# EFFECTS OF THERMOSONICATION ON WATERMELON RIND-HONEY BEVERAGE

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## ABSTRACT

This study addressed the recent interest in utilizing waste as source of natural food product to build a safer environment. The sample used was watermelon rind, a by-product of an edible fruit, often ignored for its bland taste. Thermosonication is a method of treating food beverage to improve product quality, but its effects, specifically on rind beverage has not been extensively studied. This study determined the effects of temperature and time (optimization using Response Surface Methodology) on the physicochemical, vitamin C content and microbial load of rind beverage stored under different temperatures for a week. Thermosonication at 65 ºC for 60 min significantly affected juice separation (stored under 4 ºC in a week) (11.3%), TSS (12.9 °Brix), the total color difference value (1.7) and microbial load (6.2 log CFU/mL). Themosonicated rind juice can be stored longer at temperature below 4ºC, which is beneficial to both the consumers and the country at large.

*Keywords*: physicochemical, storage, thermosonication, vitamin C, waste, watermelon rind

## 1. INTRODUCTION

The evaluation on fruit waste or fruit by-product has become a subject of interest in the food industry. The aim is to promote the use of natural waste in food application for a safer cause and a cleaner environment. Fruits such as watermelon, banana and papaya are examples of functional food with high fiber and nutrition value. Their intake helps to reduce the risk of cardiovascular disease such as coronary heart disease and stroke (WU *et al*., 2015). Previous studies have reported that nutrient contents are higher in fruit peels and seeds than in the fruit pulp (MORAIS *et al*., 2015; MOO-HUCHIN *et al*., 2015; SANTOS *et al*., 2014).

The unappealing taste of watermelon rind has been the main reason for discard despite its edibility and health-promoting content (AL-SAYED and AHMED, 2013). Recent studies claimed watermelon rind act as a good thickening, foaming and emulsifying agent, as it contains pectin and large quantity of antihypertensive and antioxidant properties due to its polysaccharides content (PETKOWITCZ *et al*., 2017; ROMDHANE *et al*., 2017). Watermelon rind was also used as wheat flour substitute in cake, pan bread, cookies and yellow noodles (AL-SAYED and AHMED, 2013; EL-BADRY *et al*., 2014; NAKNAEN *et al*., 2016; HO and DARCI, 2016). A mixture of 5% flour and 10% fat with watermelon rind powder slowed down staling and inhibited lipid oxidation and free fatty acids formation during storage (AL-SAYED and AHMED, 2013). The substitution had positive effects on human health including antioxidant activity that scavenges free-radicals (LEONG and SHUI, 2002; LEWINSOHN *et al*., 2005), converts citrulline to arginine for boosting the immune system, circulatory system and heart, as well as relaxing blood vessels in cases of cardiovascular diseases and cancer (RIMANDO and PERKINS-VEAZIE, 2005). However, research on the application of watermelon rind as juice beverage seems limited.

Honey was chosen as sweetener for the rind juice because it has better antibacterial and medicinal properties compared to sugar (MANDAL and MANDAL, 2011) as well as its common use as preservative (BOGDANOV *et al*., 2008). A milk sample that contains honey lasted longer and inhibited bacterial growth better than the sample without honey at 4ºC storage (KRUSHNA *et al*., 2006). The efficient antibacterial activity of honey in food was due to its hydrogen peroxide and polyphenol contents (WHITE, 1978; SNOWDON and CLIVER, 1996). Besides, high sugar-sweetened beverage intake contributes to heart complication, rise in blood pressure, weight-gain and cavities (CORLISS, 2016).

In beverage production, heat treatment is applied to maintain food stability and sensory quality. Pasteurization is the conventional method used to extend the shelf-life of fruit juices by applying heat that kills or inactivates certain enzymes and microorganisms (yeasts, molds and bacteria), which contribute to the juice's spoilage (POLYDERA *et al*., 2003). However, the heating effect of pasteurization was found to detract the natural quality of fruit juices, resulting in flavor loss and other changes. The thermal application also decreases the product's physicochemical and nutritional values like vitamin C and E contents, polyphenols, pH and color (DUBROVIC *et al*., 2011; GINER *et al*., 2013; SANTHIRASEGARAM *et al*., 2013). A study of the pasteurization effect on physicochemical properties of Physalis (*Physalisperuviana* L.) juice reported that pasteurization at 90ºC for 2 min significantly improved the juice's organoleptic characteristics and reduced the ascorbic acid content from 38.90 to 30.20mg/100g during storage. The ascorbic acid content of the pasteurized juice was lower than the ascorbic acid content preserved in the fresh Physalis juice during storage (RABIE *et al*., 2015). Thus, alternative technologies such as ultrasound and high hydrostatic pressure were developed to reduce the effects on product quality. Ultrasound is sound waves having frequency that exceeds 20 kHz, which can affect the physical, mechanical and chemical properties of food (AWAD *et al*., 2012). The application of ultrasound in food processing and food preservation can improve the food texture and flavor by enhancing heat and mass transfer processes. The treatment also serves as assistance to the existing thermal treatments to compensate for the loss of nutritional values caused by heat (KNORR *et al*., 2011). Previously, low power ultrasound had been used in treating vegetables and fruits in pre- and postharvest applications (AWAD *et al*., 2012).

Ultrasound treatment alone gives low germicidal effect since the process greatly depends on microorganisms' type, processing parameters and sonication medium in microbial destruction (CHENG *et al*., 2007). The combination of ultrasound and moderate heating, known as thermosonication, inactivates heat-resistant enzymes and kills microorganisms at lower temperature within a shorter period (ABDULLAH and CHIN, 2014; ABID *et al*., 2014). A study of the thermosonication effect on tomato showed that the treatment was effective in inactivating the enzyme pectin methylesterase, which degrades pectin and reduces the viscosity of tomato juice (ESKIN, 1979). There are also studies, which reported that thermosonication effectively inactivated various enzymes including polyphenoloxidase (CHENG *et al*., 2007), peroxidase (ERCAN and SOYSAL, 2010; GAMBOA *et al*., 2012) and polygalacturonase (TEREFE *et a.l*, 2009). Instead of relying on sound waves alone to initiate bubble cavitation, thermosonication involves both sound waves and temperature, where the temperature is controlled to produce maximum cavitation bubbles for juice extraction (PATIST and BATES, 2008; HOLTUNG *et al*., 2011). Thus, more polyphenols can be pertained (ABID *et al*., 2014). Thermosonication treatment also increases microbial inactivation rate in fruit juice. The killing mechanism involved is caused by thinning of cell membranes, increasing localized heat and pressure as well as increasing the production of free radicals. The treatment with combination of heat and ultrasound resulted in extensive cell damage and breakage on *E. coli* K12 cells (LEE *et al*., 2009). Examples of fruit and vegetable juices that have been studied using combined treatment include Kasturi, lime juice (BHAT *et al*., 2011), apple juice (ABID *et al*., 2013), soursop juice (DIAS *et al*., 2015), grape juice (AADIL *et al*., 2015) and carrot juice (ZOU and JIANG, 2016). They produced better results than the juice subjected to only heat treatment. Although,several studies have been done on the effect of ultrasound on fruit juices, none has been done on the optimization of thermosonication condition on watermelon rind-honey beverage.

The application of thermosonication treatment with a short processing time in juice preparation has categorized it as requiring minimal processing for freshness and health purposes. However, the study on thermosonication in improving the quality of fruit waste juice beverage is yet to be extensively conducted. The objective of this study is to determine the effect of different thermosonication condition (temperature and time) on watermelon rind beverage containing honey by evaluating the physicochemical, vitamin C content and microbiological properties of the beverage stored under different conditions for a week.

## 2. MATERIALS AND METHODS

### 2.1. Watermelon sample and honey

Approximately 2.5 kg of red seedless watermelon kept as a whole fruit at room temperature and honey were purchased from the local market in Sri Serdang, Selangor.

Both watermelon and honey were stored in a chiller of 4ºC for 2 days and a week, respectively, before analysis.

### 2.2. Proximate analysis of raw watermelon rind

The proximate analysis was carried out on seeded red watermelon rind (SRWR) and seedless red watermelon rind (SLRWR) to determine which watermelon rind is more suitable in terms of high fiber content for the development of watermelon rind beverage containing honey. The proximate compositions include, crude fiber, crude fat, and ash content, crude protein, carbohydrate and moisture content (AOAC, 2006).

### 2.3. Preliminary experiment

A preliminary experiment was done to determine the acceptable sweetness of honey in watermelon rind (v/v %). A 12 mL honey was mixed in 100 mL watermelon rind juice to get a 12% watermelon rind- honey juice. A range of 12% to 17% of honey to watermelon juice mixture was prepared and distributed to untrained panelists. The result showed that more than 80% of participants selected 13% (v/v) as the most acceptable sweetness.

### 2.4. Preparation of watermelon rind juice

The watermelon was separated into respective parts of rind, flesh and skin using a stainless-steel knife and the rind was further cut into cubes. The rind was cleaned and washed before being put into the juice blender MIX-898M (Panasonic, Japan). After blending, the rind puree was obtained. The rind puree was then transferred to a cloth sieve and squeezed to obtain the juice. For control sample, 100 mL of watermelon rind juice was weighed using a weighing balance (Scaltec, UK) and was transferred into glass bottles with metal caps sterilized in boiling water (100ºC). Such method was used for the control sample since watermelon rind juice is consumed without any heat treatment and there has been no prior study on the development of watermelon rind juice. As for sample undergoing treatment, watermelon rind juice and honey were weighed before honey was mixed manually into watermelon rind juice using a spoon with 13% (v/v) in 100 mL watermelon rind juice. Once completely mixed, the juice was transferred into sterilized glass bottles sealed with metal caps prior to thermosonication treatment. Analysis was conducted for a week but signs of mold growth was visible on the surface of the control sample on day 4 of storage.

### 2.5. Thermosonication process

Thermosonication treatment was applied at three different temperatures, 25, 45 and 65ºC for 10, 35 and 60 min respectively, using an ultrasonic cleaner bath (DC150H; Delta, China). Thermosonication treatment is regarded as a better alternative method because it has better nutrient retention capacity than the conventional method (pasteurization at 71 to 82 ºC) of treating orange and lime juice without altering or degrading the nutritional contents of the beverages (KHANDPUR and GOGATE, 2016).

The ultrasonic cleaner bath is a rectangular container (300 x 160 x 150 mm) with the maximal tank capacity of 7.5 L with frequency of 40 kHz and power W. These parameters 150 were chosen based on the study of thermosonication of grapefruit juice (AADIL *et al.*,

2015), which also used ultrasonic cleaner bath to carry out ultrasonic treatment. Six liters of distilled water was poured into the bath as transmission medium.
The ultrasound treatment sends acoustic waves that creates bubbles, whereby these bubbles induce either stable cavitation or transient cavitation phenomena (CHOWDHURY and VIRARAGHAVAN, 2009; THANGAVADIVEL *et al*, 2012). Watermelon rind beverage was kept sterilized glass bottles with metal caps at room temperature (25ºC) and chill temperature (4ºC) for further analysis.

## 2.6. Experimental design

Response Surface Methodology (RSM) was used to determine the effect of two independent variables; temperature (25 to 65ºC) and duration (10 to 60 min) of ultrasound. These variables were chosen based on parameters of ultrasound on fruit and vegetable juices (BHAT *et al*., 2011; AADIL *et al*., 2015; ZOU and JIANG, 2016). In this experiment, 14 runs (Table 2) were generated from the central composite design (CCD) with two independent variables, each with three levels of low, center and high (Table 1).

**Table 1.** Level of independent variable using CCD.

| Independent variable | Independent variable level | | |
|---|---|---|---|
| | Low (-1) | Centre (0) | High (+1) |
| Temperature (ºC) | 25 | 45 | 65 |
| Time (min) | 10 | 35 | 60 |

**Table 2.** Generated experimental runs with variable combination obtained from CCD.

| Experimental run | Blocks | Independent variable | |
|---|---|---|---|
| | | Temperature (ºC) | Time (min) |
| 1 | 1 | 25 | 10 |
| 2* | 1 | 45 | 35 |
| 3 | 1 | 65 | 60 |
| 4* | 1 | 45 | 35 |
| 5 | 1 | 65 | 10 |
| 6* | 1 | 45 | 35 |
| 7 | 1 | 25 | 60 |
| 8 | 2 | 45 | 10 |
| 9 | 2 | 25 | 35 |
| 10* | 2 | 45 | 35 |
| 11 | 2 | 45 | 60 |
| 12* | 2 | 45 | 35 |
| 13 | 2 | 65 | 35 |
| 14* | 2 | 45 | 35 |

*Centre point.

## 2.7. pH

The rind beverage pH was determined using calibrated pH meter model Jenwah 305, (Keison, UK). Approximately 50 mL of beverage was placed in a 200 mL beaker and stirred continuously while inserting the meter rode into the beaker (AOAC, 2006). The pH value was taken and comparison was done between day 1, 4 and 7 of storage.

## 2.8. Separation (%)

The rind beverage separation was determined by thoroughly mixing the beverage and then transferring 100 mL of beverage into 100 mL graduated measuring cylinder. It was left to stand for 30 min at room temperature. Then, the volume of the top clear beverage serum was recorded by reading the level of measuring cylinder. The readings of separation were recorded and comparison was done between day 1, 4 and 7 of storage. The determination of separation was done using FoodTech Method 17 (FMC, 2005).

## 2.9. Total soluble solid (°Brix)

The total soluble solid of watermelon beverage was evaluated using handheld refractometer (0-32°Brix). A drop of watermelon beverage was placed and spread on the refractometer window and the °Brix value was determined. The total soluble solid values were taken and compared between day 1, and 7 of storage. The determination of total soluble solid was done according to AOAC (2006).

## 2.10. Color

The color of watermelon beverage containing honey was determined using Hunter Lab UltraScan PRO colorimeter (Hunter Associate Laboratory, International Commission, Reston, USA) with EasyMatch QC software. The measurement L* (lightness), a* (redness) and b* (yellowness) color scale was taken with Regular Transmission (RTRAN) mode. The samples were placed in a transparent rectangular transmission cell unit until full to avoid air space inside the transmission cell that will interfere with the reading of color measurement. Analysis was done in triplicates to obtain accurate data analysis (PATHARE *et al.*, 2013; ASSAWARACHAN and NOOMHORN, 2010). Total color difference (TCD) value was also calculated using the following equation:

$$\sqrt{(L*-L_{\circ}*)^2+(a*-a_{\circ}*)^2+(b*-b_{\circ}*)^2} \quad (1)$$

where;
$L^*$ = $L^*$ for sample
$L_{\circ}^*$ = $L^*$ for control
$a^*$ = $a^*$ for sample
$a_{\circ}^*$ = $a^*$ for control
$b^*$ = $b^*$ for sample
$b_{\circ}^*$ = $b^*$ for control

## 2.11. Vitamin C content (ascorbic acid)

The analysis was conducted to estimate and compare the content of ascorbic acid between day 1, 4 and 7 of storage using the AOAC International method 967.21 (HORWITZ and LATIMER, 2006). Metaphosphoric acid-acetic acid solution was prepared by adding 100 mL deionized distilled water with 20 mL of acetic acid (Emsure, Germany). Then, 7.5 g of metaphosphroic acid (Fisher Scientific, UK) was added and stirred. Mixture was diluted to 250 mL with distilled water.

The mixture was filtered into an amber bottle with lid using a filter paper and refrigerated until further use. Ascorbic acid standard solution was prepared by weighing approximately 50 mg of ascorbic acid (Emsure, China). Thereafter, it was diluted to 50 mL with prepared metaphosphoric acid-acetic acid immediately before use.

In preparing indophenol solution-dye, 50 mL deionized water and 42 mg sodium carbonate were added in a 150 mL beaker and stirred. Then, 50 mg 2,6-dichloroindophenol sodium salt (Camlab, UK) was added. Mixture was diluted to 200 mL with deionized distilled water. The mixture was filtered into an amber bottle with lid and was refrigerated until further use. Two milliliters of standard ascorbic acid solution in 5 mL metapshosphoric acid- acetic acid solution was titrated against the dye solution until a light yet distinct rose pink color was obtained. The initial and final burette reading was recorded.

Five milliliters metaphosphoric acid- acetic acid solution and 2 mL of watermelon rind beverage was pipetted into 50 mL Erlenmeyer flask and it was titrated against the dye solution until a light yet distinct rose pink color was obtained. The initial and final burette reading was recorded. The amount of ascorbic acid was estimated using the following equation:

$$\text{mg of ascorbic acid/ g or mL of sample} = (X\text{-}B) \times (F/E) \times (V/Y) \qquad (2)$$

where;
X = average mL for test solution titration
B = average mL for test blank titration
F = mg ascorbic acid equivalents to 1.0 mL indophenol standard solution
E = sample weight (g) or volume (ml)
V = volume of initial test solution
Y = volume of test solution titrated

## 2.12. Microbiological analysis

Total plate count, yeast and mold count were determined to compare results between day 1, 4 and 7 of storage using the spread plate method. For total plate count, Plate Count Agar (PCA) was used. For yeast and mold count, Potato Dextrose Agar (PDA) was used. A series of dilutions ($10^{-1}$ to $10^{-5}$) were made from watermelon rind beverage sample in 0.1% peptone water. Then, 0.1 mL of dilution from the appropriate desired dilution series ($10^{-3}$ to $10^{-5}$) were pipetted onto the center of the agar plate surface. L-shaped glass spreader or hockey stick was dipped into alcohol and flamed over a Bunsen burner. The plates were incubated at 37ºC for 24 h. The log CFU/mL value of the sample was calculated using Eq. (A.3). The determination of PCA and PDA was achieved 21 using FDA's standard method of Bacteriological Analytical Manual (FDA, 2001).

CFU/ml = log [(no. of colonies x dilution factor of plate)/ aliquot plated] (3)

## 3. RESULTS AND DISCUSSION

### 3.1. Proximate composition of SRWR and SLRWR

Researches on nutritional content of watermelon flesh have been done widely (SABEETHA *et al.*, 2017; NONGA *et al.*, 2014; TLILI *et al.*, 2011; YAU *et al.*, 2010), however, very few research has been done on the watermelon rind. The knowledge of nutritional content of watermelon rind can reassure consumers to accept the rind as a potential food product instead of it being considered as waste (FILA *et al.*, 2013). The proximate analysis for SRWR and SLRWR was conducted to investigate which type of rind is more suitable to be applied in this study. Table 3 shows the result of proximate analysis for SRWR and SLRWR.

**Table 3.** Proximate analysis of SRWR and SLRWR.

| Proximate composition (%) | Red watermelon rind | |
|---|---|---|
| | Seeded (SRWR) | Seedless (SLRWR) |
| Moisture | 90.69a±1.31 | 87.42b±0.49 |
| Ash | 5.03a±0.78 | 5.00a±0.40 |
| Crude protein | 0.39a±0.09 | 0.57a±0.10 |
| Crude fat | 0.49a±0.29 | 0.70a±0.26 |
| Crude fiber | 2.10b±0.76 | 4.48a±0.65 |
| Carbohydrate | 1.30a±0.31 | 1.83a±0.50 |

Data are mean±SD of three replicates. Values with the same letter within a row is not significantly different at 5% level ($p<0.05$).

As shown in Table 3, there were no significant difference in ash, crude protein, crude fat and carbohydrate between SRWR and SLRWR. However, the moisture content in SRWR (90.69±1.31) was significantly higher than in SLRWR (87.42±0.49). Also, the crude fiber content in SLRWR (4.48±0.65) was significantly higher than in SRWR (2.10±0.76). Soluble fiber intake from juice makes it easier for absorption of vitamins, minerals and important phytonutrients (RUIZ-GUTIÉRREZ *et al.*, 2014). When compared with other studies, it was observed that the rind (0.30g/100g) does have higher content of fiber compared to the flesh (0.19g/100g) (FILA et al., 2013). Therefore, SLRWR was selected to be used in the development of watermelon rind beverage containing honey since it has a significantly higher crude fiber content than SRWR.

### 3.2. Fitting the RSM to significant independent variable

The effects of temperature and time of thermosonication on the watermelon rind beverage containing honey was studied using central composite design (CCD). The response variables are pH and separation. Table 4 shows the experimental data obtained for both response variables. The linear, quadratic and interaction effects of time (x1) and temperature (x2) on each response variables (yi) of watermelon rind beverage containing honey are shown in Table 4.

**Table 4.** The matrix of central composite design (CCD) and experimental data obtained for the response variables analysed; pH (Y1) and separation (Y2) (mean±SD).

| Run Order | Blocks | Independent variables<br>Temperature (°C) | <br>Time (min) | Response variables<br>pH (Y1) | <br>Separation (Y2) |
|---|---|---|---|---|---|
| 1 | 1 | 25 | 10 | 5.6±0.0 | 36.0±1.0 |
| 2* | 1 | 45 | 35 | 5.7±0.0 | 29.0±6.0 |
| 3 | 1 | 65 | 60 | 5.9±0.0 | 5.0±0.0 |
| 4* | 1 | 45 | 35 | 5.7±0.1 | 28.5±5.5 |
| 5 | 1 | 65 | 10 | 5.7±0.1 | 34.0±1.0 |
| 6* | 1 | 45 | 35 | 5.7±0.1 | 35.0±0.0 |
| 7 | 1 | 25 | 60 | 5.6±0.0 | 29.5±6.5 |
| 8 | 2 | 45 | 10 | 5.7±0.1 | 37.0±0.7 |
| 9 | 2 | 25 | 35 | 5.6±0.0 | 35.0±0.7 |
| 10* | 2 | 45 | 35 | 5.7±0.1 | 27.0±8.0 |
| 11 | 2 | 45 | 60 | 5.7±0.1 | 13.0±0.7 |
| 12* | 2 | 45 | 35 | 5.6±0.1 | 36.0±2.0 |
| 13 | 2 | 65 | 35 | 5.9±0.1 | 17.5±5.5 |
| 14* | 2 | 45 | 35 | 5.6±0.1 | 30.0±0.7 |

*Centre point.

The estimated regression coefficients for the response variables with their corresponding R1, R2 (adj), F-value and p-value of lack of fits are also included in Table 5.

**Table 5.** Regression coefficient, R2, adjusted R2, probability values and lack of fit for the final reduced models.

| Regression coefficient | pH (Y1) | Separation (Y2) |
|---|---|---|
| b0 | 5.7 | 30.2 |
| b1 | 0.1* | -7.3* |
| b2 | 0.1* | -9.9* |
| b21 | 0.0 | -1.9 |
| b22 | 0.0 | -3.2 |
| b12 | 0.1* | -5.6* |
| R2 | 0.9 | 0.9 |
| R2 (adj) | 0.8 | 0.9 |

*Significant level at $p < 0.05$ bi: the estimated regression coefficient for the main linear effects. bii: the estimated regression coefficient for the quadratic effects. bii: the estimated regression coefficient for the interaction effects. x1= time, x2= temperature

Table 5 shows the estimated regression coefficients of the final reduced model. The linear, quadratic and interaction effects of time (x1) and temperature (x2) on pH (y1) and separation (y2) were studied. The results showed that a high coefficient of determination (R2 > 0.8) was achieved for all regression models. Hence, it is concluded that more than 80% of the variation of the response can be accurately explained as a function of time and temperature of thermosonication treatment.

The experimental and predicted values for both response variables at optimum and least optimum conditions showed no significant difference ($p > 0.05$). Hence, RSM was effective in estimating the effects of thermosonication conditions on pH and separation of watermelon rind beverage containing honey.

This was seen in another study of ultrasonic treatment of soursop juice, where RSM was demonstrated to be an effective technique for investigating the effects of ultrasound intensity and processing time on polyphenol oxidase residual activity, temperature increase, phenolic compounds, ascorbic acid content and total color difference (TCD) on soursop juice. The predicted and experimental values showed no significant differences (DIAS *et al*., 2015). Similar result was observed in another study of enzymatic inactivation and antioxidant properties of blackberry juice using thermoultrasound, where time and temperature showed high correlation coefficients with the mathematical model of RSM. The predicted and experimental values for pectin methylesterase residual activity, polyphenol oxidase residual activity, diammonium salt, ascorbic acid, total phenolic compounds and anthocyanins of blackberry juice were not significantly different (CERVANTES-ELIZARRARAS *et al*., 2017).

### 3.3. Thermosonication treatment conditions

In thermosonication for juice treatment, temperature and time are examples of factors that influence the fruit juice quality. This experiment employed preliminary treatment time from a previous study of ultrasound effect on grapefruit juice, with treatment time ranging from 0 to 90 min at 20ºC to 60ºC (AADIL *et al*., 2015). The result showed that grapefruit juice sonicated for 90 min had significantly higher total carotenoids, lycopene, sugar contents and phenolic compounds with significant decrease in viscosity and microorganisms load (AADIL *et al*, 2015).

From the RSM model, the optimum condition was determined based on the pH and lowest separation (%) of watermelon rind juice. From Tables 6 and 7, the experimental and predicted values for both response variables at optimum and least optimum conditions showed no significant difference. Hence, RSM is effective in estimating the effects of thermosonication conditions on pH and separation of watermelon rind beverage containing honey.

### 3.4. Thermosonication effect on physicochemical and microbiological properties of control and treated rind beverage

Acidity is usually correlated with the measure of pH (YAU *et al*., 2010). The pH value is usually used to determine the processing requirements and for regulatory purposes (LIU *et al*., 2012). As shown in Table 8, no significant difference was observed between the pH values of control and treated beverages, indicating that thermosonication does not alter the pH of beverages. A study of ultrasound done on blackberry juice also showed no significant effect on the juice's pH value and the treatment was able to maintain the juice's pH value at 3.27 to 3.83 (RAMÍREZ-MORENO et al., 2017). The accepted range of pH for commercial non-dairy beverages is from 2.1 (lime juice concentrate) to 7.4 (spring water) (SEOW and THONG, 2005). The acidity of beverage has to be controlled as it is one of the major causes of dental erosion amongst consumers (REDDY *et al*., 2016).

**Table 6.** Values of response variables at good conditions (n=3).

| **Response** | **Watermelon rind beverage containing honey** | |
|---|---|---|
| | Predicted | Experimental |
| pH | 5.9a | 5.0a |
| Separation (%) | 5.2b | 5.3b |
| **Factors** | **Optimum condition** | |
| Time (min) | 60 | |
| Temperature (ºC) | 65 | |

Values with different letters within a row is significantly different at 5% level ($p < 0.05$).

**Table 7.** Values of response variables at the least good conditions (n=3).

| **Response** | **Watermelon rind beverage containing honey** | |
|---|---|---|
| | Predicted | Experimental |
| pH | 5.4a | 5.2a |
| Separation (%) | 39.6b | 40.5b |
| **Factors** | **Least optimum condition** | |
| Time (min) | 10 | |
| Temperature (ºC) | 25 | |

Values with different letters within a row is significantly different at 5% level ($p < 0.05$).

Separation is an important factor for consumer preference as it contributes to the physical appearance of juice. It measures the suspension degree of existing solid particles in the fruit juice to reduce layering. A comparison between juice separation (%) for treated and non-treated watermelon rind juice was done. The results in Table 8 shows that the juice separation (%) for control, optimum and least optimum are all significantly different from one another, showing temperature and time did influence juice separation (%). Watermelon rind juice treated under elevated temperature caused a significant decrease in juice separation (%). High temperature increases the kinetic movement of juice molecules which make it to collide with one another more rapidly. The mechanical forces developed from bubble cavitation disintegrate larger molecules into smaller molecules, hence, enhancing the solubility of juice molecules (DEMIRDOVEN and BAYSAL, 2008; SANTHIRASEGARAM, 2013). As thermosonication variables (time and temperature) increases, size of the particles in cantaloupe and grapefruit juice reduces, thereby providing better uniformity and stability which lead to reduced juice separation (AADIL *et al*., 2015; FONTELES *et al*., 2012).

Separation is one of the most common problems in fruit juice production, which makes it difficult to maintain the solids in suspension or dispersion in the beverage over a long period of time (DE-LEON and BOAK, 1984). It was shown that as temperature increases, the cloudiness of juices also increases (CERVANTES-ELIZARRARAS *et al.*, 2017). However, another study stated that ultrasonic helps to increase clarity in juices. Ultrasonic has been shown to activate pectinesterase, which is capable of destabilizing colloidal pectin molecules suspended in a juice, making the juice clearer (CHENG *et al.*, 2007).

The total soluble solid (TSS) of fruits depends mainly on the type of cultivar, fruit species, maturity status, agricultural practices and seasonal changes (NAYAK *et al.*, 2017; TASNIM *et al.*, 2010). As shown in Table 8, TSS of control is significantly lower than beverages treated under optimum and least optimum conditions. The significantly different TSS value may be due to the addition of honey in the treated beverages. However, there is no significant difference between the TSS value of beverage thermosonicated at optimum condition and beverage thermosonicated at least optimum condition. This indicates that thermosonication did not significantly affect the TSS of beverage. A study done on purple cactus pear juice using thermosonication reported that the treatment had minimum effect on the juice TSS (CRUZ-CASINO *et al.*, 2015). A study carried out by Abid *et al.* (2014) using thermosonication on apple juice also reported insignificant difference in the TSS of treated and non-treated apple juice. A combined treatment of thermosonication and pulse electric field on orange juice also resulted in no significant changes in TSS (WALKING-RIBEIRO *et al.*, 2009).

For color evaluation, there is no significant difference for L* value between control sample and sample thermosonicated at the least optimum conditions, but there is a significant difference for a* and b* values. The comparison between control sample and sample treated at optimum conditions showed no significant difference for b* value, whereas, there is a significant difference in L* and a* values. These results indicate that thermosonication greatly influenced the beverage color (lightness and redness).

The study shows that there is no significant difference ($p > 0.05$) in vitamin C content of beverage between control and the treated sample at the least optimum condition, while there is a significant difference ($p < 0.05$) between the vitamin C content of beverage treated under optimum condition with control beverage and beverage treated under least optimum condition. The statistic indicates that beverage treated with thermosonication at optimum conditions have lower vitamin C compared to untreated and treated beverages at least optimum conditions of thermosonication.

Vitamin C (ascorbic acid) is an important antioxidant that possess protective properties against some types of cancers (ADEKUNTE *et al.*, 2010). As shown in Table 8, there was no significant difference ($p > 0.05$) between control and thermosonicated sample at the least optimum condition. This result is similar with results obtained from studies on thermosonication of apple, watermelon and tomato juice (ABID *et al.*, 2013; RAWSON *et al.*, 2011; WALKING-RIBEIRO *et al.*, 2009). However, there was significant difference ($p < 0.05$) between control and thermosonicated sample at optimum condition similar to studies on thermosonication of orange and strawberry juice (TIWARI *et al.*, 2008; HART and HENGLEIN, 1985). Sample that was thermosonicated at optimum condition and stored at 25°C experienced complete loss of vitamin C compared to other treatment methods and storage conditions.

Microbiological analysis showed that the log CFU/mL of TPC and PDA for both sonicated samples reduced significantly compared to the control sample. This result is similar with the sonication of orange, strawberry, carrot and mango juice (ZOU and JIANG, 2016; SANTHIRASEGARAM *et al.*, 2013; TIWARI *et al.*, 2008; TOMADONI *et al.*, 2017). It was concluded that with the increase in ultrasound treatment time, destruction of microbes was greater (ZOU and JIANG, 2016). This was similar with the storage study of strawberry juice (TOMADONI, *et al.*, 2017). Ultrasound has also shown to have destructive effect on microorganisms in honey (PUTTONGSIRI and HARUENKIT, 2010). Reduction in microbial rate is a probable effect from total polyphenols increment, contributed by the release of free amino acids through the decomposing of cell structure, caused by ultrasound and thermal treatment (D'ARCY, 2017). The microbial inactivation by power ultrasound occurred due to cavitation, localised heating and free radical formation. Free radicals were formed during the application of ultrasound to liquids due to sonolysis of water, and these free radicals had a bactericidal effect.

**Table 8.** Effect of thermosonication conditions on physicochemical and microbiological properties of control beverage compared with treated beverage.

| Properties | | | Treatment | | |
|---|---|---|---|---|---|
| | | | Control | Optimum condition | Least optimum condition |
| **Physicochemical** | pH | | 5.47a±0.07 | 5.24a±0.01 | 5.49a±0.02 |
| | Separation (%) | | 51.0a±1.0 | 12.0c±2.7 | 32.7b±2.5 |
| | Total soluble solid (°Brix) | | 4.0b±0.0 | 13.0a±0.0 | 13.0a±0.0 |
| | Color values | L* | 69. 0b±2.0 | 72.7a±0.6 | 71.5ab±1.0 |
| | | a* | 2.1b±0.2 | 7.2a±0.1 | 7.0a±0.6 |
| | | b* | 30.1a±0.5 | 29.8ab±0.5 | 28.2b±1.0 |
| | | TCD | - | 6.5a±1.0 | 6.3a±0.9 |
| | Vitamin C content (mg/100 mL) | | 0.70a±0.00 | 0.00b±0.00 | 0.70a±0.10 |
| **Microbiological** | TPC (log CFU/mL) | | 7.2a±0.3 | 4.6b±0.7 | 5.3b±0.5 |
| | PDA (log CFU/mL) | | 6.5a±0.3 | 4.1b±0.1 | 4.2b±0.1 |

Data are mean±SD of three replicates Values with different letters within a row is significantly different at 5% level ($p < 0.05$).

### 3.5. Thermosonication effect on physicochemical and microbiological properties of control and treated rind beverage stored at 25°C and 4°C for a week

As shown in Table 9, all sonicated samples treated at optimum and the least optimum condition stored at 25ºC and 4ºC showed significant decrease in pH within a week of storage. This is due to the increase in lactic acid bacteria and the possible formation of hydroxyl methyl furfural throughout the storage period as well as the increase in titrable acidity (BHARDWAJ *et al*, 2005; KHANDPUR & GOGATE, 2016; WANG *et al*., 2005). The values of pH for stored watermelon rind beverage containing honey after being treated with thermosonication ranges from 3.77 to 5.49 and it has already been stated that most beverages or juice have their pH ranges between 3.5 and 5.5 during storage (PEARSON, 1995). Therefore, from the results, the pH of sonicated watermelon rind beverage containing honey is still in the acceptable range even for a week storage.
A comparison for juice separation (%) between juices stored under 25 and 4°C for 7 days was done. The separation (%) of rind juices thermosonicated at 65°C for 60 min showed significant gradual increase from day 1 to day 7 when stored under 25°C. Fruit juices have the same taste, color and aroma as their whole fruit because mechanical separation exerts minimal effect on the juices' physicochemical properties. Thus, they still contain colloids

(pectin, proteins and polyphenols) and fibers, contributing to the cloudy appearance of juice. Presence of pectin hinders the formation of aggregation of juice particles and prevents floating substances from settling (HUI *et al*., 2006). During storage under ambient temperature, the increase in separation may be due to the breaking down of pectin by pectinase into simple sugars. Hence, the juice separation is harder to maintain when stored under ambient temperature. At storage temperature of 4°C, the low temperature and high relative humidity inhibits enzyme activities, thereby, minimizing the increase of separation in juice (SINGH and MATHUR, 1983). The separation (%)of rind juice thermosonicated at optimum condition and rind juice thermosonicated at least optimum condition differed significantly, however, the increase in separation (%) through day 1 storage until day 7 storage is still temperature dependent.

The TSS of beverages for both treatment showed no significant decrease throughout the storage period under both storage conditions. A similar result was obtained from storage study of strawberry ultrasonicated at 40 kHz for 10 and 30 min, where control (untreated juice), thermally treated juice (pasteurization at 90ºC for 60 s) and ultrasonicated juice showed no significant difference in TSS after 10 days of storage at 5°C (TOMADONI *et al*., 2017).

For color analysis, the a* value significantly decreased from day 1 to day 7 for all sonicated samples. The beverage thermosonicated at optimum conditions and stored at 25 and 4oC showed decreasing TCD value during a week of storage, while beverage thermosonicated at least optimum condition and stored at 25 and 4oC showed inconsistent change in TCD value during a week of storage. This shows that low temperature and short duration of sonication and storage at 25oC has the highest negative impact on the beverage color.

Among the primary factors of consumers' evaluation on the freshness of fruits and vegetable products is the color attribute. Better color characteristics will give better appeal to the consumers (KAYS, 1999). The increase in L* was similar with studies of ultrasonic treatment for orange, strawberry, soursop, watermelon and mango juice (DIAS *et al*., 2015; RAWSON *et al*., 2011; SANTHIRASEGARAM *et al*., 2013; TIWARI *et al*., 2008; TOMADONI *et al*., 2017). The TCD value of beverage was thermosonicated at optimum and the least optimum condition was more than 2, which indicated that there were visually noticeable differences in the sonicated samples compared to control. However, on the seventh day of storage, the color difference between the beverage thermosonicated at optimum condition and the control cannot be visually perceived.

A study done on purple cactus pear showed that the color of juice thermoultrasonicated at 80% for 25 min was not visually different from control at the first day of storage only, but afterwards, the color of thermosonicated juices were visually different from control (CRUZ-CASINO *et al*., 2015). The cavitation caused by ultrasonic may accelerate chemical reactions, increasing diffusion rate, dispersion, aggregates formation and particles breakdowns which leads to color changes in treated beverages (SALA *et al*, 1995). Increase in L* value was also observed in sonicated honey, which were due to the reduction in crystal size (QUINTERO-LIRA *et al*., 2017). The L* value of sample sonicated at the least optimum condition and stored at 25ºC had significantly increased, similar to the result of sonicated orange juice after storage (TIWARI *et al*., 2008), whereas the sample sonicated at least optimum temperature and stored at 4ºC showed a decrease in day 4 and an increase in day 7. Studies showed that an increase in L* value was due to the partial precipitation of unstable suspended particles, whereas, a decrease is due to oxidative darkening (GENOVESE *et al*., 2006).

This result is similar with thermosonicated strawberry juice (HERCEG *et al*., 2013). Studies show that a decrease in a* and b* values might be attributed to the development of browning degree in the beverage (IBARZ *et al.*, 2005). The results show that low temperature and short duration of sonication and storage at 25°C has highest negative impact on color. Based on the study of thermosonication on purple cactus pear juice, changes in color may be due to acceleration of chemical reactions by the increase in temperature, dispersion, particles breakdown and formation of aggregates by cavitation of thermosonication (CRUZ-CASINO *et al.*, 2015).

The degradation of vitamin C as shown in Table 9 could be due to the formation of free radical by sonication reaction, associated with the oxidative process (AGUILAR *et al.*, 2017).

**Table 9.** Effect of thermosonication conditions on physicochemical properties of beverage stored at 25°C and 4°C for a week.

| Physicochemical properties | | Storage (Day(s)) | Storage condition 25°C | | 4°C | |
|---|---|---|---|---|---|---|
| | | | Treatment | | | |
| | | | Optimum | Least optimum | Optimum | Least optimum |
| pH | | 1 | 5.24aA±0.01 | 5.49aA±0.02 | 5.24aA±0.01 | 5.15aA±0.02 |
| | | 4 | 4.39bB±0.01 | 4.30bB±0.01 | 4.91bB±0.01 | 4.98bB±0.01 |
| | | 7 | 3.77cC±0.01 | 3.78cC±0.01 | 4.14cC±0.01 | 4.32cC±0.01 |
| Separation (%) | | 1 | 21cC±2 | 36cA±1 | 7bD±1 | 31bB±1 |
| | | 4 | 53bB±1 | 61bA±1 | 10aD±2 | 36aC±2 |
| | | 7 | 84aB±1 | 91aA±1 | 11aD±1 | 39aC±1 |
| Total soluble solid (°Brix) | | 1 | 13.0aA±0.0 | 13.0aA±0.0 | 13.0aA±0.0 | 13.0aA±0.0 |
| | | 4 | 13.0aA±0.1 | 13.0aA±0.1 | 13.0aA±0.1 | 13.0aA±0.1 |
| | | 7 | 12.9aA±0.1 | 12.9aA±0.1 | 12.9aA±0.1 | 12.9aA±0.1 |
| Color | L* | 1 | 73.0aA±0.5 | 72.8bA±2.5 | 72.4aA±0.4 | 73.4bA±0.1 |
| | | 4 | 69.9bC±0.2 | 78.1abA±2.2 | 72.0aB±0.2 | 71.1cB±0.8 |
| | | 7 | 69.0bC±0.7 | 79.0aA±1.9 | 68.9bC±0.1 | 75.6aB±0.6 |
| | a* | 1 | 8.2aAB±0.1 | 6.4aB±2.0 | 8.0aAB±0.0 | 9.2aA±0.0 |
| | | 4 | 4.9bA±0.1 | 5.2aA±0.2 | 6.3bA±1.1 | 5.7bA±0.2 |
| | | 7 | 3.1cA±0.1 | 0.3bB±0.2 | 3.1cA±0.1 | 0.1cB±0.1 |
| | b* | 1 | 30.1bAB±0.1 | 27.4aB±2.3 | 30.7cA±0.1 | 30.4cA±0.1 |
| | | 4 | 23.3cC±0.2 | 20.0bD±0.1 | 32.9aB±0.1 | 33.2aA±0.1 |
| | | 7 | 31.7aA±0.2 | 28.3aB±0.8 | 31.3bA±0.2 | 32.3bA±0.1 |
| | TCD | 1 | 7.9aA±0.9 | 6.9bA±1.3 | 6.8abA±0.2 | 8.4aA±0.0 |
| | | 4 | 7.5aB±0.2 | 13.9aA±1.4 | 5.9aBC±0.8 | 5.3cC±0.2 |
| | | 7 | 2.0bC±0.3 | 10.3abA±1.9 | 1.7bC±0.2 | 7.3bB±0.6 |
| Vitamin C (mg/100 mL) | | 1 | 0.00aB±0.06 | 0.67aA±0.02 | 0.67aA±0.02 | 0.67aA±0.02 |
| | | 4 | 0.00aA±0.00 | 0.00bA±0.00 | 0.00bA±0.00 | 0.00bA±0.00 |
| | | 7 | 0.00aA±0.00 | 0.00bA±0.00 | 0.00bA±0.00 | 0.00bA±0.00 |

Data are mean±SD of three replicates. Values with different lower case letters within a column is significantly different at 5% level ($p<0.05$) between storage day(s). Values with different capital letters within a row is significantly different at 5% level ($p<0.05$) between storage conditions.

The vitamin C content for beverage treated at least optimum condition were retained for at least a day of storage under both temperatures (25°C and 4°C). Studies also showed that the degradation of vitamin C in juices is influenced greatly by the presence of air; therefore, juice should be degassed prior to treatment for better retention of ascorbic acid (OMS-OLIU *et al*., 2009). In some cases, degradation of vitamin C occur in order to protect other compounds such as carotenoids, phenolic compounds or anthocyanins (TIWARI *et al*., 2008). This shows that ascorbic acid is an unstable compound, making it very easy to degrade. Therefore, for better retention of vitamin C, milder processing conditions should be used (BASMACI, 2017).
As shown in Table 10, all sonicated samples showed an increase in log CFU/mL of TPC and PDA within a week of storage. For all samples, on PDA, there is only growth of yeast with no growth of fungi. From this experiment, the control sample would not be compatible for human consumption starting from day 0, since the maximum limit of microorganisms in minimally processed food is 7 log CFU/mL (TOMADONI *et al*., 2017). As for sample sonicated at optimum condition, stored at both 25 and 4ºC, the watermelon rind beverage containing honey were still fit for consumption even after reaching the 7th day of storage. The application of ultrasound treatment at high temperature can regulate microbial growth in watermelon juice containing honey at a level whereby the juice shelf life could be prolonged.

**Table 10.** Effects of optimum and least optimum sonication conditions on microbiological properties of sample stored at 25 and 4 ºC for a week.

| Microbiological properties | Storage (Day(s)) | Storage condition | | | |
|---|---|---|---|---|---|
| | | 25°C | | 4°C | |
| | | Treatment | | | |
| | | Optimum | Least optimum | Optimum | Least optimum |
| TPC (log CFU/mL) | 1 | 4.6bA±0.5 | 5.3bA±0.7 | 4.6bA±0.4 | 5.3bA±0.4 |
| | 4 | 5.5bA±0.5 | 6.2abA±0.3 | 5.0abA±0.6 | 6.2abA±0.8 |
| | 7 | 6.8aA±0.3 | 7.4aA±0.5 | 6.3aA±0.6 | 7.0aA±0.2 |
| PDA (log CFU/mL) | 1 | 4.2bA±0.5 | 5.2bA±0.3 | 4.1bA±0.3 | 5.2bA±0.6 |
| | 4 | 5.1bA±0.4 | 6.1bA±0.2 | 5.0abA±0.6 | 6.1abA±0.7 |
| | 7 | 6.8aA±0.7 | 7.2aA±0.5 | 6.2aA±0.5 | 7.2aA±0.4 |

Data are mean±SD of three replicates. Values with different lower case letters within a column is significantly different at 5% level ($p < 0.05$) between storage day(s). Values with different capital letters within a row is significantly different at 5% level ($p < 0.05$) between storage conditions.

## 4. CONCLUSIONS

This study shows that the optimum condition for thermosonication process for watermelon rind-honey beverage is at 65°C for 60 min. Thermosonication has no significant effect on the pH and TSS of beverage. However, throughout the storage period, the pH of beverage decreased, depending on the storage temperature but it is still safe for consumption. The % separation, color, vitamin C content and microbial load of beverage were all significantly affected by thermosonication. The changes in beverage pH, % separation and color during storage period were temperature dependent. All the results showed that the beverage treated with thermosonication can be preserved all through the storage period of a week. Therefore, it can be concluded that watermelon rind beverage containing honey can be better treated using thermosonication method rather than thermal treatment alone to obtain longer beverage shelf life. However, further enhancement study should be done for better vitamin C retention in fruit juices using thermosonication method.

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