed. Redness and yellowness decreased at the beginning of the OD treatment, followed by a large increase of a^* and b^* values which are higher than fresh sample, then drop again after 4h treatment. This reason for this phenomenon is yet unknown. The effect of different pre-treatments and the application of PME did not shown distinctive trend. Compared to OD, Hot air drying gives a higher a^* value. Samples treated with VI had a lower b^* value compared to HP and no pre-treatment. Corzo & Alvarez (2014) stated that the decreased in b value in dehydration of mango is mainly due to the degradation of yellow pigment β -carotene. Treatment time had a significant effect ($P \leq 0.05$) on both a^* and b^* value, but no pattern can be seen on a^* values. Similar to the result of L^* , VI treated sample also resulted in lower b^* value while HPP caused the least reduction. Therefore it is believed that apply high pressure as a pre-treatment of OD, can lead to better retention of sample colour. A clearer comparison can be seen in Figure 10, which showed the difference in L^* and b^* reduction by different pre-treatment. PME addition did not cause significant differences in most samples. A study focusing on the change of biochemical contents, especially on chlorophyll and carotenoid, should be done to give more information. Fresh sample dried by hot air had an increase in both a^* and b^* value, the increase in the colour parameter values is due to the removal of water by drying process, which can increase the concentration of pigments in the raw materials (Germer, *et al.*, 2014).

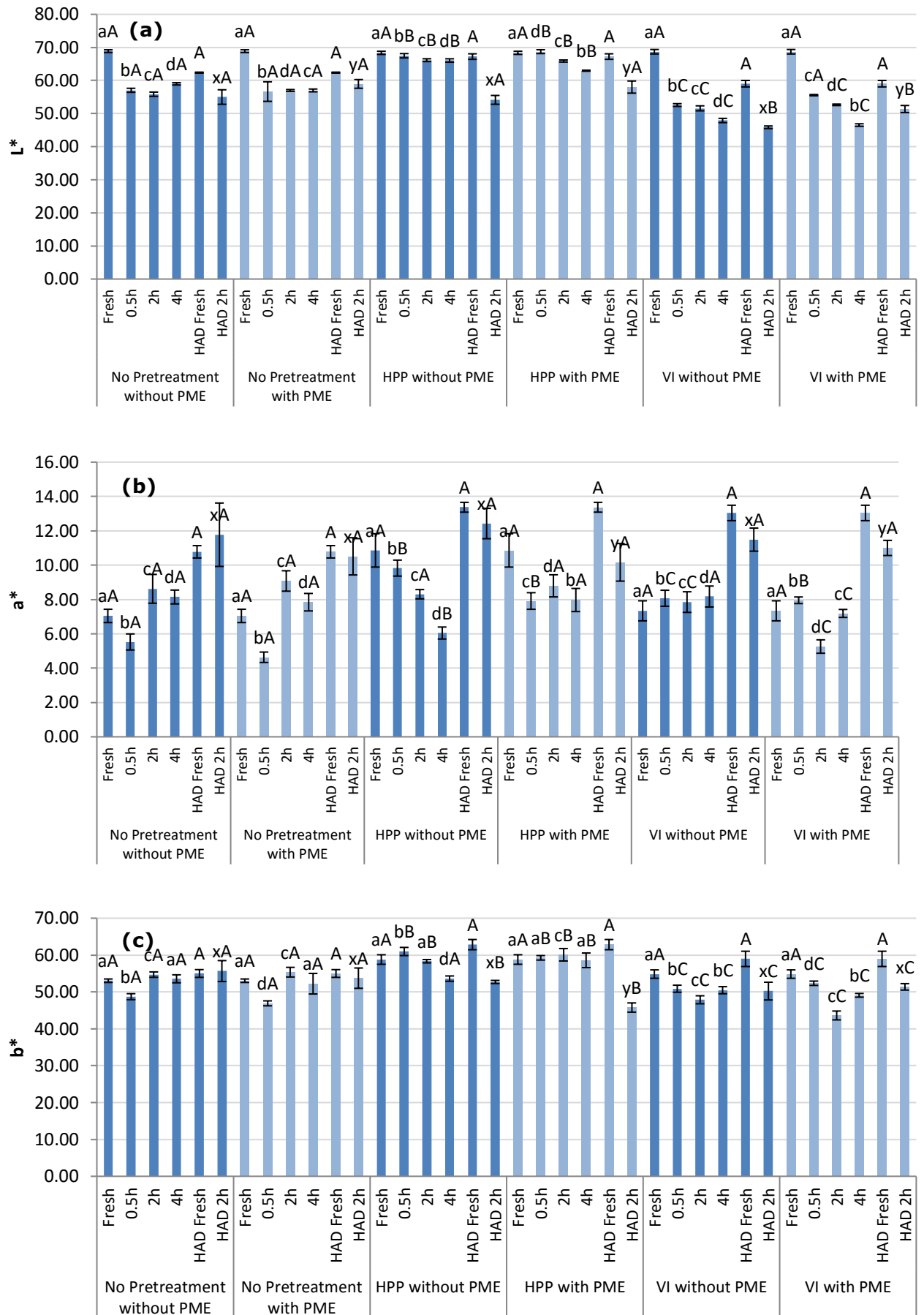


Figure 9 (a) L*, (b) a* and (c) b* values of fresh, OD and HAD mangos with different pre-treatments and time.

Mean values with different small letters (a to d for OD, x and y for HAD) are significantly different ($P \leq 0.05$) among different processing condition for the same pre-treatment. Mean values with different capital letters are significantly different ($P \leq 0.05$) among pre-treatments at the same processing condition.

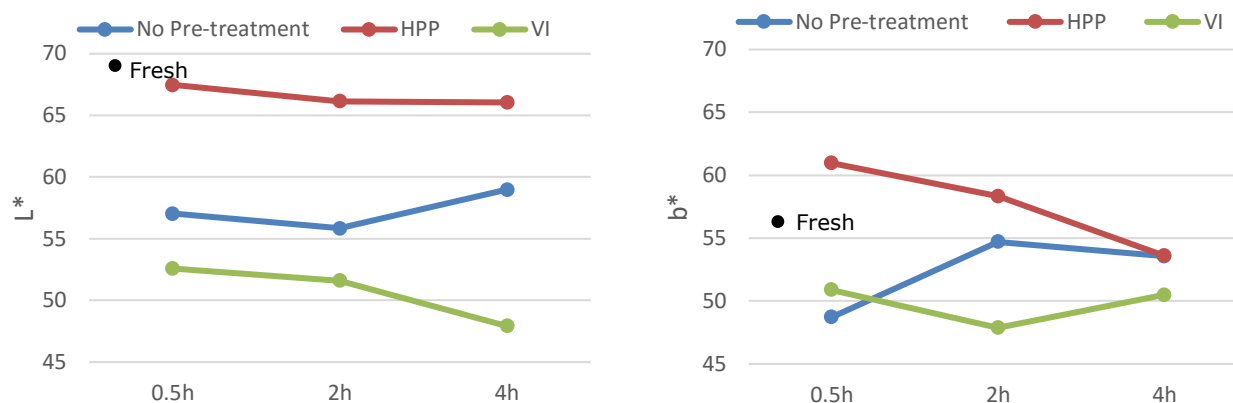


Figure 10 Difference in reduction of L* and b* values of mangos by different pre-treatments

5.5 Textural change

During processing of fruit, the mechanical behaviour of fruits tissue changes due to the alteration of structural components. The main changes affect the mechanical properties of fruits caused by OD are loss of cell turgor, alteration of middle lamella and cell wall resistance, changes in air and liquid volume fractions in the sample, loss of water, diffusion of external solute, and changes in size and shape (ALZAMORA *et al.*, 2000, Rincon & Kerr, 2010, CHIRALT *et al.*, 2001).

As the mangos are in maturity stage 1, the firmness range is between 19 to 22lbs (~84.5 to 97.9N) from a penetrometer. The result from texture analyser has given similar value, a mean firmness of 103.85N/cube in fresh sample. From Figure 11, the change of firmness did not show distinguishable pattern. Since firmness loss is associated with degradation of pectin and insoluble protopectin, as they are responsible for structural rigidity of the fruit (Ferrair *et al.*, 2013). The water loss caused by OD damage the structure of sample but the intake of sugar can increase the integrity of the fruit structure. Therefore this result may be subjected to multiple interactions among biochemical components of sample, external osmotic solution and treatment conditions. More insight can be obtained through an in-depth study on the mechanical properties of sample. Another reason for this may be due to the high variance of the data caused by raw material itself, more repetition of measurement is required to give a clearer view of the data. Hot air dried sample had a sharp decrease of firmness due to the structural collapse caused by extensive water loss.

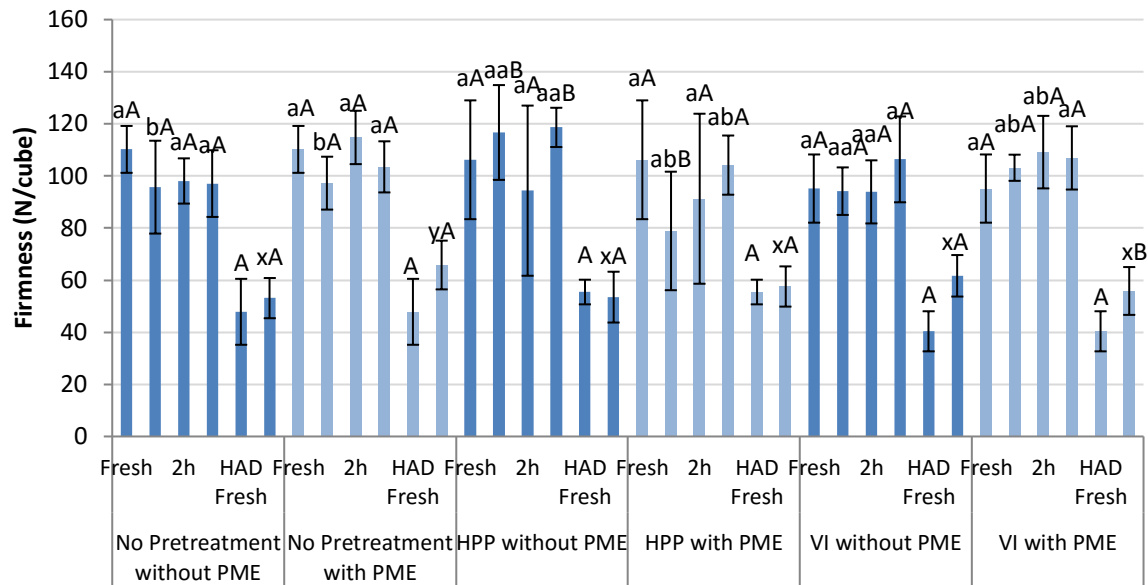


Figure 11 Firmness (N/cube) of fresh, OD and HAD mangos with different pre-treatments and time. Mean values with different small letters (a and b for OD, x and y for HAD) are significantly different ($P \leq 0.05$) among different processing condition for the same pre-treatment. Mean values with different capital letters are significantly different ($P \leq 0.05$) among pre-treatments at the same processing condition.

Work of shear is the force required to compress the sample, a certain degree of correlation between firmness of sample and work of shear is assumed since firmer sample required more work to compress. Work of shear for sample without pre-treatment is the lowest among all three pre-treatments, and the value decreased with longer treatment time. 81% less work of shear is required for the 4h sample compared to fresh sample. The effect of PME remains not significant ($P > 0.05$) on work of shear. The effect of pre-treatments shown significant difference only after 4h treatment ($P \leq 0.05$). The firmness and work of shear for HPP sample subjected to HAD, is lower than fresh sample treated with HAD, this may be a result of the cell disruption from high pressure, leading to a weaker matrix. PME addition resulted in significantly higher value of work of shear on HAD 2h ($P < 0.05$) in all three pre-treatments. The effect of PME addition on firmness only significant on HAD 2h mango with no pre-treatment.

Adhesiveness represents the work required to overcome the attractive forces between the surface of a food and the surface of other materials. From figure 12(b), it can be observed that the adhesiveness of sample decreased after OD treatment. No significant difference ($P > 0.05$) was found between sample with or without adding PME. Sample underwent HAD followed by OD has a sharp increase of adhesiveness, which is a result of the large increase of sugar content by water loss during further drying. The adhesiveness value of hot air dried fresh sample in HPP is much higher than other fresh samples. This may be caused by the longer drying time, which correspondent to lower values of a_w . Like the result of firmness and work of shear, adhesiveness had high deviations of data. Thus, the relationship between the types of pre-treatments and adhesiveness cannot be concluded, more repetition of the measurements is necessary for determining the textural attributes of fruit sample by Texture analyser

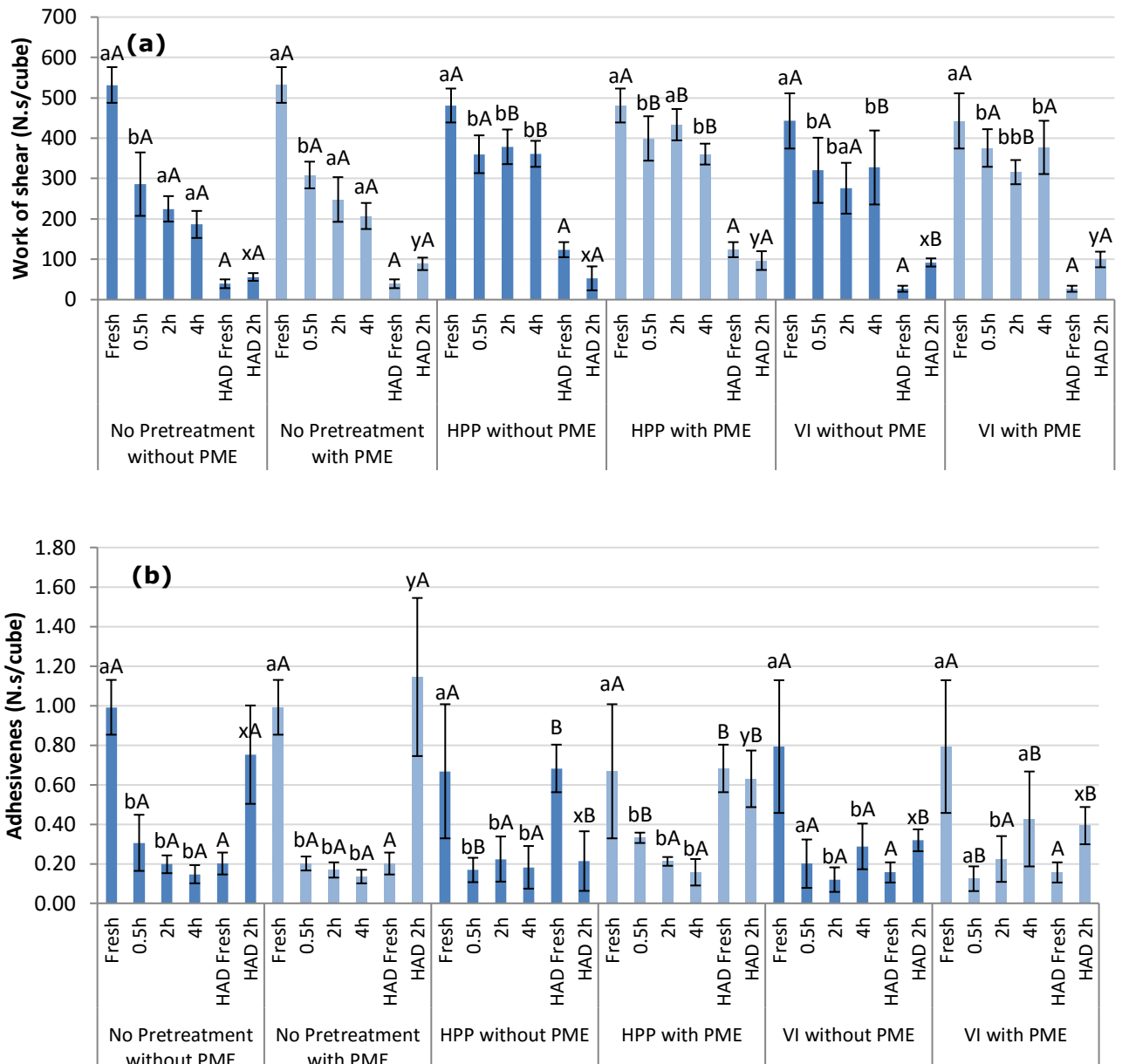


Figure 12 (a) Work of shear (N.s/cube) and (b) Adhesiveness (N.s/cube) of fresh, OD and HAD mangos with different pre-treatments and time. Mean values with different small letters (a and b for OD, x and y for HAD) are significantly different ($P \leq 0.05$) among different processing condition for the same pre-treatment. Mean values with different capital letters are significantly different ($P \leq 0.05$) among pre-treatments at the same processing condition.

5.6 Alcohol Insoluble residue (AIR)

10 g of mango sample frozen by liquid nitrogen was thawed and filtered by 95% ethanol and acetone, after drying and grinding, the AIR was obtained. However, the weight of it has brought uncertainty to the purity of AIR. According to El-Zoghbi (1994), who studied the changes in biochemical of mango during ripening, obtained AIR content of average 4.75g/100g sample when mango is mature. Several samples were analysed because of their significant difference in the effect of PME addition in terms of OD performance index. Sample treated for 4 hours without pre-treatment was the only one had significant difference with PME addition. HP 4h and 2h samples were chosen to compare the effect

of pre-treatment and time. As shown in Table 5, the weight of AIR shown large variance between samples and higher value than from literature.

Table 5 Alcohol Insoluble Residue obtained from different samples

	Sample code	AIR (g/100g sample)
Fresh	Fresh A	1.80
	Fresh B	2.39
No pre-treatment	4h No PME	7.50
	4h PME	5.29
High pressure processing	2h No PME	5.60
	2h PME	6.19
	4h No PME	5.39
	4h PME	5.19

The analysis for the determination of total sugar content according to (Dubois *et al.*, 1956) was performed on two AIR samples since they had the highest AIR amount. Three standard glucose solutions with different glucose content were prepared. The addition of 2.5% phenol and concentrated sulfuric acid led to the change of colour from transparent to brown. The same steps were also applied to AIR sample. The colour of AIR and glucose standard were visually compared. If all water-soluble sugars were removed during the AIR preparation, the colour of AIR would not change. Figure 13 (a) and (b) shows the difference of colour among HPP 2h PME and No pre-treatment 4h no PME in standard solutions. Both AIR samples are yellow/brown coloured, indicated the existence of glucose. There are two possible reasons. The first assumption is that thorough extraction was not achieved as the sample was only filtrated twice. The second possible reason is since the solubility of glucose in ethanol/water mixture decreases as the concentration of ethanol increases. In 95% ethanol, the solubility of glucose is estimated at 1.26% (Bockstanz, Buffa, & Lira, 1989), much lower than the 7.7% solubility (Alves, Silva, & Giuliatti, 2007) of another commonly used ethanol concentration which is 70%. Glucose in the sample was not entirely extracted by 95% ethanol due to its low solubility. Based on previous analysis, the DM analysis did not proceed since the amount of sample is limited and more information should be gathered.

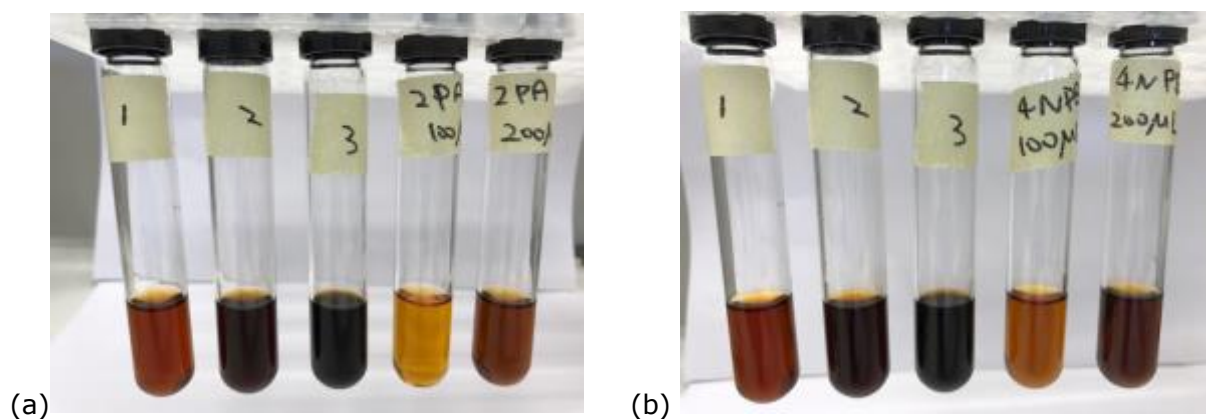


Figure 13 (a) 2h PME with high pressure as pre-treatment and (b) 4h no PME with no pre-treatment (right 1&2) with standard glucose solution (1)7.5mg (2) 15mg (3)30mg glucose in 3.5mL phenol and sulfuric acid mixture

5.7 Influence of different maturity stage on mango properties and OD performance index

A previous study on the influence of VI and HPP on osmotic dehydration of Kent mango, maturity stage 4 (Alarcón, 2016) was compared with this research. The aim is to have a general understanding of the influence of different maturity stage of mango on the properties of the material and the effect of OD. Since the addition of PME did not show significant different on the results in the case of maturity stage 1, the main focus is to compare results without PME addition.

5.7.1 Fresh sample

In Table 6, the physicochemical properties of fresh mango in maturity stage 1 and 4 are shown. The water content of mango is expected to decrease according to some research studying the changes in physical and chemical properties during mango maturation and ripening (Rincon & Kerr, 2010, Ueda *et al.*, 2000, Padma *et al.*, 2011). Although the water content of M1 is lower than M4, it cannot draw opposite conclusions since the deviation makes the value very close. The TSS increases with the maturity stage as part of starch is converted to soluble sugars (Wongmetha, Ke, & Liang, 2015). The titratable acidity decreased with the ripening of mango. Since citric acid is the main organic acid in mango, this reduction of value may be the result of the utilisation of citric acid as substrates for respiration (Medlicott & Thompson, 1985; Padma *et al.*, 2011). The decrease of firmness during mango ripening reflects the involvement of cells walls hydrolases, the degradation of cellulose and pectin components (Banjongsinsiri, Kenney, & Wicker, 2004) (Wongmetha, Ke, & Liang, 2015). The degradation of chlorophyll and accumulation of carotenoids, leads to the bright yellow-orange colour of the flesh in ripening mangos (Vasquez-Caicedo *et al.*, 2005) (Zerbini *et al.*, 2015).

Table 6 Physicochemical properties of mango with different maturity stage

Maturity Stage	Water content(%)	TSS(°Brix)	TTA(%)	Firmness (lbs)	Colour		
					L*	a*	b*
M1	84.3±1.5	13.2±0.1	0.80±0.04	20.4±1.45	68.64±0.53	8.41±0.65	55.56±0.97
M4	84.9±1.8	15.7±0.4	0.58±0.01	5.82±0.87	49.02±0.69	5.87±0.71	49.59±1.73

5.7.2 OD performance index

Table 7 compares the OD performance index with data from (Alarcón, 2016), who used the maturity stage 4 Kent mango. A pattern based on the influence of maturity stage can be seen. The OD performance index is higher when mango is riper. Longer treatment time gave a better performance index in all maturity stages when sample is subject to OD without pre-treatment. In the case of using vacuum impregnation as pre-treatment, the performance index decreased. This is due to the higher rate of soluble solid gain compared to water loss at longer treatment time. A much higher OD performance index was observed for maturity stage 4 mango employing high pressure and PME. This indicated better response for PME and high pressure for riper mango due to the differences in structure and chemical properties.

Table 7 OD performance index with different maturity stage*

Treatment time		30m		2h		4h	
Maturity Stage		M1	M4	M1	M4	M1	M4
No Pre-treatment	No PME	1.8	8.3	2.9	7.9	4.8	9.4
	PME	2.6	6.1	2.8	6.1	3.5	10.6
Vacuum Impregnation	No PME	1.1	8.1	1.1	8.1	1.2	7.1
	PME	0.6	6.0	1.1	6.5	1.1	9.8
High Pressure	No PME	1.5	6.2	1.6	9.3	1.5	7.7
	PME	1.7	11.7	1.6	13.2	1.6	13.7

* All values are presented in average

6. Conclusion

The effect of two different pre-treatments prior to osmotic dehydration of mango cubes was studied, including vacuum impregnation and high pressure processing. Osmotic dehydration without pre-treatment was also carried out for comparison. Results showed that OD sample treated without pre-treatment had the highest water loss and lowest soluble solid gain, given an overall highest osmotic dehydration performance index. Application of pre-treatments result in higher soluble solid gain and lower water loss of mango. Vacuum caused more structural changes in mango tissue, which is reflected by higher rate of soluble solid gain after 4h treatment. The application of high pressure led to a lower OD performance index, but higher than sample applied with vacuum. Vacuum impregnated sample gave highest soluble solid content among treatments. Treatment time had a significant effect on reduction of water activity of treated sample, but the effect of different pre-treatments was not significant. Change of colour profile was different among pre-treatments as the application of vacuum resulted in decreased lightness caused by the substitution of air by impregnation solution in the tissue pores. Application of high pressure as a pre-treatment of OD can lead to a better retention of sample colour. The textural attributes of mango including firmness, work of shear and adhesiveness is investigated, a conclusion for firmness change cannot be drawn due to no clear pattern and large variance of the data sets. A lower work of shear and adhesiveness is observed with treated sample, in which sample without pre-treatment needs the least work to compress the sample, indicating weaker matrix of the sample structure.

The effect of PME addition in the osmotic dehydration was also researched, but no significant effect is observed in water loss, soluble solid gain, water activity, hot air drying time and colour. There were some exceptions, which PME addition had a significant effect, however, a lack of pattern in these results made them difficult to be interpreted. Further microscopic analysis is required to have more insight. The large data variance in mango texture led to unclear results which cannot be concluded.

Part of the osmotic dehydrated samples are followed by further drying by hot air, which given higher solid content and lower water content. The drying time is longer for OD sample compared with fresh sample for achieving the same water activity, owing to less unbound water in the tissue thus require more energy despite lower water content. Hot air dried sample gave more redness to sample colour but no strong effect on lightness and yellowness. The firmness and work of shear of samples decreased considerably after

hot air drying because of loss of matrix strength corresponding to the large degree of shrinkage seen in sample pictures.

A comparison of the result was carried out with same variety, but different maturity stage of mango (maturity stage 4) treated with same methods. Riper mango led to higher OD performance index in all treatments. HP treated sample had the highest OD performance index in riper mango but in the case of mango in maturity stage 1, sample without pre-treatment had the highest OD performance index. The difference in physicochemical and structural properties between mangos with different maturity stage is significant, thus causing the large distinction towards processing.

7. Recommendation

- For textural attributes analysis, more repetition of measurements is necessary to reduce variance.
- A purification step of PME is preferable to ensure the material be free of contamination enzyme.
- The method of the degree of methylation determination should be revised, especially the extraction of AIR.
- In order to have a better understanding of the structural and physicochemical changes during sample treatment, a proper microscopic analysis is recommended.

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9. Appendices

9.1 Pictures of osmotic dehydrated mango cubes with no pre-treatment

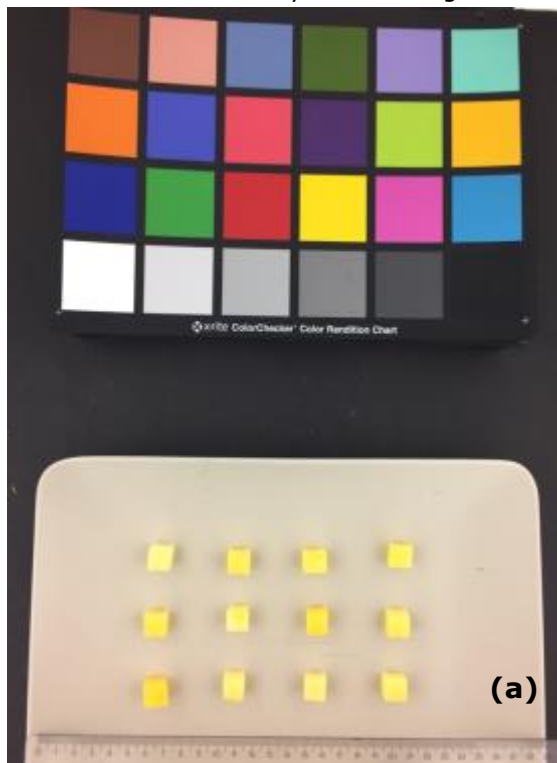


Figure 9.1 (a) Fresh

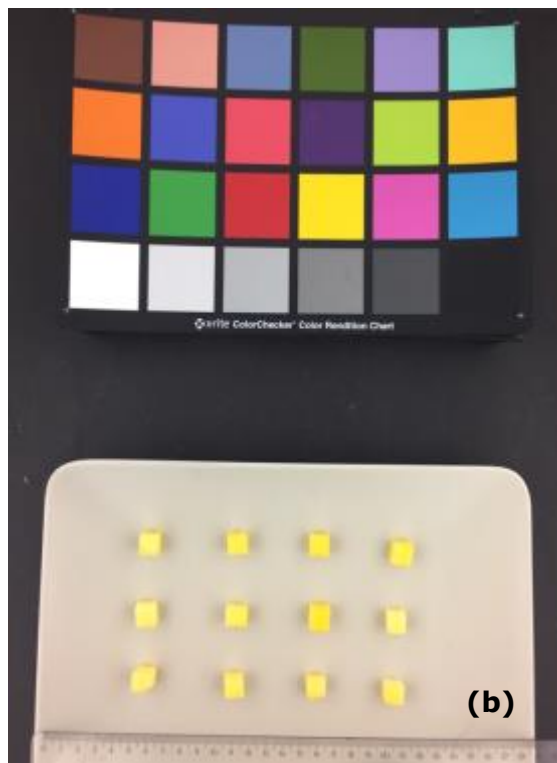
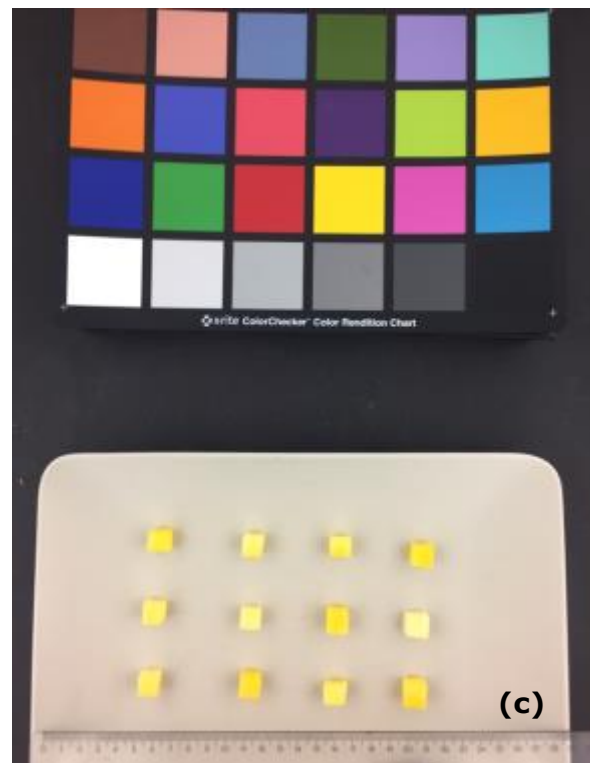


Figure 9.1 (b) 0.5h no PME



(c) 0.5h PME

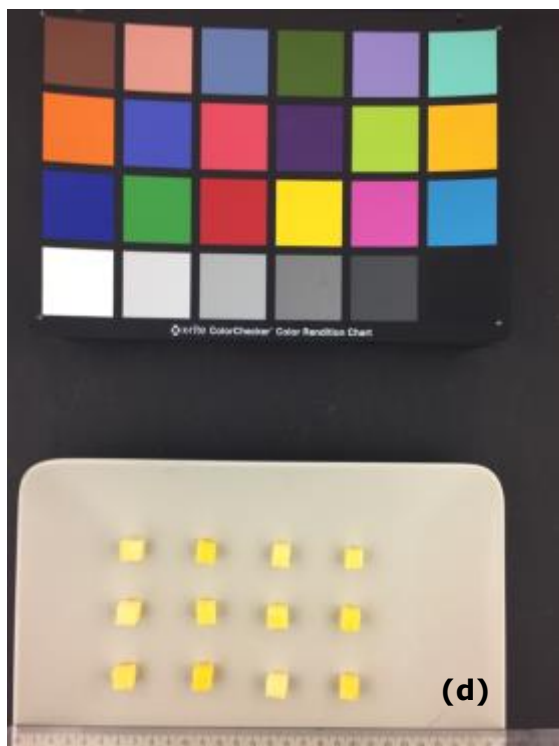
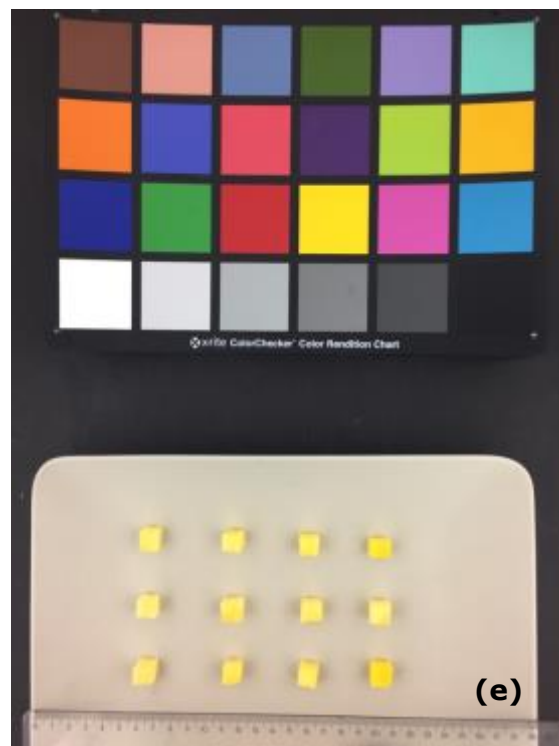


Figure 9.1 (d) 2h no PME



(e) 2h PME

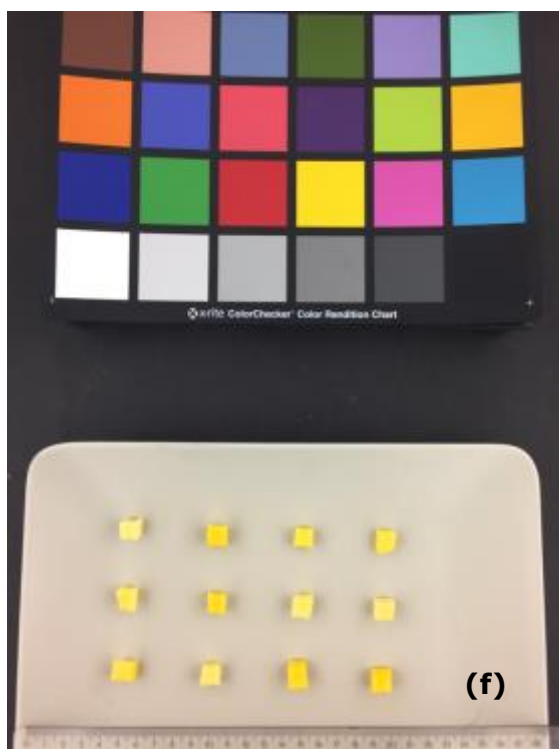
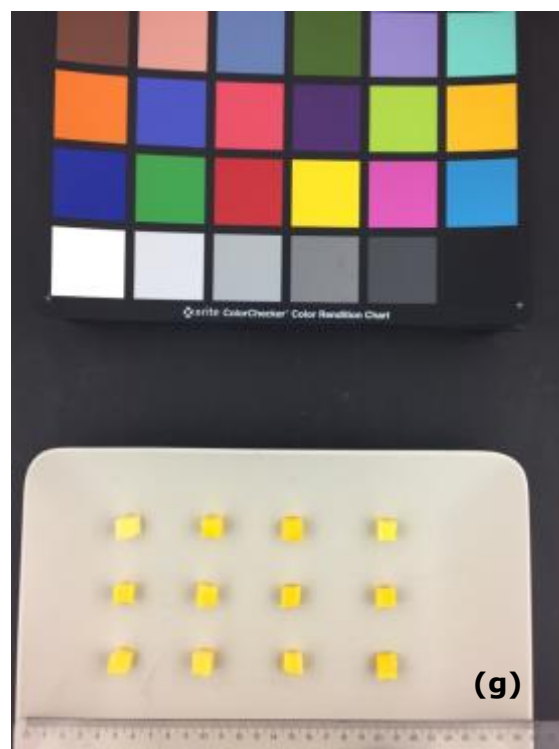


Figure 9.1 (f) 4h no PME



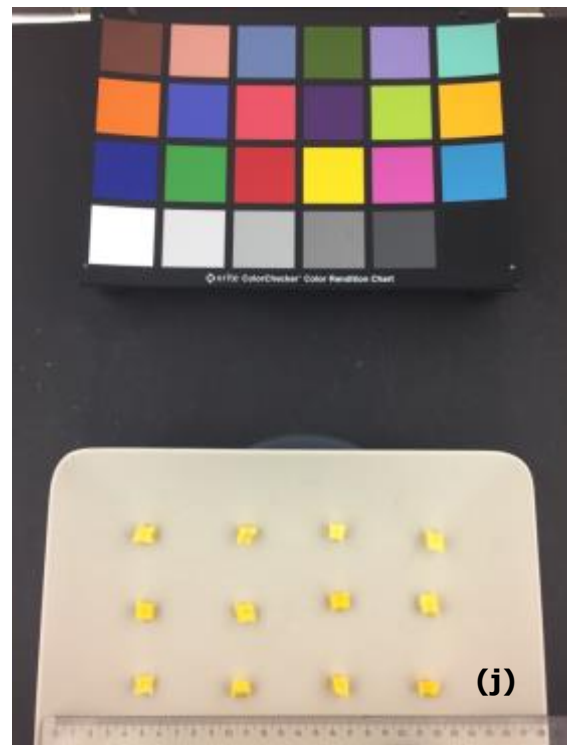
(g) 4h PME



Figure 9.1 (h) HAD Fresh



Figure 9.1 (i) HAD 2h no PME



(j) HAD 2h PME

9.2 Pictures of osmotic dehydrated mango cubes with HPP

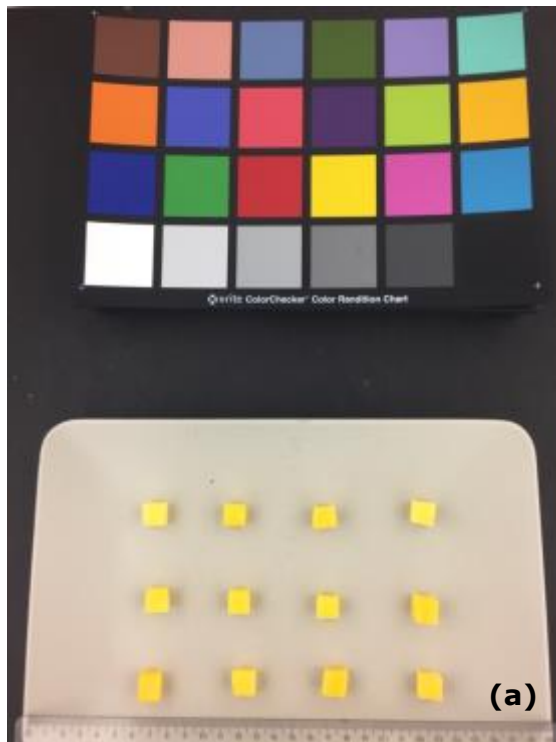


Figure 9.2 (a) Fresh

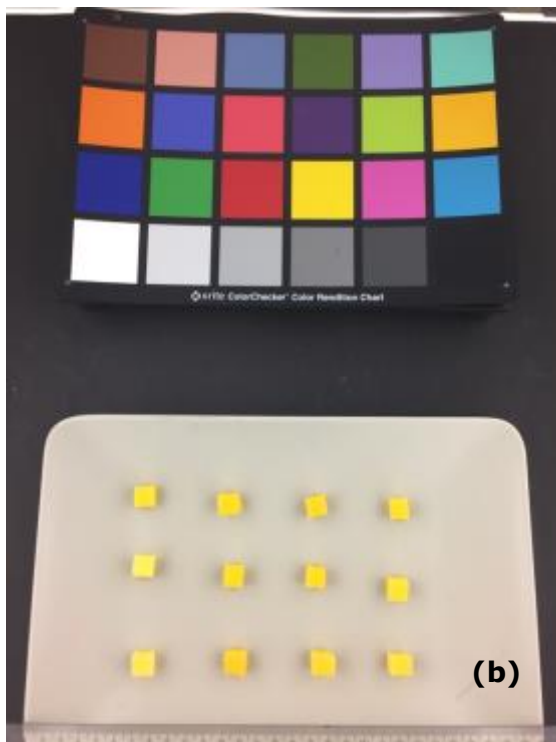
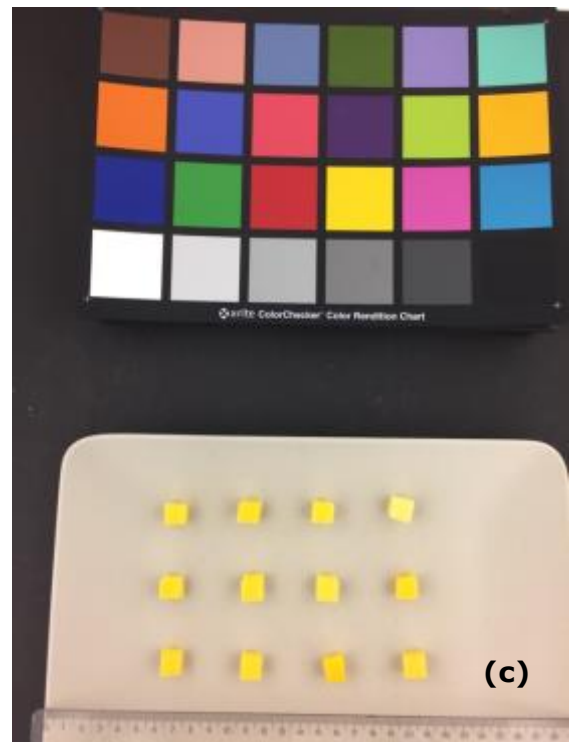


Figure 9.2 (b) 0.5h no PME



(c) 0.5h PME



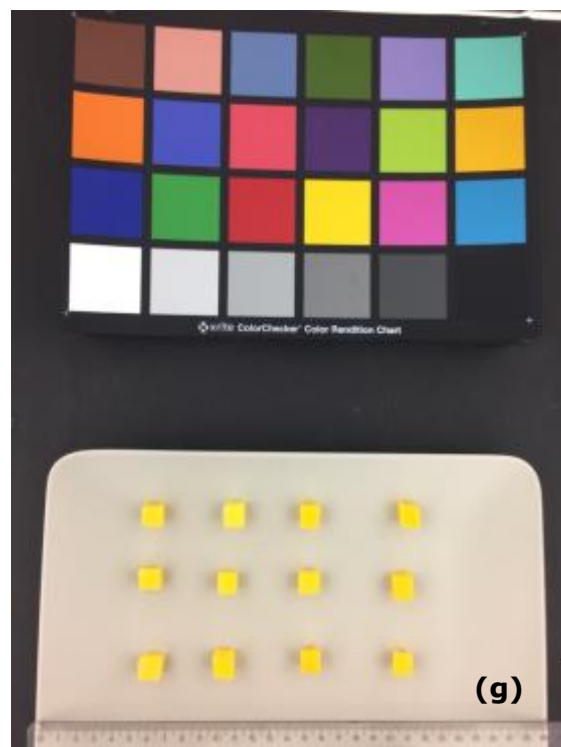
Figure 9.2 (d) 2h no PME



(e) 2h PME



Figure 9.2 (f) 4h no PME



(g) 4h PME

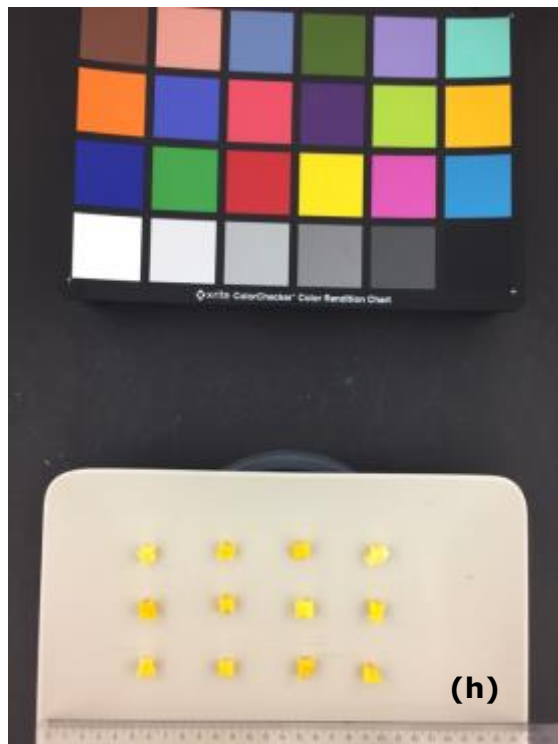


Figure 9.2 (h) HAD Fresh

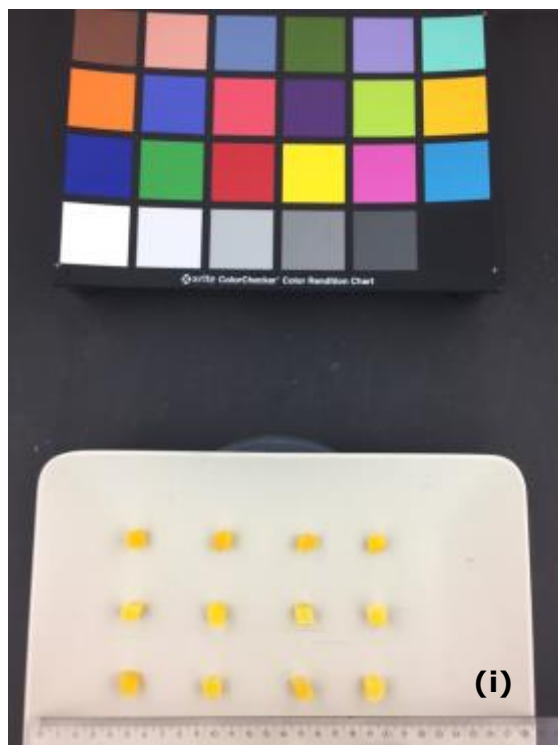
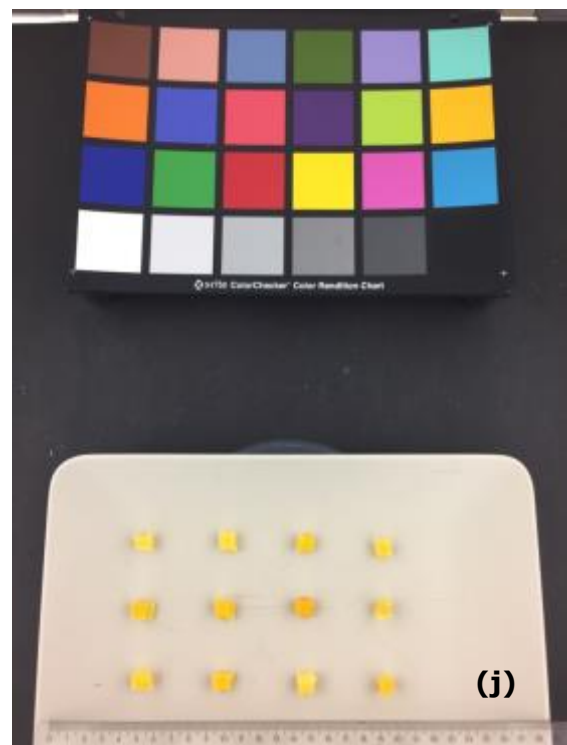


Figure 9.2 (i) HAD 2h no PME



(j) HAD 2h PME

9.3 Pictures of osmotic dehydrated mango cubes with VI

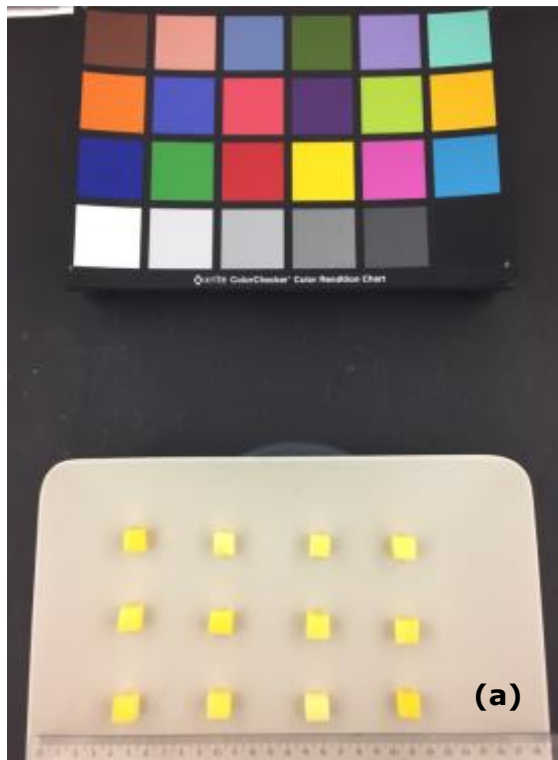


Figure 9.3 (a) Fresh

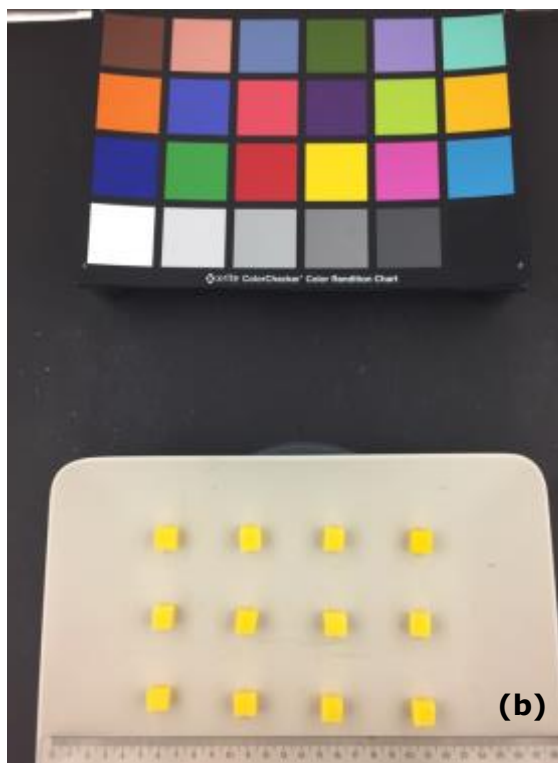


Figure 9.3 (b) 0.5h no PME



(c) 0.5h PME

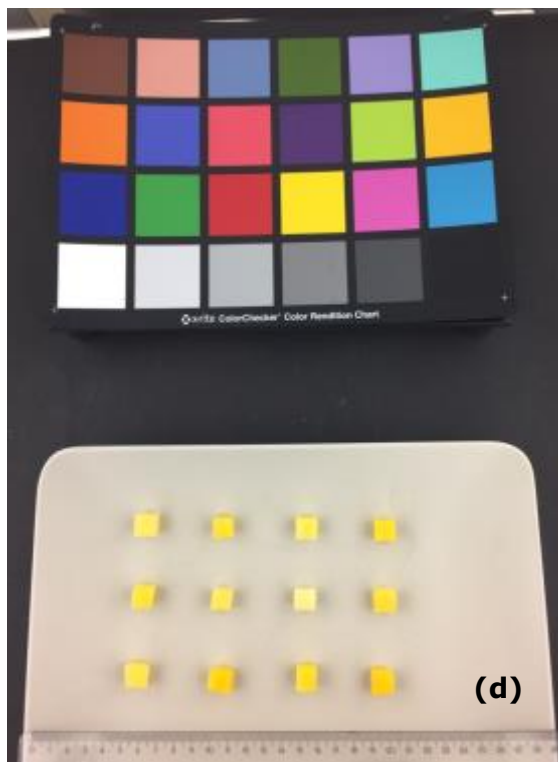
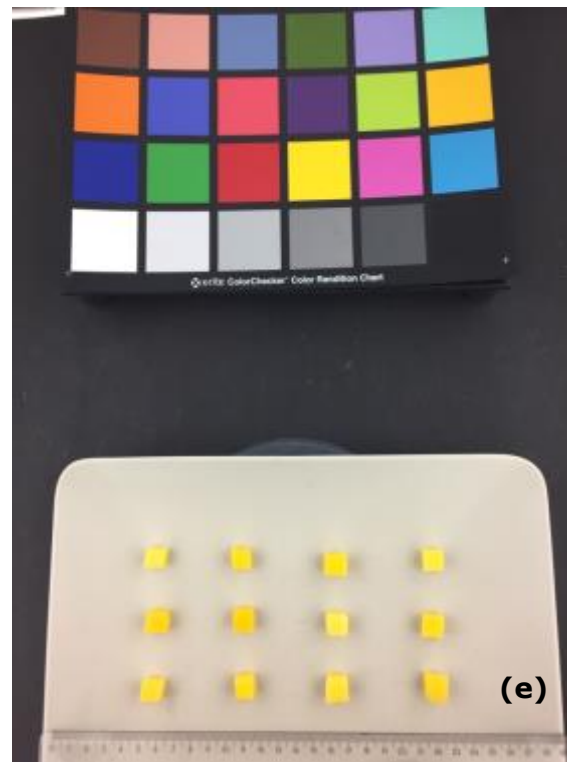


Figure 9.3 (d) 2h no PME



(e) 2h PME

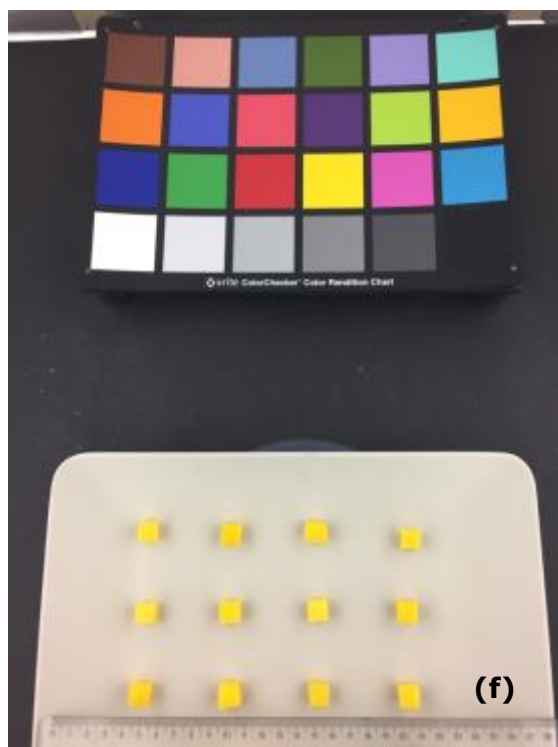
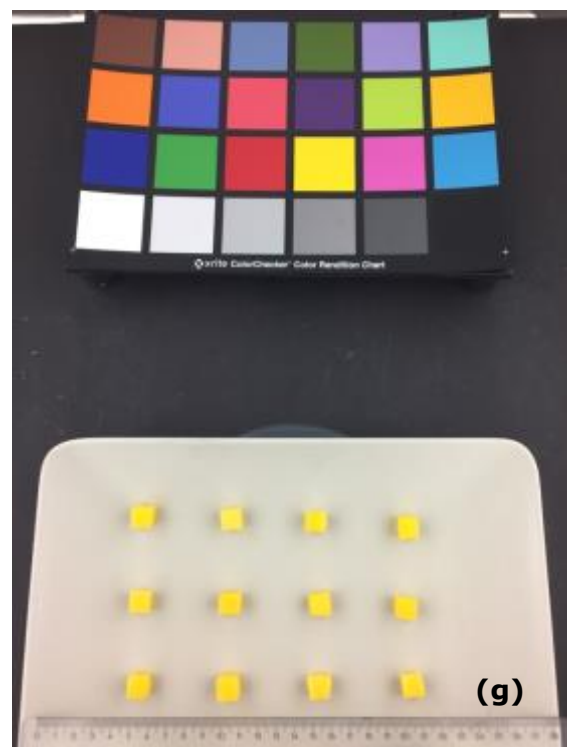


Figure 9.3 (f) 4h no PME



(g) 4h PME

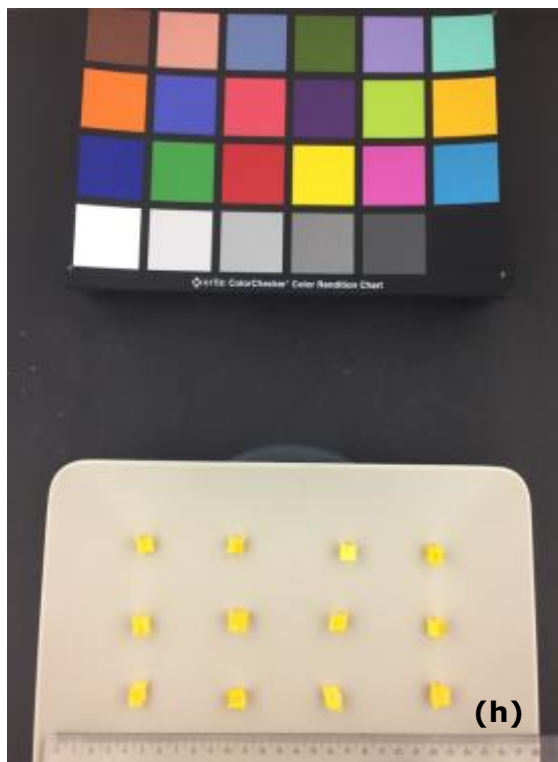


Figure 9.3 (h) HAD Fresh

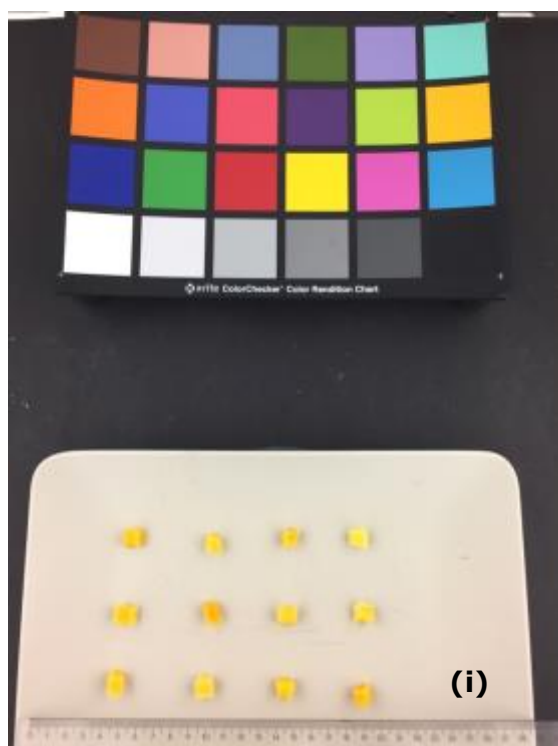
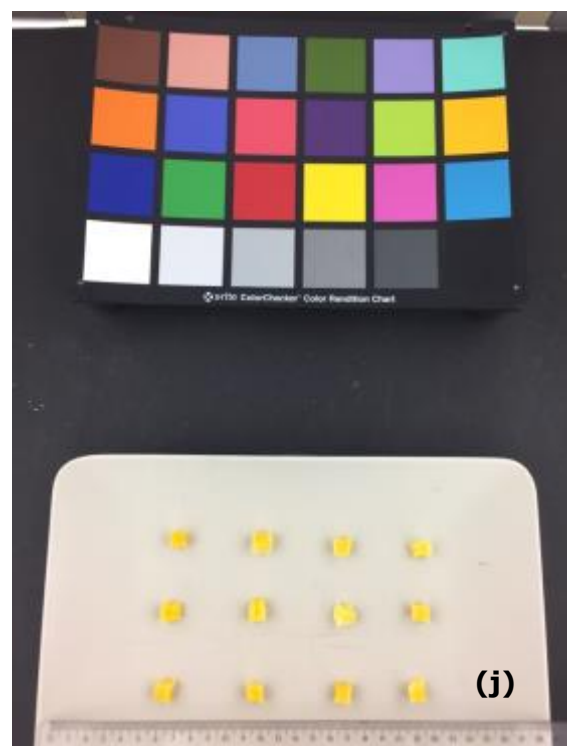


Figure 9.3 (i) HAD 2h no PME



(j) HAD 2h PME