Development of *Etlingera elatior* pearl for drinks and cocktail

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Abstract. Herbs such as *Etlingera elatior* (torch ginger flower) is well known in Malaysia and one of the main sources of phytochemicals with strong antioxidant activity. A recent study is done to develop herbs into new confectionery products, pearls in drinks and cocktails. In this research, the screening process is done to select the best pearl formulation, which described the desired viscosity and pH. The mixture variables are different types of starch (xanthan gum, modified cassava flour, hanjeli flour, and modified suweg flour). Five out of 20 pearl formulations are chosen for texture profile, antioxidant analysis, and sensory analysis. The result shows pearl formulation 2 (467.62 g force) has the highest hardness of the pearl, while the stickiest is pearl formulation 4 (-9.24 g force). The pearl formulation 2 has the highest antioxidant capacity when tested with TPC (6.61 μgGAE/ml), TFC (177.42 μgRE/ml) and ABTS (56.74 %). Contradict to this result, the highest DPPH antioxidant properties are pearl formulation 1 (69.98 %). The sensory analysis by 9-point hedonic scale result shows that the average mean of overall acceptance is formulation 2.

Keywords. torch ginger flower, xanthan gum, mocaf, hanjeli flour, modified suweg flour

1. Introduction

Herbs are primary sources of various phytochemicals, which dominate strong antioxidant activity, leading to antioxidant defence [1]. Herbs such as torch ginger flower (*E. elatior*) are usually used as the main ingredient in dishes among Malay people in Malaysia. It is locally known in Malaysia as kantan, bunga kantan, bunga siantan, and recognized as bunga kecombrang or honje in Indonesia [2], [3]. Torch ginger has pink to red flowers, and the plant's inflorescence is borne on an erect stalk of the plants that produced sweet fragrance. They are mainly used to add fragrance and elevate the taste of many dishes such as Penang laksa, nasi kerabu and nasi ulam [4]. Nowadays, Malaysian people tend to ignore these beneficial herbs due to modern lifestyles. Thus, it is vital to presents these herbs in a new, exciting way, which is herbs pearl. Developers can produce the pearl by using one of the molecular gastronomy techniques known as reverse spherification.

Spherification is an old technique in the world of modernist cuisine, was pioneered at El Bulli in 2003 [5]. The traditional spherification method has been improvised to the reverse technique. It utilizes a calcium source to be added to the edible liquid and is cooked in a sodium alginate bath. Reverse
spherification techniques will only encapsulate the liquid's outer layer, forming the pearls containing liquid inside it, and it will explode when it is compressed, or pressure is applied. The pearls are added to the drinks and cocktails as a topping to enhance the drinks' flavour. Nowadays, the topping is served explicitly in hot or cold beverages where the topping is invented to be applicable to float in liquids [6]. It is also important to maintain the colour of the pearl to keep appearance [7]. Therefore, this study is conducted to create alternatives topping and substituting with the beneficial herb, which is a torch ginger flower (E. elatior). The objective of this study is to develop E. elatior pearls by reverse spherification. Next, to investigate the physical properties of the pearls and evaluate the sensory acceptance and antioxidant activity of the beneficial herb.

2. Materials and methods

2.1 Preparation of alginate solution and herbs-calcium mixture

It involved two steps, the first step involved preparing alginate solution as setting bath and followed by preparation of herbs-calcium mixture as sphere base. Alginate solution was prepared with 1% concentration (10 g sodium alginate in 1000 ml distilled water) and mixed using a homogenizer.

For the herbs-calcium mixture, torch ginger was used as the herb in calcium mixture. For this mixture, torch ginger flower was washed and cut into smaller pieces. Then, the torch ginger pieces were made into juice via juice extractor. Four types of starch, xanthan gum, modified cassava flour, hanjeli flour, and modified suweg flour were selected to be mixed with herbs juice with a different formulation (Table 1). Finally, a 4% concentration of calcium lactate was added (6 g calcium lactate in 150 ml herbs juice) completing the herbs-calcium mixture. A total of 20 formulated pearls was prepared (Table 1).

2.2 Physical analysis

The alginate solutions' pH values and the herbs-calcium mixture were measured using a digital pH meter (pH 700, Eutech Instruments, Thermo Fisher Scientific Inc.). The alginate solutions' viscosity and the herbs-calcium combination were determined using a Brook Field Viscometer (model LVF 69726, Brookfield, ametek). Texture profile analysis of the pearl was measured using a texture analyzer (Stable micro system TA.XT plus).

2.3 Reverse spherification- sample preparation

Alginate solution was made as setting bath and herbs-calcium mixture as sphere base. Both solutions were prepared. Droplets of sphere base, which was an herbs-calcium mixture, were released into the setting bath to form the sphere pearl in pea-size. They were left for 1 to 3 minutes to allow them to develop a skin. The newly formed pearl was removed from the setting bath and rinsed with water. A total of 20 formulated pearls was prepared (Table 1).

2.4 DPPH scavenging activity

Antioxidant activity was performed by DPPH (2,2-diphenyl-1-picrylhydrazyl) as a radical scavenger. DPPH was estimated according to the procedure with slight modification by Brand-Williams [8]. The prepared samples were as the formulation in Table 1. Reagent solutions were prepared by mixing approximately 0.5 ml of 0.2g/ml of sample and were mixed with 2.7 ml of methanolic 0.024 mg/ml DPPH radical solution. The mixture was vigorously shaken and was incubated for 15 minutes prior to the measurement of absorbance at 517 nm using UV-Vis spectrophotometer. The radical scavenging activity (RSA) was calculated using the equation below.

%RSA = (absorbance (control) - absorbance (sample)/ absorbance (control)) x 100
2.5 **ABTS scavenging activity**

ABTS assay was measured by a slightly modified method of Roberta [9]. The stock solution was prepared by dissolving the ABTS in water to 7 mM concentration. ABTS radical cation (ABTS+) was produced by reacting ABTS stock solution with 2.45 mM of potassium persulfate and left in the dark at room temperature for 12-16 hours. 1ml of ABTS+ solution diluted with 60ml distilled water to obtain an absorbance of 0.700 (± 0.020) at 734 nm using the spectrophotometer. 2 ml of ABTS+ radical solution was mixed with 100μl of the sample solution, and absorbance was measured after 6 minutes. The reagent blank was prepared by adding the ABTS+ radical+ethanol instead of the sample. Percent inhibition of absorbance at 734 nm was calculated using the equation below.

\[
\text{ABTS+ scavenging effect (\%)} = \left( \frac{\text{absorbance (ABTS radical + ethanol)} - \text{absorbance (ABTS radical + sample)}}{\text{absorbance (ABTS radical + ethanol)}} \right) \times 100
\]

2.6 **Total phenolic content**

100 μl of the sample was mixed with 2 ml sodium carbonate (2 g in 100 ml distilled water) and was left for 2 minutes at room temperature. Then, mixed with the same Folin-Ciocalteu reagent and was left for 30 minutes. The absorbance reading was taken at 750 nm using UV-Vis Spectrophotometer (U-3900H Hitachi High-Tech Science Corporation). The absorbance value was compared to the gallic acid standard curve.

2.7 **Total flavanoid content**

The content of flavonoids was determined by a pharmacopeia method (1989) with few modifications by Shehata, Mahmoud & Abdou [10] using rutin as a reference compound. 1 ml of the sample was mixed with 4 ml of distilled water and 0.3 ml of 5 % sodium nitrite (NaNO₂) and was left for 6 minutes. Then, 0.3 ml of 10 % aluminium chloride hexahydrate (AlCl₃·6H₂O) added and left for 5 minutes. After that, 2 ml of 1 M NaOH added and left for 5 minutes. The mixture was mixed by using vortex-mix. The absorbance was read at 510 nm immediately after mixing. The content of flavonoid was calculated by comparing the absorbance value against the rutin standard.

2.8 **Sensory analysis**

Thirty semi-trained panellists evaluated the sensory analysis of the samples by using the 9-points hedonic scale. This 9-point hedonic scale was used to evaluated flavour, texture, colour, odour, sweetness, and overall acceptability of the pearl. Panellists were asked to evaluate the pearl and choose the acceptability on a structured hedonic scale (9=most like, 5=neither like nor dislike and 1=most dislike) [11].

2.9 **Screening analysis**

Screening analysis involved the preparation of the pearl formulation consisting of mixing the xanthan gum, modified cassava flour (mocaf), hanjeli flour and modified suweg flour with the torch ginger flower juice. There are twenty different formulations of pearl solution that were used for screening purpose. The screening analysis was used to evaluate the effect of viscosity and pH towards the formation of a pearl. The only formulations that were successfully forming a pearl were used for further analysis.

3. **Result and discussion**

3.1 **Statistical screening design**

In this design, the total amount of *E. elatior* pearl formulation is fixed, and the factors settings were proportions of the total sum to one as can be seen in Table 1. Fifteen different formulations were tested for optimization while the other five formulations were repetition for formulation 1, 5, 6, 8 and 10 and were used for lack of fit test. The value of "Prob F" for responses viscosity and pH are < 0.0001 and
0.0036 respectively, which is less than 0.05. The lack of fit for the dependent variables was insignificant. This is desirable, as we want a model that fits. The $R^2$ value calculated for viscosity at 100 rpm and pH was 0.9998 and 0.8282 respectively, reasonably close to 1, which is accepted. The ANOVA table for viscosity was shown in Table 2, while ANOVA table for pH was shown in Table 3.

Table 1. Design layout and experimental result for viscosity and pH of pearl formulation.

| Std run no. | Factor 1 | Factor 2 | Factor 3 | Factor 4 | Response variables |
|-------------|----------|----------|----------|----------|-------------------|
|             | Xanthan gum (% w/v) | Modified cassava flour (% w/v) | Hanjeli flour (% w/v) | Modified Suweg flour (% w/v) | Viscoysity (mPas) 100 rpm | pH |
| 1           | 1.00     | 0.00     | 0.00     | 0.00     | 1037.66           | 4.42 |
| 2           | 0.50     | 0.50     | 0.00     | 0.00     | 503.83            | 4.36 |
| 3           | 0.50     | 0.00     | 0.50     | 0.00     | 935.33            | 4.35 |
| 4           | 0.50     | 0.00     | 0.00     | 0.50     | 581.83            | 4.36 |
| 5           | 0.00     | 1.00     | 0.00     | 0.00     | 48.7              | 4.42 |
| 6           | 0.00     | 0.50     | 0.50     | 0.00     | 70.8              | 4.37 |
| 7           | 0.00     | 0.50     | 0.00     | 0.50     | 58.5              | 4.37 |
| 8           | 0.00     | 0.00     | 1.00     | 0.00     | 48.7              | 4.42 |
| 9           | 0.00     | 0.00     | 0.50     | 0.50     | 48.2              | 4.36 |
| 10          | 0.00     | 0.00     | 0.00     | 1.00     | 53.6              | 4.45 |
| 11          | 0.63     | 0.13     | 0.13     | 0.13     | 629.6             | 4.36 |
| 12          | 0.13     | 0.63     | 0.13     | 0.13     | 93.73             | 4.37 |
| 13          | 0.13     | 0.13     | 0.63     | 0.13     | 73.4              | 4.37 |
| 14          | 0.13     | 0.13     | 0.13     | 0.63     | 80.67             | 4.36 |
| 15          | 0.25     | 0.25     | 0.25     | 0.25     | 108.4             | 4.35 |
| 16          | 0.00     | 1.00     | 0.00     | 0.00     | 43.3              | 4.45 |
| 17          | 0.00     | 0.00     | 1.00     | 0.00     | 39.2              | 4.37 |
| 18          | 1.00     | 0.00     | 0.00     | 0.00     | 1053.67           | 4.49 |
| 19          | 0.00     | 0.00     | 0.00     | 1.00     | 55.5              | 4.49 |
| 20          | 0.00     | 0.50     | 0.50     | 0.00     | 80.8              | 4.42 |

Table 2. ANOVA table for viscosity and pH.

| Response Variables | Sum of square | Degree of freedom | Mean square | F value | Probability |
|--------------------|---------------|-------------------|-------------|---------|-------------|
| Viscosity          | 95875.06      | 4                 | 23968.77    | 341.04  | < 0.0001    |
| Regression         | 421.68        | 6                 | 70.28       |         |             |
| Residual           | 182.01        | 1                 | 182.01      | 3.80    | 0.1089      |
| Lack of fit        | 239.67        | 5                 | 47.93       |         |             |
| Pure error         | 2.521E+00     | 19                |             |         |             |
| Cor total          | 6             | 1                 |             |         |             |
| $R^2$              |               |                   |             |         | 0.9998      |
| Adeq Precision     |               |                   |             |         | 0.9995      |

0.0036 respectively, which is less than 0.05. The lack of fit for the dependent variables was insignificant. This is desirable, as we want a model that fits. The $R^2$ value calculated for viscosity at 100 rpm and pH was 0.9998 and 0.8282 respectively, reasonably close to 1, which is accepted. The ANOVA table for viscosity was shown in Table 2, while ANOVA table for pH was shown in Table 3.
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| pH- Quadratic |       |           |       |     |
|---------------|-------|-----------|-------|-----|
| Regression    |       |           |       |     |
| Residual      | 0.030 | 6         | 4.917E-003 | 7.17 | 0.0036 | significant |
| Lack of fit   | 6.208E-003 | 6 | 0.11 | 0.9862 | insignificant |
| Pure error    | 003   | 5         | 1.035E-003 | 0.11 | 0.9862 | insignificant |
| Cor total     | 6.557E-003 | 5 | 0.03 | 0.8282 | 0.6735 |
| R²            | 004   | 13        | 1.311E-003 | 0.8282 |
| Adeq Precision| 003   | 003       | 1.240E-003 | 7.066 |

### 3.2 pH

Alginic acid is a pH-sensitive hydrogel where at a low pH environment, it shrinks and converts into an insoluble polymer, which called as alginic acid skin [12]. Alginic skin converted into soluble polymer once transformed into a high pH environment, which results in the hardeness of alginate pearl decreasing [13]. Based on the result obtained from Table 1, it shows that the pH range of the pearl solution is around pH 4 to 5. Low pH will result in rapid release of calcium which makes the calcium ions diffused faster. Moreover, the low pH condition is likely to contribute to an increase in hardeness of the pearl.

### 3.3 Viscosity

Viscosity is crucial in determining the texture of the pearl. Different type of starch with different percentage formula was used to achieve the desired viscosity. Based on the results obtained from Table 3.1, the pearl solution requires high enough viscosity to avoid deformations while forming the spherical shape. The hardness of the pearl also increased with increasing viscosity of solution used. The viscosity range of the herbs-calcium mixture that is successful in developing pearl is around 503.83 to 1037.66 mPa per second while the viscosity of the alginate solution at 1% is around 230 mPa per second—the viscosity which below the range were failed to formed spherical shape. Thus, only five formula which is 1, 2, 3, 4 and 11 were used for further analysis.

### 3.4 Texture profile analysis

Hardness is regarded as one of the most important physical properties of hydrogel capsules because it indicates the pearl stability under processing and gelation efficiency between ionic biopolymer and ions [14], [15]. High hardness could prevent the liquid-core hydrogel pearl from bursting during transportation and storage. The average hardness of all pearl sample was found to be within the range of 104.505 g force to 467.615 g force as shown in Table 3 which is comparable to the existing market product as control reference which its average hardness is at 186.932 g force. The used of the starch affected the stickiness of the pearls as the combination of starch results in terms of minimal stickiness of the pearls where its stickiness range from -2.86 g to -9.24 g, lower than control product which is -5.56 g force.

### 3.5 DPPH scavenging activity

The antioxidant activity using DPPH radicals is related to the intensity reduction of the purple radicals to yellow [16]. The results of antioxidant activity shown in Figure 1. It shows that the highest percentage is formula 1 with 69.98% while the lowest is formula 11 with 65.72%. Based on the literature, it stated that the percentage DPPH of fresh torch ginger flower is 76.4% [17]. There is a reduction value in the result which may happen because of different herbs have variable compositions of phytochemicals and bioactive compounds.
3.6 ABTS Scavenging activity
For ABTS assay, the results of antioxidant activity were illustrated in Figure 2. The value varied from 51.00 to 56.74 % inhibition. It shows that the highest percentage is formula 2 with 56.63% while the lowest is formula 1 with 51.00 %. High percentage inhibition indicates high antioxidant properties towards radical scavenging activity.

The percentage of inhibition between DPPH and ABTS radical scavenging activities contradict each other. In this study, it was observed that the percentage inhibition of DPPH was the highest with formulation 1; likewise, ABTS showed the lowest value. However, there had been several similar cases due to three possibilities acting upon the differences [18]. First, it might be due to differences in wavelength measurements for both assays. ABTS was measured at wavelength 734 nm and DPPH at 515 nm. This underestimation of samples by DPPH radicals was to be expected since the visible region, with coloured compounds, presented in the test sample, may have the spectra that overlap with DPPH at 515 nm and thus, interfere with the measurements. Second, this can be influenced by the reactions mechanisms of DPPH and free radical scavengers which are also affected by structural conformations of antioxidants [19]. In their study, it was claimed that the small molecules that have better access to the radicals have a higher apparent antioxidant activity. Thirdly, another reason relate to DPPH reactions with certain phenols and its derivatives, being reversible, thus resulting in low readings of antioxidant activity [20]. These reasons were believed to be a few of the main reasons for this contradiction.

Table 3. Average hardness and stickiness of the pearl.

| Formulation | Hardness (g) | Stickiness (g) |
|-------------|--------------|----------------|
| Control     | 186.93±16.02 | -5.56±1.67     |
| 1           | 104.50±8.15  | -5.79±0.94     |
| 2           | 467.62±34.81 | -5.24±1.39     |
| 3           | 154.01±14.83 | -7.50±2.86     |
| 4           | 156.78±3.12  | -9.24±4.31     |
| 11          | 193.94±12.27 | -2.86±0.81     |

*Mean values ± standard error

3.7 Total phenolic content
The gallic acid standard curve is shown in Figure 3. Figure 4 shows the TPC of the pearl formulation. The total phenolic contents were calculated using the standard curve of gallic acid varied from 4.74 to 6.61 μgGAE/ml. The highest total phenolic content is formulation 2 (6.61 μgGAE/ml) followed by...
11, 4, 1, and 3. In previous research, it stated that the highest total phenolic compound of fresh torch ginger flower in his studies was 618.9 mg/100 g DM [17].

3.8 Total flavanoid content
The rutin standard is shown in Figure 5, and Figure 6 illustrated the contents of flavanoid that were calculated using the standard curve of rutin varied from 153.96 to 177.42 μgRE/ml. The highest formulation is formulation 2, followed by 4, 5, 3 and 1. In previous research, it stated that the highest total flavanoids compound of fresh torch ginger flower in his studies was 354.2 mg/100 g DM [17].

| Formulation | Mean | Color | Odor | Texture | Sweetness | Overall acceptance |
|-------------|------|-------|------|---------|-----------|--------------------|
| 2           | 7.30 | 4.87  | 6.53 | 5.63    | 6.57      |
| 4           | 7.30 | 4.93  | 6.20 | 5.87    | 6.13      |
Figure 7. Spider web graph of 5 different attributes

4. Conclusion
Screening analysis was carried out to identify the viscosity and pH of the pearl solution to produce the desired shape of the pearl. The analysis showed significant (P <0.05) relationship between the independent variables and the response variables, with R² values, 0.9998 to 0.8282 for viscosity and pH, respectively. The formulation that is successful in forming pearl is formulation 1,2,3,4 and 11. The texture profile analysis shows that the pearl from all different formulation was comparable to the existing market product. It also revealed that the pearl also has high antioxidant capacity due to its ability to scavenging radical. Other than that, the sensory analysis shows that it can be stated the panellist preference based on the average mean of overall acceptance is formulation 2.

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