INTRODUCTION

Nowadays, nutraceutical products have been widely developed to prevent and treat disease, as well as to maintain public health. A 1999-2000 National Health and Nutrition Examination Survey showed that more than 5,000 of the 10,000 respondents consumed a nutraceutical product regularly. Nutraceutical products can be food or food-related, such as dietary supplements, herbal products, cereals, soups, and healthy drinks. The therapeutic areas covered by nutraceutical products include cancers, osteoporosis, hypertension, cholesterol, depression, and diabetes [1-3]. A previous study showed that mulberry leaf extract had an anti-diabetic effect in diabetes-induced Wistar rats [4,5]. Mulberry leaves (Morus alba L.) contain 1-deoxynojirimycin and its derivatives, an intestinal glucosidase inhibitor that prevents the hydrolysis of carbohydrates to monosaccharide, and therefore, suppresses the blood glucose level [6]. Mulberry leaf extract has been formulated as an effervescent tablet, whitening cream, and capsule.

Beads are a spherical dosage form containing an active substance. They can enhance the bioavailability and stability of drugs, provide a uniform distribution of drugs, and have an interesting appearance as a dosage form. Hydrogel beads are able to swell when in water because the hydrophilic polymer of the hydrogel is crosslinked with its cross linker. Ionic gelation is the method used to cross-link polymer and its cross linker to produce hydrogel beads.

Hydrogel beads can be made from natural and synthetic polymers. Much research efforts have been concentrated on the development of hydrogel beads using natural polymers, as they have good solubility in water, a high swelling ability, a low toxicity level, and high biodegradability and biocompatibility. Pectin as a natural polymer to make and evaluate mulberry leaf extract hydrogel beads with pectin as a polymer and zinc acetate as a cross linker to produce a nutraceutical product in a bead dosage form.

MATERIALS AND METHODS

Materials

Dry mulberry leaf extract (Huamao Biotechnology, Yongzhou, China), mulberry leaf extract (Ballito, Bogor, Indonesia), pectin (Danisco, Grindsted, Denmark), zinc acetate (Merck, Darmstadt, Germany), and distilled water (Brataco, Jakarta, Indonesia) were purchased.

Equipment

An EB-330 analytical balance (Shimizu, Kyoto, Japan), magnetic stirrer (IKA, Staufen, Germany), freeze dryer (Vacuubrand, Wertheim, Germany), Nikon model Eclipse E 200 optical microscope (Nikon model Eclipse E 200, Tokyo, Japan), AMB 50 moisture analyzer (Adam, Connecticut, United States), XT2i texture analyzer (Stable Micro System, Godalming, England), Vernier caliper (Tricle Brand, Shanghai, China), pH meter type 510 (Eutech Instrument, Singapore, Singapore), and Brookfield viscometer (Brookfield, Wisconsin, United States) were used.

Methods

To ensure optimum conditions, formula optimization was conducted beforehand. Five parameters have been optimized, as shown in Table 1. The formula of the beads can be seen in Table 2 with variations in zinc acetate and pectin concentrations.

Mulberry leaf extract hydrogel beads were prepared by the ionic gelation method. Pectin and mulberry leaf extracts were solubilized in distilled water and then mixed until they became homogenous. After that, the mixture of mulberry extract and pectin was dropped into the zinc acetate solution by a hose with a diameter of 2.5 mm. The cross-linking time was 15 minutes with mechanical stirring, after which the
beads were filtered and washed with distilled water. Finally, the beads were dried using a freeze dryer.

Evaluations of mulberry leaf extract hydrogel beads

Yield

The rendemen was calculated using the following equation:

$$\text{Yield} = \frac{W_m}{W_t} \times 100\% \quad (1)$$

Where \(W_p\) is the yield, \(W_m\) is the total weight of the beads after formulation, and \(W_t\) is the total weight of beads being prepared before formulation.

Shape and morphology

An optical microscope was used for the shape and morphology examination.

Particle size distribution

The diameters of 300 randomly selected beads from each formula were measured using a Vernier caliper.

Swelling ability

The 100-mg beads from each formula were placed in weighing dishes. After, 10 mL of distilled water was added. Then, the swelling of the beads in room temperature was observed. Beads were weighed using an analytical balance at 5, 10, 15, 30, 60, 90, and 120 minutes, and swelling ability was calculated by the following equation:

$$\text{Swelling ability} (\%) = \frac{W_2 - W_1}{W_1} \times 100\% \quad (2)$$

Swelling time

The 10 mL of distilled water was added to the 100-mg beads from each formula were placed in weighing dishes. Then, the swelling of the beads was observed in terms of time.

Moisture content

Moisture content was determined using a moisture analyzer. The equipment was prepared to reach a temperature of 105°C before use. Then, 1-g beads were spread on a plate, and the moisture analyzer was turned on to show the beads’ moisture content.

Gel strength

The gel strength of hydrogel beads was measured using a texture analyzer. Samples were placed on a plate, and the probe was pressed to the sample to determine the gel strength of each sample.

RESULTS AND DISCUSSION

Optimization

To ensure optimum conditions, formula optimization was conducted beforehand. The optimization results of the cross-linker concentration were 2.5% and 5% because those concentrations created spherical beads with no tail. The shape of the spherical beads was caused by the process of cross-linking between pectin and zinc acetate. The dry extract was chosen as the best kind of extract to formulate hydrogel beads because it makes attractive beads with a brown color, whereas the wet extract makes dark-green beads with black freckles. The best extract and polymer ratios were 1:2 and 1:3 because the mixture of the extract and pectin can flow into a hose and form spherical beads. 15 minutes was chosen as the best cross-linking time because it results in beads having a good swelling ability. The best drying method was freeze-drying because it allowed the beads to retain their same shape and size as before drying, even though there were some deformations on some sides of the beads. The beads before and after the drying process are shown in Fig. 1.

Table 1: Parameters of formula optimization

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc acetate concentration</td>
<td>2.5%, 5% (pH 1.3, 3.0, and 6.3)</td>
</tr>
<tr>
<td>Kind of extract</td>
<td>Dry or wet extract</td>
</tr>
<tr>
<td>Extract to pectin ratio</td>
<td>1:1, 1:2 and 1:3</td>
</tr>
<tr>
<td>Cross-linking time</td>
<td>15, 30, 45, 60 and 120 minutes</td>
</tr>
<tr>
<td>Drying method</td>
<td>Room temperature and freeze dryer</td>
</tr>
</tbody>
</table>

Table 2: Formulation of mulberry leaf extract hydrogel beads

<table>
<thead>
<tr>
<th>Materials</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mulberry leaf extract (g)</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Pectin (g)</td>
<td>2.4</td>
<td>2.4</td>
<td>3.6</td>
<td>3.6</td>
</tr>
<tr>
<td>Distilled water (mL)</td>
<td>48.0</td>
<td>48.0</td>
<td>72.0</td>
<td>72.0</td>
</tr>
<tr>
<td>Zinc acetate solution (%)</td>
<td>2.5</td>
<td>5.0</td>
<td>2.5</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Table 3: Average diameter of mulberry leaf extract hydrogel beads

<table>
<thead>
<tr>
<th>Formula</th>
<th>Average diameter of beads (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.59±0.04</td>
</tr>
<tr>
<td>2</td>
<td>3.93±0.11</td>
</tr>
<tr>
<td>3</td>
<td>3.55±0.03</td>
</tr>
<tr>
<td>4</td>
<td>3.81±0.03</td>
</tr>
</tbody>
</table>
hydrogel beads is <100%, whereas the swelling ability of mulberry leaf extract was better, at more than 200% [8].

Swelling time
Swelling time was tested by observing the time needed for the beads to swell the first time in distilled water. The swelling time test was done visually by watching the beads as they began to sink into the distilled water from their initial floating position in distilled water.

Table 4 shows the result of the swelling times of mulberry leaf extract hydrogel beads from formulas 1 until 4, respectively. Using Formula 1, the beads first swell in water at 30 s, and they swell at 35 s using Formula 2, 23 s using Formula 3, and 16 s using Formula 4. The fastest swelling time was with Formula 4, while Formula 2 had the slowest swelling time. This was because Formulas 3 and 4 used an extract-to-polymer ratio of 1:3. The more polymers cross linked with an extract, the larger the cross-linked hydrogel beads became, so more and more hydrogen bonds were formed. With the great number of hydrogen bonds, the beads swelled and bonded with water faster. This result could be explained by the fact that the cross-linking time was not long (15 minutes), so it caused the beads to swell quickly [5,6]. Furthermore, pectin was used as the polymer, and it is a hydrophilic polymer, so it can absorb water.

Moisture content
The results of the moisture content test are shown in Table 5.

Table 5 shows the results of the test of the moisture content of mulberry leaf extract hydrogel beads, which were 21.09%, 26.52%, 26.44%, and 23.64% for Formulas 1–4, respectively. This was due to the polymer in the beads, which was pectin, a hydrophilic polymer that can absorb water and high humidity.

Gel strength
The gel strength test aimed to determine the ability to maintain the shape of the hydrogel beads. Gel strength test results can be seen in Table 6.

Table 6 shows that the gel strength of the hydrogel beads before drying was stronger than after drying. In all formulations, the gel strength of the beads after drying decreased compared to before drying. However, the decrease was not too large, except in Formula 2, where the gel strength of the beads before drying was 551.4 gf, and it was 274.9 gf after drying. The decrease in the gel strength of the dry beads occurred because the beads before drying had a chewy texture and could break if pressed, but the beads after drying had a texture that when pressed, the beads would not only break but also deflate. In Formula 2, the gel strength of the beads was more decreased before to after drying. This could be because the dry beads tested in Formula 2 were deflated, so when the probe was pressed, the beads deflated directly, resulting in lesser gel strength.

CONCLUSION
The results of this study indicate that the hydrogel beads had spherical shape with an unsmooth surface, were able to swell in water, and were able to maintain their form. In addition, Formula 1 was chosen as the best formula for mulberry leaf extract hydrogel beads to ensure a proper spherical shape and brown color, an average diameter of 3.6 mm, a swelling ability of 210.68%, a swelling time of 30 seconds, and a yield value of 82.25%.
Table 6: Gel strength of mulberry leaf extract hydrogel beads

<table>
<thead>
<tr>
<th>Formula</th>
<th>Hardness (gf) Beads before drying</th>
<th>Hardness (gf) Beads after drying</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>493.1</td>
<td>461.3</td>
</tr>
<tr>
<td>2</td>
<td>551.4</td>
<td>274.9</td>
</tr>
<tr>
<td>3</td>
<td>382.8</td>
<td>323.4</td>
</tr>
<tr>
<td>4</td>
<td>331.6</td>
<td>356.7</td>
</tr>
</tbody>
</table>

REFERENCES


