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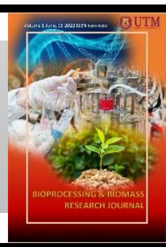

Research Article

Optimisation of Flour Composition Ratio, Baking Temperature, and Time for Papaya Biscuit Production and Evaluation of Biscuit Stability During Storage

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ABSTRACT

The underutilised papaya from agroindustry can cause adverse impact towards economy and environment. Papaya is a highly nutritious fruit however it is prone to decay and injury during storage. Thus, it is important to find solutions for this underutilised papaya. One of the solutions is to convert fresh papaya into papaya powder, and use it as functional ingredient in food and beverages. The objectives of this study were to optimise the flour composition ratio, baking temperature and time for biscuit production, and evaluate the stability of physicochemical properties of optimised papaya biscuit during storage. The optimised condition with desirability of 53.7% produced biscuits with moisture content of 3.83 ± 0.08%, antioxidant activity of 85.95 ± 0.86%, hardness of 1256.33 ± 93.11 N, 43.52 ± 0.57 of *L** value, 19.91 ± 0.27 of *a** value, and 21.47 ± 0.63 of *b** value. Flour composition ratio, baking temperature and time have significant impacts on the properties studied. Kinetic study shows that the optimised papaya biscuit was more stable during storage since the kinetic rate constants of the antioxidant, moisture content, hardness and colour were lower than that of the control biscuit.

INTRODUCTION

Fruits are an essential part of a healthy diet because fruits contain essential nutrients and fibres required by human body. According to the Food and Agriculture Organization, 400 billion dollars, or 14% of the world's food supply, is lost before it is even sold. Fruits are normally discarded due to several reasons such as spoilage due over-maturity or diseases, overproduction, irregular shape and size, and inefficient storage and processing methods. Fruit waste is a threat to the economy and environment. Over the past few decades, various research studies have been published to increase human understanding on the valorisation of fruit wastes into health beneficial products. Fruit wastes (flesh, peel, rind and seed) contain abundance of bioactive compounds such as vitamins, minerals, carotenoids, phenolic acids, flavonoids, and dietary fibres (Hasan et al., 2024). Fruit wastes may find applications as innovative medications or as additives in foods. They can also lessen food insecurity and promote food sustainability, particularly in developing nations. Value addition and the use of agricultural byproducts have been on the rise lately. Thus, it is necessary to develop profitable ways to employ these underutilised wastes (Abdel-Hameed et al., 2023).

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Carica papaya, commonly known as papaya, is currently a highly significant and popular fruit on a global scale with a total of 11.22 metric tons, or 15.36% of the overall production of tropical fruits. Globally papaya is estimated to be produced in over 60 countries, with emerging tropical nations producing the majority of the crop. Between 2000 and 2017, the total production of this fruit increased from 7.25 metric tons to 13.02 metric tons, a significant increase over the 17 years. The top five countries producing papaya are Mexico, Brazil, Indonesia, Nigeria and India with 38.61, 17.5, 6.89, 6.79, and 6.18%, respectively. Imbalance between supply and demand, economic crisis, disease susceptibility, and inefficient transportation and storage, cause abundance of unutilised papaya in the market that eventually being discarded in the landfill (Tan et al., 2022).

One way of salvaging the unutilised papaya is by converting the fruits into powder. The papaya powder can be used as a functional ingredient for functional food or beverages. These days, people all around the world are becoming more interested in lowering the risk of illness by eating foods that promote health and meet their fundamental nutritional needs. Modern foods are expected to do more for their consumers than merely satisfy hunger and provide necessary nutrients. Food should also prevent diseases associated with malnutrition and enhance human physical and mental health. In this regard, functional foods offer a fantastic chance to improve product quality. The health benefits of plant-based diets are mostly due to organic micronutrients, including carotenoids, polyphenolics, tocopherols, vitamins C, minerals, organic acids, and others, in addition to dietary fibres. Particularly by scavenging reactive oxygen species and protecting against degenerative illnesses like cancer and cardiovascular disorders, phenolics and carotenoids may offer health benefits. Consumers' desire for wheat-based products, such as pasta and noodles, has increased over the past few decades as they see the added value that comes from combining both plant and animal ingredients (Bekele & Emire, 2023). Wheat-based products are generally high in carbohydrates but low in fibres, protein, minerals, vitamins, and phenolic compounds. As a result, customers often experience nutrient imbalances after taking wheat products.

There were two objectives of this study. The first objective was to optimise the flour composition, baking temperature and time to obtain optimised papaya biscuit with high antioxidant, high fibre, low moisture content and acceptable hardness and colour. The second objective was to evaluate the stability of the optimised papaya biscuits during storage through kinetic study of moisture content, antioxidant content, hardness and colour of the biscuits.

MATERIALS AND METHOD

Materials

Wheat flour, matured papaya, eggs, corn flour, monk fruit sweetener, milk powder, and butter were procured from local markets. All chemicals used were purchased from local suppliers.

Design of Experiment and Statistical Analysis

The experimental design and statistical analysis for the optimisation study were performed using Design Expert Version 13 (Stat-ease Inc., USA). Central Composite Rotatable Design (CCRD) was applied for this optimisation study. The experiment consists of three factors (baking temperature (°C), baking time (min), flour composition ratio (g/g) as shown in **Table 1**. The Low and High values were determined based on previous study, while the +alpha and –alpha values were calculated by the software. The responses of this study were moisture content (%), antioxidant activity (%), hardness (N) and colour (*L* value) of the papaya biscuits.

Table 1 Factors and levels for the papaya biscuit experiments based on CCRD

Factors	-alpha	Low	High	+alpha
A-Baking temperature (°C)	143.18	150	170	176.82
B-Baking time (min)	9.89	15	30	35.11
C-Flour composition ratio (g/g)	3.07	15	50	61.93

The experimental data obtained were analysed using Analysis of Variance (ANOVA) from the software. The optimisation point was determined based on the desirability factor in the software. Kinetic modeling approach was used for biscuit stability study, and the modeling and analyses were carried out using Microsoft Excel Software (Microsoft Office Professional Plus 2019, USA).

Papaya Flour Preparation

The papaya flour was prepared according to Pathak et al. (2018), with modifications. Firstly, the fresh papaya fruits were washed and sliced to the required thickness. Then, the fresh papaya slices were dried in a hot air dryer (China) for 24 h at 70 °C. To obtain papaya flour, dried papaya slices were ground with a Tefal BL3071 dry grinder (France). The papaya flour was then stored in an air-tight container in the refrigerator until further use.

Papaya Biscuit Preparation

The original recipe of the biscuit utilises 200 g of wheat flour, 50.00 g of corn flour, 80.00 g of sugar, 6.29 g of milk powder, 150.00 g of butter and 1 egg. For preparing the papaya biscuit, the 200.00 g of wheat flour was substituted with different ratios of papaya powder flour to wheat flour (3.07 - 61.93%) as shown in **Table 1,** while other ingredients remained unchanged. The control sample was prepared using the original recipe with 200.00 g of wheat flour. The first step to prepare the biscuits was to mix all the ingredients in a bowl using Khind SM 220 mixer (Malaysia) until dough was formed. Then the dough was placed on a clean flat surface, and by using a roller pin, the dough was rolled and flattened to a final thickness of approximately 6 – 8 mm. A circular biscuit mold was used to cut the dough into circular form. Then the circular dough was put on the baking tray. The dough was baked according to baking time and baking temperature as set in **Table 1**. The biscuits were left to cool for 30 minutes before being subjected to subsequent analyses, or stored in air-tight container.

Chemical analysis

Antioxidant Activity using DPPH Inhibition Method

The antioxidant activity of papaya biscuits was obtained using DPPH inhibition method as described in Frezzini et al. (2019). To prepare a 0.1 mM DPPH solution, approximately

4.00 mg of DPPH reagent was mixed with 100.00 mL of 99% ethanol in a conical flask. Then, the flask was covered by aluminium foil and kept in the dark at room temperature. To prepare the extracted sample, 3.00 g of biscuits were mixed with 50.00 mL of 99% ethanol. This extract was then filtered using Whatman filter paper. To measure the antioxidant activity of the biscuit, 1.00 mL of the biscuit extract was mixed uniformly with 3.00 mL of 0.1 mM DPPH solution and 6.00 mL of 99% ethanol, and the mixture was kept in the dark for 30 min at room temperature. The absorbances of the samples were measured at wavelength 517 nm using Jenway 7305 spectrophotometer (Netherlands). Ethanol was used as the blank solution. The antioxidant activity was determined through DPPH inhibition % calculation as in Equation **(1)** :

$$DPPH\ inhibition\ \% = \frac{A_0 - A_s}{A_0} \times 100\% \qquad \textbf{(1)}$$

where,
A_0 = absorbance of the blank solution
A_s = absorbance of the biscuit extract

Moisture Content Determination
Moisture content of the biscuits was determined using the moisture analyser (OHAUS MB27, China). The analysis was carried out according method described in OHAUS MB27 moisture analyser manual. For drying the sample, the temperature of the analyser was set at 105 °C. 5.00 g of the ground biscuit sample was placed on the analyser test pan. The weight of the sample was displayed on the screen. During the drying process, the value on the screen reduced by time. The drying process stopped automatically when the weight of the sample became constant. The moisture content of the sample was calculated using Equation **(2)**:

$$\%MC_{(wb)} = \frac{w_i - w_f}{w_f} \times 100\% \qquad \textbf{(2)}$$

Where,
$MC_{(wb)}$ = moisture content (wet basis),
w_i = the initial weight (g) of the sample before drying
w_f = the final weight (g) of the sample after drying

Physical Analysis

Biscuit Hardness Evaluation
The hardness of the biscuits was determined using Brookfield CT3 Texture Analyser (USA). A total of 63 samples were subjected to puncture tests to evaluate the hardness and texture of the biscuit. The hardness and texture of each sample were recorded.

Colour Evaluation
The colour of papaya biscuits was obtained from the Konica Minolta Chroma Meter CR-400 (USA). The colour was measured based on CIELAB colour system where *L** value refers to lightness, *a** value represents the redness value, and *b** value refers to yellowness value on the CIELAB colour chart (Chung et al., 2014).

Kinetic Modeling and Kinetic Rate Constant
The stability of the biscuits was evaluated through three weeks of biscuit storage at room temperature. The common reaction rate equation in Equation **(3)** was used as in Nambi et al. (2016):

$$-\frac{d[C]}{dt} = k[C]^n \qquad \textbf{(3)}$$

$where,$
$C = concentration\ of\ quality\ factor\ at\ time$
$k = reaction\ rate\ constant$
$n = order\ of\ the\ reaction$

For 1st order reaction, the general reaction rate equation was modified to Equation **(4)**:

$$C = C_0 exp(-kt) \qquad \textbf{(4)}$$

$where,$
$C_0 = initial\ value\ of\ parameter$
$k = reaction\ rate\ constant$
$t = time$

Then Equation **(3)** was simplified to Equation **(5)**.

$$In\left(\frac{C}{C_0}\right) = -kt \qquad \textbf{(5)}$$

$where,$
$C = value\ of\ parameter\ at\ time\ t$
$C_0 = value\ of\ parameter\ at\ time\ zero$
$k = reaction\ rate\ constant\ for\ stability$
$of\ parameter\ (week^{-1})$
$t = time\ (week)$

The reaction rate constant (k) value was obtained from the slope of graph of $In\left(\frac{C}{C_0}\right)$ against t.

RESULTS AND DISCUSSION

From CCRD, 19 experiments were proposed and the experiments were carried out in randomized order. The experimental design and results were tabulated in **Table 2**.

Table 2 Experimental design and data for papaya biscuits optimisation study

	Independent variable			Dependent variable					
Run	Baking temperature (°C)	Baking time (min)	Flour ratio (g/g)	Moisture content (%)	Antioxidant (%)	Hardness (N)	*L**	*a**	*b**
1	150	15.00	15.00	6.48	7.77	343	61.82	12.96	30.95
2	150	30.00	50.00	4.42	50.90	994	41.92	19.60	21.11
3	150	15.00	50.00	7.36	36.97	463	44.55	16.28	23.83
4	160	22.50	32.50	5.82	40.75	742	52.13	20.54	33.07
5	160	22.50	61.93	3.71	80.12	1010	40.06	18.10	17.84
6	160	22.50	3.07	3.76	6.43	527	65.37	13.07	33.39
7	176.82	22.50	32.50	3.58	40.29	1484	45.03	18.63	23.48
8	170	15.00	15.00	4.84	13.78	666	60.32	17.20	33.45
9	150	30.00	15.00	4.47	15.22	709	58.50	17.21	33.05
10	160	22.50	32.50	6.10	31.28	786.67	51.99	20.25	30.65
11	170	30.00	50.00	1.90	85.95	2606	33.54	15.35	8.21
12	160	35.11	32.50	3.48	70.65	2267	39.72	16.41	15.76
13	160	22.50	32.50	5.65	28.21	982	53.73	19.19	32.22
14	160	9.89	32.50	7.32	27.25	384	49.86	16.16	28.82
15	160	22.50	32.50	5.54	24.81	767	53.13	19.24	32.00
16	143.18	22.50	32.50	7.41	33.21	540	52.12	18.40	30.43
17	160	22.50	32.50	5.75	35.95	491	51.32	20.12	30.32
18	170	30.00	15.00	1.87	31.90	1756	44.11	17.05	22.08
19	170	15.00	50.00	4.81	73.15	806	46.77	20.70	25.92

Effects of baking temperature, baking time and flour composition ratio on antioxidant activity of papaya biscuits

The effects of the parameters on antioxidant activity can be seen in **Figure 1**. As the baking temperature and time increased, the percentage of antioxidant activity also increased (**Figure 1a**). A study on the effect of baking temperature on black corn bread by Blanch & Ruiz del Castillo (2021) showed that the antioxidant activity of the bread significantly increased in bread baked between 180 °C to 200 °C. Two plausible reasons for this are the preservation of bound phenolics at higher temperature and formation of antioxidant during baking due to Maillard reaction (Alfeo et al., 2020). Alide et al. (2020) stated that the total phenolic content and total flavonoid content levels are unaffected by cooking time. This study agrees with Abacan et al. (2017), who also stated that the total phenolic content and antioxidant activity of edible mushrooms were unaffected by the cooking time. For flour composition ratio, biscuits with higher content of papaya powder have higher antioxidant activity (**Figure 1b**). As the papaya flour increases, the amount of beta-carotene and other phytochemicals also increases, thus increasing the antioxidant level of the flour. **Table 3** shows the ANOVA for the antioxidant activity of the biscuits. All three factors have significant impact ($p < 0.05$) on the antioxidant activity of the biscuits after baking (**Table 3**). As suggested by ANOVA, the antioxidant activity of papaya biscuit is best described using linear model.

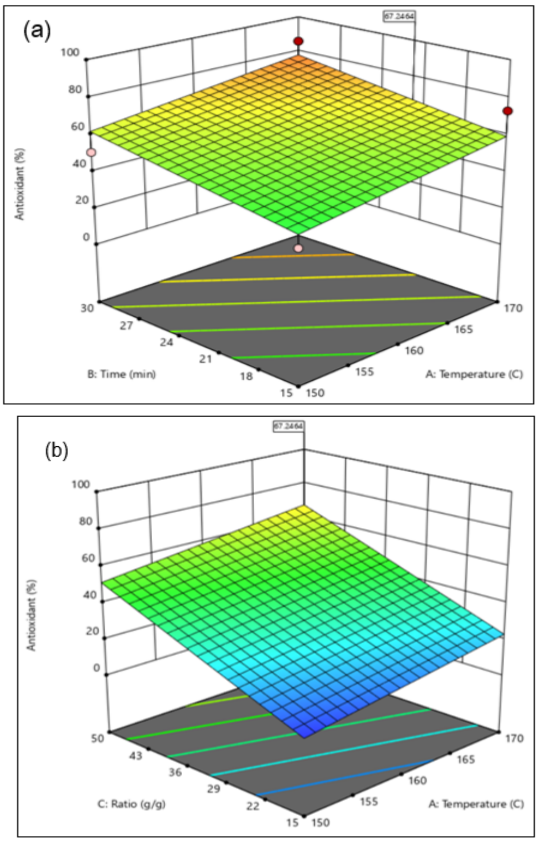


Figure 1 Effect of (a) baking temperature and baking time and (b) baking temperature and flour composition ratio on the antioxidant of the papaya biscuits

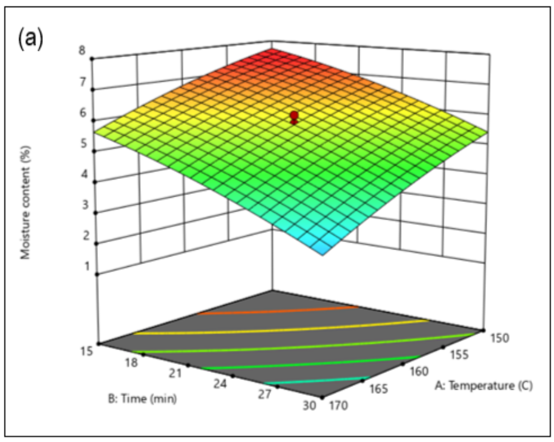
Table 3 ANOVA for antioxidant activity of papaya biscuits

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	8669.06	3	2889.69	31.21	< 0.0001	significant
A	831.25	1	831.25	8.98	0.0090	
B	1149.32	1	1149.32	12.41	0.0031	
C	6688.50	1	6688.50	72.25	< 0.0001	
Residual	1388.67	15	92.58			
Lack of Fit	1230.12	11	111.83	2.82	0.1643	Not significant
Pure Error	158.54	4	39.64			
Cor Total	10057.73	18				

Effects of baking temperature, baking time and flour composition ratio on moisture content of papaya biscuits

Siddiqui & Nasreen (2014) reported that the baking temperature and baking time have a significant effect on the shelf-life of biscuits. The baking temperature and baking time have a role in reducing the moisture content of the biscuits (Azmi et al., 2019). Referring to **Figure 2a**, as the baking temperature and time increased, the moisture content of the biscuits decreased. This is because the higher the baking time and temperature, the higher the amount of moisture is evaporated from the papaya biscuits during baking. Low moisture content is important in biscuits to avoid microbial attack and to ensure that the biscuits have longer shelf-life during storage. The flour composition ratio has quadratic impact on the moisture content of the biscuits (**Figure 2b**).

This is because papaya flour has two characteristics called water-holding capacity and oil-holding capacity (Varastegani et al., 2015). As the papaya flour ratio increases, plausibly the moisture content of the papaya biscuit also increases due to the water-holding capacity. Table 4 shows the ANOVA for the moisture content of the biscuits. All three factors have significant impact ($p < 0.05$) on the moisture content of the biscuits of the biscuits after baking. As suggested by ANOVA, the moisture content of papaya biscuit is best described using quadratic model.



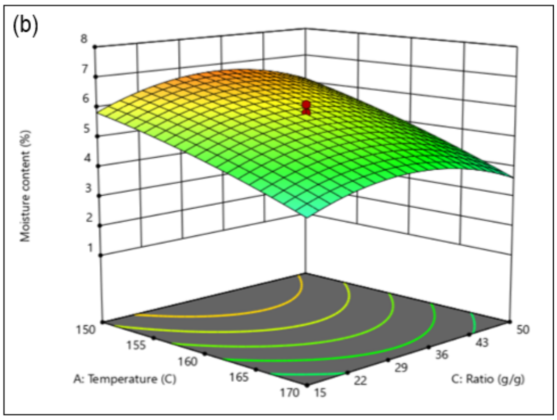


Figure 2 Effect of (a) baking temperature and baking time (b) baking temperature and flour composition ratio on the moisture content of the papaya biscuits

Table 4 ANOVA for moisture content of papaya biscuits

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	48.9400	9	5.4400	64.9300	< 0.0001	significant
A	18.3200	1	18.3200	218.7400	< 0.0001	
B	21.8900	1	21.8900	261.3200	< 0.0001	
C	0.0407	1	0.0407	0.4865	0.5031	
AB	0.1081	1	0.1081	1.2900	0.2852	
AC	0.0861	1	0.0861	1.0300	0.3371	
BC	0.0946	1	0.0946	1.1300	0.3155	
A^2	0.4206	1	0.4206	5.0200	0.0518	
B^2	0.5064	1	0.5064	6.0500	0.0362	
C^2	8.3500	1	8.3500	99.6600	< 0.0001	
Residual	0.7537	9	0.0837			
Lack of Fit	0.5747	5	0.1149	2.5700	0.1910	Not significant
Pure Error	0.1791	4	0.0448			
Cor Total	49.7000	18				

Effects of baking temperature, baking time and flour composition ratio on hardness of papaya biscuits

Figure 3a shows that as the baking temperature and time increased, the hardness of the papaya biscuits also increased. According to Saxena & Rao (1996), the baking temperature has a significant effect on the hardness of biscuits because it stimulates the migration of water from the core of the biscuit which results in a stiffer and harder texture that is unsuitable for producing a higher quality product. According to Sharma et al. (2016), as the fibre content increases, the hardness of the biscuit also increases. This is due to the increase in the water-holding capacity of papaya flour. Meanwhile, Coura et al. (2020) stated that papaya is a tropical fruit that contains high amount of fibres. Insoluble fibres also have a significant contribution in altering the texture of the biscuit to which they are added (Koppel et al., 2015). Mechanical experiments have shown that increasing the amount of fibres causes a concentration-dependent increase in hardness. The baking time also has a significant effect on hardness due to the amount of moisture loss from the papaya biscuits. Due to the loss of water from the papaya biscuit, the fibre frame becomes more rigid. Furthermore, because long-term heat treatment changes the fibre characteristics, the interplay between fibre weight and baking time is critical. When the flour ratio of papaya powder increased, the hardness increased (**Figure 3b**). All three factors have significant impact ($p < 0.05$) on the hardness of the biscuits after baking (**Table 5**). As suggested by ANOVA, the hardness of the papaya biscuit is best described using quadratic model.

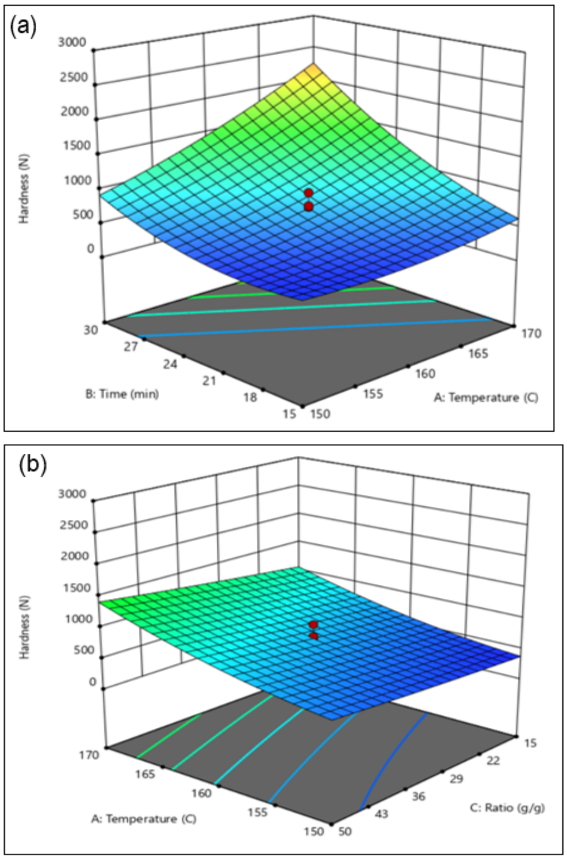


Figure 3 Effect of (a) baking temperature and baking time (b) baking temperature and flour ratio on the hardness of the papaya biscuit

Table 5 ANOVA for hardness of papaya biscuits

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	6.923E+06	9	7.692E+05	29.09	< 0.0001	significant
A	1.795E+06	1	1.795E+06	67.91	< 0.0001	
B	3.541E+06	1	3.541E+06	133.92	< 0.0001	
C	3.568E+05	1	3.568E+05	13.49	0.0051	
AB	4.965E+05	1	4.965E+05	18.78	0.0019	
AC	42778.13	1	42778.13	1.62	0.2352	
BC	95703.13	1	95703.13	3.62	0.0895	
A^2	1.214E+05	1	1.214E+05	4.59	0.0607	
B^2	5.403E+05	1	5.403E+05	20.44	0.0014	
C^2	43.01	1	43.01	0.0016	0.9687	
Residual	2.379E+05	9	26438.12			
Lack of Fit	1.154E+05	5	23082.02	0.7535	0.6252	Not significant
Pure Error	1.225E+05	4	30633.24			
Cor Total	7.161E+06	18				

Effects of baking temperature, baking time and flour composition ratio on colour (L* value) of papaya biscuits

Figure 4a shows the effect of baking temperature and time on the *L** value or lightness value of the papaya biscuit surface. Both factors have quadratic effect on the *L** value. The surface colour of the biscuit changes due to the browning process during baking (Gallagher et al., 2003). As the baking temperature increases, the colour of the biscuits becomes more intense (Ho & Pulsawat, 2020). The browning process consists of caramelization, dextrinization, and the Maillard reaction (Ameur et al., 2007). However, among all baking factors, temperature is the major factor impacting the colour of biscuits. This is because the Maillard reaction is more prevalent at higher baking temperatures. The presence of reducing sugars is influencing the colour of baked products (Lerici et al., 1990). Kamelia et al. (2019) stated that papaya contains glucose, which is a reducing sugar. Therefore, as the flour composition ratio increases, the colour of papaya biscuit becomes more intense due to the Maillard reaction. In addition, the papaya flour contains a large amount of beta-carotene, which is an orange-red pigment that contributes to the orange-red papaya flour. As the composition of papaya flour increases, the orange-red colour of papaya biscuits will be more intense (Bogacz-Radomska & Harasym, 2018). Chung et al. (2014) stated that the *L** parameter expresses the CIE lightness value. **Figure 4b** shows that the flour composition ratio has a significant linear effect on the colour of papaya biscuits. As the flour ratio of papaya flour increased, the *L** value also increased. *L** value represents the lightness value, a decrease in the value of the *L** value means that the papaya biscuits surface colour is becoming darker. **Table 6** shows the ANOVA for *L** value or lightness value of the papaya biscuits surface. All three factors have significant impact ($p < 0.05$) on the *L** value of the biscuits after baking. As suggested by ANOVA, the *L** value of the papaya biscuit is best described using quadratic model.

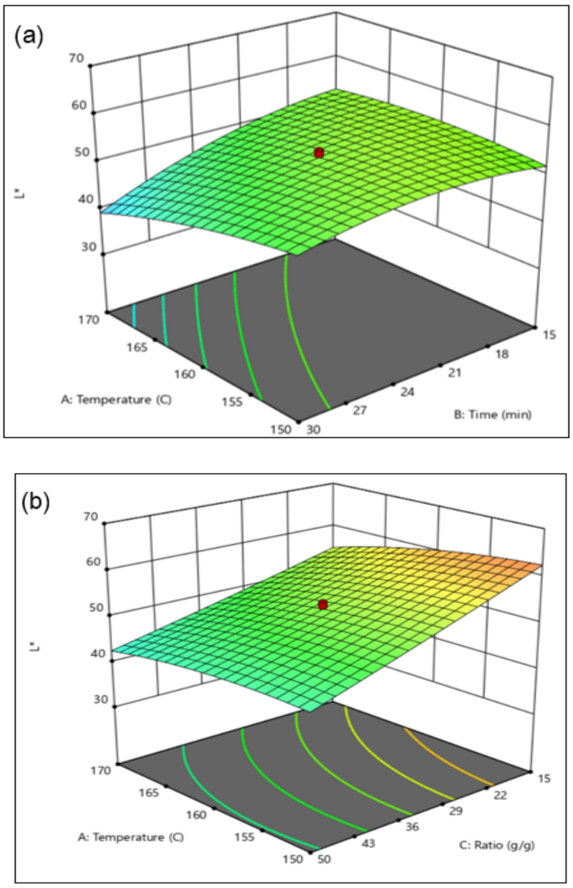


Figure 4 Effect of (a) baking temperature and baking time and (b) baking temperature and flour ratio on the *L** value of the papaya biscuits

Table 6 ANOVA for colour (*L** value) of papaya biscuits

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1219.50	9	135.50	93.48	< 0.0001	significant
A	86.87	1	86.87	59.93	< 0.0001	
B	201.40	1	201.40	138.94	< 0.0001	
C	740.11	1	740.11	510.60	< 0.0001	
AB	68.97	1	68.97	47.58	< 0.0001	
AC	11.83	1	11.83	8.16	0.0189	
BC	1.68	1	1.68	1.16	0.3092	
A^2	23.00	1	23.00	15.87	0.0032	
B^2	92.06	1	92.06	63.51	< 0.0001	
C^2	0.5937	1	0.5937	0.4096	0.5381	
Residual	13.05	9	1.45			
Lack of Fit	9.35	5	1.87	2.03	0.2566	Not significant
Pure Error	3.69	4	0.9228			
Cor Total	1232.55	18				

Optimisation, Verification and Comparison Study

The selection of the optimised condition was done based on the desirability value. Since the objective of the study was to create biscuits with high antioxidant and fibre content, and at the same time the biscuit must have low moisture content and acceptable hardness and colour values, the highest desirability value calculated by Design Expert was only 53.70%. This moderate value was obtained because of the conflicting biscuit requirements. The optimised baking temperature, time and flour composition ratio to obtain the abovementioned biscuit were 170 °C, 21.33 min and 50 g/g, respectively. **Table 7** shows the results for comparison between theoretical data that was obtained from RSM and actual experimental data from the present study. **Table 8** shows the results for comparison between the control sample and optimised papaya biscuits.

Table 7 Comparison between theoretical data from RSM and experimental data

Responses	Optimum Conditions		Percentage Error (%)
	Theoretical Prediction Data	Experimental Data	
Moisture Content (%)	3.84	3.83±0.08	0.2604
Hardness (N)	1321.12	1256.33±93.11	4.9042
Antioxidant (%)	67.25	85.95±0.86	27.8067
*L** value	43.55	43.52±0.57	0.0689
*a** value	19.38	19.91±0.27	2.7348
*b** value	21.70	21.47±0.63	1.0599

Table 8 Comparison between control sample and optimised papaya biscuit

Responses	Control sample	Optimized Papaya Biscuit
Moisture Content (%)	2.78±0.03	3.83±0.08
Hardness (N)	896.00±22.61	1256.33±93.11
Antioxidant (%)	8.93±3.62	85.95±0.86
*L** value	61.23±0.79	43.52±0.57
*a** value	13.08±0.34	19.91±0.27
*b** value	29.39±0.65	21.47±0.63
Fibre (%)	1.5	2.4

Optimised papaya biscuit contains 50% of papaya powder thus the biscuit has higher antioxidant activity and more fibre than the control sample. These are some of the desired characteristics for functional biscuits. However, the drawbacks are the higher moisture content, harder texture and darker surface colour in optimized papaya biscuits than the control samples (**Figure 5**).

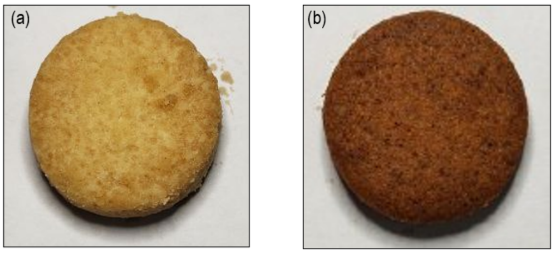


Figure 5 Surface colour of (a) control biscuit and (b) optimised papaya biscuit

Stability of Moisture Content, Hardness, Antioxidant Activity and Colour of the Papaya Biscuits during Storage

The stability of the moisture content, hardness, antioxidant activity and colour of the papaya biscuits during three weeks storage at room temperature was evaluated using kinetic study. This study is important as to understand the behaviour of the moisture content, hardness, antioxidant and colour changes during storage, thus appropriate modifications can be done in future research to improve the stability of these parameters. The stability of the biscuit affects the shelf-life and sensory acceptance of the biscuits. **Table 9** shows the kinetic rate constant, *k*, of the parameters studied. The stability of the optimised papaya biscuits was higher than that of the control sample during storage since the values of *k* for all parameters in papaya biscuits were lower. **Figure 6a** illustrates that the moisture content of the control sample and optimised papaya biscuits increased as storage time increased. However, the moisture content stability was higher for optimised papaya biscuits (k = 0.0129 week^{-1} at room temperature) than the control sample (k = 0.0291 week^{-1} at room temperature). Meanwhile, **Figure 6b** illustrates the decreasing trend of hardness for the control sample and optimised papaya biscuits during storage. However, the hardness stability was higher in papaya biscuits (k = 0.0343 week^{-1} at room temperature) than in the control sample (k = 0.1059 week-*1* at room temperature). **Figure 6c** illustrates that the antioxidant content of the control sample and optimised papaya biscuits decreased as storage time increased. However, antioxidant stability was higher in papaya biscuits (k = 0.0267 week^{-1} at room temperature) than in the control sample (k = 0.0523 week^{-1} at room temperature). The loss of antioxidants in the papaya biscuits was slower than in the control sample, which may be due to the synergistic effect between antioxidants and fibre content in papaya flour.

Table 9 The kinetic rate constant of factors on control sample and optimised papaya biscuit

Factors	Kinetics Rate Constant, *k* (week^{-1})	
	Control Biscuit	Optimised Papaya Biscuit
Moisture Content	0.0291	0.0129
Hardness	0.1059	0.0343
Antioxidant	0.0523	0.0267
*L**	0.0529	0.0142
*a**	0.1626	0.0209
*b**	0.0247	0.0213

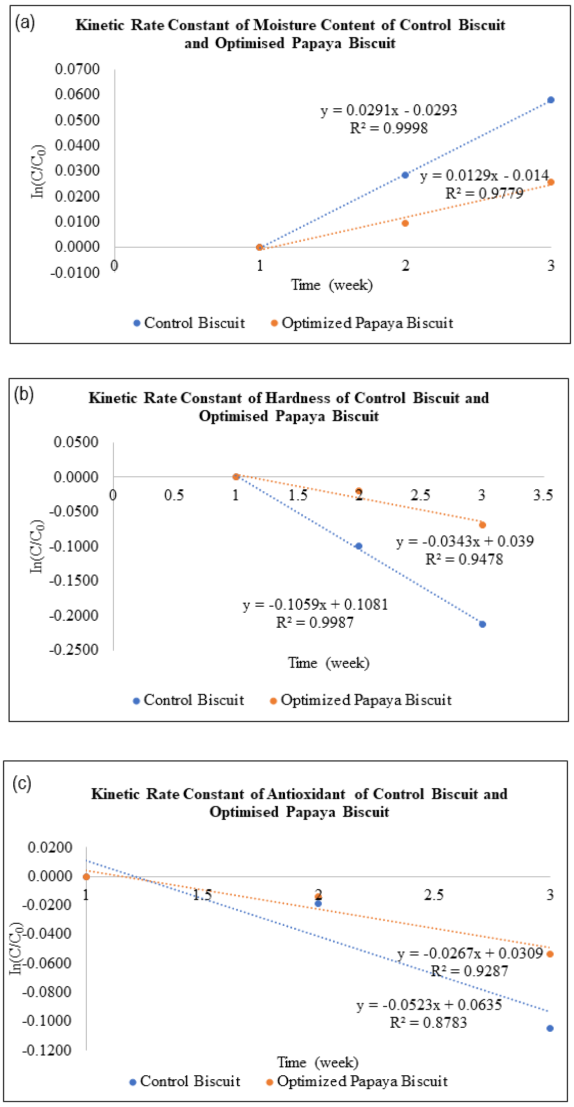


Figure 6 Kinetic rate constants of (a) moisture content, (b) hardness and (c) antioxidant of control sample and optimised papaya biscuits

Figure 7a shows that the *L** values of the control sample and optimised papaya biscuit decreased as storage time increased. However, the stability of the *L** value was higher in the optimised papaya biscuits (k = 0.0142 week^{-1} at room temperature) than in the control sample (k = 0.0529 week^{-1} at room temperature). The *a** value has the same decreasing trend with time during storage (**Figure 7b)**, similar to the *L** value. The stability of the *a** value was higher in the optimised papaya biscuits (k = 0.0209 week^{-1} at room temperature) than in the control sample (k = 0.1626 week^{-1} at room temperature). On contrary, the *b** value of the control sample and papaya biscuit increased as the storage time increased (**Figure 7c)**. However, the difference between the stability of *b** value of the optimised papaya biscuits (k = 0.0213 week^{-1} at room temperature) and the control sample (k = 0.0247 week-1 at room temperature) was not significant.

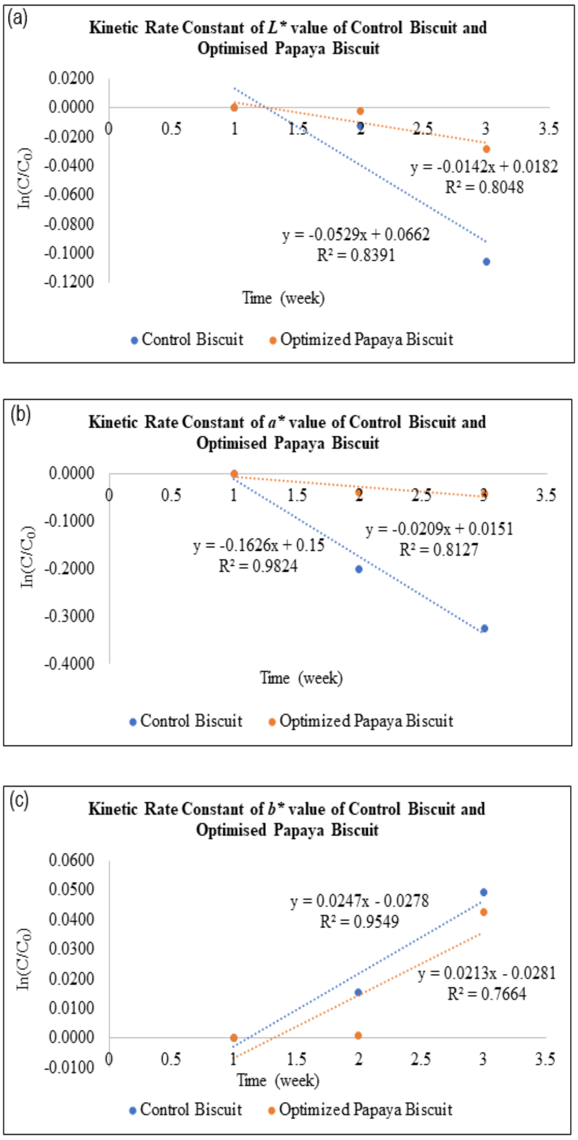


Figure 7 Kinetic rate constants of (a) L^* value, (b) a^* value and (c) b^* value of control sample and optimised papaya biscuit

CONCLUSION

In this study, response surface methodology (RSM) was successfully utilised to optimise the flour composition ratio, baking temperature and time for the production of papaya biscuits with high antioxidant and fibre content, and acceptable moisture content, hardness and colour. ANOVA shows that flour composition ratio, baking temperature and time significantly affect the antioxidant, moisture content, hardness and colour of the optimised papaya biscuits. Kinetic study was used to evaluate the stability of the biscuits during storage. Based on the low kinetic rate constant values obtained for moisture content, hardness, antioxidant and colour of the optimised papaya biscuits, thus it can be inferred that these biscuits were more stable than the control sample during the three weeks of storage. The low kinetic rate constant values also indicate the slow changes in the optimised papaya biscuits during storage. The findings support the potential for this papaya biscuit formulation to be marketed as a substitute functional meal in the future. However, further modifications may be required to improve the stability of the biscuits for a longer shelf-life period.

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References

Abacan, S. F., Hurtada, W. A., & Devanadera, M. A. R. 2017. Effects of Cooking Time, Temperature, and Salt Concentration on the Phenolic Content and Antioxidant Activity of Selected Edible Mushrooms. International Food Research Journal, *24*(5).

Abdel-Hameed, S. M., Abd Allah, N. A. R., Hamed, M. M., & Soltan, O. I. A. 2023. Papaya Fruit By-Products as Novel Food Ingredients in Cupcakes. Annals of Agricultural Sciences. *68*. 60-74. https://doi.org/10.1016/j.aoas.2023.05.003

Alfeo,V., Bravi, E., Ceccaroni, D., Sileoni, V., Perreti, G. & Marconi, O. 2020. Effect of Baking Time and Temperature on Nutrients and Phenolic Compounds Content of Fresh Sprouts Breadlike Products. Foods, *9*, 1447.

Alide, T., Wangila, P., & Kiprop, A. 2020. Effect of Cooking Temperature and Time on Total Phenolic Content, Total Flavonoid Content and Total In Vitro Antioxidant Activity of Garlic. BMC Research Notes, *13*(1), 1-7.

Ameur, L. A., Mathieu, O., Lalanne, V., Trystram, G., & Birlouez-Aragon, I. 2007. Comparison of the Effects of Sucrose and Hexose on Furfural Formation and Browning in Cookies Baked at Different Temperatures. Food Chemistry, *101*(4), 1407-1416.

Azmi, M. M. Z., Taip, F. S., Kamal, S. M. M., and Chin, N. L. 2019. Effects of Temperature and Time on the Physical Characteristics of Moist Cakes Baked in Air Fryer. Journal of Food Science and Technology, *56*(10), 4616-4624.

Bekele, D. W., and Emire, S. A. 2023. Formulation Optimization and Characterization of Functional Kemesh. Heliyon. *9*. e20829. https://doi.org/10.1016/j.heliyon.2023.e20829

Blanch, G.P. and Ruiz del Castillo, M.L. (2021). Effect of Baking Temperature on the Phenolic Content and Antioxidant Activity of Black Corn (*Zea mays*) Bread. Foods *2021, 10*, 1202.

Bogacz-Radomska, L., and Harasym, J. 2018. β-Carotene—Properties and Production Methods. Food Quality and Safety, *2*(2), 69-74.

Chung, H. J., Cho, A., & Lim, S. T. 2014. Utilization of Germinated and Heat Moisture Treated Brown Rices in Sugar-Snap Cookies. LWT-Food Science and Technology, *57*(1), 260-266.

Coura, G. L. C., Freire, R. T. S., Dos Santos, J. C., De Oliveira, L. Á., Scarpa, F., & Panzera, T. H. 2020. Tensile and Flexural Properties of Epoxy Laminates with Natural Papaya Bast Fibre Cellular Layers. Composites Part C: Open Access, *2*, 100017.

Frezzini, M. A., Castellani, F., De Francesco, N., Ristorini, M., & Canepari, S. 2019. Application of DPPH assay for Assessment of Particulate Matter Reducing Properties. Atmosphere, *10*(12), 816.

Gallagher, E., O'brien, C. M., Scannell, A. G. M., & Arendt, E. K. 2003. Evaluation of Sugar Replacers in Short Dough Biscuit Production. Journal of Food Engineering, *56*(2-3), 261-263.

Hasan, M. H., Islam, M. R., Haque, A. R., Kabir, M. R., Khushe, K. J., & Hasan, S. M. K. 2024. Trends and Challenges of Fruit By-Products Utilization: Insights Into Safety, Sensory, and Benefits of the Use for the Development of Innovative Healthy Food: A Review. Bioresources and Bioprocessing. *11*:10. https://doi.org/10.1186/s40643-023-00722-8

Ho, L. H., and Pulsawat, M. M. 2020. Effects of Partial Sugar Replacement on the Physicochemical and Sensory Properties of Low Sugar Cookies. International Food Research Journal, *27*(3).

Kamelia, M., Widiani, N., and Damayanti, I. (2019, October). The Analysis of Glucose and Vitamin C on Papaya (Carica papaya L.) with Different Time Storage. In Journal of Physics: Conference Series (Vol. 1338, No. 1, p. 012026). IOP Publishing.

Koppel, K., Monti, M., Gibson, M., Alavi, S., Donfrancesco, B. D., and Carciofi, A. C. 2015. The Effects of Fiber Inclusion on Pet Food Sensory Characteristics and Palatability. Animals*, 5*(1), 110-125.

Lerici, C. R., Barbanti, D., Manzano, M., & Cherubin, S. 1990. Early Indicators of Chemical Changes in Foods Due to Enzymatic or Non-Enzymatic Browning Reactions. I, Study on Heat Treated Model Systems. Lebensmittel-Wissenschaft Technologie*, 23*(4), 289-294.

Nambi, V.E., Gupta R.K., Kumar, S. and Sharma, P.C. (2016). Degradation Kinetics of Bioactive Components, Antioxidant Activity, Colour and Textural Properties of Selected Vegetables During Blanching. Journal of Food Science and Technology, *53*(7), 3073-3082.

Pathak, R., Thakur, V., & Gupta, R. K. 2018. Formulation and Analysis of Papaya Fortified Biscuits. Journal of Pharmacognosy and Phytochemistry, *7*(4), 1542-1545.

Tan, G.H., Ali, A. & Siddiqui, Y. 2022. Current Strategies, Perspectives and Challenges in Management and Control Pf Postharvest Diseases of Papaya. Scientia Horticulture, 301, 111139.

Saxena, D. C., and Rao, P. H. 1996. Optimization of Ingredients and Process Conditions for The Preparation of Tandoori Roti using Response Surface Methodology. International Journal of Food Science & Technology, *31*(4), 345-351.

Sharma, S. K., Bansal, S., Mangal, M., Dixit, A. K., Gupta, R. K., & Mangal, A. K. 2016. Utilization of Food Processing By-Products as Dietary, Functional, and Novel Fiber: A Review. Critical Reviews in Food Science and Nutrition, *56*(10), 1647-1661.

Siddiqui, A. A., and Nasreen, L. 2014. Effects of the Baking Temperature and Time on the Quality of Biscuits. Journal of State University of Bangladesh*, 5*(1), 1-12.

Varastegani, B., Zzaman, W., and Yang, T. A. 2015. Investigation on Physicochemical and Sensory Evaluation of Cookies Substituted with Papaya Pulp Flour. Journal of Food Quality*, 38*(3), 175-183.