WALAILAK JOURNAL
http://wjst.wu.ac.th Agricultural Technology and Biological Sciences

Characterization of Anti-Oxidative Cassava Starch Based Film Supplemented with *Anacardium occidentale* L. Leaf Extract

Supasit CHOOKLIN[*], Karoon REHBENMOOD, Jirassaya BUNMAK and Weerapong CHIANGSONG

Department of Food Science and Technology, Faculty of Agro-Industry, Rajamangala University of Technology, Srivijaya, Nakhon Si Thammarat Campus, Nakhon Si Thammarat 80240, Thailand

([*]Corresponding author's e-mail: supasit_c@yahoo.co.th, supasit.c2015@gmail.com)

Received: 12 April 2016, Revised: 18 March 2017, Accepted: 20 April 2017

Abstract

The objective of this research was to optimize cassava starch based film production using a response surface methodology (RSM) experimental design. It was found that solution weight, drying time and drying temperature contributed significantly to tensile strength and elongation at break. The following conditions: 15 g film solution, 36 h drying time and 50 °C drying temperature gave optimal tensile strength and elongation at break of 0.62 MPa and 107.74 %, respectively. Modelling helps to explain the correlation between response and regression variables ($P < 0.05$). Results also showed that the highest total phenolic content and antioxidant activity in the cashew leaf extract were 189.39 mg (gallic acid equivalent/g dried weight, GAE/g DW) and 1,093.90 mg vitamin C equivalent/g DW, respectively. Total phenolic content and antioxidant activity in cassava starch based film increased with an increase of the cashew leaf extract content in solid or liquid form compared with the control sample. An increase of the cashew leaf extract content in solid or liquid form compared with the control sample increased tensile strength and the b* value of cassava starch based film but decreased the elongation at break, L*, a* and transparency values of the cassava starch based film.

Keywords: Anti-oxidative film, cassava starch, phenolic compound, cashew leaf, response surface methodology

Introduction

An oxidative reaction is one among many important reactions that cause many chemical alterations in foods and food products e.g. flavor changes (rancidity), nutritional loss of many essential fatty acids and vitamins, shortened shelf-life and many other newly formed substances that are toxic to consumer's health [1]. So far, the food industry has been continuously endeavoring to find naturally occurring antioxidants rather than chemically synthetic antioxidants. Many reports have claimed toxicity of synthetic antioxidants in experimental animals and also organ dysfunction and eventually tumorigenic, carcinogenic and tetrarogenic substances when persistently exposed or overdosed [2-4]. Many synthetic antioxidants that were rather constantly used in foods and food products are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG). Therefore, in order to avoid and/or reduce the potential of its toxicity, synthetic antioxidants have been restricted both qualitatively and quantitatively and many naturally-occurring crude and extracted antioxidants have been proposed and investigated [5]. Many biologically active substances e.g. phenolic compounds, carotenoids play important roles to mop up free radicals. In addition, these antioxidants also possess anti-bacterial, anti-inflammatory, anti-mutagen and anti-cancer properties as well [6].

Naturally occurring antioxidants or phytonutrients are abundantly available in fruits and vegetables. In vegetables, there are many continuously extensive researches in relation to this topic. Thailand is located in the northern tropical area, and has high biological abundance and thus diverse sources of antioxidants, phytonutrients, dietary fiber and other vital nutrients [7]. There are numerous studies in relation to anti-oxidative activity derived from natural extracts based on various vegetables in many regions of Thailand [8]. In Southern Thailand, anti-oxidative potency of various native vegetables of various portions has been investigated. Among these high anti-oxidative vegetables with high phenolic content is a cashew leaf.

Anacardium occidentale L. (family Anacardiaceae) or cashew is a small-sized tree with a dome-shaped crown. The bark is brown or grey with longitudinal fissures. Leafs are simple, alternate, narrowly to broadly ovate with a rounded apex. When young, they are pliable and reddish, and are dark green and leathery with prominent yellow veins when mature. Traditionally, cashew leafs have also been used to treat rheumatic disorders and hypertension [9]. Phenolic compounds are a large group phytochemicals derived from secondary metabolism in plants that serve as powerful antioxidants. Antioxidant properties based on phenolic content and antioxidant activity of fresh leafs of cashew are 3,890 mg GAE/100 g and 6,620 mg ascorbic acid/100 g [9]. Moreover, the total polyphenol content of young cashew leafs, indigenously grown vegetables from Southern Thailand, is 4,075.79 mg GAE/100 g [10].

Most studies focuses on its direct supplementation in foods and results in retarding or hindering oxidative reactions. The direct addition of antioxidants in products, especially in foods, in one large initial dose is limited by the potential for rapid depletion of the antioxidants, in addition to very high initial concentration. Other interesting methods involve fortification as a part of packaging materials to extend their shelf-life by reducing related parameters e.g. oxygen concentration, light exposure, moisture content and temperature [11]. Past experiments on cassava based films revealed that the film is superior to other plant-based films in terms of its physical parameters e.g. turbidity, solubility, elongation and tensile strength. Possible mechanisms behind its superiority are its main components: flour that is made of amylose and amylopectin molecules strongly and continuously linked together to form a continuous molecular network and resulting in a vigorous film [12] and suitable for industrial applications.

Statistical experimental design techniques, especially the response surface methodology (RSM), are very useful tools for the optimizing process parameters. They can provide statistical models which help us to understand interactions among the parameters at varying levels and to calculate the optimal level of each parameter for a given target [13]. Therefore, this research aims to produce anti-oxidative films based on cassava starch, a proper addition of anti-oxidative substances from cashew leaf extract and investigate optimal conditions for film production by experimental design and results interpretation by means of RSM.

Materials and methods

Determination of an optimal condition for film production by RSM

A Box-Behnken design [13] with 3 variables including solution weight, drying time and drying temperature at 3 levels was followed to determine the response pattern and also to determine synergy of the variables. According to this design, 17 runs (**Table 1**) were conducted containing 3 replications at the central point for estimating the purely experimental uncertainty variance.

Films were prepared according to the casting technique. Briefly, 5 g of cassava starch was weighed, 1.5 g of glycerol (30 % of initial cassava starch weight) and 100 mL distilled water were mixed homogeneously. The mixture was allowed to stand at 80 °C for 15 min, then cooled to approximately 50 - 60 °C and poured into a mold by determining 3 levels of 3 parameters; 15, 30 and 45 g (20, 40 and 60 % by solution weight), mold size of 99×15 mm^2; 36, 48 and 60 h (drying time) and 40, 50 and 60 °C (drying temperature). These parameters were taken into account and were used to determine the series and experimental design [14]. After that, films were made as determined by the experimental design mentioned earlier and the films using various conditions were produced and their physical properties e.g. tensile strength and elongation at break were measured.

The relationship of the variables was determined by fitting a second order polynomial equation to data obtained from the 17 runs. Response surface analysis was based on the multiple linear regression which takes into account the main, quadratic and interaction effects, in accordance with the following equation;

$$Y = \beta_0 + \Sigma\beta_i x_i + \Sigma\beta_{ii} x_i^2 + \Sigma\beta_{ij} x_i x_j \quad (1)$$

where Y is the predicted response, x_i and x_j represent the variables or parameters, β_o is the offset term, β_i is the linear effect, β_{ij} is the first order interaction effect and β_{ii} is the squared effect. The goodness of fit of the model was evaluated by the coefficient of determination (R^2) and the analysis of variance (ANOVA). Response surface plots were developed to indicate optimum conditions using the fitted quadratic polynomial equations obtained by holding one of the independent variables at a constant value and changing the levels of the other 2 variables.

A preparation of cashew leaf extract

Fresh indigenous young cashew leafs were collected from Phatthalung province, Southern Thailand where these vegetables are abundantly available. Two to 3 kg of each food was purchased from local markets in the province. Samples were transported on ice covered or in a cool box to the Food Chemistry Laboratory, Department of Food Science and Technology, Faculty of Agro-Industry, Rajamangkala University Technology of Srivijaya, Nakhon Si Thammarat province within 24 h and were thoroughly washed, dried in air and then weighed. Analysis of moisture content was performed by an infrared moisture tester (Kett, FD-620, Japan). Then, the cashew leafs were tray-dried (OFM, SO-12, Thailand) at 60 °C until moisture content was below 10 %. Afterwards, samples were ground and sieved through 0.25 mm mesh, graded by their size and kept in aluminum foil laminated packaging at ambient temperature.

Determination of total phenolic content and anti-oxidative activity of the cashew leaf extract

3 g of cashew leafs were weighed and put into a 250 mL conical flask. 150 mL of 40 % (vv^{-1}) ethanol was added at a sample:solution ratio of 1:50 (wv^{-1}). Samples were ultrasonically extracted (CREST, 690DAE, 120 W, 45 MHz), at 30 °C, for 20 min [15] and after that the sample was centrifuged (HERMLE, Z36HK, 2,620 g·force, 4 °C, 5 min) and its supernatant and sediments were separated. Afterwards, the supernatant was evaporated on a rotary evaporator (100 - 150 mbar, 40 °C) to give a liquid extract. Phenolic content was analyzed by Folin Ciocalteu's method [16] and results expressed by comparison with a standard curve of mg gallic equivalent/g dried weight. Antioxidant potency was analyzed with the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method [17] and expressed as mg vitamin C equivalent /g dried weight and the results compared with a standard curve of mg vitamin C equivalent /g dried weight. The supernatant was dehydrated in a freeze-drier and used in its solid form.

DPPH radical scavenging activity

DPPH scavenging activity was determined using a modified method of Ohnishi *et al*. [17]. Free radical scavenging activity of crude extracts was tested, indicated by bleaching of the stable DPPH. A diluted extract of the right concentration, 0.3 mL of sample, was added to 1.5 mL of 0.1 mM ethanolic DPPH solution. The mixture was vortexed and allowed to stand at 37 °C. After 40 min, the absorbance was recorded at 517 nm. A control consisted of 0.3 mL of 99 % aqueous ethanol and 1.5 mL of 0.1 mM DPPH solution. DPPH % scavenging activity (%SA) was calculated %SA = (C–X)*100/C, where C was the absorbance of the control and X was the absorbance of the extract.

Antioxidant capacity

Interpretation of antioxidant capacity was made based on the ability of the crude extract to scavenge free radical DPPH compared to a standard antioxidant. In order to express antioxidant activity of crude extracts in familiar terms, antioxidant capacity as mg vitamin C equivalent/g dry weight was introduced. A standard curve of vitamin C (ascorbic acid, Fisher Scientific) was obtained from DPPH %SA (x) plotted against various vitamin C concentrations (y). Prepared concentrations of vitamin C solution were 10, 20, 30, 40 and 50 $mg \cdot L^{-1}$ distilled water. The regression line was $y = 1.9367x – 1.9826$.

Determination of total phenolic content

Total phenolics were determined using Folin-Ciocalteu's reagent, adapted from Yingngam *et al*. [16]. 0.2 mL of sample extracts was transferred and reacted with 0.2 mL of Folin-Ciocalteu's reagent (previously diluted 10-fold with distilled water) in test tube. After that, 2 mL of 7.5 % sodium carbonate was added and mixed and kept in the dark for 90 min. Color was developed and its absorbance was measured at 765 nm. A standard curve was prepared using 0, 50, 100, 150 and 200 $mg \cdot L^{-1}$ of gallic acid stock solution in a test tube. Regression line between absorbance (y) and gallic acid content (x) was $y = 0.0025x + 0.0064$. Results were expressed as mg gallic acid equivalent (GAE) /g dry weight.

Determination of cashew leaf extract in cassava starch based film

After extraction, the cashew leaf extract both in the solid and liquid form were added into a cassava starch based film (optimal conditions for the film involved adding 0.1 and 1.0 % (wv^{-1}, a solid portion) and 1 and 5 % (vv^{-1}, a liquid portion). The film was physically and chemically tested to identify total phenolic content, DPPH anti-oxidative potency, tensile strength and elongation at break, color and transparency.

Determination of film properties

Film thickness

The thickness of the film was measured using a micrometer (Mitutoyo, Model ID-C112PM, Serial No. 00320, Mitutoyo Corp., Kawasaki-shi, Japan). Five random locations around each film for 10 film samples were used for average thickness determination.

Mechanical properties

Tensile strength and percentage of elongation at break were determined by using a Texture Analyzer (Stable Micro System TA.XT-plus, UK). Six rectangular strips (20×60 mm^2) were prepared from each film to determine their mechanical properties. The average thickness of each film strip was used to estimate the cross-sectional area of the sample. Initial grip separation and mechanical crosshead speed were set at 30 mm and 1 $mm \cdot s^{-1}$, respectively. The nominal tensile strength was calculated by dividing the maximum load by an original minimum cross-sectional area of the specimen (related to minimum thickness). Nominal percent of elongation at break was calculated by dividing the extension at the moment of rupture of the specimen to its initially gauged length and multiplied by 100. All formulations were evaluated in triplicate.

Color and Transparency

Film samples were subjected to color measurement using a CIE colorimeter (Hunter associates laboratory, Inc., VA, USA). D65 (day light) and a measuring cell with 30 mm opening orifice was used. Color of the films was expressed as L* value (lightness), a* value (greenness/redness), b* value (blueness/yellowness) and transparency (%).

Total phenolic content and Antioxidant activity

To obtain the extracts, 10 g of each film were placed in a capped centrifuge tube and 50 mL methanol was added, after that the mixture was homogenized for 3 min at 23,580 g·force. The tube was

then centrifuged at 2,739 g for 20 min at 4 °C and the supernatant was transferred to a round bottomed flask. Total phenolic content and antioxidant potency of the extracts were analyzed as mentioned above.

Results and discussion

Determination of an optimal condition of producing cassava starch based film by RSM

Results from 17 treatments of cassava starch based film using 3 factors with 3 different levels of individual factors: solution weight (15, 30 and 45 g), drying time (36, 48 and 60 h), and drying temperature (40, 50 and 60 °C) as designed and interpreted by DESIGN-EXPERT software version 10 using a Box-Behnken design experiment are shown in **Table 1**.

Table 1 Experimental ranges and levels of the 3 independent variables used in RSM in terms of coded and actual factors and experimental data for the 3-factors with 2-level response surface analysis.

Treatments	Parameters (X)			Film thickness (mm)	Response (*Y*)	
	Solution weight (X_1)(g)	Drying time (X_2)(h)	Drying temperature (X_3)(°C)		Tensile strength (Y_1)(MPa)	Elongation at break (Y_2)(%)
1	1(45)	1(60)	0(50)	0.27±0.01	4.03±0.06	55.83±3.54
2	0(30)	0(48)	0(50)	0.24±0.00	0.63±0.17	134.17±8.25
3	-1(15)	0(48)	1(60)	0.14±0.00	0.71±0.00	123.34±11.79
4	1(45)	0(48)	1(60)	0.31±0.00	4.92±0.18	59.17±2.60
5	0(30)	0(48)	0(50)	0.24±0.00	1.59±0.10	130.83±10.60
6	0(30)	1(60)	-1(40)	0.21±0.00	1.51±0.11	85.56±5.50
7	1(45)	0(48)	-1(40)	N/A	N/A	N/A
8	-1(15)	0(48)	-1(40)	0.13±0.01	0.68±0.11	148.33±2.36
9	0(30)	1(60)	1(60)	0.13±0.01	1.82±0.22	138.33±2.35
10	1(45)	-1(36)	0(50)	0.34±0.01	4.19±0.10	13.08±3.18
11	0(30)	-1(36)	-1(40)	N/A	N/A	N/A
12	-1(15)	-1(36)	0(50)	0.11±0.01	0.52±0.03	106.67±11.79
13	0(30)	0(48)	0(50)	0.25±0.01	1.45±0.12	84.17±1.18
14	0(30)	0(48)	0(50)	0.25±0.01	1.25±0.06	134.99±9.42
15	-1(15)	1(60)	0(50)	0.24±0.01	0.48±0.02	154.33±6.12
16	0(30)	0(48)	0(50)	0.25±0.01	0.74±0.17	137.50±10.61
17	0(30)	-1(36)	1(60)	0.24±0.01	0.62±0.01	121.67±11.79

Values are given as mean±standard deviation (n = 3), N/A: not detectable

If suitability of experimental design was taken into account by means of analysis of variance at the confidence level of 95 %, it revealed that the *P* value of tensile strength and elongation break was equivalent to 0.0291 and 0.0081 (or $P \leq 0.05$). When the *P* values of the parameter related to the film solution weight are lower than 0.05, it shows that the parameter significantly affects tensile strength and elongation break. To illustrate the interactive effects of the independent variables on tensile strength and elongation at break of the cassava starch based film, a 3D response surface and contour plots are displayed in **Figures 1** and **2** The plots were generated by plotting the response (tensile strength and elongation at break) using the z-axis against 2 independent variables while the other independent variable is fixed. **Figure 1A** shows the response surface plot for the interactive effect of solution weight and drying time on the response values at a fixed drying temperature. An increase in solution weight led to rapid enhancement of tensile strength reaching a peak value a 45 g while tensile strength increased slightly in accordance with the increase in drying time from 36 to 60 h. Imprapai *et al*. [18] have reported that the interactions between amylose-amylose, amylose-amylopectin and amylopectin-amylopectin that occur during film drying reduces the amount of polar groups available to interact with water. For higher cassava starch concentration a high amount of non-polar groups are found (amylose), which leads to improved tensile strength. **Figure 1B** shows the response surface plot for the interactive effect of solution weight and drying temperature on the response value at a fixed drying time. Increasing the solution weight from 15 to 45 g improved tensile strength, while the tensile strength increased slightly with an increase in drying temperature from 40 to 60 °C. **Figure 1C** shows the response surface plot for the interactive effect of drying temperature to drying time on the response value at a fixed solution weight. The tensile strength increased as the drying temperature was increased reaching a peak value at 60 °C, while drying time almost did not change tensile strength. Similar results have been reported by Wiset *et al*. [19] who showed that the tensile strength increased significantly with increasing drying temperature (45 - 55 °C). During the filmogenic solution drying process, water evaporation occurs, which leads to the formation of a starch network. Denser and more non-homogeneous structures were observed at high drying rates (high temperature and low RH) [20]. Andrade-Mahecha *et al*. [21] found that intermediate drying conditions such as drying temperature (50 - 55 °C) provided tougher achira flour films.

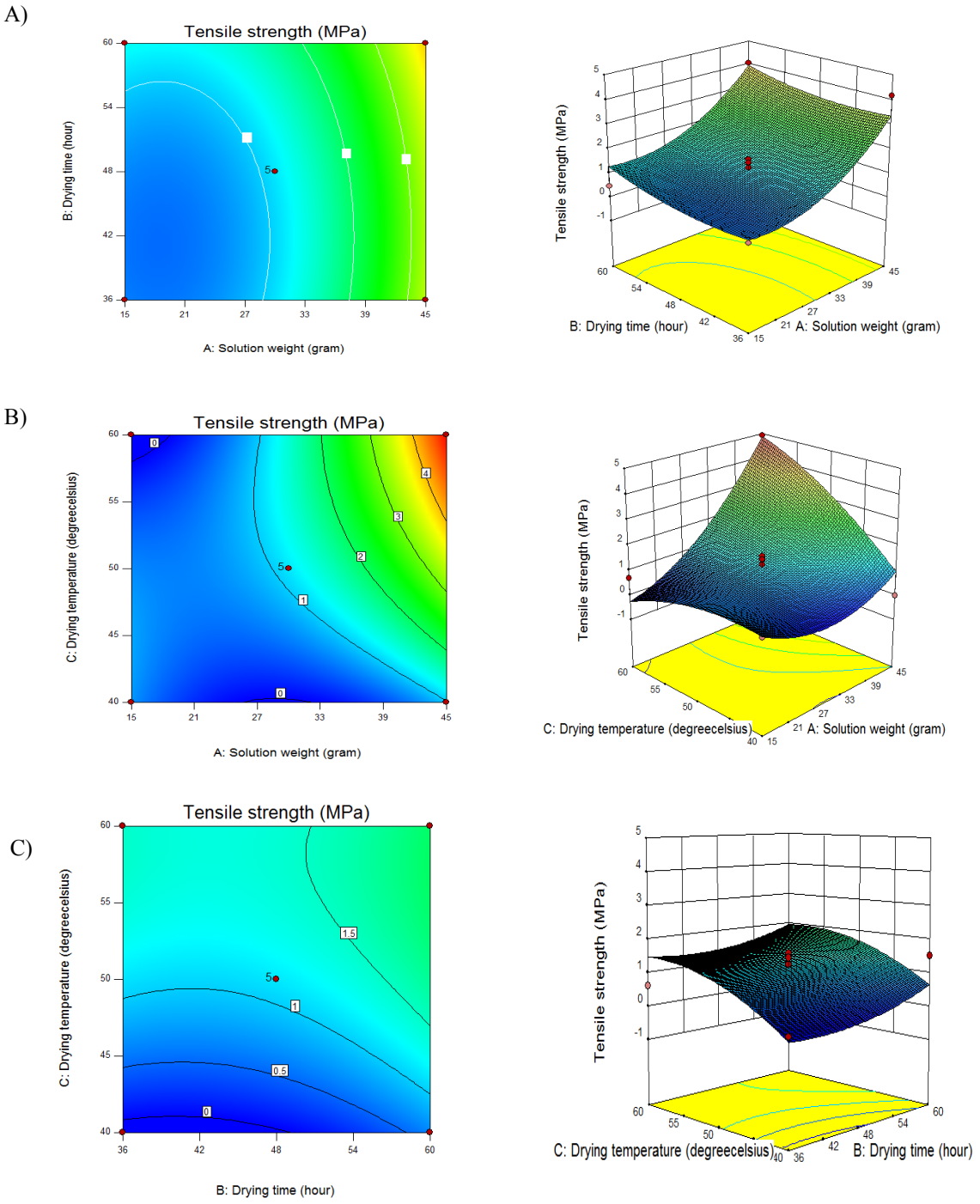


Figure 1 Contour plot and response plot of 3 parameters, where X_1 = solution weight (15, 30 and 45 g), X_2 = drying time (35, 48 and 60 h) and X_3 = drying temperature (40, 50 and 60 °C) for cassava starch based film on tensile strength.

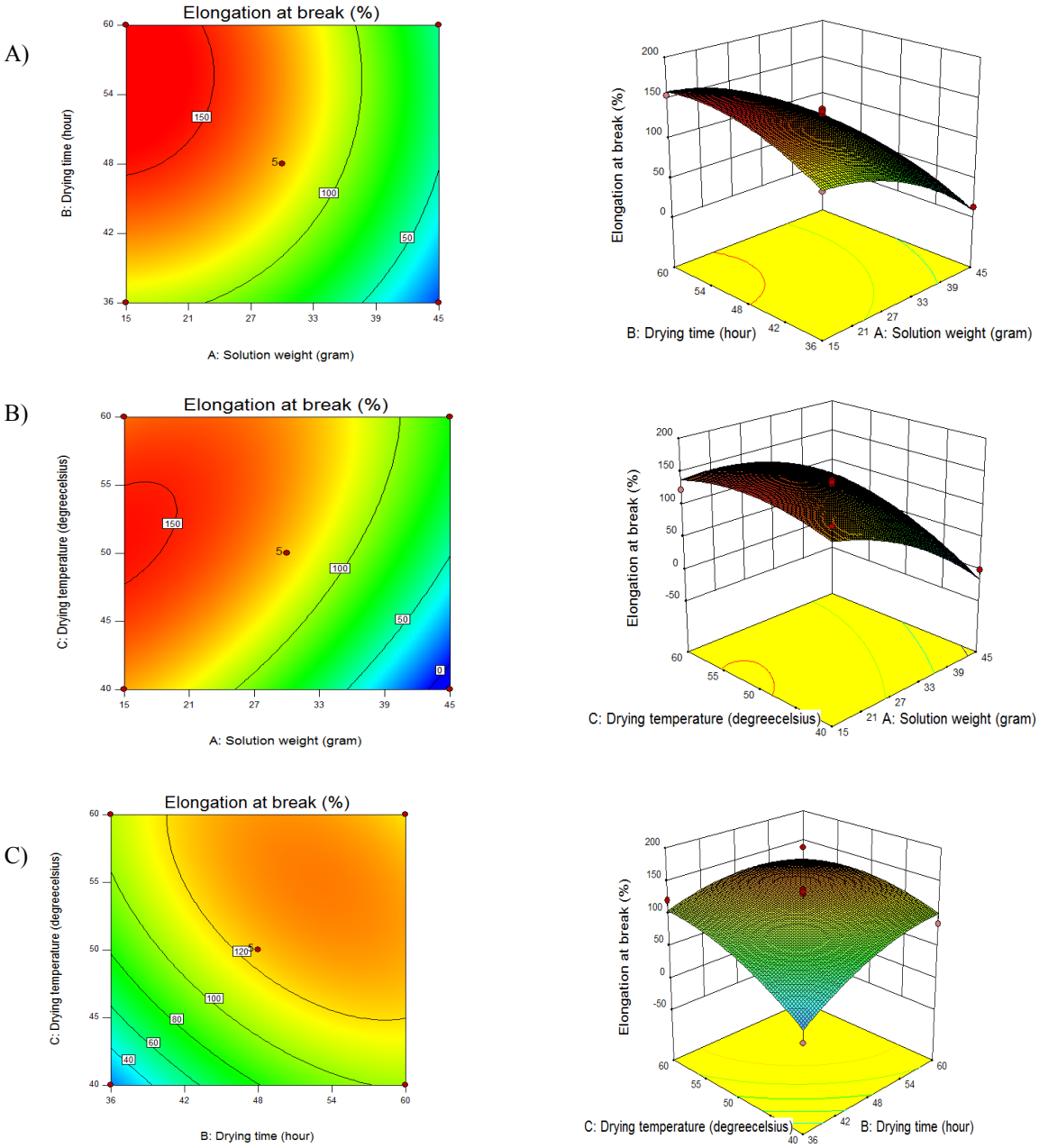


Figure 2 Contour plot and response plot of 3 parameters where X_1 = solution weight (15, 30 and 45 g), X_2 = drying time (36, 48 and 60 h) and X_3 = drying temperature (40, 50 and 60 °C) for cassava starch based film produced on elongation at break (%).

Figure 2A shows the response surface plot for the interactive effect of solution weight and drying time on the response value at a fixed drying temperature. An increase in solution weight led to a decrease of elongation at break while elongation at break increased as drying time increased from 36 to 60 h. **Figure 2B** shows the response surface plot for the interactive effect of solution weight and drying temperature on the response value at a fixed drying time. Increasing the solution weight from 15 to 45 g decreased the elongation at break, while elongation at break increased slightly by increasing the drying temperature from 40 to 60 °C. **Figure 2C** shows the response surface plot for the interactive effect of drying temperature and drying time on the response value at a fixed solution weight. The elongation at break increased with an increase in both drying temperature and drying time and reached a peak value at 60 °C and 60 h, respectively. Jangchud and Chinnan [22] have reported that the tensile strength increased from 0.40 to 4.02 MPa and elongation at break from 73.74 to 146.49 %, when the film forming temperature was increased from 70 to 90 °C.

Results based on an investigation of optimal conditions for cassava starch based film produced by means of a Box-Behnken experimental design and analysis using the RSM program showed that the correlation of the various parameters and co-parameters could be expressed as a quadratic equation. The results obtained by the Box-Behnken design were analyzed by ANOVA (**Table 2**).

Table 2 Regression of coefficients and analysis of variance of the second order polynomial for response variables.

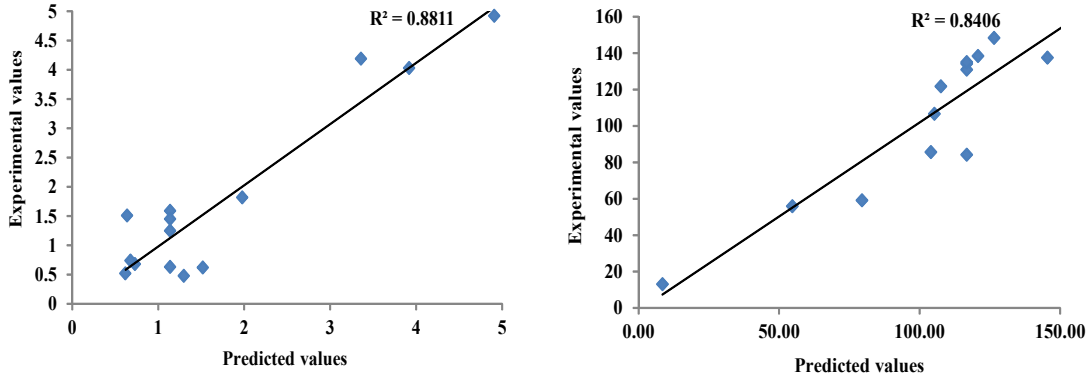
Coefficient	**Tensile strength, Y_1**	**Elongation at break, Y_2**
β_0	1.14	116.77
Linear		
X_1	1.34	-49.56
X_2	0.31	24.30
X_3	0.75	26.08
Interaction		
X_1X_2	-0.03	-1.19
X_1X_3	1.22	21.04
X_2X_3	-0.08	-17.71
Quadratic		
X_1^2	0.88	-20.87
X_2^2	0.28	-14.71
X_3^2	-0.42	-13.95
Variability		
R^2 of model	0.8444	0.8959
F value of model	4.22	6.70
$P > F$	0.0354	0.0101

$$\text{Tensile strength } (Y_1) = 1.14 + 1.34*X_1 + 0.31*X_2 + 0.75*X_3 + 0.88*X_1^2 + 0.28*X_2^2 - 0.42*X_3^2 - 0.03*X_1X_2 + 1.22*X_1X_3 - 0.08*X_2X_3 \quad (2)$$

$$\text{Elongation at break } (Y_2) = 116.77 - 49.56*X_1 + 24.30*X_2 + 26.08*X_3 - 20.87*X_1^2 - 14.71*X_2^2 - 13.95*X_3^2 - 1.19*X_1X_2 + 21.04*X_1X_3 - 17.71*X_2X_3 \quad (3)$$

Resultant models fitted satisfactorily with the experimental data as indicated by their goodness of fit expressed by R^2 and P values (**Table 2**). The R^2 values of the models for Y_1 and Y_2 were 0.84 and 0.89, respectively. This indicated that up to 84 - 89 % of the variations in solution weight, drying time and drying temperature can be explained by these equations. The P values of models for tensile strength and

elongation at break were 0.035 and 0.010, respectively. The *P* values of the models (≤ 0.05) indicate the significance of the coefficients. A correlation of experimental data and the predicted data for tensile strength and elongation at break was determined using normal probability plot. Experimental values of tensile strength and elongation at break were fitted with a straight line (**Figures 3A** and **3B**). The R^2 (multiple termination coefficient) value was 0.8811 and 0.8406, respectively. This indicated the aptness of the model that was able to explain 88.11 and 84.06 % of the results.



(A) (B)

Figure 3 Linear correlation between experimental data and predicted data of tensile strength (A) and elongation at break (B) of cassava starch based film.

On the basis of data analysis in order to find out the optimal condition for production of a cassava starch based film through Box Behnken design using following parameters: 15 - 45 g solution weight, heating time (40 - 60 h) and drying temperature (40 - 60 °C), it was found that the optimal conditions for cassava starch based film making were a solution weight of 15 g, drying time of 36 h and drying temperature of 50 °C. These conditions were selected from the RSM program to give a tensile strength of 0.62 MPa and 107.74 % elongation at break. The desirability was 1.000 under the optimal conditions.

Determination of anti-oxidative potency of cassava starch based film supplemented with the cashew leaf extract

The extract preparation based on cashew leafs

It showed that phenolic content and anti-oxidative activity in the crude extract were 189.39 mg gallic acid equivalent/ g sample, and 1,093.90 mg vitamin C equivalent/ g sample (data not shown), respectively. Compared to a study conducted by [23], the extracted phenolic content was 282.48 mg gallic acid equivalent/ g sample and its anti-oxidative capacity was 729.95 mg vitamin C equivalent/ g sample.

Textural characteristics of anti-oxidative cassava starch based film supplemented with the cashew leaf extract

After the cassava starch based film was mixed with glycerol (30 % of initial weight of cassava starch) and heated to 80 °C, solution gelatinized with a clear but viscous appearance, matching a previous report by [24]. It appeared that the resultant film would be clear and viscous, glossy and its surface was comparatively even, flexible and delicately soft. After plasticizer was added, the film was softer due to the fact that the molecular cohesive force of polymers in the adjacent area was weakened. After that, the sample was further boiled for 14 min, the temperature reduced to 60 °C and the cashew leaf extract was fortified as 0.1 and 1 % (wv^{-1}) for the solid portion and 1 and 5 % (vv^{-1}) for the liquid portion, and then

the sample was rigorously stirred. Air was eliminated from the sample by using ultrasonication, molded into a thin film plate using a 20 % mold weight, hot-air dried at 50 °C for 36 h giving a thin film as shown in **Figure 3**. It was found that homogeneous, thin and flexible cassava starch films were obtained. They could be readily removed from the plastic plates after drying. Visually, all films were colorless and slightly opaque.

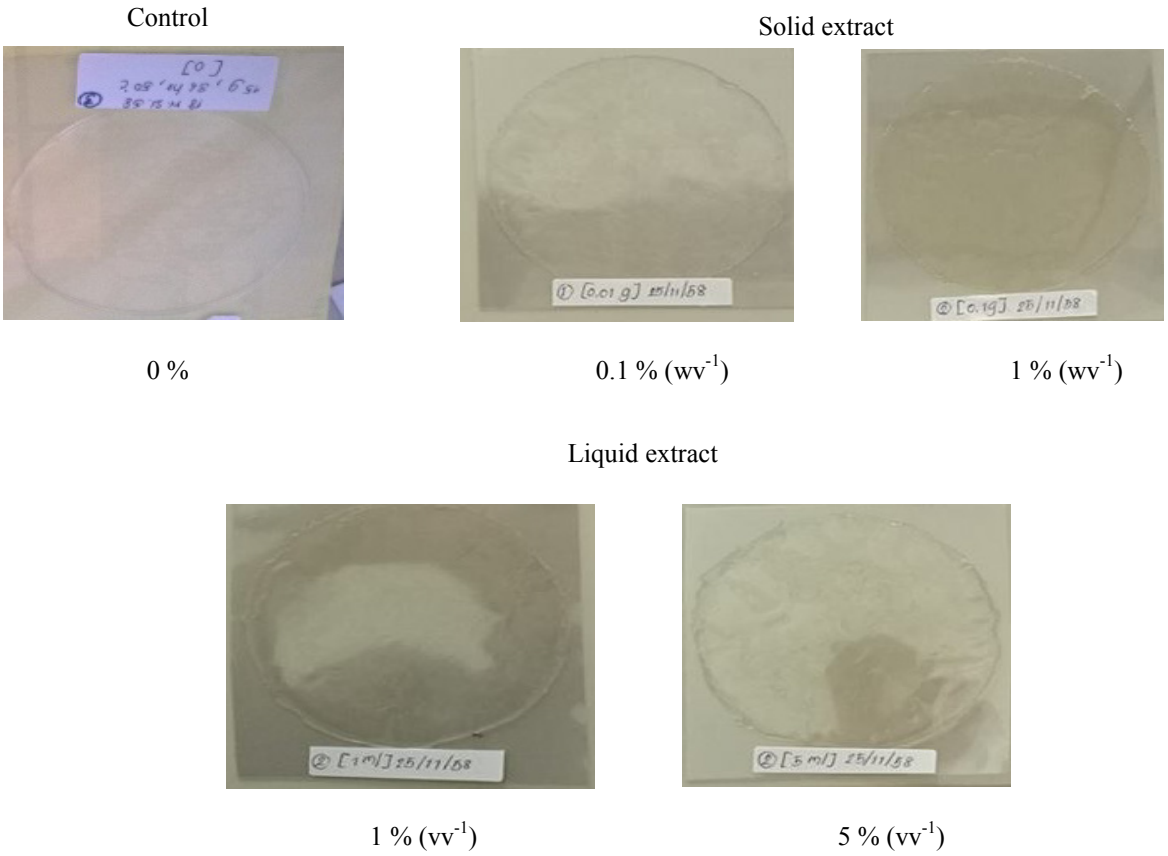


Figure 3 Anti-oxidative cassava starch based film supplemented with the extract of cashew leafs.

Determination of chemical properties of anti-oxidative cassava starch based film supplemented with the cashew leaf extract

Cassava starch based film supplemented with cashew leaf extract in form of 0.1 and 1.0 % (wv^{-1}) solid extract and 1 and 5 % (vv^{-1}) as a liquid extract was cut into small pieces (1 g per a piece), 20 mL of distilled water was added and allowed to stand at room temperature for 3 h. The sample was finely homogenized and centrifuged at 26.20 g·force for 5 min. After that, the sample was filled into a microcentrifuge tube (1 mL per a tube) and 1 mL 99.99 % ethanol added, before being recentrifuged at 2,620 g·force for 10 min. Later, 300 µL supernatant was filled in a test tube and 1.5 mL 1,1-diphenyl-2-picrylhydrazyl (DPPH) was added and stirred, allowed to stand in the dark and its absorbance measured at 765 nm as shown in **Table 3**.

Table 3 Total phenolic content and anti-oxidative activity by DPPH method of the cassava starch based film supplemented with the cashew leaf extract.

The cashew leaf extract	Total phenolic content (mg GAE/g DW)	Anti-oxidative activity (mg vitamin C equivalent/g DW)
0	0.00±0.00[e]	0.00±0.00[d]
Solid content (mg·L^{-1})		
0.1	0.52±0.00[d]	1.08±0.02[c]
1	4.09±0.02[a]	8.59±0.08[a]
Liquid content (mL·L^{-1})		
1	0.75±0.01[c]	1.30±0.03[c]
5	2.83±0.03[b]	6.09±0.20[b]

[a-e]Means ± SD of triplicate measurements with different superscript letters in each column are significantly different ($P \leq 0.05$)

Antioxidant activities of the cassava starch films containing the cashew leaf extract were assessed (**Table 3**). The cassava starch film without the extract had no antioxidant activity, whereas the films containing the extracts exhibited increased total phenolic content and anti-oxidative activities with an increase in the extract content. In general, the antioxidant activities of plant extracts are affected by the nature and concentration of polyphenolic compounds. Research by [25] described gelatin films fortified with green tea extracts which showed improved potency of anti-oxidation due to an increase in concentration of the green tea extract as the most fortified films, 10 % (solid) and 5 % (liquid), showed the highest anti-oxidative potency. Moreover, a report by [26] concluded that DPPH anti-oxidative potency was proportionally related to extractable phenolic concentration and also corresponded to a report that phenolic concentration could be employed as an indicator of anti-oxidative possibility. Nonetheless, extractable phenolic concentration may vary according to vegetable varieties, field and climatic conditions and portions used in the experiment [27].

Physical properties of anti-oxidative cassava starch based film supplemented with the cashew leaf extract

Tensile strength is a measurement of the maximum force a film can withstand against applied tensile stress and percent of elongation represents the ability of a film to stretch [28]. The physical properties of the anti-oxidative cassava starch based film supplemented with the cashew leaf extract are given in **Table 4**. The results showed that both form and concentration of extract significantly affected ($P < 0.05$) the physical properties.

Table 4 Tensile strength and elongation at break values of anti-oxidative cassava starch based film supplemented with the cashew leaf extract.

The cashew leaf extract	Tensile strength (MPa)	Elongation at break (%)
0	0.68±0.21[c]	125.00±0.00[a]
Solid content (mg·L^{-1})		
0.1	1.52±0.06[b]	86.67±0.07[c]
1	2.29±0.14[a]	41.67±0.36[d]
Liquid content (mL·L^{-1})		
1	1.39±0.04[b]	103.33±0.43[bc]
5	0.72±0.63[c]	104.17±0.25[b]

[a-d]Means ± SD of triplicate measurements with different superscript letters in each column are significantly different ($P \leq 0.05$)

Based on the information in **Table 4**, the results showed that increasing the solid (1 mg·mL^{-1}) and liquid (5 mL·mL^{-1}) content of the leaf extract caused an increase in the tensile strength by 3.37 and 1.06 times, respectively compared to the control sample but a decrease in the elongation at break (3.00 and 1.20 times in a solid and a liquid form, respectively). The result was similar to that of [25] who found that polyphenolic compounds contain many hydrophobic groups, which can form hydrophobic and hydrogen bonds with gelatin, leading to film strengthening. However, increasing the liquid content of the leaf extract caused a decrease in tensile strength, and there was no statistical difference in elongation at break. Thus, the cashew leaf extract concentration of both solid and liquid forms have a significant effect on the mechanical properties of the film. Improvement of mechanical properties of films incorporated with the leaf extract may be attributed to the interaction between the polysaccharide matrix and polyphenolic compounds from the cashew leaf extract.

Colour values of anti-oxidative cassava starch based film supplemented with the cashew leaf extract are shown in **Table 5**. (L* = intensity and lightness, when L* is high, it demonstrated that the sample is brighter, a* = colour changed in the range of green to red colour and b* demonstrated colour changes in the range of blue to yellow).

Table 5 Colour and transparency of anti-oxidative cassava starch based film supplemented with the cashew leaf extract.

The cashew leaf extract	Colour			Transparency (%)
	L*	a*	b*	
0	96.56±0.00^{a}	0.05±0.00^{a}	0.69±0.01^{c}	91.36±0.00^{a}
Solid content (mg·L^{-1})				
0.1	95.88±0.13^{b}	0.02±0.01^{ab}	1.17±0.01^{b}	89.71±0.33^{b}
1	95.10±0.05^{c}	(-0.53)±0.05^{c}	3.37±0.24^{a}	87.84±0.12^{c}
Liquid content (mL·L^{-1})				
1	96.32±0.02^{a}	(-0.03)±0.01^{b}	1.30±0.01^{b}	90.76±0.04^{a}
5	95.73±0.22^{b}	(-0.59)±0.01^{d}	3.57±0.04^{a}	89.35±0.53^{b}

$^{a-d}$Means ± SD of triplicate measurements with different superscript letters in each column are significantly different ($P \leq 0.05$)

In **Table 5**, it was discovered that the content of the cashew leaf extract affected colour values with lightness or L* decreasing as the phenolic content is increased. The films also change colour from colourless to greenish yellow as shown in **Table 5**. Whereas a* would decrease, b* increases in proportion to the concentration of the cashew leaf extract. Colour values in this experiment were consistent with an investigation undertaken by [29] who produced films based on chitosan and green tea extracts finding that L* would decrease as a* and b* would decrease. Y transmission which is defined as the transparency of the film was found to decrease as the phenolic concentration from cashew leaf extract increased. In general, the concentration of the cashew leaf extract affected both colour and transparency. Results in this experiment corresponded to a study carried out by [25] in which gelatin film fortified with green tea extract was compared to film without fortification and showed that the fortified film was less transparent compared to control.

Conclusions

Parameters relating to the production of cassava starch based films were solution weight, drying time, and drying temperature. These all affect tensile strength and elongation at break with the optimal conditions to make a film being 15 g, 36 h and 50 °C for the solution weight, drying time, and drying temperature, respectively. The resulting tensile strength and elongation at break were 0.62 MPa and 107.74 %, consecutively.

A production of anti-oxidative cassava starch based film fortified with cashew leaf extract in solid and liquid forms showed a significant change in the chemical and physical properties of the film. The incorporation of cashew leaf extract into a cassava starch film may have supplementary applications in food packaging to improve the stability of foodstuffs.

Acknowledgements

The author would like to express deep gratitude towards the Faculty of Agro-Industry, Rajamangala University of Technology Srivijaya, Nakorn Si Thammarat of support of equipment, facilities and research fund.

References

[1] MG Lindley. The impact of food processing on antioxidants in vegetable oil, fruits and vegetables. *Trends Food Sci. Tech*. 1998; **9**, 336-40.
[2] S Jeong, S Kim, D Kim, S Jo, K Nam, D Ahn and S Lee. Effect of heat treatment on the antioxidant activity of extracts of citrus peels. *Food Chem*. 2004; **52**, 3389-93.
[3] A Padmashee, N Roopa, AD Semwal, GK Sharma, G Agatian and AS Bawa. Star-anise (*Illicium verum*) and black caraway (*Carum nigram*) as natural antioxidants. *Food Chem*. 2007; **104**, 59-66.
[4] C Chotimarkon, S Benjakul and N Silalai. Antioxidant components and properties of five long: Grained rice bran extracts from commercial available cultivars in Thailand. *Food Chem*. 2008; **111**, 636-841.
[5] NS Hettiarachchy, KC Glenn, R Gnanasambandam and MG Johnson. Natural antioxidant extracts from fenugreek (Trigonella foenumgraecum) for ground beef patties. *J. Food Sci*. 1996; **61**, 516-9.
[6] N Chantima, P Sasamol and T Puraput. 2012, Anti-oxidative Potency of Crude Extract of Limonia (*Limonia acidissima*). Special Project for Bachelor of Science. Rajapat University of Walailongkorn under the Royal Patronage, Thailand.
[7] W Aomjai. Native vegetables and foods for therapeutic effect purpose. Available at: http://culture.pn.psu.ac.th/3spdb/RDOC/omjai2.pdf, accessed January 2016.
[8] K Kesinee and S Janpen. Potential of anti-oxidative power of Thai native vegetable extracts. *Food* 2000; **30**, 164-76.
[9] YP Tan and EWC Chan. Antioxidant, antityrosinase and antibacterial properties of fresh and processed leaves of Anacardium occidentale and piper betle. *Food Biosci*. 2014; **6**, 17-23.
[10] R Kongkachuichai, R Chareonsiri, K Yakoh, A Kringkasemsee and P Insung. Nutrients value and antioxidant content of indigenous vegetables from southern Thailand. *Food Chem*. 2015; **173**, 838-46.
[11] M Supasil and P Thammanoon. *A Development of Film and Coating Based on Polysaccharide of Weissella Confuse NH0$_2$ Mixed with Microbial Preservatives to Extend Shelf-life of Chilled Pork*. Research Report for Government Fund Research, Faculty of Agro-Industry, Prince of Songkhla University, 2010.
[12] P Panadda. 2007, A Preparation of Edible Film Based on Cassava Flour and Its Potential Applications. A Master Degree Thesis, Kasetsart University, Thailand.
[13] C Saenge and B Cheirsilp. Potential use of oleaginous red yeast *Rhodotorula glutinis* for the bioconversion of crude glycerol from biodiesel plant to lipids and carotenoids. *Process Biochem*. 2011; **46**, 210-8.

[14] G Box and DW Behnken. Some new three level designs for the study of quantitative variables. *Technometrics* 1960; **2**, 455-75.
[15] C Supasit. Ultrasound-assisted extraction of phenolic compounds from brown rice and their antioxidant activities. *Kasetsart J. (Nat. Sci.)* 2013; **47**, 864-73.
[16] B Yingngam, M Monschein and A Brantner. Ultrasound-assisted extraction of phenolic compounds from *Cratoxylum formosum* ssp. leaves using central composite design and evaluation of its protective ability against H_2O_2-induced cell death. *Asian Pac. J. Trop. Med*. 2014; **7**, 497-505.
[17] M Ohnishi, H Morishita, H Iwahashi, S Toda, Y Shirataki, M Kimura and R Kido. Inhibitory effects of chlorogenic acids on linoleic acid peroxidation and hemolysis. *Phytochemistry* 1994; **36**, 579-83.
[18] K Imprapai, O Kerdchoechuen and N Laohakunjit. Investigation physical and mechanical properties of edible film from cassava starch and rice starch. *Agri. Sci*. *J.* 2010; **41**, 609-12.
[19] L Wiset, N Poomsa-ad and P Jomlapeeratikul. Effects of drying temperatures and glycerol concentrations on properties of edible film from konjac flour. *J. Med. Bioeng*. 2014; **3**, 171-4.
[20] D Tapia-Blacido, PJ Sobral and FC Menegalli. Effects of drying temperature and relative humidity on the mechanical properties of amaranth flour films plasticized with glycerol. *Braz. J. Chem. Eng*. 2005; **22**, 249-56.
[21] MM Andrade-Mahecha, DR Tapia-Blacido and FC Menegalli. Development and optimization of biodegradable films based on achira flour. *Carbohyd. Polym*. 2012; **88**, 449-58.
[22] A Jangchud and MS Chinnan. Peanut protein film as affected by drying temperature and pH of film forming solution. *J. Food Sci*. 1999; **1**, 153-7.
[23] B Hamida. 2014, Ultrasound Assisted Extraction of Phenolic Content of Thai Southern Native Vegetables. A Special Project for Bachelor of Science. Rajamangala University of Technology, Srivijaya, Thailand.
[24] I Kasidith, K Orapin and L Nut-tha. Physical and mechanical properties of edible film based on cassava flour and *Oryza sativa*. *Agri. Sci.* 2010; **41**, 609-12.
[25] J Wu, S Chen, S Ge, J Miao, J Li and Q Zhang. Preparation, properties and antioxidant activity of an active film silver carp (*Hypophthalmichthys molitrix*) skin gelatin incorporated with green tea extract. *Food Hydrocoll*. 2013; **32**, 42-51.
[26] J Gómez-Estaca, B Giménez, P Montero and MC Gómez-Guillén. Incorporation of antioxidant borage extract into edible films based on sole skin gelatin or a commercial fish gelatin. *J. Food Eng*. 2009; **92**, 78-85.
[27] I Poonsup and K Rachanee. Biodiversity of Types, Nutritional Values and Applications of some Native Vegetables Emphasizing on Conventional Food Preparation of Thai Southern People. In Research Report on Government Research Budget. Faculty of Agro-Industry, Rajamangala University of Technology, Srivijaya, Thailand, 2009.
[28] P Park, J Je and S Kim. Free radical scavenging activities of differently deacetylated chitosans using an ESR. *Carbohydr. Polym.* 2014; **55**, 17-22.
[29] Y Peng, Y Wu and Y Lia. Development of tea extracts and chitosan composite films for active packaging materials. *Int. J. Biol. Macromol*. 2013; **59**, 282-9.