Original Article

Screening of Electrospray-operating Parameters in the Production of Alginate–Royal Jelly Microbeads Using Factorial Design

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Introduction: Royal jelly (RJ) has been consumed as food or as a supplement because of its high nutritional and medicinal values. A fresh harvested RJ is yellowish to whitish in color and contains proteins, free amino acids, lipids, vitamins, and sugar. Without proper storage conditions, such as at 4°C, the color of RJ changes to much darker yellow and produces a rancid smell. To prolong its shelf life, RJ is usually mixed with honey. Alginate, a natural and edible polymer derived from seaweed, is commonly used to encapsulate drugs and food due to its ability to form gels by reacting with divalent cations. However, there is a lack of research on the microencapsulation of RJ in alginate using electrospray. The electrospray technique has the advantage in producing consistent size and shape of alginate microbeads under optimum parameters. Aim: This research aimed to optimize electrospray-operating parameters in producing alginate–RJ microbeads. Materials and Methods: Optimization of alginate–RJ microbeads electrospray parameters was carried out using 2⁴ factorial design with three center points (19 runs). The studied parameters were flow rate, high voltage, nozzle size, and tip-to-collector distance, whereas the responses were particle size, particle size distribution, and sphericity factor. The responses of each run were analyzed using Design-Expert software. Results: Nozzle size is a significant parameter that influences the particle size. Flow rate is a significant parameter influencing the sphericity factor. Conclusion: Screening of the electrospray-operating parameters paves the way in determining the significant parameters and their design space to produce consistent alginate–RJ microbeads.

Keywords: Alginate, electrospray, factorial design, royal jelly

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Introduction

RJ is produced by the hypopharyngeal glands of young worker bees (Apis mellifera) and to be fed to the queen bee for the rest of her life.[1] Honeybee products such as RJ have been consumed as food and medicine because of its high nutritional and medicinal values. A fresh harvested RJ is yellowish to whitish in color and contains abundant amounts of proteins, free amino acids, lipids, vitamins, and sugars.[2] Major RJ proteins (MJRPs) such as royalisin and apisimin are the main bioactive compounds of RJ. Not only that, one of the essential proteins in RJ is 10-hydroxy-2-(E)-decanoic acid (10-HDA), which is used as a quality marker.[3] Owing to its benefit and nutritional values for general human health, there are many commercial RJ products sold in the market.

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Without proper storage conditions such as 4°C, its color changes to a much darker yellow, and it produces a rancid smell. Protein such as 10-HDA will also denature at high temperature conditions (e.g., 40°C). The stability issue makes it difficult for the consumers of RJ during travelling and administration. Owing to its instability under certain temperatures, one of the methods used is to mix pure RJ with honey to prolong its shelf life. Encapsulation methods are among the several techniques used to enhance the stability of drugs. This study was conducted to encapsulate RJ with alginate beads so that later the beads can be used for longer duration and stability under high temperature.

Alginate is an edible polymer that is naturally extracted from seaweed of the marine polysaccharides, and it is most abundant in marine brown algae. Alginate is commonly used to encapsulate drugs because of its unique properties such as biocompatible, nontoxic, and ease of gelation. The unique feature of alginate is its ability to form gels by reacting with divalent cations such as Ca²⁺, Sr²⁺, and Ba²⁺ under ambient temperature. However, there is a lack of research on the microencapsulation of RJ in alginate using electrospray. The electrospray technique has the advantage in producing consistent size and shape of alginate microbeads under ambient temperature. Technique such as spray drying requires high temperature (e.g., more than 40°C). Such high temperature can jeopardize the RJ stability.

Electrospray is a technique of atomizing a polymeric solution that applies stronger electrostatic forces than its surface tension. During the process of electrospray, a droplet of polymeric solution at the tip of a capillary nozzle is exposed to a high electric field, and then it will undergo a shape deformation to a cone. Because of high internal electrostatic repulsions and external attractive Coulombic forces, the beads jet that is emitted from the tip will break up into fine droplets. In the electrospray solution, the polymer solution is sprayed as microdrops into a collecting bath at voltages above its surface tension. There are several parameters involved in the electrospraying, such as nozzle size diameter, applied voltage, the viscosity of the solution, and flow rate.

To investigate the relationship between the formation of the RJ–alginate beads using electrospray with those parameters mentioned before, we used the design of experiment (DOE) analytical methods. The DOE usage in producing statistical models enables researcher to determine significant parameters affecting a studied process. Hence, the DOE method allows us to recognize all aspects that we should control during the project implementation.

Thus, this research aimed to microencapsulate RJ in alginate beads and optimize RJ beads formulation using a quality-by-design (QbD) approach, which is DOE. This research also sought to characterize the formulation of RJ beads. Besides, beads loaded with RJ in micrometer size will ease administration, especially in elderly and children who have difficulties in swallowing a big capsule of RJ.

**MATERIALS AND METHODS**

**Materials**

The materials used in this study are RJ (Giant B Honey, Kuala Lumpur, Malaysia), calcium chloride (Merck, Darmstadt, Germany), and sodium alginate (FMC Biopolymer, Philadelphia, Pennsylvania). The sample solutions were prepared using distilled water.

**Methods**

**Fabrication of royal jelly in alginate beads using electrospray**

The fabrication of RJ in alginate beads using electrospray adopted methods as reported in the literature with modifications. Briefly, 10g of RJ is dissolved in 2% wt/vol sodium alginate solution. The mixture was then pumped out from the metal capillary attached to the syringe into 1% wt/vol calcium chloride solution at a flow rate of 0.6, 0.9, and 1.2mL/min using the syringe pump. The electrospray metal capillary (18, 22, and 26 G) was connected to a high voltage supplying 2.0, 2.9, and 3.8 voltage, respectively. The distance between the tip and the surface of calcium chloride solution was set up to 1, 1.5, and 2cm. The beads were formed through ionic gelation. The beads were left for approximately 20 min for hardening in the calcium chloride solution with stirring before harvesting the beads. Fabricated beads were filtered using a mesh and were washed with distilled water. Next, all the wet beads were dried using a freeze dryer for 24 h at -40°C.

**Screening of electrospray-operating parameters using design of experiment**

DoE was successfully executed in this study to investigate the effects of electrospray-operating parameters on the characteristics of RJ in alginate beads formulation. A factorial design consisted of four factors at two levels, which were flow rate (X1), applied voltage (X2), nozzle size (X3), and tip distance between metal capillary and calcium chloride solution (X4) were investigated using DoE software (Design-Expert 8, Stat-Ease, Minneapolis, Minnesota). The characteristics of the beads such as particle size (Y1), particle size distribution (Y2), and sphericity factor (Y3) were studied. The statistical analysis and predicted model developed by the DoE software would determine the critical electrospray parameters and their levels in achieving intended beads characteristics.
Particle size and particle size distribution measurement
Approximately 30 freeze-dried beads of each run were visualized using a light microscope. The average diameter of the beads was calculated from the captured image using image analysis software (Image J, National Institutes of Health, Bethesda, Maryland).

The particle size distribution was calculated using the following equation [Equation 1]:

\[
\text{Particle size distribution} = \frac{\text{Average of 30 beads diameter}}{\text{Standard deviation of 30 beads diameter}}
\]  

Sphericity factor measurement
Sphericity factor is a measurement of whether the beads are spherical. The sphericity factor measurement was calculated using the following equation [Equation 2]:

\[
\text{SF} = \frac{(d_{\text{max}} - d_{\text{min}})}{(d_{\text{max}} + d_{\text{min}})}
\]  

where the \(d_{\text{max}}\) and \(d_{\text{min}}\) are the maximum and minimum bead diameter, respectively; the value below 0.05 is considered spherical.[12]

Statistical analysis
The statistical analysis was done using Design-Expert 8 (Stat-Ease). The significant electrospray parameters were determined using analysis of variance (ANOVA) at \(P < 0.05\).

RESULTS AND DISCUSSION
Screening of influenced factors on particle size
Table 1 shows the observed and predicted value of each response obtained from the experiment and the model, respectively. It was found that the particle size ranged from 0.59 mm (run 15) to 1.16 mm (run 11). The final equation of the fitted model for the influence factors on the particle size is as follows:

\[
Y_i = 1.8143 - 0.043764X_3
\]

From the ANOVA result, the significant factor (\(P < 0.05\)) affecting the particle size was the nozzle size with \(P\) value < 0.0001. The statistical analysis for the model to predict the particle size is shown in Table 1. The model \(F\) value of 47.28 implied that the model was significant, with 99% confident interval (Prob > F = 0.0001) [Table 1]. The model was significantly affected by the factors selected by the model. Hence, it could be used to predict the effect of the significant factors on particle size. The particle diameter decreases with the decrease in nozzle diameter. The results are in agreement with the studies reported by other researchers.[13,14]

| Table 1: Particle size, particle size distribution, and sphericity factor of royal jelly alginate beads |
|---|---|---|---|---|---|---|---|---|---|
| Run | Flow rate (mL/min) \((X_1)\) | Applied voltage (kV) \((X_2)\) | Nozzle size (G) \((X_3)\) | Tip distance (cm) \((X_4)\) | Particle size (mm) \((Y_1)\) | Particle size distribution \((Y_2)\) | Sphericity factor \((Y_3)\) |
| R1 | 1.20 | 2.00 | 26.00 | 1.00 | 0.69 | 0.85 | 7.34 | 6.40 | 0.11 | 0.12 |
| R2 | 0.60 | 3.80 | 18.00 | 1.00 | 1.13 | 1.03 | 11.40 | 7.60 | 0.13 | 0.10 |
| R3 | 0.60 | 3.80 | 26.00 | 1.00 | 1.03 | 0.85 | 6.55 | 6.40 | 0.10 | 0.12 |
| R4 | 0.60 | 3.80 | 18.00 | 2.00 | 1.06 | 1.03 | 10.24 | 8.61 | 0.11 | 0.14 |
| R5 | 1.20 | 3.80 | 18.00 | 1.00 | 1.09 | 1.03 | 10.03 | 9.70 | 0.16 | 0.14 |
| R6 | 0.90 | 2.90 | 22.00 | 1.50 | 0.61 | 0.68 | 5.58 | 5.71 | 0.16 | 0.14 |
| R7 | 0.60 | 3.80 | 26.00 | 2.00 | 0.69 | 0.68 | 9.28 | 9.26 | 0.08 | 0.10 |
| R8 | 1.20 | 3.80 | 26.00 | 2.00 | 0.77 | 0.68 | 7.36 | 7.63 | 0.08 | 0.10 |
| R9 | 1.20 | 2.00 | 18.00 | 1.00 | 0.89 | 1.03 | 5.85 | 5.71 | 0.10 | 0.14 |
| R10 | 0.60 | 2.00 | 18.00 | 1.00 | 0.69 | 0.68 | 5.66 | 6.04 | 0.14 | 0.10 |
| R11 | 0.60 | 2.00 | 18.00 | 2.00 | 1.16 | 1.03 | 8.43 | 6.18 | 0.15 | 0.14 |
| R12 | 1.20 | 3.80 | 26.00 | 1.00 | 0.59 | 0.68 | 6.99 | 8.61 | 0.15 | 0.14 |
| R13 | 1.20 | 2.00 | 18.00 | 2.00 | 0.99 | 1.03 | 7.91 | 7.63 | 0.15 | 0.10 |
| R14 | 0.90 | 2.90 | 22.00 | 1.50 | 0.76 | 0.68 | 3.93 | 6.18 | 0.16 | 0.14 |
| R15 | 1.20 | 3.80 | 18.00 | 2.00 | 0.59 | 0.68 | 9.38 | 9.70 | 0.13 | 0.14 |
| R16 | 1.20 | 2.00 | 26.00 | 2.00 | 0.70 | 0.68 | 3.80 | 7.60 | 0.08 | 0.10 |
| R17 | 0.90 | 2.90 | 22.00 | 1.50 | 0.82 | 0.85 | 5.30 | 6.40 | 0.15 | 0.12 |
| R18 | 0.60 | 2.00 | 26.00 | 2.00 | 1.00 | 1.03 | 6.42 | 6.04 | 0.05 | 0.10 |
| R19 | 0.60 | 2.00 | 26.00 | 1.00 | 0.91 | 1.03 | 9.24 | 9.26 | 0.05 | 0.10 |

\(R^2\) 0.7471 0.3899 0.3176
\(F\) value 47.28 0.98 7.45
Prob > \(F\) <0.0001 0.4929 0.0148

Bold indicates a significant model (\(P < 0.05\))
Screening of influenced factors on sphericity factor

The observed and predicted values of each response obtained from the experiment and the model are given in Table 1. It was found that the sphericity factor ranged from 0.05 (run 18 and 19) to 0.16 (run 5, 6, 11, and 14). The final equation of the fitted model for the influence factors on the sphericity factor is as follows:

\[ Y_3 = 0.030 + 0.011111X_1 \]

The statistical analysis for the model to predict the particle size is shown in Table 2. To estimate the accuracy of the predicted model, Fisher’s F test was applied. The model F value of 7.45 implied that the model was significant, with around 98% confident interval (Prob > F = 0.0148). The P = 0.0148, was also smaller than 0.05, which shows the high significance of the model affected by the flow rate. Hence, it could be used to predict the effect of the significant factor on the sphericity factor. The result from this sphericity factor model is in agreement with other studies that a slower flow rate of electrospray will produce a more spherical alginate bead.[12,14]

**Conclusion**

Screening of the electrospray-operating parameters paves the way in determining the significant parameters and their design space to produce consistent alginate-RJ microbeads. Formation of RJ in alginate beads is through the gelation method by extruding the solution via electrospray, into calcium chloride solution. The electrospray-operating parameters were screened using DoE. Nozzle size is a significant parameter that influences the particle size. Flow rate is a significant parameter influencing the sphericity factor. The model for particle size distribution is not significant. Thus, this study shows that different factors affect different characteristics of the RJ in alginate beads.

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**Conflicts of interest**

There are no conflicts of interest.

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**Table 2: Statistical analysis of particle size (Y_1), particle size distribution (Y_2), and sphericity factor (Y_3)**

| Coefficient | P value | Coefficient | P value | Coefficient | P value |
|-------------|---------|-------------|---------|-------------|---------|
| Constant    | 0.85    | Constant    | 7.59    | Constant    | 0.12    |
| Nozzle size (X_3) | -0.180 | Flow rate (X_3) | -0.040 | Flow rate (X_3) | 0.022   |
|             |         | High voltage (X_3) | -0.600 | 0.9429      |         |
|             |         | Tip distance (X_3) | 0.070  | 0.2937      |         |
|             |         |              |         | 0.0902      |         |

Bold indicates significant value (P < 0.05)

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