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# Enhancing physicochemical and leaching properties of grass jelly formulation by incorporation of gelatine

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## Abstract

Grass jelly is a popular black jelly used in preparing various drinks and desserts. Nonetheless, preservatives and additional colourants are often used by food industries to maintain the properties of grass jelly and minimize discolouration as well as its leaching occurrence. These additives are linked with deleterious health effects and loss of vitamins in foods. The use of gelling agents such as gelatine in making grass jelly could improve its physiochemical and leaching properties. This study was designed to enhance the physicochemical (pH, colours, Brix) and textural properties of grass jelly using bovine gelatine. A texture profile analysis was conducted to analyse the hardness, springiness, gumminess and chewiness of the grass jelly. This study was also conducted to study the effect of gelatine addition on the leaching and floating properties of the grass jelly. The absorbance value of the grass jelly drink was measured in 10-minute intervals to determine its leaching occurrence while the floating analysis was done by recording the floating time. Various grass jelly formulations (GJF) were designed using a D-optimal mixture design in Design Expert® software. Data from commercial grass jelly were used as a reference during the optimization process. The amount of gelatine added to the formulations was in the range of up to 50 g (~17%). The results showed that the addition of gelatine at approximately 12.5 g (~4%) was effective in maintaining most of the physiochemical properties (L, a, b, Brix) with an overall desirability function of 0.63. This formulation was selected as the optimum grass jelly formulation (OGJF). The leaching properties and floating duration were measured on the OGJF and commercial grass jelly for 90 mins and 60 mins, respectively. The results indicated that OGJF showed a significantly ($p$<0.05) lower leaching rate and floating time ($p$<0.05). Hence, further optimization is needed to increase the floating duration of gelatine-based grass jelly.

## 1. Introduction

Recent years have shown increasing interest in using natural functional food as a tool to improve health and reduce the risk of non-communicable diseases (Su *et al*., 2022). *Mesona chinensis* (grass jelly) is a plant that belongs to the genus *Liliaceae* and is used in Chinese folk medicines to prevent hypertension, diabetes and other non-communicable diseases (Sasmita and Ling, 2017; Xiao *et al*., 2019). Grass jelly is low in fat and highly nutritious resulting from its extract (Wulandari *et al*., 2019). In addition, the grass jelly has numerous medicinal benefits such as antioxidant (Widyaningsih *et al*., 2018; Huang *et al*., 2021), antihyperlipidemic (Thao *et al*., 2019), antihyperglycemic (Lim *et al*., 2017), anti-inflammatory, and immunomodulatory (Widyaningsih *et al*., 2017; Widyaningsih *et al*., 2020) activities and hepatoprotective effect (Hong *et al*., 2022). These bioactivities and health-promoting effects of *M. chinensis* have been correlated with the presence of polyphenols such as kaempferol, apigenin, caffeic acid

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and many more. The plant also contains polysaccharides, terpenes, and vitamins which explains its use as a functional food (Huang *et al.*, 2021).

Traditionally, black grass jelly is prepared from *M. chinensis* leaves using an alkaline extraction method. The solution is heated, then tapioca starch (2.5 - 3.7%) and sugar are added with constant stirring, cooled and the gel is formed (Juen, 2020; Boonjing and Ruttarattanamongkol, 2020). The extracts of *M. chinensis* have low viscosity in an aqueous solution, while the additions of small amounts of starch increase the viscosity and form a gel (Lim *et al*., 2018). Starch is widely used in food products as a gelling agent, and thickener as well as to retain water. Natural starches such as tapioca, wheat and sweet potato starch are commonly used in food products, however, their disadvantages are short shelf-life, thermal instability and loss of water (Ren *et al*., 2021). This was also found to be true in black grass jelly where tapioca starch is used in jelly preparation and was reported to have a high syneresis rate and unstable texture. This results in a shorter shelf life, especially at room temperature (Juen, 2020).

Texture properties play a major role in determining consumer acceptance of jelly products. Among texture properties, hardness is the main factor that affects the initial perception of jelly (Huang *et al*., 2021). Gelling agents such as gelatine and carrageenan have been previously proven to be effective in improving the texture of jelly products. Kreungngern and Chaikham (2016) investigated the effect of 3% and 6% (w/v) concentrations of various gelling agents (potato flour, agar and carrageenan) on the rheological, textural, colour and sensory properties of grass jelly. The results showed that the 3% potato flour addition to grass jelly improved all tested parameters. Gelatine also significantly improved the hardness and chewiness of gummy jelly; however, the jelly was only formed at concentrations above 4% gelatine. The study also showed that above 10%, the jelly product becomes too hard and unacceptable (Zhou *et al*., 2021).

Instead of optimizing the various components and preparation steps involved in black grass jelly preparation, the addition of commercial gelling agents might improve the texture with minimal changes to its other physiochemical properties. The extract of *M. chinensis* used in making grass jelly contains pigments that give it its dark brown or black (Yuris *et al*., 2019). One of the most important factors that determine customer acceptability is colour or appearance. Leaching could cause the black colour to be drawn out resulting in discolouration of the grass jelly. As a result, this may affect the colour quality of the grass jelly drink and reduce customer acceptance. As grass jelly is recognised for its black colour, it is crucial to keep the original black colour to meet consumer expectations (Foroni *et al*., 2016). The addition of gelatine was effective in preventing the leaching of water-soluble compounds, proteins and peptides in aquaculture feed (Guo *et al*., 2021), fish ball (Feng *et al*., 2017) and meat products (Antoniewski *et al*., 2007). However, commercial gelatine ranges from bright or pale yellow to dark amber (Silva *et al*., 2017). Hence, this research aimed to improve the physicochemical and textural properties of grass jelly with the addition of gelatine using Design Expert®. The floating and leaching rate of gelatine-based grass jelly was also studied.

## 2. Materials and methods

### *2.1 Raw materials*

Dried herbs (stem and leaves) of *M. chinensis* were purchased from a Chinese herbal market at Pasir Gudang, Johor. Gelling agents such as gelatine (Food grade; Bovine source; Halagel Products Sdn. Bhd.), sweet potato (Riang Ban Hung Sdn. Bhd.) flour and corn (Barkath Foods Sdn. Bhd) flour were purchased from the respective supplier. Sugar and sodium carbonate (Food grade; Future Foods) were also purchased for the grass jelly preparation.

### *2.2 Grass jelly extraction*

The grass jelly extraction was done based on Kreungngern and Chaikham (2016), by extracting its polysaccharide gum from the dried herb of *M. chinensis* (100 g) and modified accordingly. Washing of the dried herbs was performed to remove any impurities and dirt. Then, 0.25% (w/v) of sodium carbonate was put into purified water (3 L) and soaked for 15 mins. The soaking period may allow the interaction of sodium carbonate with the gum as well as provide an alkaline medium. The soaked mixture was then boiled for one hour and frequently stirred. The boiled extract was then filtered using a clean muslin cloth to remove any solid particles and kept for the grass jelly production.

### *2.3 Preparation of grass jelly agar*

The traditional formulation of the grass jelly with ingredients such as water (25 g), sweet potato flour (10 g), corn flour (2.5 g) and grass jelly extract was added with gelatine at equal interval amounts of up to 50 g (0, 12.5, 25, 37.5 and 50 g), approximately 0 to 17%. The total amount of GJF was 287.5 g. All the ingredients were mixed and cooked (~15 mins) until the mixture became thick. The thickened mixtures were poured into heat-stable containers (flat bottom) and allowed to solidify at room temperature. The solidified grass jelly

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was stored at 4°C until further used (Kreungngern and Chaikham, 2016).

### 2.4 Preparation of sugar syrup

The sugar syrup was prepared at 11°Brix whereby 1° Brix is equal to 1 g of sucrose in 100 g of liquid. The 11° Brix of sugar syrup was prepared by dissolving 110 g of sugar in 1 L of water.

### 2.5 Preparation of commercial grass jelly drink

The grass jelly agar was sliced into tiny cubes of 2 × 2 cm and weighed. The commercial grass jelly drink was prepared by mixing the sugar syrup (11°Brix) and the sliced grass jelly agar in 250 mL of a glass bottle.

### 2.6 Colour analysis

The colour of the grass jelly samples was analysed using a Konica Minolta Chroma Meter (CR-400). The parameters tested were in the notation of L*, a* and b*. Lightness was indicated by L* while redness and yellowness were indicated by a* and b*, respectively. The shade of grass jelly colours was determined by these three notations (Prakoso, 2013) and the darkest shade of grass jelly was identified.

### 2.7 Texture profile analysis

The texture of grass jelly was analysed using a TA.XTplus Texture Analyser (Stable Micro System, London, England) at ambient temperature. The texture profile analysis (TPA) was conducted using a 10 kg load cell with a compression platen of 75 mm diameter (SMS P/75). About 75% of the sample height was compressed in two cycles of the compression tests. The experiments were performed in triplicates and Texture Exponent Software (Stable Micro System, London, England) was used for data analysis of the grass jelly texture (Nishinari *et al.*, 2013).

### 2.8 pH analysis

The pH value of the grass jelly was analysed using a checker pH meter (APH-20) to examine the effect of gelatine addition since changes in pH may affect the taste as well as consumer acceptability. The pH of grass jelly was tested by taking the liquid produced from its crushed samples. The pH values were recorded for all samples.

### 2.9 Brix analysis

The excess of liquid produced by the grass jelly during its production was analysed using a Portable Refractometer (Cole-Parmer) to determine the Brix value. In the Brix analysis, a refractive index of the grass jelly was measured. This measurement was based on the density of the samples and the concentrations of dissolved solids were determined.

### 2.10 Experimental design

The experimental design was constructed and analysed by Design Expert® 7.0 (Stat-Ease Inc.), with a D-optimal mixture design and no blocking. The physicochemical and texture of the grass jelly (colour, pH, Brix, hardness, springiness, gumminess, and chewiness) formulations were set as dependent variables or responses while parameters that affected the responses were set as independent variables (Gelatine: 0-50 g; Extract: 200-250 g). The enhanced formulation was further evaluated for floating and leaching properties.

### 2.11 Leaching rate analysis

In determining the leaching rate of grass jelly, the absorbance value of the grass jelly drink (containing sugar syrup and grass jelly agar) was measured every 10 mins by UV-Vis spectrophotometer at 309 nm. The leaching of grass jelly was observed for 90 mins. The absorbance value of grass jelly with gelatine addition was compared to commercial grass jelly.

### 2.12 Floating duration analysis

Floating analysis of the grass jelly was done by recording the floating time. The time was immediately recorded after the grass jelly was mixed in the sugar syrup and stopped recording upon sinking. The grass jelly drink with the longest duration of floating was determined. The floating duration of grass jelly with gelatine addition was compared to commercial grass jelly.

## 3. Results and discussion

In the preliminary analysis of the Design Expert®, all responses showed a straight-line plot for the normal probability of residuals indicating a normal distribution of error as plotted in Figures 1 and 2. Adequacy of model prediction is indicated by small residual values and presented by predicted vs actual plots in which all the data were close to the diagonal line as observed in Figures 3 and 4. Hence, all the models are well-fitted to predict the responses. As for the correlation analysis, positive linear correlations were observed for all responses except for pH which showed negative linear correlations (Figures 5 and 6). The correlation value between experimental and predicted data is represented by the $R^2_{adj}$ value with the best value being near 1 (Asfaram *et al.*, 2015). In this study, high $R^2_{adj}$ values of all response models were observed ranging from 0.9927 to 0.9998. Meanwhile, $R^2_{pre}$ indicates the capability of the model to predict the responses. The difference

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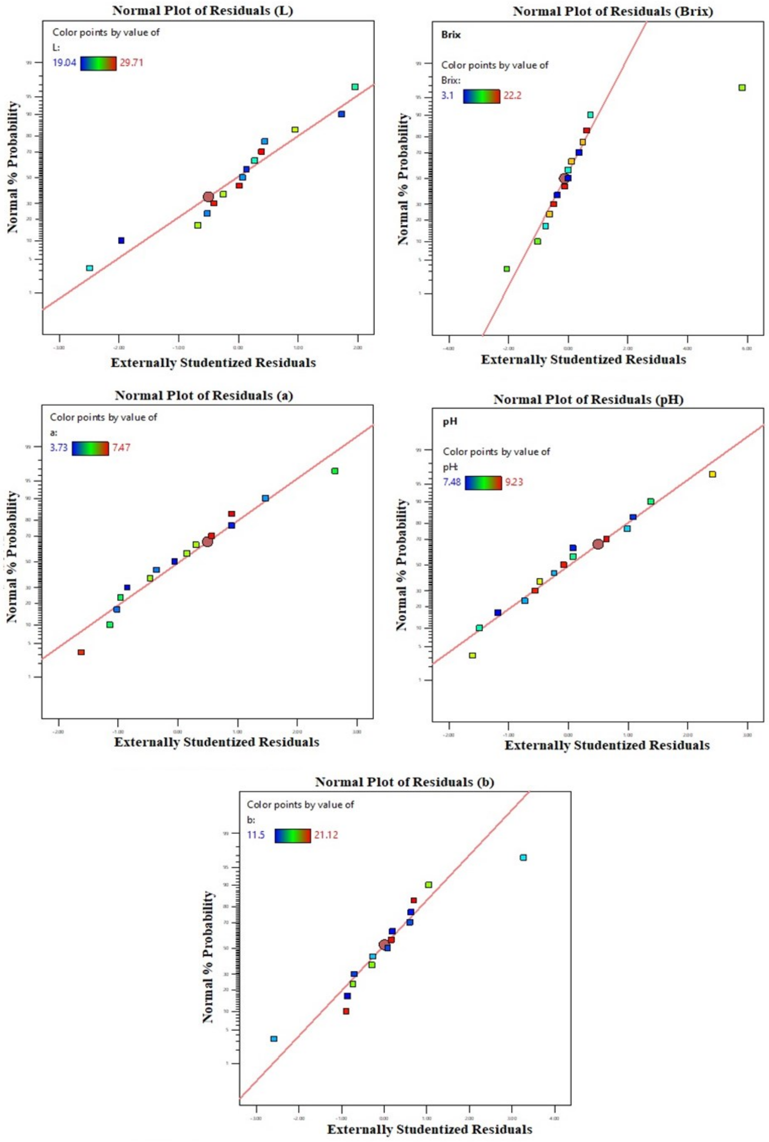


Figure 1. Normal probability plots of residual for physicochemical analysis.

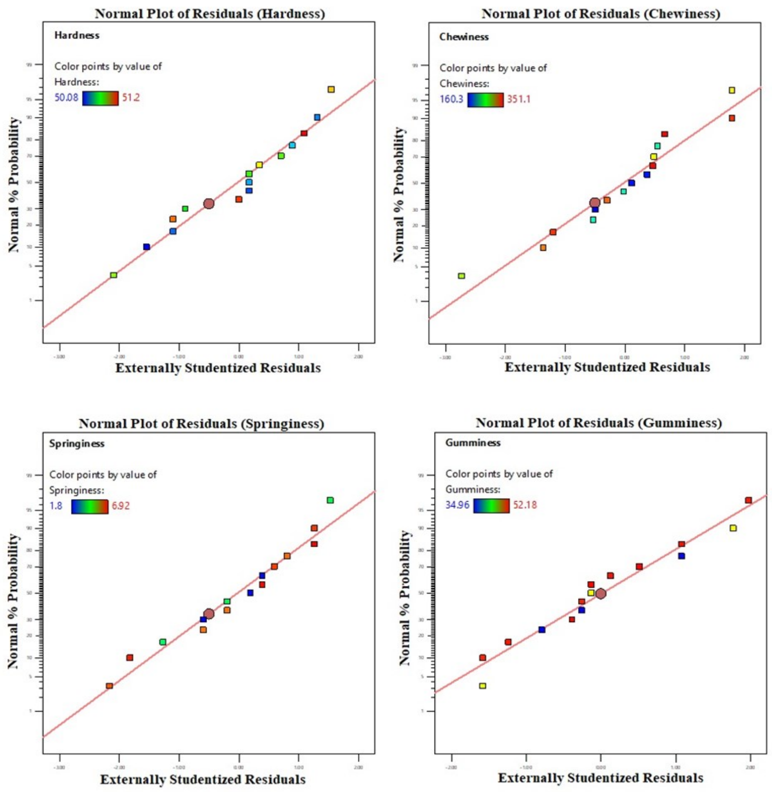


Figure 2. Normal probability plots of residual for textural analysis.

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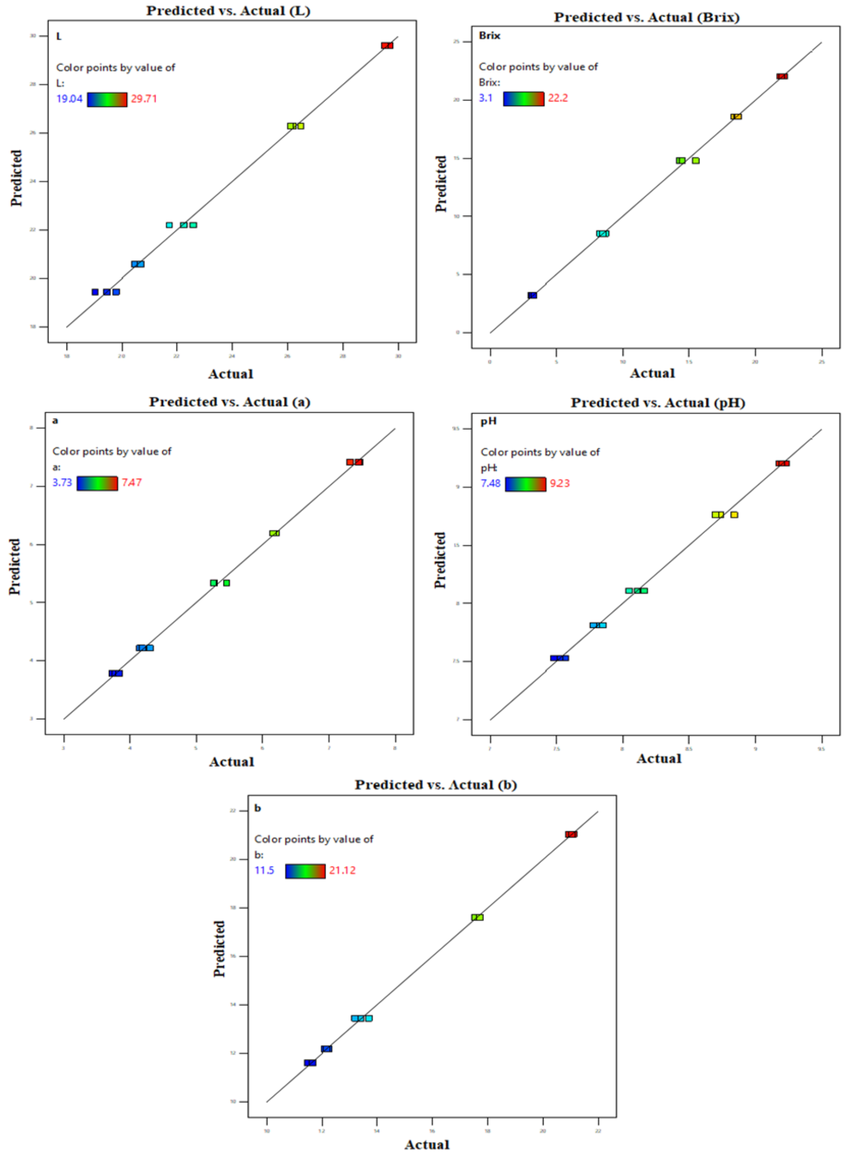


Figure 3. Predicted vs. Actual plots for physicochemical analysis.

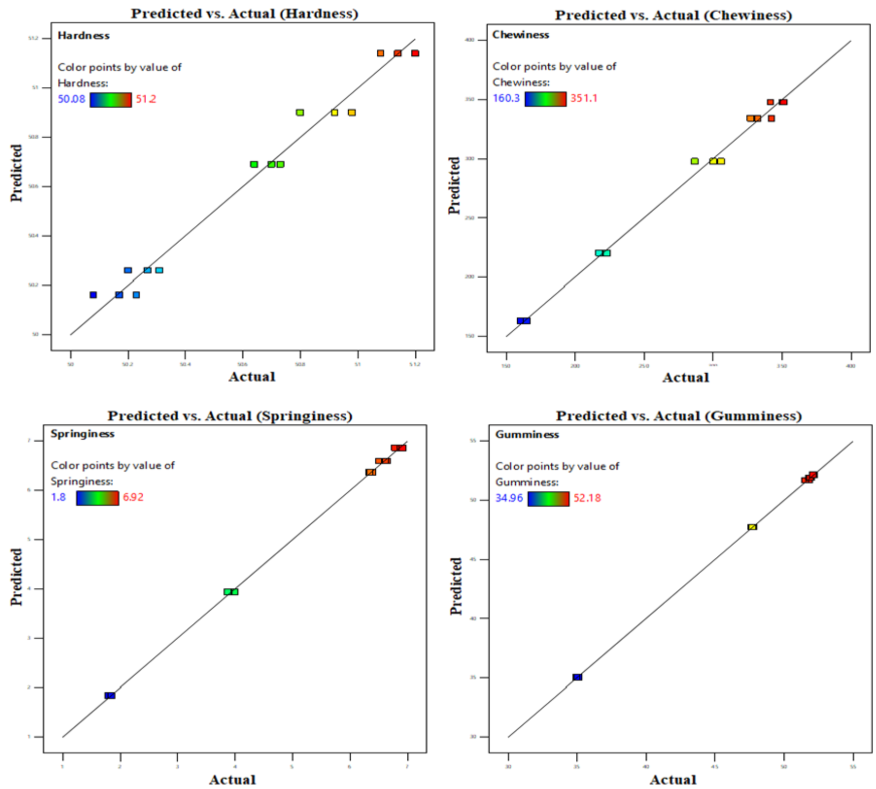


Figure 4. Predicted vs. Actual plots for textural analysis

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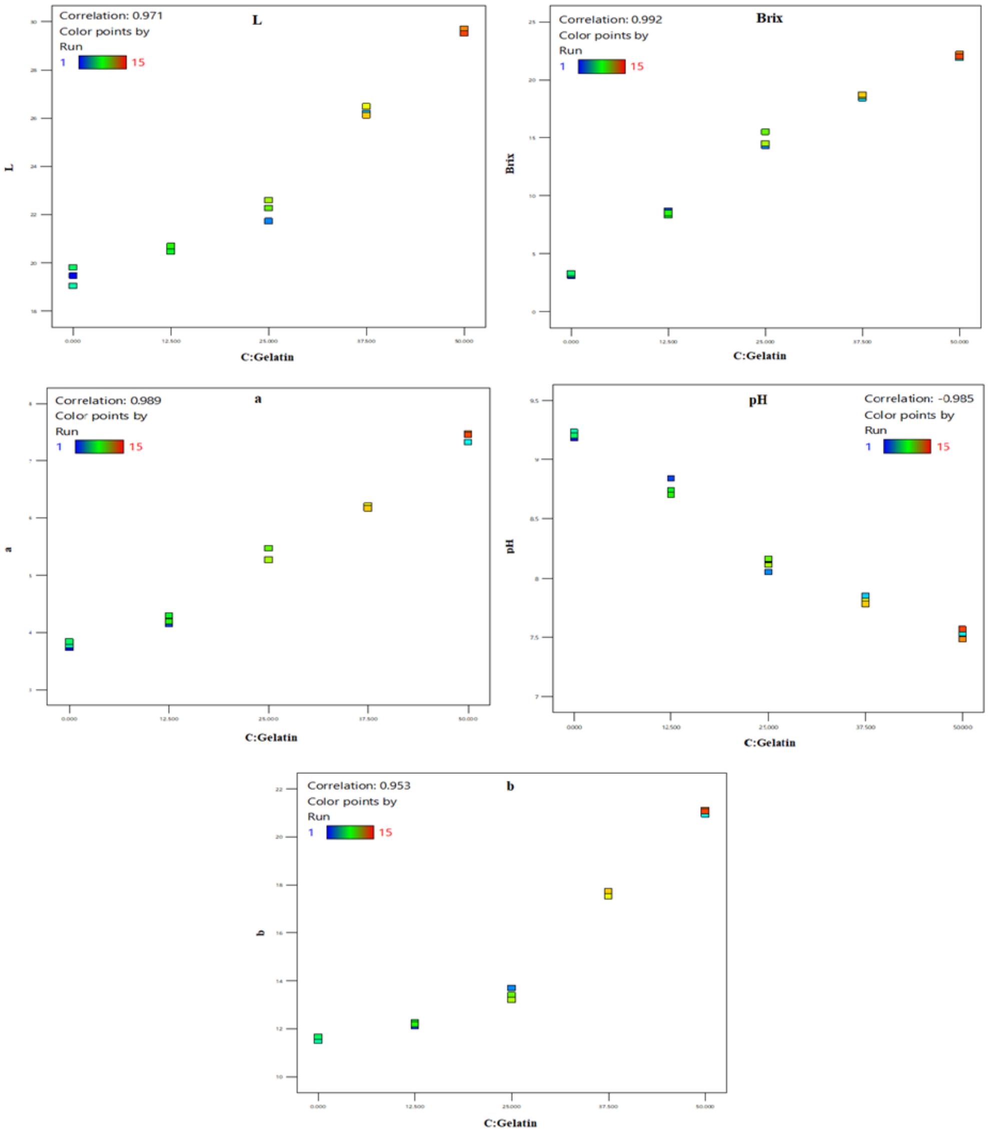


Figure 5. Correlation plots of physicochemical analysis.

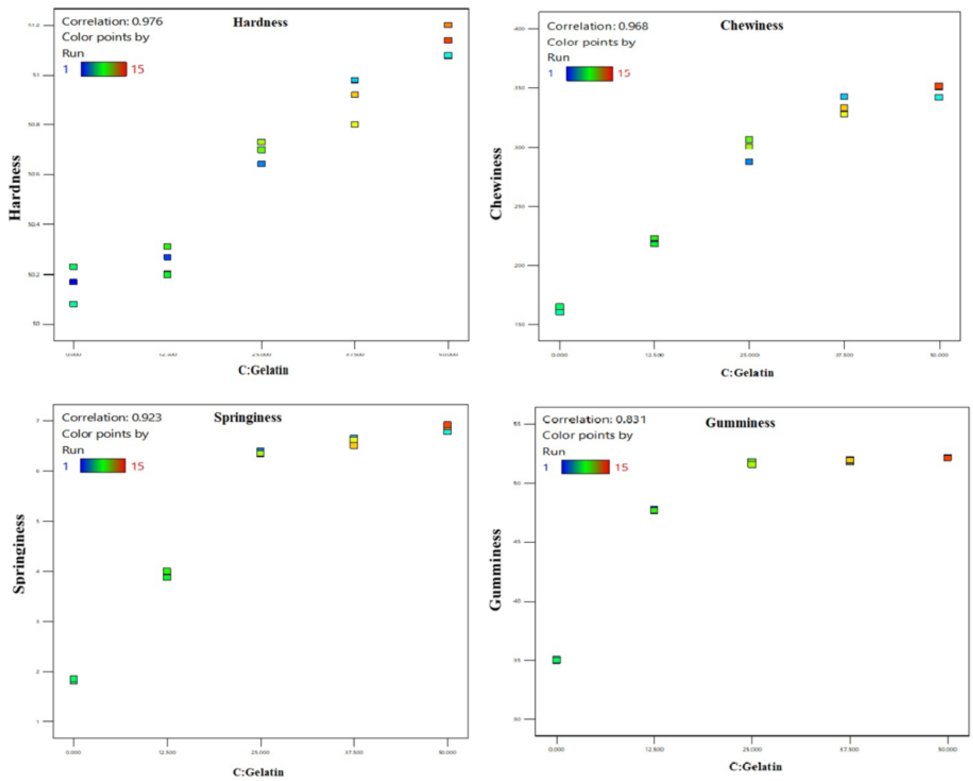


Figure 6. Correlation plots of textural analysis.

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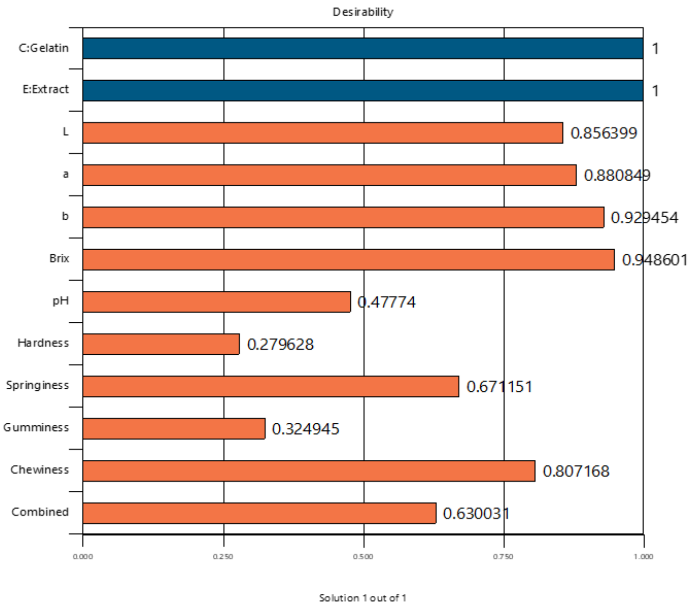


Figure 7. Desirability graph of individual factors, responses and combination of all responses.

between $R^2_{adj}$ and $R^2_{pre}$ value should be less than 0.2 (Fadhlina *et al*., 2020) which was observed in all models (L = 0.9918; a = 0.9950; b = 0.9979; c = 0.9967; pH = 0.9904; Hardness = 0.9516; Springiness = 0.9986; Gumminess = 0.9997; Chewiness = 0.9882). Another crucial indicator is the coefficient of variation (CV) for each model to estimate the relative variability in relation to the mean and expressed as a percentage. For designing an excellent model, the CV value should be less than 10%, however, the model is considered acceptable if the CV value is less than 20% (Fadhlina *et al*., 2020). A correlation coefficient of 0.7 and 1.0 also reflects a strong and linear relationship between variables (Laith *et al*., 2021). In the present study, all models showed less than 10% of CV value in a range of 0.13 to 2.38 which was ideal (Granato and de Araújo Calado, 2014; Fadhlina *et al*., 2020).

The desirability function was first proposed by Harrington (1965) to assess the overall desirability of a multifactorial experiment. The desirability function used is based on Derringer's desirability function which ranges from 0 (non-desirable) to 1 (very desirable) (Pourmortazavi *et al*., 2019). The score is obtained by combining the desirability score for each response to obtain an overall desirability score. This makes result interpretation complex as changes in the independent variable (factors) affect the studied responses differently (Chen *et al*., 2016). The desirability results (Figure 7) showed that the designated optimum setting was more desirable for certain factors (e.g. colour lightness), but less desirable for others (e.g. hardness). Previous studies also reported that when the optimal outcome of all the studied responses does not overlap, it is harder to obtain a high desirability score and a compromise needs to be made (Amdoun *et al*., 2018). In this study, the objectives were to modify the physicochemical properties of grass jelly in order to limit leaching and retain its black colour. Based on the results, the desirability functions of colour metrics (L, a and b) were ≥ 0.85 which is very favourable. Hence, the addition of approximately 12.5 g of gelatine could retain the black colour and reduce leaching. In a previous study, gelatine was used to coat aquaculture feed to prevent leaching of water-soluble compounds, reduce sinking velocity and increase pellet durability (Guo *et al*., 2021). Meanwhile, discolouration was also reported to be reduced in meat products coated with gelatine (Antoniewski *et al*., 2007).

The desirability function of hardness and gumminess was ≤ 0.3 which was relatively low. Previous studies reported that a desirability score below 0.7 indicates a need for further optimization (Granato and de Araújo Calado, 2014). The range of hardness for gelatine-based formulations was very small. The difference between the highest and the lowest readings was 2% (50.08 - 51.2 $N/m^2$). This small margin indicated the high sensitivity of this parameter and might have led to lower desirability. The commercial jelly showed an average hardness of 50.67 $N/m^2$, while the enhanced grass jelly formulation (GJF) showed a hardness of 50.27 $N/m^2$ (predicted = 50.245 $N/m^2$) as presented in Table 1. This showed the accuracy of the model to achieve the target despite the small range of the tested response. In terms of gumminess, the gelatine-based grass jelly ranged from 34.96 to 52.18 $N/m^2$. The approximately 12.5 grams of gelatine suggested by the model had a low (0.32) desirability. This is due to the predicted value (47.43 N/m2) being ~20% higher than the target (37.59 N/m2) as shown in Figure 8. The actual gumminess of enhanced GJF was 47.83 N/m2, which was similar to the predicted value of 47.43 N/m2. Despite the accuracy of the model in predicting the outcome, the enhanced GJF had a high gumminess. Firstly, gumminess is associated with hardness as it is a product of multiplying hardness and cohesiveness (Tireki *et al*., 2021), while hardness also had low desirability and was discussed previously. Secondly, increasing gelatine concentration was previously reported to increase the gumminess and hardness of candies due to increased intermolecular bonds (Mutlu *et al*., 2018). Gelatine also significantly affected the gumminess of gummy confections when starch (0% and 1.5%) and gelatine (3% and 6%) were used. Regression analysis showed that starch and gelatine had a significant interaction effect on gumminess, while removal of starch was recommended for stability (Tireki *et al*., 2021). Other responses that had lower desirability were pH and springiness. Springiness is associated with hardness and gumminess, hence, its low desirability as both hardness and gumminess had low desirability. Moreover, enhanced

 

Table 1. Predicted and observed results for optimum conditions.

| Response | Predicted Mean | Observed | Commercial | Std Dev | SE Mean | 95% CI low for Mean | 95% CI high for Mean |
|---|---|---|---|---|---|---|---|
| L | 20.57 | 20.61 | 16.76 | 0.28 | 0.17 | 20.20 | 20.94 |
| a | 4.18 | 4.15 | 0.81 | 0.08 | 0.05 | 4.07 | 4.28 |
| b | 12.18 | 12.10 | 5.69 | 0.14 | 0.08 | 12.00 | 12.36 |
| Brix | 8.26 | 8.7 | 7.50 | 0.32 | 0.19 | 7.83 | 8.68 |
| pH | 8.79 | 8.84 | 8.30 | 0.05 | 0.03 | 8.72 | 8.85 |
| Hardness ($N/m^2$) | 50.25 | 50.27 | 50.67 | 0.07 | 0.04 | 50.16 | 50.33 |
| Springiness (m) | 3.82 | 3.92 | 2.30 | 0.06 | 0.04 | 3.74 | 3.90 |
| Gumminess ($N/m^2$) | 47.44 | 47.83 | 37.59 | 0.09 | 0.05 | 47.32 | 47.56 |
| Chewiness ($J/m^3$) | 217.19 | 220.10 | 185.20 | 6.27 | 3.73 | 208.89 | 225.50 |

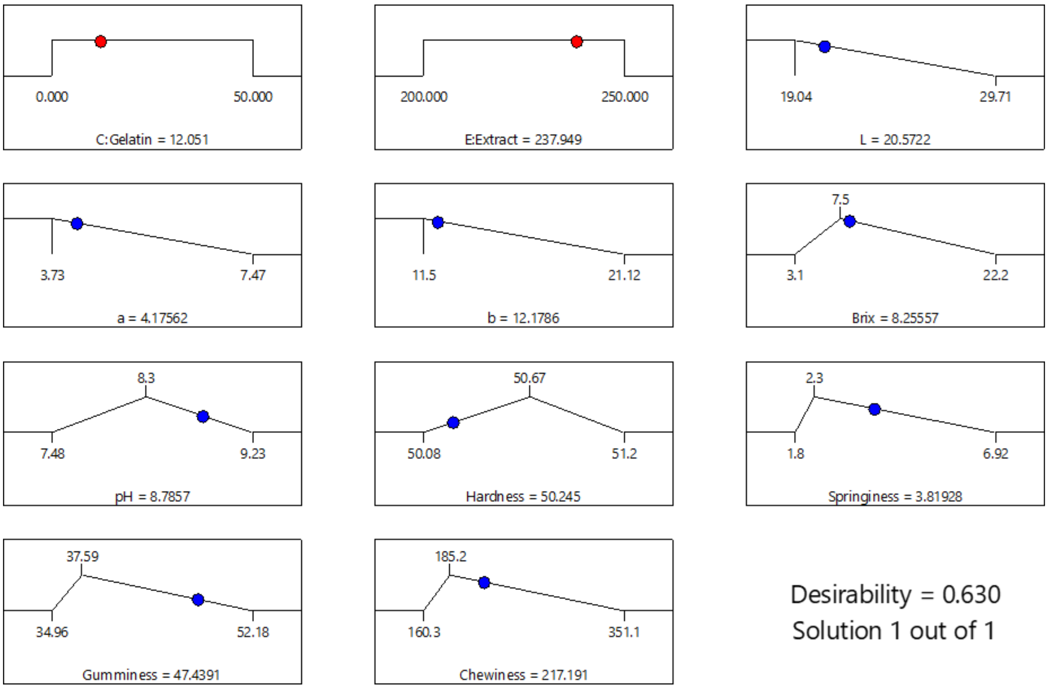


Figure 8. Desirability ramp of optimum parameters.

GJF had a springiness of 3.92 which was >40% higher than the commercial/target value of 2.3. A previous study also showed that the addition of gelatine to marshmallows led to a more visco-elastic texture with high springiness (Periche *et al*., 2015). In terms of pH, the pH of gelatine-based grass jelly had a very small range of 7.48 to 9.23. Approximately 12.5 grams of gelatine suggested by the model had a low (0.47) desirability despite the predicted value of 8.78 being only ~5% higher than the target (8.3). This indicated that this parameter was very sensitive in terms of modelling due to its small range. However, the actual pH of enhanced GJF was 8.84. This can be considered acceptable as there was only a slight decrease (<5%) of pH compared to the control (without gelatine) which was 9.18. This slight drop in the pH may be attributed to the pH of gelatine (type B gelatine: bovine source) used in this study which ranges from 4.7 and 5.4 (Gimat *et al*., 2021).

The Design Expert® suggested the addition of approximately 4% (12.5 g) of gelatine to GJF to minimize the leaching rate. This was validated by comparing the enhanced GJF with commercial grass jelly in terms of optical density. The optical density measured over time reflected the leaching rate. An increase in optical density is expected in a leaching formulation and vice versa. The results showed that the enhanced GJF showed significantly less optical density across time compared to commercial grass jelly (Table 2). The enhanced formulation was stable between 20 mins and 60 mins, but leached significantly ($p<0.05$) in the first 10 mins and after 60 mins. On the other hand, commercial jelly was only stable in the first 20 mins but leached significantly and consistently from 20 mins to the end of the experiment (90 mins). Figure 9 shows the leaching observation of the grass jelly drinks after 90 mins. The highest leaching was observed in the commercial grass jelly drink which is indicated by a darker colour. These findings correlated with colour metrics (L, a and b) in this study. *Mesona chinensis* is rich in nutrients such as polyphenols, polysaccharides, terpenes, and vitamins which are often hydrophilic (Huang *et al*., 2021). Previous studies supported that gelatine can prevent the leaching of water-soluble compounds (Guo *et al*., 2021), proteins and peptides

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Table 2. Leaching rate and floating duration of the grass jelly samples

| Leaching rate | No Gelatine | Enhanced GJF | Commercial |
|---|---|---|---|
| Time (min) | Absorbance (309 nm) | | |
| 10 | 0.385±0.001[b] | 0.122±0.0006[c] | 0.465±0.00[a] |
| 20 | 0.444±0.002[b] | 0.164±0.004[c] | 0.467±0.0006[a] |
| 30 | 0.573±0.003[b] | 0.177±0.002[c] | 0.627±0.0006[a] |
| 40 | 0.592±0.001[b] | 0.189±0.0006[c] | 0.744±0.001[a] |
| 50 | 0.645±0.0006[b] | 0.193±0.003[c] | 0.929±0.0006[a] |
| 60 | 0.666±0.002[b] | 0.207±0.002[c] | 1.015±0.003[a] |
| 70 | 0.717±0.002[b] | 0.248±0.001[c] | 1.097±0.001[a] |
| 80 | 0.747±0.001[b] | 0.266±0.009[c] | 1.164±0.0006[a] |
| 90 | 0.780±0.002[b] | 0.398±0.024[c] | 1.247±0.0006[a] |
| Floating duration | Time (min) | | |
| | 90.00±5.195[d] | 50.00±4.359[e] | 120.00±16.00[f] |

Values are presented as mean±SD of triplicates. Values with different superscripts are statistically significantly different ($p<0.05$).

(Feng *et al*., 2017) in various food products. The last parameter studied was the floating rate, as the addition of ingredients such as gelatine might lead to higher density and lower the floating time. The enhanced GJF showed significantly lower floating time compared to commercial grass. A previous study where various gelatine types (skin, bladder and head) were used as a binder for the common carp's feed pellets. The results showed that all gelatine-containing samples had a higher density than the control (0% gelatine). However, gelatine -based diets had higher floating times and lower sinking velocities (Al-Dubakel *et al*., 2014). Hence, further optimization is needed to prolong the floating time such as using different sources of gelatine and excluding either corn or potato starch to reduce the grass jelly density thus increasing the floating time.

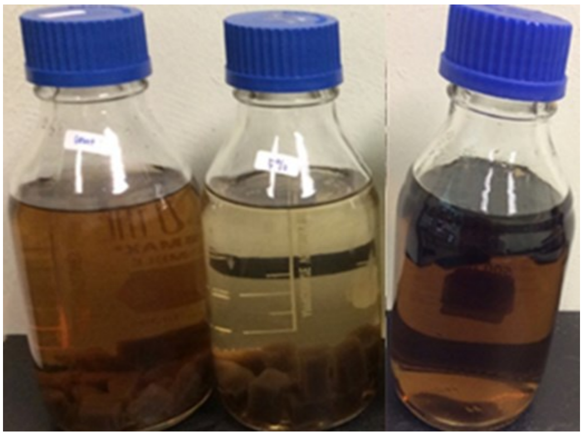

Figure 9. Leaching observation in grass jelly drinks (From left: No gelatine, with gelatine (12.5 g), and commercial grass jelly).

## 4. Conclusion

From this study, it is possible to mention that the development of foods with optimum conditions is feasible by using a statistical approach, Design Expert®. An optimum condition of GJF with the addition of approximately 12.5 g gelatine was suggested to be added to the grass jelly. At this optimum condition, some of the physicochemical properties (L, a, b, Brix) were effectively maintained and minimized the leaching rate compared to the commercial grass jelly. The addition of gelatine in the grass jelly formulation may replace the use of colourants or additives found in commercial grass jelly. Further optimization is needed in order to obtain a formulation with a longer floating duration of the grass jelly.

## Conflict of interest

The authors declare no conflict of interest.


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