

## Starch Film Incorporated with Cinnamon Oils Optimally Prepared by using Response Surface Methodology

(Penyediaan secara Optimum Filem Kanji dengan Minyak Kayu Manis menggunakan Kaedah Rangsangan Permukaan)

UMMI HABIBAH ABDULLAH<sup>1</sup>, MUHAMMAD JEFRI MOHD YUSOF<sup>1</sup>, AINON HAMZAH<sup>2</sup> & ISHAK AHMAD<sup>1\*</sup>

<sup>1</sup>*Department of Chemical Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan, Malaysia*

<sup>2</sup>*Department of Biological and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan, Malaysia*

Received: 22 December 2021/Accepted: 12 April 2022

### ABSTRACT

Recent progress in the food industry emphasizes on active packaging that integrates antimicrobial feature into food packaging made from biodegradable films. In this study, antimicrobial films were prepared from starch biopolymer incorporated with cinnamon oil as antimicrobial agent. Many studies have reported on the increasing antimicrobial properties of biofilms when the concentration of antimicrobial agent is increased, yet their tensile strength would be lowered. Therefore, the preparation of these films requires comprehensive optimization to ensure optimum properties of the resulting films. The aim of this study was to optimize the mechanical and antimicrobial property of film after adding cinnamon oil using response surface methodology (RSM). The optimization of variables to prepare the films was successfully accomplished by manipulating cinnamon oil loadings and mixing temperature as proposed by RSM. Several models were generated to associate those parameters with the responding variables such as tensile strength and antimicrobial activity against *Bacillus cereus*, *Bacillus subtilis*, *Aspergillus niger*, and *Aspergillus brasiliensis* with  $R^2$  values of 0.9835, 0.9816, 0.9709, 0.9930, and 0.9950, respectively. Subsequently, the optimum conditions for the preparation of starch/cinnamon oil films were found to be at 24.02% of cinnamon oil and 49.35 °C of mixing temperature. Our study has demonstrated a novel, statistical experimental design and elaborate discussion on the effects of processing parameters in preparing films composing of starch and cinnamon oil. In summary, the active films prepared from this study have displayed promising qualities as potential food packaging against most common food borne microorganism during the food storage.

Keywords: Cinnamon oil; films; optimization; response surface methodology; starch

### ABSTRAK

Kajian terkini dalam industri makanan menekankan pembungkusan aktif yang memasukkan ciri antimikrob ke dalam pembungkus makanan diperbuat daripada filem biodegradasi. Dalam kajian ini, filem antimikrob disediakan daripada biopolimer kanji yang dimasukkan minyak kayu manis sebagai agen antimikrob. Kebanyakan penyelidikan lepas melaporkan peningkatan sifat antimikrob biofilem apabila kepekatan agen antimikrob ditingkatkan, namun, kekuatan regangan biofilem tersebut akan menurun. Oleh itu, penyediaan filem ini memerlukan pengoptimuman yang komprehensif bagi menghasilkan filem dengan ciri yang optimum. Dalam kajian ini, filem kanji/minyak kayu manis disediakan menggunakan kaedah tuangan larutan sebagai potensi bahan pembungkus. Pengoptimuman pemboleh ubah berjaya dilaksanakan dengan memanipulasi jumlah minyak kayu manis dan suhu campuran sebagaimana yang dicadangkan oleh kaedah rangsangan permukaan (RSM). Beberapa model dijana bagi mengaitkan parameter yang dikaji tersebut dengan kekuatan regangan filem dan aktiviti antimikrob filem terhadap *Bacillus cereus*, *Bacillus subtilis*, *Aspergillus niger* dan *Aspergillus brasiliensis* dengan nilai  $R^2$  masing-masing sebanyak 0.9835, 0.9816, 0.9709, 0.9930 dan 0.9950. Seterusnya, keadaan optimum bagi penyediaan filem kanji/minyak kayu manis ini ialah 24.02% minyak kayu manis dan 49.35 °C suhu campuran. Kesimpulannya, kajian kami telah membuktikan reka bentuk uji kaji yang novel secara statistik berserta perbincangan menyeluruh berkenaan kesan parameter pemprosesan ke atas ciri filem yang terdiri daripada kanji dan minyak kayu manis ini. Ringkasnya, filem aktif yang disediakan dalam kajian ini telah menampilkan kualiti sebagai pembungkus makanan yang berpotensi melawan serangan mikroorganisma perosak makanan ketika tempoh penyimpanan makanan.

Kata kunci: Filem; kanji; kaedah rangsangan permukaan; minyak kayu manis; pengoptimuman

## INTRODUCTION

The application of starch as plastic substitute has been widely explored for its easy availability and low cost. For packaging purposes, starch has promising remarks as potential edible films due to its biocompatibility, high extract yield, biodegradability, and non-toxic. These films are current alternative in food packaging industry to improve food preservation, shelf life extension and minimize the environmental problems caused by synthetic plastics (Muller et al. 2017). In contrast, when compared with conventional petroleum-based packaging, the mechanical, thermal and barrier properties of starch films are found to be poor in quality (Raigond et al. 2019). Several active compounds have been incorporated into starch films to improve the functionality of starch as packaging material such as active nanoparticles, antioxidant compounds, phenolic extracts, and antimicrobial polymers. Ghasemlou et al. (2013) reported that their corn-starch-based films were brittle and not suitable for conventional packaging due to lack of mechanical integrity. After addition of essential oils from *Zataria multiflora* Boiss and *Mentha pulegium*, the elongation at break of the films was increased as well as displaying antimicrobial effects against *E. coli* and *S. aureus* (Ghasemlou et al. 2013).

The addition of essential oils can be associated with elasticity properties enhancement of films as essential oil behave like plasticizing agent (Vianna et al. 2021). Meanwhile, the effects of reaction temperature on the films would display the compatibilizing manner of starch and cinnamon oil. In addition, some active compounds in natural products especially cinnamon oil are prone to restructure depending on the environmental temperature. The synergy of optimizing reaction temperature and cinnamon oil loading would aid in providing better mechanical properties of the prepared films (Sapper & Chiralt 2018).

The addition of essential oils can significantly alter the physical-chemical properties and provide functional antioxidant and antimicrobial activities to maximize the film performance. Nevertheless, the decrease in tensile strength with the incorporation of essential oil was reported by Zhang et al. (2021). There have been several similar studies conducted on the films preparation of starch with cinnamon oil, e.g., Diaz-Galindo et al. (2020), Souza et al. (2013), and Zhou et al. 2021. Their main studies revolved on the effects of cinnamon oil on the starch films without considering the parameter optimization. Similarly, other essential oils also possess same outcomes such as starch-orange oil (do Evangelho

et al. 2019) and starch- *Zanthoxylum bungeanum* oil (Wang et al. 2020).

It is crucial to produce films that have been optimized in terms of antimicrobial and mechanical properties so that they are practical to be used. To the best of our knowledge, no previous studies have been reported on the optimizing formulation of starch films incorporated with cinnamon oil. For instance, articles studied by Diaz-Galindo et al. (2020) and Souza et al. (2013) only focused on certain microbes, i.e., fungi *Penicillium commune*, *Eurotium amstelodami*, and *Botrytis cinerea*, respectively. On the other hand, this study investigated the antimicrobial effects of starch/cinnamon oil films against the common food borne pathogens, namely bacteria of *Bacillus cereus* and *Bacillus subtilis* and fungi of *Aspergillus niger* and *Aspergillus brasiliensis*.

In this study, we reported on the preparation of starch/cinnamon oil films using casting method as potential edible films that exhibit high mechanical properties and antimicrobial properties. One of the approaches to set an experimental design is to employ response surface methodology (RSM). The experimental design suggested by RSM is beneficial in preparing the optimum conditions of starch/cinnamon oil films based on the loading percentage of cinnamon oil and reaction temperature during mixing. By using RSM, the experimental runs to optimize the resulting films could be systematically designed and statistically conducted. This approach offers better and more precise optimization study as opposed to traditional one-variance-at-time (OVAT) optimization technique. OVAT technique is a randomized optimization study that fails to illustrate the optimization trends, nor fully confirms the best optimized parameters (Busu et al. 2021). Alzate et al. (2016) used RSM to investigate the effects of antimicrobial agents (potassium sorbate and carvacrol) on the colour, mechanical properties, water solubility, water vapor permeability and antimicrobial effectiveness of the films. The optimized films had better surface hydrophobicity and improved antimicrobial defense against *Z. bopillii*, *L. plantarum*, and *P. fluorescens*. Meanwhile, Mustapha et al. (2019) managed to identify the significant impacts of coating thickness and volume of turmeric oil in producing inhibition zone of *A. niger* for their cassava/turmeric oil films using RSM optimization. The findings would be practical for the films to be used as a safe and environmental-friendly food packaging that is well accepted by consumers, owing to the selection of materials used.

## MATERIALS AND METHODS

### MATERIALS

Cassava starch (ABC Sdn. Bhd. Malaysia), cinnamon oil, glycerol and polysorbate-80 (Tween 80) were purchased from Sigma-Aldrich and used without further purification. The physical properties of the cinnamon oil used were yellow to dark brown in colour, refractive index of  $n_{20/D}$  1.53 and flash point at 87 °C.

### PREPARATION OF STARCH/CINNAMON OIL FILMS

Starch/cinnamon oil films were prepared via solution casting method. All ingredients were 10 g of starch was mixed with 5 g of glycerol as plasticizer and 180 g of water. The mixture was heated to gelatinize the starch. Tween 80 was added into cinnamon oil at the ratio of 1:0.02 to ensure homogenous dispersion of cinnamon oil later in the mixing with gelatinized starch. The emulsion of starch and cinnamon oil was poured onto Perspex plate, followed by drying in the vacuum oven at 50 °C for 24 h. Finally, the starch/cinnamon oil films were kept in dried cabinet for 5 days at relative humidity 30% and ambient temperature 25 °C before further analysis.

### OPTIMIZATION USING RSM

The preparation of starch/cinnamon oil films were conducted at different percentage of cinnamon oil and mixing temperature that required 13 sets of experiments as suggested by RSM (Design Expert software 7.1.5). Central composite rotational design (CCRD) 5-level-2-factor was employed to study the optimization of processing parameters in preparing these films. This CCRD design consisted of 13 sets of experiments with four factorial point, four axial point, and five central point. Analysis of variance (ANOVA) was conducted to determine the significant difference among variables. The precision of the statistical models was confirmed by comparing those experimental and predicted values. Next, the models were validated through a series of validation experiments.

### CHARACTERIZATION FILM CHARACTERIZATION

The films were evaluated based on their (1) tensile strength, (2) antimicrobial properties, and (3) colour and light transmittance barrier.

### MECHANICAL TESTING

The tensile strength of starch/cinnamon oil films was evaluated using Universal Testing Machine (Instron model 5566, USA) at room temperature according to ASTM D882 protocol. A crosshead speed of 50 mm/min and load cell of 50 N were used for this test. The film thickness was determined with a digital caliper at five points. The average thickness of the samples was  $0.20 \pm 0.02$  mm. The average value of seven replicates for each sample was taken and tensile test characteristics were calculated by using Bluehill software.

### ANTIMICROBIAL PROPERTIES

The antimicrobial properties of starch/cinnamon oil films were determined using disc diffusion method against *Bacillus cereus* (BC), *Bacillus subtilis* (BC), *Aspergillus niger* (AN) and *Aspergillus brasiliensis* (AB). The film was cut into 20 mm diameter discs that were placed on agar plates, which had been previously spread with the working culture. The growth of microorganism on the films discs was visually examined. A ruler was used to measure the diameter of the growth inhibition zones including the film discs. All samples were tested in triplicate.

### COLOUR, LIGHT TRANSMITTANCE AND MORPHOLOGY OF FILMS

The colour of the films was being determined physically. The light transmission through the films was recorded between 200-400 nm (UV region) using ultraviolet-visible (UV-Vis) spectrophotometer (Mettler Toledo) by measuring the absorbance of the film.

## RESULTS AND DISCUSSION

### MODELLING AND ANALYSIS OF VARIANCE (ANOVA)

The comparison between experimental and predicted values of responding variables of starch/cinnamon oil films is tabulated in Table 1. The values were obtained from model fitting feature in RSM/CCRD. The overall interaction among cinnamon oil percentage (*A*) and mixing temperature (*B*) that affected the tensile strength and inhibition zone for each microbes of starch/cinnamon oil films can be presented in mathematical equation as follows:

$$\text{Tensile strength, MPa} = 2.50 - 0.65A + 0.084B + 0.038AB - 0.41A^2 - 0.27B^2 \quad (1)$$

$$\text{Inhibition zone for BC, mm} = 27.46 + 3.83A + 1.75B + 0.21AB - 0.44A^2 - 1.54B^2 \quad (2)$$

$$\text{Inhibition zone for BS, mm} = 28.70 + 4.25A + 1.74B - 0.25AB - 6.25e^{-3}A^2 - 1.63B^2 \quad (3)$$

$$\text{Inhibition zone for AN, mm} = 37.32 + 7.55A + 2.86B + 0.48AB - 1.73A^2 - 3.43B^2 \quad (4)$$

$$\text{Inhibition zone for AB, mm} = 47.90 + 8.63A + 3.88B + 0.13AB - 4.67A^2 - 5.42B^2 \quad (5)$$

In these equations (1 - 5), when the coefficient is positive in value, it remarks synergistic effects while

its negative value signifies antagonistic effects for each variable.  $R^2$  coefficient value for these models were 0.9835, 0.9816, 0.9709, 0.9930, and 0.9950 for equations 1 - 5, representing 98.35%, 98.16%, 97.09%, and 99/30% for each equation outcome of the total variations in the optimization were greatly influenced by the manipulating variables. In statistics, models with  $R^2 > 0.9$  indicate high correlation values among variables (Yusof et al. 2018). In this study, high  $R^2$  value obtained (in the range of 0.9709 - 0.9950) clearly disclosed in the accuracy between experimental and predicted data.

Table 2(a) - 2(e) shows the ANOVA for each generated model. These models showed significant  $p$  value ( $p < 0.01$ ) and insignificant lack of fit ( $p = 0.1518$ ). A model is considered to be accepted when it has significant  $p$  value ( $p < 0.01$ ) and insignificant lack-of-fit ( $p > 0.01$ ) (Thakur et al. 2017).

TABLE 1. CCRD design

Run	Cinnamon oil (%)	Temperature (°C)	Tensile, MPa		BC, mm		BS, mm		AN, mm		AB, mm	
			Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.
1	16.00	60.00	2.4	2.50	23.67	23.17	25	24.81	26	26.41	32.5	32.94
2	30.00	40.00	1	1.05	27.5	27.35	29	29.82	37.1	36.37	43	42.43
3	16.00	40.00	2.35	2.43	20	20.10	20	20.82	22	22.23	25	25.43
4	23.00	50.00	2.5	2.50	27.8	27.46	29	29.82	37.8	37.32	48	47.90
5	13.10	50.00	2.7	2.60	21	21.15	23	22.68	24	23.19	27	26.36
6	23.00	50.00	2.4	2.52	26.5	27.46	28	28.70	36.8	37.32	46.5	47.90
7	30.00	60.00	1.2	1.29	32	31.26	33	32.80	43	43.05	51	50.45
8	32.90	50.00	0.83	0.76	31.5	31.99	35	34.69	44	44.54	50	50.76
9	23.00	64.14	2.2	2.08	26.1	26.84	27.5	27.90	35.2	34.51	42.5	42.55
10	23.00	35.86	1.9	1.85	22	21.90	24	22.97	26	27.00	31.5	31.57
11	23.00	50.00	2.6	2.50	28	27.46	30	28.70	38	37.32	49	47.90
12	23.00	50.00	2.5	2.50	27.5	27.46	28.5	28.70	37	37.32	48	47.90
13	23.00	50.00	2.5	2.50	27.5	27.46	28	28.70	37	37.32	48	47.90

TABLE 2(a). Analysis of variance (ANOVA) for tensile strength

Source	Sum of squares	df	Mean square	F-value	P-value
Model	4.93	5	0.99	83.43	< 0.0001
Percentage of cinnamon oil, <i>A</i>	3.37	1	3.37	285.45	< 0.0001
Temperature, <i>B</i>	0.057	1	0.057	4.81	0.0644
<i>AB</i>	5.625E-003	1	5.625E-003	0.48	0.5124
<i>A</i> <sup>2</sup>	1.17	1	1.17	98.96	< 0.0001
<i>B</i> <sup>2</sup>	0.50	1	0.50	42.13	0.0003
Residual	0.083	7	0.012		
Lack-of-fit	0.063	3	0.021	4.18	0.1003
Pure error	0.020	4	5.000E-003		
Corrected total	5.01	12			

TABLE 2(b). Analysis of variance (ANOVA) for BC inhibition zone

Source	Sum of squares	df	Mean square	F-value	P-value
Model	159.22	5	31.84	74.78	< 0.0001
Percentage of cinnamon oil, <i>A</i>	117.65	1	117.65	276.27	< 0.0001
Temperature, <i>B</i>	24.39	1	24.39	57.27	0.0001
<i>AB</i>	0.17	1	0.17	0.40	0.5450
<i>A</i> <sup>2</sup>	1.37	1	1.37	3.23	0.1156
<i>B</i> <sup>2</sup>	16.59	1	16.59	38.96	0.0004
Residual	2.98	7	0.43		
Lack-of-fit	1.65	3	0.55	1.65	0.3128
Pure error	1.33	4	0.33		
Corrected total	162.20	12			

TABLE 2(c). Analysis of variance (ANOVA) for BS inhibition zone

Source	Sum of squares	df	Mean square	F-value	P-value
Model	187.64	5	37.53	46.64	< 0.0001
Percentage of cinnamon oil, <i>A</i>	144.25	1	144.25	179.29	< 0.0001
Temperature, <i>B</i>	24.32	1	24.32	30.23	0.0009
<i>AB</i>	0.25	1	0.25	0.31	0.5946
<i>A</i> <sup>2</sup>	2.717E-004	1	2.717E-004	3.377E-004	0.9859
<i>B</i> <sup>2</sup>	18.51	1	18.51	23.01	0.0020
Residual	5.63	7	0.80		
Lack-of-fit	2.83	3	0.94	1.35	0.3775
Pure error	2.80	4	0.70		
Corrected total	193.27	12			

TABLE 2(d). Analysis of variance (ANOVA) for AN inhibition zone

Source	Sum of squares	df	Mean square	F-value	P-value
Model	615.70	5	123.14	198.21	< 0.0001
Percentage of cinnamon oil, <i>A</i>	455.78	1	455.78	733.64	< 0.0001
Temperature, <i>B</i>	65.61	1	65.61	105.61	< 0.0001
<i>AB</i>	0.90	1	0.90	1.45	0.2673
<i>A</i> <sup>2</sup>	20.79	1	20.79	33.46	0.0007
<i>B</i> <sup>2</sup>	81.78	1	81.78	131.64	< 0.0001
Residual	4.35	7	0.62		
Lack-of-fit	3.18	3	1.06	3.63	0.1225
Pure error	1.17	4	0.29		
Corrected total	620.05	12			

TABLE 2(e). Analysis of variance (ANOVA) for AB inhibition zone

Source	Sum of squares	df	Mean square	F-value	P-value
Model	1031.56	5	206.31	277.47	< 0.0001
Percentage of cinnamon oil, <i>A</i>	595.59	1	595.59	801.01	< 0.0001
Temperature, <i>B</i>	120.56	1	120.56	162.14	< 0.0001
<i>AB</i>	0.063	1	0.063	0.084	0.7803
<i>A</i> <sup>2</sup>	151.63	1	151.63	203.93	< 0.0001
<i>B</i> <sup>2</sup>	204.26	1	204.26	274.71	< 0.0001
Residual	5.20	7	0.74		
Lack-of-fit	2.00	3	0.67	0.84	0.5405
Pure error	3.20	4	0.80		
Corrected total	1036.77	12			

Manipulating variables involved in this optimization were not dependent on one another based on the insignificant *p* values for cinnamon oil percentage (*A*) and mixing temperature (*B*). However, all responding variables were significantly affected by mixing temperature (*B*<sup>2</sup>) in quadratic manner. Similarly, quadratic parameter of cinnamon oil percentage (*A*<sup>2</sup>) affected all responding variables except for inhibition zone of BS and BC.

#### EFFECTS OF MANIPULATING VARIABLES

Three dimensional (3D) plotted graph from RSM/CCRD model is useful to explain the interaction of parameters involved in optimization (Idris et al. 2018). This 3D graph can also be used to determine the optimum conditions. Z-axis in the 3D graph denotes the responding variable (tensile strength and inhibition

zone) while the manipulating variables (cinnamon oil percentage, *A* and mixing temperature, *B*) are plotted against it at y-axis and x-axis. Figure 1(a)–1(e) illustrates the 3D graphs showing the interactions among those manipulated variables with responding variables.

Notably, further addition of cinnamon oil loadings would only decrease the tensile strength of the films. Similar findings were reported by Ahmed et al. (2016) and Ma et al. (2016) who incorporated cinnamon oil into polylactic acid and chitosan films, respectively. Regardless of the reaction temperature, the tensile strength was reduced drastically. The lowest tensile strength was recorded (0.83 MPa) when the percentage of cinnamon oil used was the highest (32.9%). Meanwhile, the highest tensile strength observed (2.4 MPa) was showed at the lowest percentage of cinnamon oil (16.0%). As expected, cinnamon oil behaves like plasticizing agent

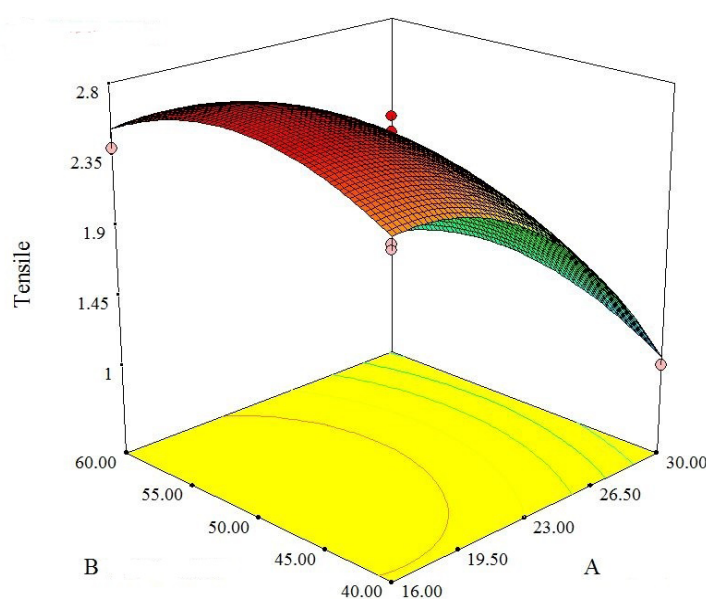


FIGURE 1(a). 3D graph of tensile strength against manipulating variables



which improved the flexibility and reduced the tensile strength of the film (Vianna et al. 2021).

In contrast, the antimicrobial properties of starch/cinnamon oil films followed different trends. Better dispersion of cinnamon oil when the mixing temperature was increased had permitted better coverage on inhibiting the growth of microbes. Dong et al. (2020) also observed better antimicrobial properties of their polyurethane films incorporated with carvacrol and cinnamyl aldehyde when the reaction temperature

was increased to further disperse the coverage of the antimicrobial agents. Correspondingly, the escalation of antimicrobial agents in cinnamon oil when the percentage was increased, had shown substantial antimicrobial activity when the inhibition zone for each microbe was widened. Iamareerat et al. (2018) reported analogous findings for their meatball packaging films made up from cassava starch and cinnamon oil that exhibited better antimicrobial activity against *E. coli* and *S. typhimurium* as they increased the cinnamon oil loadings.

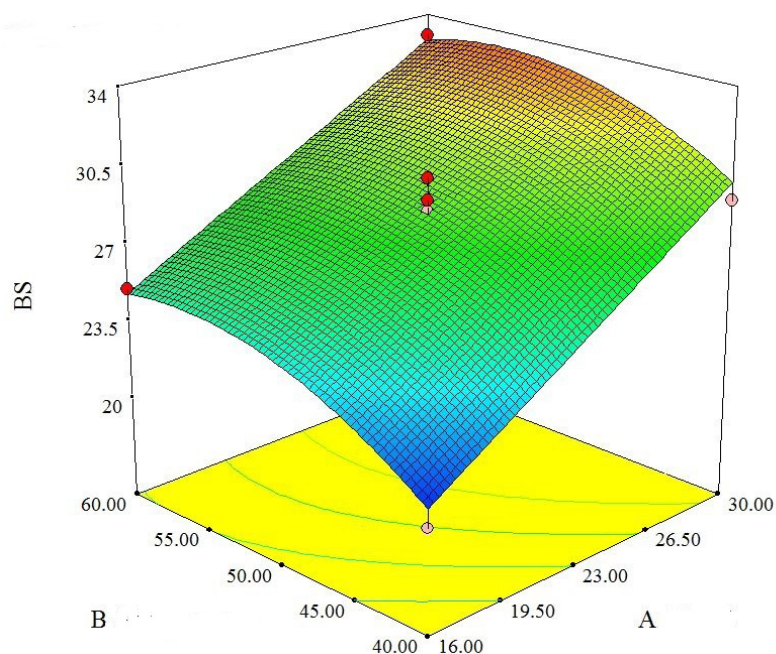


FIGURE 1(b). 3D Graph of BS inhibition zone against manipulating variables

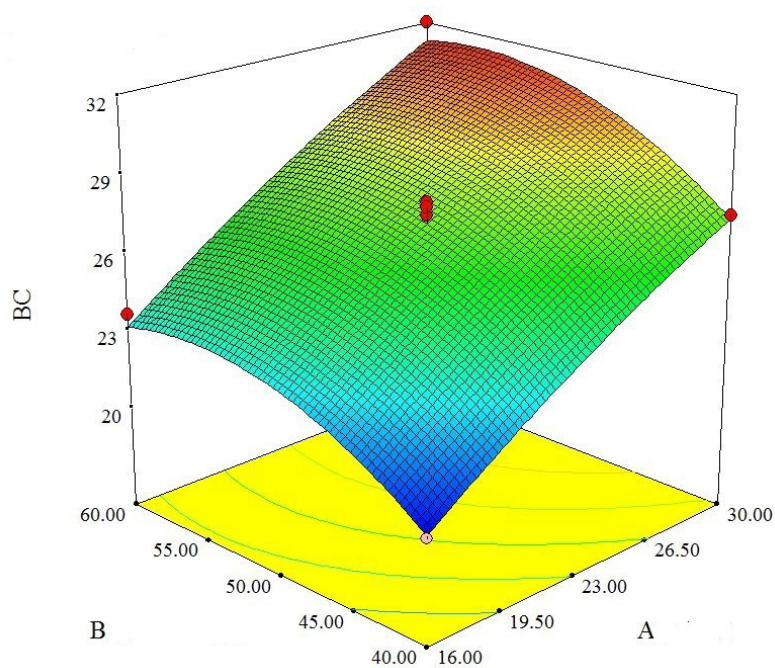


FIGURE 1(c). 3D Graph of BC inhibition zone against manipulating variables

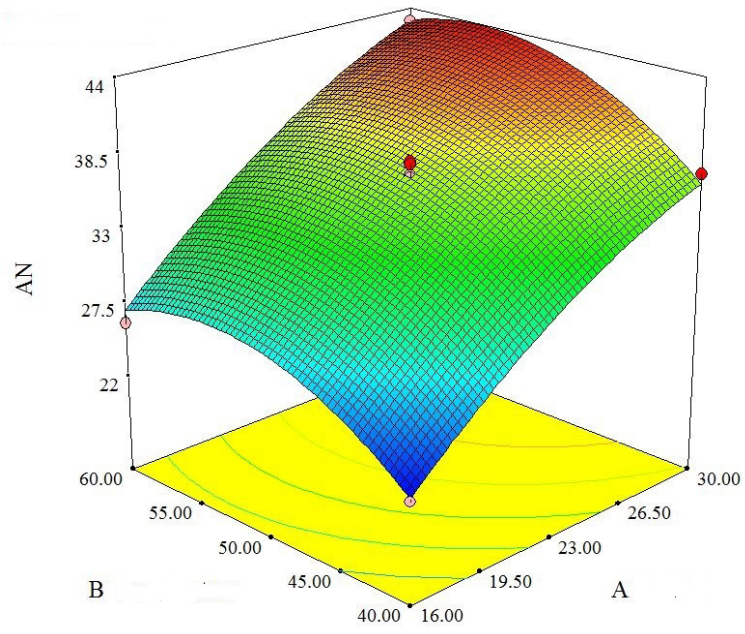


FIGURE 1(d). 3D Graph of AN inhibition zone against manipulating variables

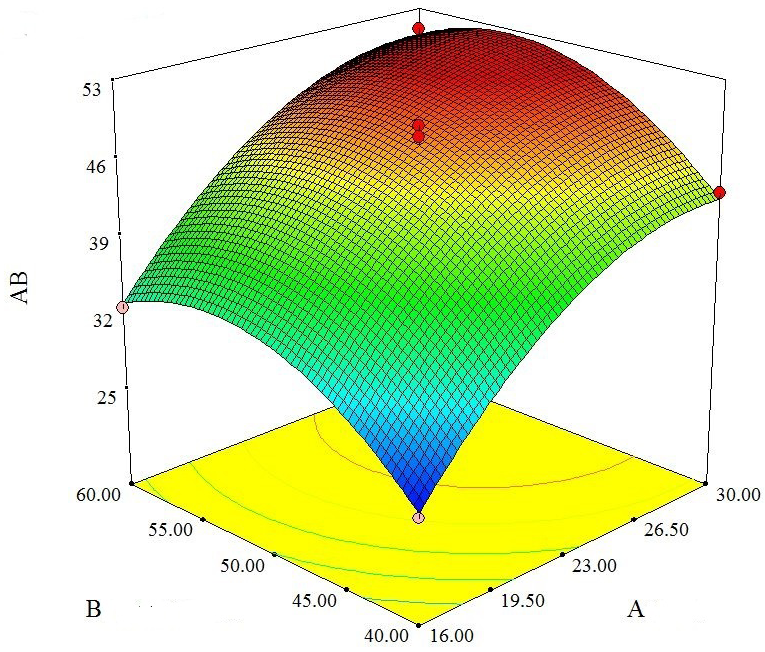


FIGURE 1(e). 3D Graph of AB inhibition zone against manipulating variables



## OPTIMUM CONDITIONS

The prediction of the optimum conditions in preparing starch/cinnamon oil films was conducted using the 'Optimization' feature in *Design Expert* software. One set of experiment was performed according to the proposed optimum conditions to validate the suggestion. As a result, both predicted and experimental data for optimum conditions recorded nearly similar values as can be seen in Table 3. Finally, in order to validate the

RSM models, validation experiments (at least one) needs to be performed by randomly executing experiments with values within the range of the manipulating variables (need to be different from the initial set of CCRD design) (Firdaus et al. 2017). Table 4 compares between predicted and experimental data for those validation experiments. The narrow deviation values between predicted and experimental data verified the acceptance of these models.

TABLE 3. Optimum conditions as simulated by RSM

Variables		Tensile, MPa		Inhibition zone BC, mm		Inhibition zone BS, mm		Inhibition zone AN, mm		Inhibition zone AB, mm	
Cinnamon oil, %	Temperature, °C	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.
24.02	49.36	2.39	2.40	27.89	28	29.20	29	38.18	39	48.78	47

TABLE 4. Model validation

Variables		Tensile, MPa		Inhibition zone BC, mm		Inhibition zone BS, mm		Inhibition zone AN, mm		Inhibition zone AB, mm	
Cinnamon oil, %	Temperature, °C	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.
26	50	2.15	2.10	29.02	28	30.52	30	40.23	39	50.74	48
18	40	2.43	2.35	21.35	22	22.11	20	25.09	26	30.14	30
20	50	2.70	2.60	25.73	26	26.88	27	33.77	31	43.34	42
24	60	2.22	2.30	28.23	27	29.38	28	37.87	35	47.52	45

## COLOUR AND LIGHT TRANSMISSION

The effects of incorporating cinnamon oil on the physical appearance of starch films are shown in Figure 2. From an aesthetic point of view, with the addition of cinnamon oil, the films became slightly yellow, but they still remained transparent. The intensity rate of yellowish colour was increased with the increase of concentration of cinnamon oil. In contrast, the film without the presence of cinnamon oil were transparent.

Starch films without cinnamon oil had no peak appearance in the UV absorption spectra. The dual absorption peaks of cinnamon oil appeared at ~220 and ~290 nm attributed to aldehyde group and aromatic ring, respectively. The absorptions gradually increased at 220 and 290 nm with the increasing of cinnamon oil

concentration. This behaviour can be considered as the proof of cinnamon oil incorporating into starch matrix. Besides, the peaks changes indicated incorporation of cinnamon oil into supramolecular host.

UV light is one of the common degradation initiators in food that causes lipid oxidation. Therefore, it is one of the important indicators in the process of studying suitable food packaging materials. The addition of cinnamon oil had interrupted the light transmittance and improved dispersion of light. This led to the reduction of light transmittance (UV region) and increasing hindrance towards UV light. Moreover, high concentration of cinnamon oil had disrupted the microstructure networks in the films that heavily depended on the particle size in dispersion phase (Figure 2). This is in line with

Noshirvani et al. (2017) and Zhao et al. (2022), where the addition of cinnamon oil into biopolymers could block UV radiation in this region. In other words, this

type of films exhibits barrier properties against UV light and could be applied to potentially preserve food from oxidation.

## CONCLUSION

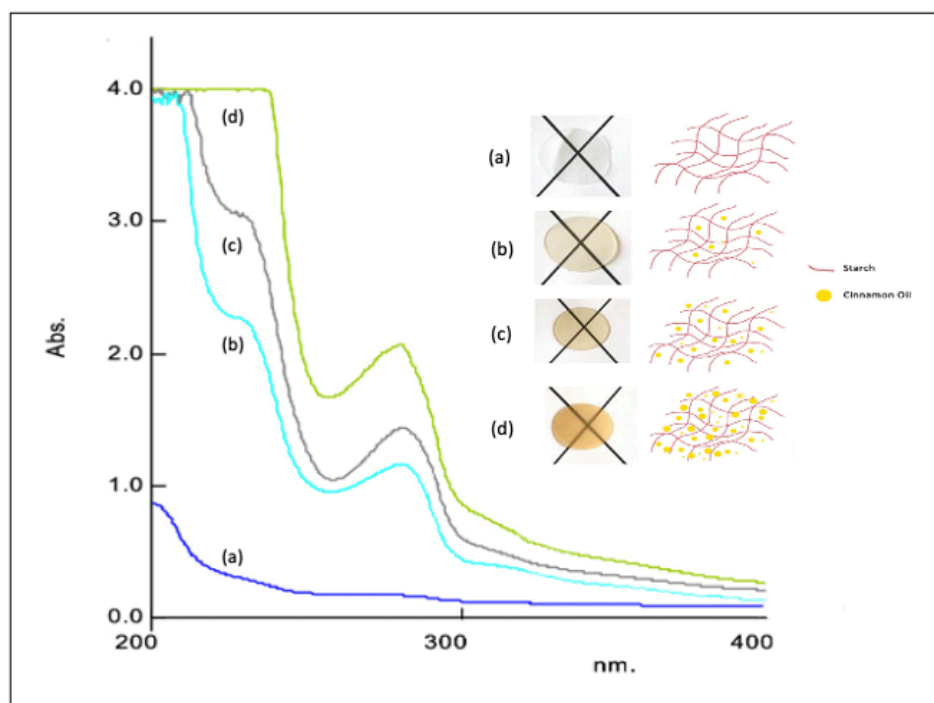


FIGURE 2. The UV absorption spectra of starch film with cinnamon oil; (a) starch film (b) starch-16% cinnamon oil, (c) starch-24.02% cinnamon oil, (d) starch-32.9% cinnamon oil

The preparation of starch/cinnamon oil films was successfully optimized using RSM based on the manipulation of cinnamon oil percentage and mixing temperature. High  $R^2$  value for all models had certified the precision of experimental and model predicted data. The generated model was accepted and could be used to predict the tensile strength and antimicrobial properties for these starch/cinnamon oil films at any given values within the precedent range of manipulating variables. Furthermore, the tensile strength of starch/cinnamon oil films could be maximized to 2.7 MPa and inhibition zone of 32, 35, 44, and 51 mm for *Bacillus cereus*, *Bacillus subtilis*, *Aspergillus niger*, and *Aspergillus brasiliensis*, respectively. The optimum conditions for preparation of starch/cinnamon oil films were 24.02% of cinnamon oil and 49.35 °C of mixing temperature, that resulted in starch/cinnamon oil films with tensile strength of 2.4 MPa and inhibition zone of 28, 29, 39 and

47 mm for *Bacillus cereus*, *Bacillus subtilis*, *Aspergillus niger*, and *Aspergillus brasiliensis*, respectively. For future study, more variables can be discovered to optimize the preparation conditions of starch/cinnamon oil films such as reaction time and starch loading.

## ACKNOWLEDGEMENTS

The authors would like to thank Universiti Kebangsaan Malaysia under research funding GUP-2018-028 and Center for Research and Instrumentation (CRIM) for providing facilities and instrumentations.

## REFERENCES

- Ahmed, J., Mulla, M.Z. & Arfat, Y.A. 2016. Thermo-mechanical, structural characterization and antibacterial performance of solvent casted polylactide/cinnamon oil composite films. *Food Control* 69: 196-204.

- Alzate, P., Miramont, S., Flores, S. & Gerschenson, L.N., 2016. Effect of the potassium sorbate and carvacrol addition on the properties and antimicrobial activity of tapioca starch-hydroxypropyl methylcellulose edible films. *Starch-Stärke* 69(5-6): 1600261.
- Busu, W.N.W., Chen, R.S., Shahdan, D., Yusof, M.J.M., Saad, M.J. & Ahmad, S. 2021. Statistical optimization using response surface methodology for enhanced tensile strength of polyethylene/graphene nanocomposites. *International Journal of Integrated Engineering* 13(6): 109-117.
- Díaz-Galindo, E.P., Nesic, A., Bautista-Baños, S., Dublan García, O. & Cabrera-Barjas, G. 2020. Corn-starch-based materials incorporated with cinnamon oil emulsion: Physico-chemical characterization and biological activity. *Foods* 9(4): 475.
- do Evangelho, J.A., da Silva Dannenberg, G., Biduski, B., El Halal, S.L.M., Kringel, D.H., Gularte, M.A., Fiorentini, A.M. & da Rosa Zavareze, E. 2019. Antibacterial activity, optical, mechanical, and barrier properties of corn starch films containing orange essential oil. *Carbohydrate Polymer* 222: 114981.
- Dong, H., He, J., Xiao, K. & Li, C. 2020. Temperature-sensitive polyurethane (TSPU) film incorporated with carvacrol and cinnamyl aldehyde: Antimicrobial activity, sustained release kinetics and potential use as food packaging for Cantonese-style moon cake. *International Journal of Food Science & Technology* 55(1): 293-302.
- Firdaus, F., Azhar, N.H.A., Idris, M.S.F. & Yusoff, S.F.M. 2017. Pengoptimuman tindak balas penghidrogenan getah asli cecair melalui kaedah rangsangan permukaan. *Sains Malaysiana* 46(12): 2469-2475.
- Ghasemlou, M., Aliheidari, N., Fahmi, R., Shojaee-Aliabadi, S., Keshavarz, B., Cran, M.J. & Khaksar, R. 2013. Physical, mechanical and barrier properties of corn starch films incorporated with plant essential oils. *Carbohydrate Polymers* 98(1): 1117-1126.
- Iamareerat, B., Singh, M., Sadiq, M.B. & Anal, A.K. 2018. Reinforced cassava starch based edible film incorporated with essential oil and sodium bentonite nanoclay as food packaging material. *Journal of Food Science and Technology* 55(5): 1953-1959.
- Idris, M.S.F., Rasid, H.M., Firdaus, F. & Yusoff, S.F.M. 2018. Hydrogenation of liquid natural rubber using 2, 4, 6-trimethylbenzenesulfonylhydrazide: optimisation of reaction parameters via response surface methodology. *Polymer Korea* 42(4): 544-550.
- Ma, Q., Zhang, Y. & Zhong, Q. 2016. Physical and antimicrobial properties of chitosan films incorporated with lauric arginate, cinnamon oil, and ethylenediaminetetraacetate. *LWT-Food Science and Technology* 65: 173-179.
- Muller, J., Quesada, A.C., González-Martínez, C. & Chiralt, A. 2017. Antimicrobial properties and release of cinnamaldehyde in bilayer films based on polylactic acid (PLA) and starch. *European Polymer Journal* 96: 316-325.
- Mustapha, F.A., Jai, J., Nik Raikhan, N.H.N., Sharif, Z.I.M. & Yusof, N.M. 2019. Response surface methodology analysis towards biodegradability and antimicrobial activity of biopolymer film containing turmeric oil against *Aspergillus niger*. *Food Control* 99: 106-113.
- Raigond, P., Sood, A., Kalia, A., Joshi, A., Kaundal, B., Raigond, B., Dutt, S., Singh, B. & Chakrabarti, S.K. 2019. Antimicrobial activity of potato starch-based active biodegradable nanocomposite films. *Potato Research* 62(1): 69-83.
- Sapper, M. & Chiralt, A. 2018. Starch-based coatings for preservation of fruits and vegetables. *Coatings* 8(5): 152.
- Souza, A.C., Goto, G.E.O., Mainardi, J.A., Coelho, A.C.V. & Tadini, C.C. 2013. Cassava starch composite films incorporated with cinnamon essential oil: Antimicrobial activity, microstructure, mechanical and barrier properties. *LWT-Food Science and Technology* 54(2): 346-352.
- Thakur, R., Saberi, B., Pristijono, P., Stathopoulos, C.E., Golding, J.B., Scarlett, C.J., Bowyer, M. & Vuong, Q.V.V. 2017. Use of response surface methodology (RSM) to optimize pea starch-chitosan novel edible film formulation. *Journal of Food Science and Technology* 54(8): 2270-2278.
- Vianna, T.C., Marinho, C.O., Júnior, L.M., Ibrahim, S.A. & Vieira, R.P. 2021. Essential oils as additives in active starch-based food packaging films: A review. *International Journal of Biological Macromolecules* 182: 1803-1819.
- Wang, B., Sui, J., Yu, B., Yuan, C., Guo, L., Abd El-Aty, A.M. & Cui, B. 2020. Physicochemical properties and antibacterial activity of corn starch-based films incorporated with *Zanthoxylum bungeanum* essential oil. *Carbohydrate Polymers* 254: 117314.
- Yusof, M.J.M., Tahir, N.A.M., Firdaus, F. & Yusoff, S.F.M. 2018. Pengoptimuman parameter untuk penurunan diimida getah asli cecair dalam sistem hidrazin hidrat/hidrogen peroksida menggunakan kaedah rangsangan permukaan (RSM). *Sains Malaysiana* 47(9): 2055-2061.
- Zhao, R., Guan, W., Zhou, X., Lao, M. & Cai, L. 2022. The physicochemical and preservation properties of anthocyanidin/chitosan nanocomposite-based edible films containing cinnamon-perolla essential oil pickering emulsions. *LWT-Food Science and Technology* 153: 112506.
- Zhang, B., Liu, Y., Wang, H., Liu, W., Cheong, L. & Teng, B. 2021. Characterization of seaweed polysaccharide based bilayer films containing essential oils with antibacterial activity. *LWT* 150: 111961.
- Zhou, Y., Wu, X., Chen, J. & He, J. 2021. Effects of cinnamon essential oil on the physical, mechanical, structural and thermal properties of cassava starch-based edible films. *International Journal of Biological Macromolecules* 184: 574-583.