Optimisation of formulation in development of candied musk lime peel using response surface methodology (RSM)

[Pengoptimuman formulasi dalam penghasilan halwa kulit limau kasturi menggunakan Kaedah Reaksi Permukaan (RSM)]

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Abstract
The development of candied musk lime peel was carried out using Response Surface Methodology (RSM) and ECHIP software. In this study, a new product of candied musk lime peel (CMLP) with the optimum formulation of ingredient was developed. The CMLP development involved pickling, candying and drying processes. Discarded musk lime peel, a by-product from enzymatic peeling of candied musk lime fruit was used in this study. The three main ingredients responsible in the development process were sugar syrup, sorbitol and citric acid. Several formulations were obtained through RSM analysis using the three main ingredients. The peels were treated with each formulation separately and the developed products were then evaluated by a group of trained sensory panellists.

Sensory results using RSM analysis and ECHIP software produced the 'target value' and the 'optimum value' for the ingredients. The optimum value for each ingredient was 56.3 °Brix sugar syrup, 7.0% (w/v) citric acid and 10% (v/v) sorbitol. The optimum value was the suggested optimum formulation from RSM analysis for the development of CMLP, however, verification process had to be done to confirm the optimum formulation. The sensory profiles for the product acceptance were done by a group of trained panellists and the values obtained were interpreted as target value and experimental value. Comparing of target value with experimental value was called a verification of product. In this study, the verification process has proved that the developed product using optimum formulation has been achieved. Therefore, the product development of candied musk lime peel using RSM optimisation was satisfactory.

Introduction

Candied peel is a dehydrated product obtained by treating the peel with sugar, glycerol or sorbitol and can also contain approved preservatives (Food Act 1983 and Food Regulation 1985). The most recognised citrus candied peel is made from sweet citrus fruit such as mandarin orange *(Citrus suhuiensis)*, but none from sour citrus fruit such as musk lime *(Citrus mitis)* and sour lime *(Citrus aurantifolia)*. However, grapefruit and lemon are not recommended because they turn bitter when dried (Wagner et al. 2002). Normally, musk

lime was preserved as a candied fruit where the whole fruit was used in the process (Yeoh et al. 1990).

Currently, there is no candied musk lime peel being produced yet. This phenomenon maybe related with the limitation in the fruit physiological characteristics (Allen 1975) that is due to its small size and also thin and smooth peel that is hard to peel especially manually or by using mechanical peeler. However, in this study, the musk lime peel was obtained from the enzymatic peeling process (Hazniza et al. 2002), where this peel was considered as a waste of the process.

In order to develop a new by-product of candied musk lime peel (CMLP), a specific methodology is required. The methodology will direct the creation of candied peel for the musk lime peel in order to model the effect of ingredient level (Rossi 2001). In this study, Response Surface Methodology (RSM) technique (Rossi 2001) was carried out to determine the best formulation for the CMLP development process. The RSM has been chosen because it is very economical and it allows the developers to predict the result by using different combinations of ingredients. RSM is explained as a system of experimental design and analysis that allows simultaneous examination of multiple addition or factors in a single experiment (Currall 1992), where a response in this method is considered to be the result whereas a factor is considered to be the ingredient.

There are three phases of product development cycle in using RSM method. The phases are (i) selecting the useful or important ingredients; (ii) determining the significance of ingredients towards sensory attributes; and (iii) to try out the suggested mixes and produce the exact properties required that best match a target product's sensory profile (Currall 1992).

This study was conducted to optimise the ingredient formulation in developing candied musk lime peel using RSM method. In every new product development, a

Plate 1. Discarded peel of musk lime fruit by enzymatic peeling

sensory evaluation is required to obtain a consumer preference and acceptability (Carr et al. 2001) during the process of product development. The sensory profile obtained from the sensory evaluation is a tool for describing and quantifying perception that is frequently used in the food industry profile as an operational tool for improving the product properties and for their development (Monrozier and Danzart 2001).

Materials and methods
Source of peels
The musk lime *(C. mitis)* peels used were obtained from enzymatic peeling process of musk lime fruits *(Plate 1)*. The peels which were by-products (waste) of the enzymatic peeling process were washed with tap water twice and drained to remove excessive water.

Raw materials
Coarse table sugar and salt were purchased from a grocery in Sri Serdang, Selangor. Sorbitol (Flavoroma) and anhydrous citric acid (Flavoroma) were purchased from Damah Trading Sdn. Bhd., Kuala Lumpur. Stock solution of sugar solution (syrup) and salt solution (brine) were prepared separately at 80 °Brix and 24% (g/100 g of NaCl), respectively (Siti Shahrul Bariah, UPM, pers. comm. 2000). Stock solutions for syrup and brine were prepared by boiling the sugar and salt, respectively, in water until saturated.

Processing of candied musk lime peel
The method for processing of candied musk lime peel was adopted from the combination

method of fruit pickling (Yeoh et al. 1990) and fruit candying processes (Zakaria et al. 1986), with some modifications to suit the candying process for musk lime peel. In this study, the process was divided into four stages which were pickling, candying, drying and coating *(Figure 1)*.

In the pickling process, the peel was first boiled for an hour to denature residual enzymes from the enzymatic process. The peel was drained and immersed in brine stock solution (24%, g/100 g of NaCl). The concentration of the salt was measured daily using a salometer (Atago, S-28E, Japan). The brine concentration was also measured daily until the concentration became constant. Then, the peel was removed, soaked in tap water (30 min), then washed twice with tap water and drained prior to candying process.

In the candying process, the peel was immersed in the syrup with sorbitol as humectant. The brix of the syrup was

measured daily using a refractometer (Atago, ATC-1, Japan) and stopped when the reading reached the required value (45, 52.5 or 60 °Brix) until the value was not altered and remained constant. Sorbitol and citric acid were added after the syrup concentration reached a constant reading. After 3 days of soaking, the solution was drained and the peel was arranged on a wooden tray with metal sieve (35 x 50 cm) and dried (50 ± 1 °C, 4–6 h, 2–3 days) in a locally fabricated cabinet dryer (40 x 60 x 60 cm) as shown in *Plate 2*. Finally, the peel was coated with fine sugar powder and packed in a plastic container.

Experimental design

A single stage optimisation process was carried out using Response Surface Methodology (RSM) (Roststein et al. 1997). RSM uses an experimental paradigm such as Central Composite Design (CCD) with a quadratic model to fit a model by the least square technique (Chiang et al. 2001). In this study, the ECHIP software for RSM was used to provide the experimental design, calculate equations, evaluate statistic and print out data (Whesler 1993). The designation of ingredient and formulations were done using RSM with ECHIP software to verify the amount of selected ingredients. The ingredients or so called 'variables' in RSM terminology will be used in the development of candied musk lime peel.

Data for all variables will be run automatically by the software to obtain a
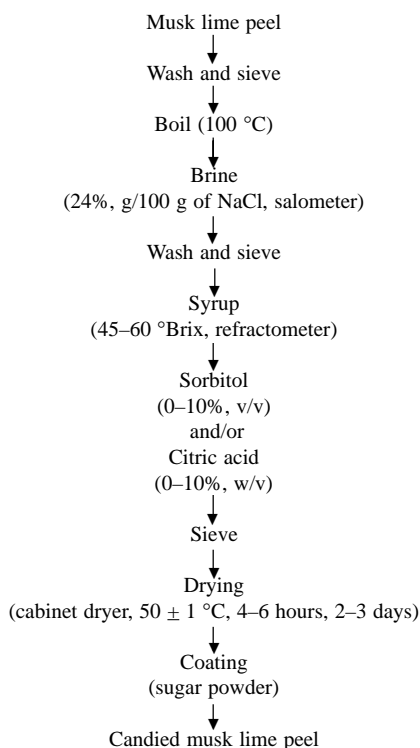
Musk lime peel
↓
Wash and sieve
↓
Boil (100 °C)
↓
Brine
(24%, g/100 g of NaCl, salometer)
↓
Wash and sieve
↓
Syrup
(45–60 °Brix, refractometer)
↓
Sorbitol
(0–10%, v/v)
and/or
Citric acid
(0–10%, w/v)
↓
Sieve
↓
Drying
(cabinet dryer, 50 ± 1 °C, 4–6 hours, 2–3 days)
↓
Coating
(sugar powder)
↓
Candied musk lime peel
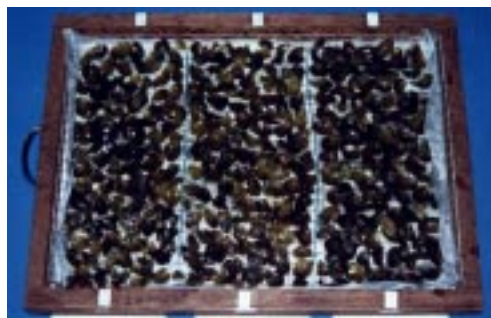
Figure 1. Preparation of candied musk lime peel

Plate 2. Musk lime peels arranged in a wooden tray for drying process

Table 1. Formulation of ingredients based on syrup concentration using RSM in development of candied musk lime peel

Formulation	Syrup (°Brix)	Sorbitol (%, v/v)	Citric acid (%, w/v)
1	45.0	10	10
2	45.0	10	0
3	45.0	5	5
4	45.0	5	0
5	45.0	0	10
6	45.0	0	0
7	52.5	10	5
8	52.5	10	0
9	52.5	5	10
10	52.5	0	0
11	60.0	10	10
12	60.0	10	5
13	60.0	5	10
14	60.0	5	0
15	60.0	0	5

suggested formulation for each ingredient. The concentration for the three variables selected, namely syrup, sorbitol and citric acid were ranged between 45–60 °Brix, 0–10% (v/v) and 0–10% (w/v), respectively. The concentration for each variable was the minimum and maximum values that were obtained from the preliminary study (unpublished data) on the product ingredients during the early stage of this study. A total of 15 combinations of formulations were designed randomly by the software as shown in *Table 1*. Six formulations were 45 °Brix syrup concentration, four formulations were 52.5 °Brix syrup concentration and five formulations were 60 °Brix syrup concentration.

All formulations were grouped based on syrup concentration prior to syrup soaking in initial preparation *(Figure 1)* of the candied musk lime peel. The grouping purpose is to ease conducting the sensory test for all the formulations on a same day. However, each group was evaluated randomly in a different period of time. In sensory evaluation, panellists were not recommended to analyse too many samples simultaneously to avoid them from feeling

stress and fatigue thus influencing the product evaluation (Lyon et al. 1992). All sensory evaluations for each formulation were carried out in duplicate and in randomized order within each replication.

The candied musk lime peels (CMLP) were then prepared using each formulation, simultaneously, and were then evaluated by a sensory test. The prepared CMLP were evaluated using QDA (Qualitative and Descriptive Analysis) test. The collected data from QDA test were then analysed using ECHIP software. The QDA results enabled elucidation of interaction between ingredients coefficient and sensory attributes. Coefficient of P value with highly significance level determined the variables that would be affecting the sensory attributes in the product development process. The contour maps obtained from the sensory results (QDA test) provide a Correx Reading for each sensory attribute. The Correx Reading shows the contour maps for each sensory attribute. Superimpose of all contour maps enabled the determination of the Optimum Value *(OV)* for each ingredient and the Target Value *(TV)* for ingredient limit. All results were obtained using ECHIP software automatically by pointing the values at the centre of the acceptable area (area from superimposed contour maps).

The Optimum Value *(OV)* is explained as the optimum concentration or intensity for each ingredient which was most preferred by the panellists through sensory evaluation. The *OV* for each ingredient will then be used in the preparation of optimum CMLP. The Target Value *(TV)* is explained as the optimum ingredient limit with low to high intensity of acceptance towards the product which was most preferred by the panellists through sensory evaluation and mostly acceptable for the production of new product.

The *TV*, however, will be used only after the optimum CMLP has been tested (sensory) and the obtained results ('Experimental Value', *EV*) would be compared with the *TV* using verification
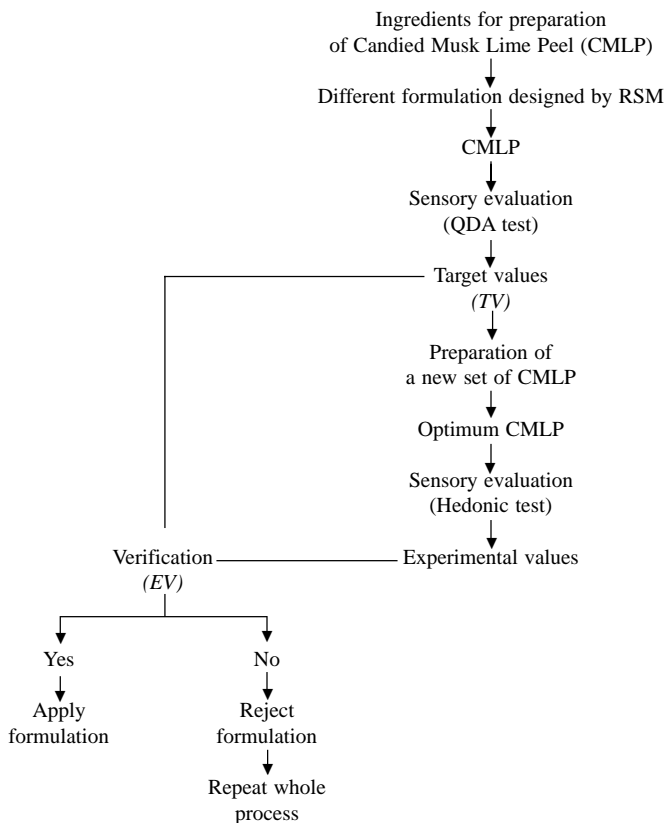
Ingredients for preparation
of Candied Musk Lime Peel (CMLP)
↓
Different formulation designed by RSM
↓
CMLP
↓
Sensory evaluation
(QDA test)
↓
Target values
(TV)
↓
Preparation of
a new set of CMLP
↓
Optimum CMLP
↓
Sensory evaluation
(Hedonic test)
↓
Verification —————————— Experimental values
(EV)
↓   ↓
Yes   No
↓   ↓
Apply   Reject
formulation   formulation
                     ↓
                    Repeat whole
                    process

Figure 2. Optimisation of formulation in development of candied musk lime peel

process. The *OV* for each ingredient was then used in the making of optimum CMLP. The prepared product was then evaluated using Hedonic test and the obtained results were analysed using ECHIP software. The results showed the *EV* for each sensory attribute in the tested product.

The *EV* is the acceptance value for each sensory attribute in the development of optimum product that is more preferred by the sensory panellists. The *EV* obtained for each sensory attribute would then be compared with the *TV* previously obtained from the QDA test. The comparison of both *EV* and *TV* was called a verification process. The process is to determine the acceptability of the developed formulation as preferred by the sensory panellists (representative consumer).

Application of the *OV* in the making of the other product (optimum) will produce an acceptance profile (*EV*) for each sensory attribute as most preferred by the sensory panellists. Therefore, the acceptance profile should be in the range of *TV* in order to accept the formulation for the development of optimum product. Thus, the verification process was used to determine either the *EV* was within the range of the *TV*. If the acceptance values for each sensory attributes were within the limit, it verified that the optimum formulation for the development of CMLP using RSM model was adequate and applicable, and vice versa. The experimental design for the development of CMLP using RSM model is shown in *Figure 2*.

Sensory analysis
The sensory analysis was done by 20 trained panellists comprising the staff and postgraduate students of Faculty of Science and Food Technology, Universiti Putra

Malaysia, Serdang, Selangor. In this study, the panellists were required to evaluate the developed product. The panellists were trained for their capability of recognising competency in recognising the basic tastes and in describing taste (Hazniza 2003). This group of panellists was trained to have a standard capability in recognising and describing the taste in every sensory evaluation of the developed product.

Two sensory tests were used in this study, a Hedonic and QDA tests. A scale from 1 (dislike extremely) to 7 (like extremely) rating was used for Hedonic test (Hazniza 2003) and a 15 cm scale of horizontal unstructured line was used for QDA test (Hazniza 2003). In QDA test, the horizontal unstructured line scale was anchored at the left end with 'least acceptable' or 'weak' and at the right end with 'most acceptable' or 'extremely'.

Panellists were required to evaluate the product between scales 1 and 7 in the Hedonic test to show their sensory preference on the presented products. However, more details on product preference were needed. Therefore, a QDA test was required to measure the product preference on the presented product. In this study, the sensory evaluation was carried out in duplicate and in randomised order within each replication.

Results and discussion
Regression analysis on sensory attributes
The results of Hedonic test were analysed using ECHIP software as illustrated in *Table 2*. The table shows the significant factors and the interaction between the ingredients coefficient, namely, syrup, sorbitol and citric acid, and sensory attributes such as colour, odour, taste, texture and overall acceptability. Three types of significant levels were presented by this model such as 0.05 ($p < 0.05$), 0.01 ($p < 0.01$) and 0.001 ($p < 0.001$). These results also indicated the regression coefficient for each of the dependent variables with their corresponding coefficients of determination (R^2) and probability (P) of F values.

Table 2. Significant factors and the interactions between the ingredients coefficient and sensory attributes

Coefficient	Colour	Odour	Taste	Texture	Overall acceptability
β_0	4.89892	4.92168	4.78187	5.25439	4.95187
β_1	0.0188251	0.0246028*	0.0470788**	0.0177489	0.0378016***
β_2	0.020766	0.0212841	0.0164348	0.0566448**	0.0288512**
β_3	0.11174***	0.0664384***	0.0897603**	0.189033***	0.143802***
$\beta_1{}^2$	-0.000828095	0.00261586	-0.000448825	0.00133595	0.00166332
$\beta_1{}^3$	0.00291349	0.000521366	0.00490586	-0.00355811	-0.0011349
$\beta_2{}^3$	0.00653658	0.00410105	0.00485198	0.00731234	0.00406688*
β_{11}	-0.00363698	-0.00622267*	-0.00368952	-0.00577438	-0.00463985**
β_{22}	-0.00355925	-0.00396771	0.00779792	-0.0037263	0.00436978
β_{33}	-0.0297847***	-0.0221782**	-0.0353429**	-0.062882***	-0.048806***
R^2	0.919	0.919	0.891	0.975	0.990
P value	0.0002***	0.0002***	0.0009***	0.0000***	0.0000***

$_0$Value is coefficient for scale
$_1$Value is coefficient for sugar syrup
$_2$Value is coefficient for sorbitol
$_3$Value is coefficient for citric acid
*Significant factor of P value at 0.05 level ($p < 0.05$)
**Significant factor of P value at 0.01 level ($p < 0.01$)
***Significant factor of P value at 0.001 level ($p < 0.001$)

The closer the value of R^2 is to unity, the better the empirical model fits the actual data. The smaller the R^2, the less relevance the empirical model fits the behaviour variation (Mendehall 1975). The R^2 of more than 0.75 was statistically considered accurate enough for developing a model for the developed product. According to Henika (1982), R^2 lower than 0.75 means a significant lack of fit, whereas, Yusof et al. (1988) who studied the optimisation of guava concentrate process had taken only R^2 greater than 0.90 for prediction purposes. The value of R^2 for colour, odour, taste, texture and overall acceptability responses were 0.919, 0.919, 0.891, 0.975 and 0.990, respectively and with significant P values 0.0002, 0.0002, 0.0009, 0.000 and 0.0000, respectively. This indicates that amounts of syrup, citric acid and sorbitol were highly significant in affecting all the sensory attributes of candied musk lime peel.

Optimisation of ingredients

The development of candied musk lime peel involved the optimisation process of ingredient formulation. At this stage, the OV was a mixture of each sensory attribute obtained from superimposed contour maps from the QDA results. *Figure 3 (a-f)* shows the Correx Reading of contour maps for each sensory attribute (colour, odour, taste, texture and overall acceptability) in the development of candied musk lime peel. The contour lines in the diagrams are lines of equal response (syrup versus citric acid), in the same way that contour lines on a map are lines of equal height. Interactions may be identified, in those cases, where the pattern of response at the left hand edge of a diagram differs from the response at the right hand edge. The fact that the contour lines are more or less equally spaced in these diagrams shows that the response is linear (Currall 1992).

Superimposed contour maps for all the sensory attributes are constructed to determine the optimum formulation (Rossi 2001) that best matched the target for the

development of the candied musk lime peel. Acceptable area of superimposed contour map revealed that colour, taste, texture and overall acceptability were the limiting factors in attaining the optimum value for the product's target formulation *(Figure 4)*. Using the RSM with ECHIP software, the OV and TV can be obtained from the superimposed contour maps *(Table 3)*. The OV obtained for the sugar syrup was 56.33 °Brix, while for citric acid and sorbitol were 7.03% (w/v) and 10% (v/v), respectively.

The TV is the computed factor scores for the target current product formulation (Rossi 2001). The obtained TV can give effective direction when the product development goal is to match the sensory profile of a target product (Currall 1992). The low and high values of EV that are closer to the TV for the specific component of sensory attributes give more weight to that component. In this study, the low and high TV obtained from the ECHIP Software were 4.37 and 6.20, respectively. The average TV obtained was 5.29. After obtaining the OV and the TV, another set of candied musk lime peel was developed using the OV of ingredient, and a sensory evaluation of Hedonic test was done to verify the results with the TV.

### Verification of optimum formulation (Target Value)

Results for the Hedonic test on the newly developed candied musk lime peel (CMLP) using the ingredients optimum values were obtained as in *Table 4*. The attribute values obtained were known as the experimental values *(EV)*. In the verification process, the optimum formulation for the development of CMLP can be accepted if the EV were similar or within the range of the TV. However, if the EV falls outside the TV range, the optimum formulation is rejected. From the table, the EV for each attribute such as colour, odour, taste, texture and overall acceptability are 5.27, 5.00, 4.95, 4.64 and 4.59, respectively. All values were within the target value range that is between
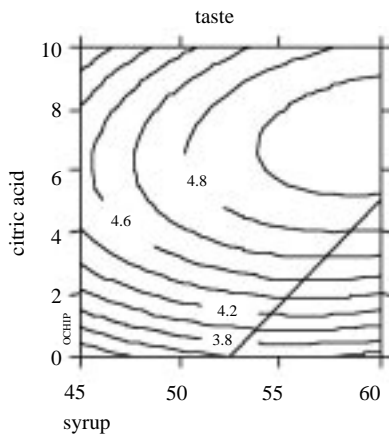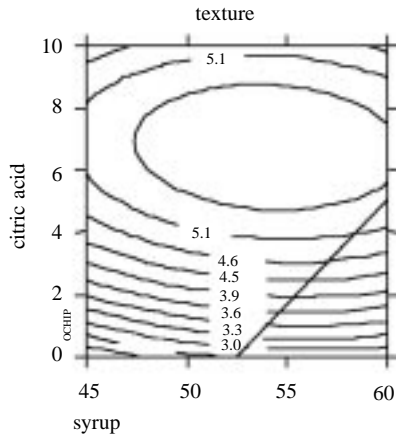
a) Colour
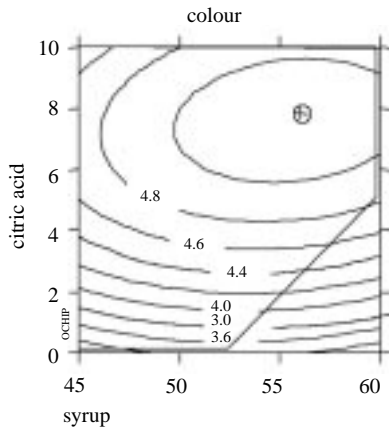
b) Odour
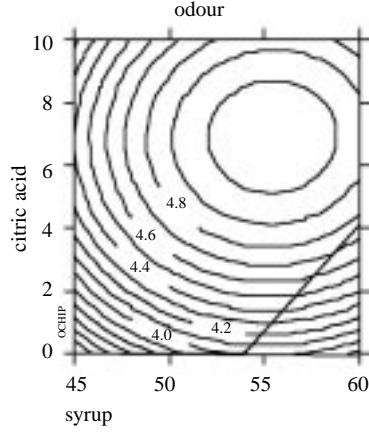
c) Taste
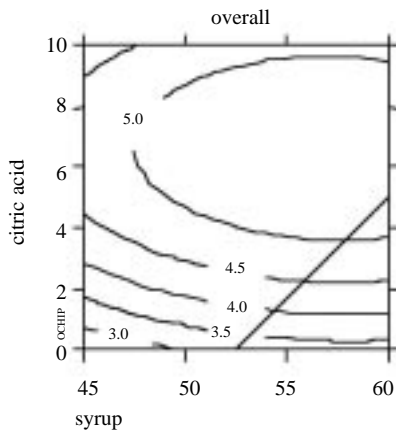

d) Texture

e) Overall Acceptability

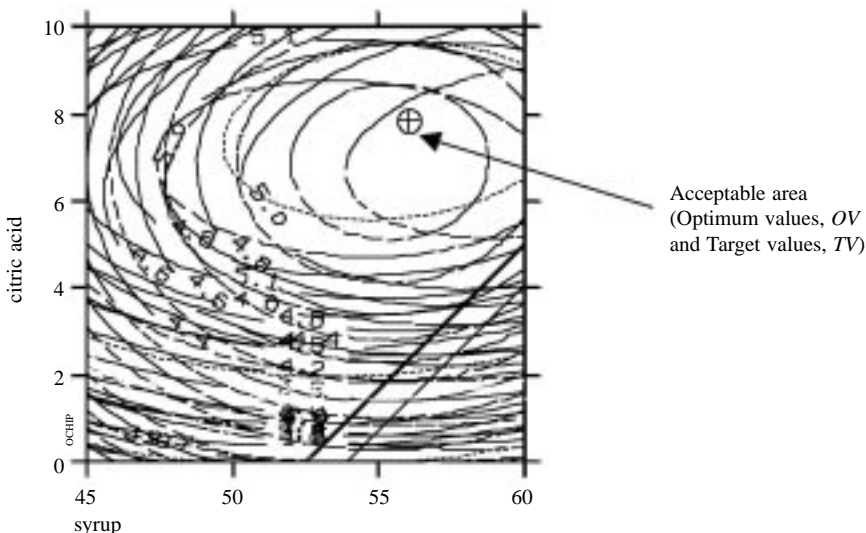*Figure 3 (a-e). Effect of sensory attributes on Correx Reading*

Figure 4. Superimposed contour maps for all sensory attributes in determination of optimum and target values

Table 3. The optimum and target values of candied musk lime formulation by overlapping process of sensory contour maps

Ingredient Limit	Optimum Value *(OV)*	Ingredient Limit	Target Value *(TV)*
Sugar syrup	56.33 °Brix	Low	4.37
Citric acid	7.03 (w/v)	High	6.20
Sorbitol	10% (v/v)		

Table 4. Experimental value obtained from Hedonic profiles of sensory attributes in development of candied musk lime peel using optimum values

Sensory attribute	Experimental Value *(EV)*
Colour	5.27
Odour	5.00
Taste	4.95
Texture	4.64
Overall acceptability	4.59

4.37 (low) and 6.20 (high). This verification showed that the optimum processing range of dependent variables (sugar syrup, sorbitol and citric acid) for the development of optimum formulation for CMLP by RSM model was adequate and applicable.

Conclusion and recommendation

Development of Candied Musk Lime Peel (CMLP) using optimum formulation with 56.3 °Brix of sugar syrup, 7.0% (w/v) of citric acid and 10% (v/v) of sorbitol was satisfactory. The application of Response Surface Methodology (RSM) with ECHIP software aided the optimisation process of ingredients for the development of CMLP. Several formulations were produced and selection of optimum formulation achieved was obtained through sensory evaluation using trained panellists. The verification process is the final stage in the development of CMLP. A verification process is necessary in order to indicate either the result obtained achieved the target product. Therefore, further research such as physico-chemical characteristics should be conducted on the final product that are potentially to be commercialised.

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Abstrak

Pembangunan halwa kulit limau kasturi telah dijalankan dengan menggunakan Kaedah Reaksi Permukaan (RSM) dan perisian ECHIP. Dalam kajian ini, satu produk baru halwa kulit limau kasturi (CMLP) dengan formulasi ramuan yang optimum telah dihasilkan. Penghasilan CMLP melibatkan proses penjerukan, penghalwaan dan pengeringan. Kulit limau kasturi yang terbuang, hasil sampingan daripada proses pengupasan berenzim buah limau kasturi telah digunakan dalam kajian ini. Tiga ramuan utama yang penting dalam proses penghasilan ini ialah sirap gula, sorbitol dan asid sitrik. Beberapa formulasi telah diperoleh melalui analisis RSM dengan menggunakan ketiga-tiga ramuan utama tersebut. Kulit limau tersebut telah dirawat dengan setiap formulasi secara berasingan dan produk yang dihasilkan kemudiannya dinilai oleh sekumpulan juri uji rasa terlatih.

Analisis daripada keputusan uji rasa menggunakan RSM dan perisian ECHIP telah menghasilkan 'nilai sasaran' dan 'nilai optimum' untuk ramuan. Nilai optimum untuk setiap ramuan ialah 56.3 °Brix sirap gula, 7.0% (berat/ isipadu) asid sitrik dan 10% (isipadu/isipadu) sorbitol. Nilai optimum ialah formulasi optimum yang dicadangkan daripada analisis RSM untuk penghasilan CMLP, walau bagaimanapun proses pengesahan perlu dilakukan untuk memastikan formulasi optimum. Profil uji rasa untuk penerimaan produk telah dilakukan oleh sekumpulan juru uji rasa terlatih dan nilai-nilai yang diperoleh ditafsirkan sebagai nilai sasaran dan nilai uji kaji. Perbandingan nilai sasaran dengan nilai uji kaji dikenali sebagai proses pengesahan produk. Dalam kajian ini, proses pengesahan telah membuktikan bahawa produk yang dihasilkan menggunakan formulasi optimum telah dicapai. Maka, penghasilan produk halwa kulit limau kasturi dengan menggunakan pengoptimuman RSM telah berjaya.