Isolation of inulin oligomer from white yam tuber (*Dioscorea rotundata*) for preparation of curcumin–inulin nanoparticles

Nurdila¹, Imran¹, L.O. Kadidae¹, L.A. Kadir¹, and L.O.A.N. Ramadhan¹

¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, Halu Oleo University, Indonesia

e-mail: laode.ramadhan@uho.ac.id

Abstract. Research on the isolation of inulin oligomers from white tuber yam (*Dioscorea rotundata*) for the manufacture of curcumin–inulin nanoparticles has been conducted. The purpose of this study is to find out the results of inulin isolation from white yam (*Dioscorea rotundata*) as an ingredient in making curcumin–inulin nanoparticles. Based on the results of the isolation, the rendement of inulin obtained was 10.2%. The solubility tests revealed that inulin had 89% solubility and for curcumin–inulin particles, either with 1:2 or 1:3 ratio had the same solubility, i.e., 92 g/mL. The functional groups of inulin were analyzed by FTIR spectrophotometer. The IR spectrum of inulin showed the availability of OH bonds, CH bending, C=C bonds, C-O-C bonds, and C-OH bonds. The IR spectra of curcumin–inulin of 1:2 and 1:3 ratio, on the other hand, indicated the existence of OH bonds, CH bending, carbonyl strain, C-O strain, C-O-C strain, CH₃ bending, and H-C=O bond. The morphologies of inulin and curcumin–inulin particles were analyzed using SEM. The surface shape of inulin was semi-spherical, which are different from the morphology of curcumin–inulin particles spreading over a size range of 169-283 nm. With this particle size, the curcumin–inulin particles tend to form nanoparticles.

1. Introduction

Recently, technological developments could not be separated from nanomaterial. Increasing industrial demand for smaller particle size causes nanoparticles to progress rapidly. The use of nanoparticles is widely applied in the fields of pharmacy and biotechnology [1]. The material can be expressed as a nanoparticle if it has a size range from 1 to 100 nm [2]. Applications of nanoparticles are various including diagnosis, wound healing, drug delivery, molecular imaging, water treatment, catalysis, cosmetics, clothing industry, food industry, sunscreen, and many more [3].

One of the examples of nanoparticle material is nanocurcumin. Nanocurcumin was first reported [4], by making nano-size of curcumin using micellar aggregates of cross-linked and random copolymers of N isopropylacrylamide (NIPPAM), N-vinyl-2-pyrrolidone (VP) and polyethylene glycol monoacrylate (PEG-A). The physical-chemical characterization of polymeric nanoparticles was analyzed by laser on an electron microscope which produced the size of 50 nm.

One of the nanocurcumin variants that are potential to be developed is nano curcumin–inulin for pharmacology applications. Regarding inulin sources for the synthesis of curcumin–inulin nanoparticles, [5] previously carried out inulin isolation from dahlia flower tuber (*Dahlia sp*). [6] also carried out inulin isolation from shallots (*Allium cepa Linn*). Furthermore, [7] also carried out isolation of inulin from the tubers of Jerusalem (*Artichokes*).
Inulin consists of polymers from fructose units with glucose terminal groups. Fructose units in inulin are linked by glycosidic β-(2,1) bonds. Inulin from plants usually contains 20 to several thousand fructose units. The smallest molecule of inulin is called fructooligosaccharide (FOS), which contains two molecules of fructose and one molecule of glucose [8]. In this study, inulin was isolated from white yam tuber, which was obtained from Muna Island of Southeast Sulawesi Province, Indonesia.

2. Methods

2.1 Material
The materials used in this study were, white yam tuber (Dioscorea rotundata) obtained from Liangkobori Village, Muna Regency, Southeast Sulawesi province, curcumin (Okushi Biotek, Jakarta), ethanol (96%), and distilled water.

2.2 Apparatus
The tools used in this study are blender, Buchner funnel, centrifugation device (Buchi), vortex, evaporation device (Buchi), common flasks used in laboratory, filter paper, knife, hot plate (IKA @ C-MAG H57), aluminium foil, stirrer, spatulas, desiccators, analytical scales (CHETAH-Dh S16-A), scanning electron microscope (SEM), Fourier Transform Infrared (FTIR) and refrigerators.

2.3 Procedures
The procedures in this study are as follows:

2.3.1 Preparation of white yam tuber
As much as 2 kg of the yam tuber sample, obtained from the village of Liangkobori, Muna Regency, Southeast Sulawesi province was prepared and washed it in running water. The samples were then drained in a basket so that the water content was slightly reduced and then cut them into small pieces.

2.3.2 Isolation of inulin
As much as 250 g of white yam tuber was placed into a blender, added with 500 mL of distilled water (1:2; b/v), blended. After that, the resulting mixture was heated using a water bath for 40 minutes at 90 °C. After being cold, the mixture was then filtered using a Buchner funnel. The resulted filtrate was dissolved in ethanol (96%), with the solvent volume used was about 40% of the filtrate volume. The solution is then stored in the freezer for 18 hours to solidify. After that, the solid material was then left at room temperature to melt. The resulted solution was centrifuged for 15 minutes with a speed of 4500 rpm to form a white deposit (wet inulin). The wet inulin was collected and subsequently dried in an oven at the temperature range of 50-60 °C for about 7 hours. After that, the dried material was mashed to powder [5].

2.3.3 Synthesis of Inulin-curcumin Nanoparticles
Curcumin (1 g) was dissolved in 50 mL ethanol in 100 mL of measuring flask and inulin (2 g) from white yam tuber was dissolved in 50 mL of distilled water. The curcumin-ethanol solution was gradually added to the inulin solution, the pH of the binary ethanol-water solution was adjusted 7.0 and left to mix thoroughly at room temperature for 1 hour and then the solution was mixed using vortex. The resulting mixture was poured into a flask and then put it into a water bath and heated it at 60 °C to evaporate the ethanol producing a thick solution of inulin-curcumin nanoparticles. The nanoparticles were collected and placed in a container and then dried. Products obtained were a 1:2 ratio, then characterized with FTIR and SEM. For a 1:3 ratio, it was employing the same procedure to make 1:3 except the inulin used was 3 g. And the characterization of the1:3 was employed the same procedure as well [9].
2.4 Characterization of the isolated inulin and the product of curcumin-inulin synthesis
Both the inulin that has been isolated from the sample and curcumin-inulin as the product of synthesis were then assessed by employing two main instruments, FTIR and SEM.

2.4.1 Characterization with FTIR
FTIR absorption analysis was conducted to determine the functional groups contained in inulin and curcumin-inulin samples. Inulin powder, mixed with KBr, was converted to pellet by applying high pressure. The pieces of cells that contain the sample are placed in the cell where the beam is always passed. The recordings are carried out from wave numbers 4000 to 400 cm⁻¹, and the spectra of the samples were analyzed by identifying the existing functional groups and compared to the functional groups of a known compound [9]. The same procedure was also applied to curcumin-insulin samples.

2.4.2 Characterization with SEM
The characterization with SEM was carried out to determine the morphology of the inulin and curcumin-inulin powder. Before doing the scanning process of the SEM, inulin powder was placed on top of the gold plate. After scanning, the images were obtained as the surface morphology of the inulin powder [9]. The same procedure was also applied to curcumin-insulin sample.

2.5 Solubility Test
Solubility and water absorption are two important characteristics of inulin. The solubility of inulin in water is commonly dependent on the way that inulin is recrystallized. Inulin recrystallized with ethanol has greater solubility than that of recrystallized with water [5].

3. Results and Discussions

3.1 Isolation of Inulin
Isolation is a method of extracting beneficial compounds from a natural compound. Data obtained on the extraction of inulin from white yam tuber at a temperature of 85 °C are presented in Table 1.

| Treatment                              | Results   |
|----------------------------------------|-----------|
| Initial sample weight                  | 250 grams |
| Wet inulin weight                      | 92 grams  |
| The final weight of the sample (dry inulin) | 27 grams |
| Rendement of inulin                    | 10.8 %    |

From Table 1, it is obvious that the final product was 27 grams of 250 grams of raw material. Therefore the amendment of inulin is 10.8%. The results of the extraction process itself are influenced by several factors, including heating time, filtering process, less effective drying, or even the amount of inulin contained in the sample itself [10].

The temperature used in the extraction process was 85 °C, and the heating process was about 40 minutes. This condition may affect the amendment since a good process of degradation of inulin is at the temperature around 130 °C [11]. The isolated product of inulin was then tested its solubility. It was found that inulin from white yam tuber has an 89% solubility, which is comparable with inulin extracted from Dahlia tuber, namely 90% [5].
Figure 1. Function group (cm$^{-1}$) (a) inulin, (b) curcumin, (c) inulin-curticumin 1:2, and (d) inulin-curticumin.

The FTIR spectrum of inulin is presented in Figure 1 (A). OH, (hydroxyl) groups were shown at a wavenumber of 3410 cm$^{-1}$ with a relatively broad band. A quite sharp intensity at 2906 is assigned to the absorption of C-H stretching of CH$_2$, while another sharp intensity at 1658 is assigned to C=O carbonyl. All these functional groups are related to insulin functional groups and agree with the reference [11].

Figure 2. Morphology of inulin (a), the morphology of curcumin (b), the morphology of curcumin-inulin 1:2 (c) and morphology of curcumin-inulin 1:3 (d).
The results of SEM analysis can be seen in Figure 2. The results of morphological analysis at 5000 times magnification of inulin (a) has a form of semi-rectangular. This is different from the morphology of curcumin (b) using the same magnification of 5000 times.

3.2 Synthesis of Inulin-Curcumin Nanoparticles

Inulin-curcumin was prepared by mixing curcumin with inulin. Both materials were dissolved in different solvents in which curcumin was dissolved in ethanol while inulin was dissolved in water. In this study, the curcumin-inulin was provided in two different ratios, 1:2 and 1:3 (1 gram of curcumin: 2 grams of inulin and 1 gram of curcumin: 3 gram of curcumin). This variation was carried out to seek for the best possible ratio of the starting materials to make inulin-curcumin nanoparticles. However, in the FTIR spectrum point of view, there is not any significant difference between the two material produced from those different combinations. The same outcome was also obtained when their solubility was tested. Their solubility was identical, which is about 90%. As can be seen from Figure 1, the hydroxyl group (OH) appears at 3425 for 1:2 and at 3427 cm\(^{-1}\) for the 1:3 ratio. There is a slight shift, but less significant. The absorption of C-H stretching of CH\(_2\) is even identical for both materials, at 2926 cm\(^{-1}\), but this wavenumber is having a significant difference from the inulin one. Some absorptions were assigned to the curcumin moieties, and the sole curcumin spectrum is shown in Figure 1 (B).

Characterization of the surface morphology was utilized SEM. The results of this characterization are presented in Figure 2 c and d. At a glance, the morphology of curcumin-inulin 1:2 and 1:3 are identical. Nonetheless, the size of the inulin-curcumin particles (1:2) has the smallest size 169-283 nm using a magnification of 5000 times. While the smallest size of 1:3 inulin-curcumin particles using the same magnification 271-775 nm. This is the only distinguishing characteristic of the two, but it is providing quite priceless information. Based on the particle size, the particles manufactured using a ratio of 1:2 of curcumin-inulin is tending to form nanoparticles.

4. Conclusion

White yam tuber (Dioscorea rotundata) contains inulin of 10.8%, which has 89% solubility. FTIR spectrum confirmed the existence of all the functional groups that may be possessed by inulin, such as the presence of OH (hydroxyl) groups at the wavenumber 3410 cm\(^{-1}\) with a fairly broad intensity band. Besides OH, there are also CH groups (alkanes), stretching C=C trans (alkenes), stretching C=O and stretching alkyl-COC aryl ether. Analysis using SEM showed a semi-spherical surface morphology. Inulin-curcumin nanoparticles had 92% solubility for both the ratio of inulin-curcumin synthesized in this study.

FTIR results showed the presence of functional groups in inulin-curcumin nanoparticles, for instance the presence of OH (hydroxyl) groups at the wavenumber of 3425 cm\(^{-1}\), CH (alkane) groups, C = C (alkene) stretches, CH bending, HC=O bonds aliphatic aldehyde, COC anhydride bond, CO bond, and C-OH bond and ROC stretching. Based on SEM characterization results, inulin-curcumin with 1:2 ratio had more tendency to form nanoparticles than the 1:3 ratio. This was supported by its smallest particle size about 169-283 nm compared to 271-775 nm for 1:3 ratio.

5. Reference

[1] M. Jahanshahi, M. H. Sanati, and Z. Babaei. 2008. Optimization of parameters for the fabrication of gelatin nanoparticles by the Taguchi robust design method. Journal of Applied Statistics, 2008, vol. 35, issue 12, 1345-1353

[2] Sietsma, J. R. A., J. D. Meeldijk, J. P. den Breejen, M. Versluijs-Helder, A. J. van Dillen, P. E. de Jongh, and K. P. de Jong. 2007. The Preparation of Supported NiO and Co3O4 Nanoparticles by the Nitric Oxide Controlled Thermal Decomposition of Nitrates. Angew.Chem. Int. Ed. 2007, 46, P. 4547 –4549.

[3] Martien, R., Adhyatmika, Irianto, K. D. I., Farida , V., Sari, P. D., 2012, Perkembangan Teknologi Nanopartikel Sebagai Sistem Penghantaran Obat, Majalah Farmaseutik, 8 (1);
133-145.

[4] Savita Bisht, Georg Feldmann, Sheetal Soni, Rajani Ravi, Collins Karikar, Amarnath Maitra and Anirban Maitra. 2007. Polymeric nanoparticle-encapsulated curcumin ("nanocurcumin"): a novel strategy for human cancer therapy. Journal of Nanobiotechnology, 5(3).

[5] Sundari, E., Erda, R.D., Munas, M dan Erti, P. 2014. Identifikasi Dan Kondisi Ekstraksi Inulin Dari Umbi Dahlia Di Sumatra Barat. Prosiding SNSTL. ISSN 2356-4938; 174-179.

[6] Hartono., Cut, M dan Andi, I.A. 2013. Pengaruh Ekstrak Senyawa Inulin Dari Bawang Merah (Allium Cepa Linn.) Terhadap Pertumbuhan Bakteri Probiotik Lactobacillus acidophilus. Jurnal Bionature. 14 (1); 61-73.

[7] Barkhatova, T.V., Nazarenko, M.N., Kozhukhova, M.A dan Khripko, I.A. 2015. Obtaining and identification of inulin from jerusalem artichoke (helianthus tuberosus) tubres. Foods and materials. 3 (2); 75-92.

[8] Roberfroid MB. 2005. Introducing inulin-type fructans. Br J Nutr. 93 :13-25.

[9] Fares, M.M., and Salem, S.M., 2015, Dissolution enhancement of curcumin via curcumin–prebiotic inulin nanoparticles, Research Article, ISSN: 0363-9045, Vol. 41(11); 1785-1792.

[10] Lakshminarayana, T.S., And Madhusudhan, B., 2017, Development and Evaluation Of Inulin-Loaded Ethyl Cellulose Nanoparticles As Oral Prebiotic Supplement for Selective Eubiosis, International Journal of Pharma and Bio Sciences, 8 (1), 17-21.

[11] Winarti, S., Harmayani, E., dan Nurismanto, R., 2011, Karakteristik Dan Profil Inulin Beberapa Jenis Uwi (Dioscorea Spp.), Agritech, 31 (4); 378-393.