Measurement Method of Nanofibrils Length

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Abstract. Nanofibril is a bio material in the form of fibrils and nano-sized. These fibrils can be made from protein; one of them is soy protein isolate (SPI). Nanofibrils can be used in the fields of food and medicine, for example, shells of microcapsules, food thickener, and food texture makers. To utilize this nanofibril need to know the characteristics of nanofibrils. One of the important characteristics of nanofibrils is the diameter and length of nanofibrils. Based on the shape of the fibrils that can be taken from the image with TEM (Transmission Electron Microscopy) visible irregular curved fibrils. Therefore we need a measurement method that can measure the length of the nanofibrils. One such method is to trace the image of the fibril and convert it to a certain scale. Methods that can be used to trace and measure the length of the curve are using the GDG Measure It to DRAW X4; X5, X6 which is nested in Corel DRAW X4 (Evaluation Version). Based on the results of calibration and trials show this method can be used to measure the length of nanofibrils with an error of about 0.17%.

1. Introduction

Nanofibrils are nano-sized fibrils made from biological material. Fibrils can be made from vegetable and animal. The ingredients of nanofibrils made from animals include egg yolk albumin, whey protein. One material from vegetable is soy protein isolate [1-11]. These nanofibrils can be used as ingredients for artificial meat, food texture and microcapsules [8,9,12]. The maximum utilization of nanofibrils requires the characteristics of nanofibrils. Important characteristics include length, diameter or thickness, shape and charge of the fibrils. Purwanti et al. [9] reported the thickness or diameter of the fibrils largely determines the size of the microcapsules formed. Warji et al. [10] reported the form of curved and branched SPI nanofibrils having a higher viscosity compared to Whey Protein Isolate (WPI) which has the form of a straight shape which is not branched. The length of the fibrils formed is also thought to affect its viscosity; this is important to know so that it can utilize these nanofibrils in food and medicine.

Nanofibrils have a nano size and irregular shape, in the form of a curve, this makes it difficult to know the exact size. So far, the measurement carried out is the observation method. One measurement that can be used is to make lines that can follow the contours of nanofibrils. The contour or shape of the nanofibril made used is TEM image. Nanofibrils in TEM image is followed by contours by forming a new curve. This curve is known for its length and can represent the length of the nanofibrils in the TEM image. Tool kit that can be used to measure the length of the curve is "GDG Measure It". This tool kit can be installed on Corel Draw. This study aims to utilize the GDG Measure It tool kit that is attached to the Corel Draw Tool to measure the length of the nanofibrils in the TEM image.
2. Materials and Methods

2.1. Materials
GDG Measure It for DRAW X4, X5, X6 is nested in Corel Draw X4 (evaluation version). TEM image used is the result of Warji et. al. [8].

2.2. Validation of Length
The length of nanofibrils validation done by measure 10 lines with length 1 to 10 μm and 5 circles with diameter 1 to 5 μm.

2.3. Nanofibrils Measurement
The measurement process is carried out on the SPI nanofibril TEM image. The TEM image is opened in the Corel Draw program, the GDG Measure It for DRAW X4, X5, X6 menu is selected to create a new curve that follows each nanofibril contour on the TEM image. The length of the fibrils is listed on the GDG Measure It menu. Finally, the length of the curve that represents the length of the nanofibrils with the scale of the image is drawn on the TEM image.

3. Results and Discussions

3.1. Nanofibrils of SPI
Fig. 1 shows the TEM images of soy protein isolate var. Grobogan [8] and local black soybean [9]. The nanofibrils show irregular shapes. Nanofibril shaped branching curve. At first glance, there are long nanofibrils and some are short. There are thick nanofibrils and thin ones. The length of a nanofibril is difficult to determine because it is irregular (not straight). The length of each nanofibril in this TEM image can be determined by knowing the length of the curve that follows each nanofibril. These nanofibril contour curves can be made using the "GDG Measure It" tool kit.

3.2. Validation of Length
Fig. 2 shows the correlations for actual length and measurement length of linear line and curve of nanofibrils. The correlation actual length and measurement length are $R^2=1$ both for line and circle validation, which showed that the length of the nanofibrils is comparable to the contour length that measured using line and curve measurer tool kit. The percent error of lines about 0.15%, and circle about 0.18%. The average of both errors is 0.17%.

3.3. Length of Nanofibrils
Fig. 3 shows TEM images of nanofibrils before (a) and after measurement. Measurements made by making curves that follow each nanofibril contour produce the size of each nanofibril. Short nanofibrils produce short nanofibril contour curves as well as long ones that produce long contour curves.

![Figure 1. TEM images of SPI (a) var. Grobogan [8], (b) local black soybean [9].](image-url)
Figure 2. Correlation actual length and measurement length of ( ) linear line and ( ) curve.

Figure 3. Tem images before (a) and after (b) measurement of length

Fig. 4 shows the size of the SPI nanofibrils on the captured TEM image. The length of the nanofibrils varies from 0.1 ± 0.00017 micrometers (µm) to 3.8±0.00646 µm. Cumulatively 95% nanofibrils are less than 1.7 µm, and accumulatively 50% nanofibrils are less than 0.3 µm.
4. Conclusions
Nanofibril length measurements can be done with the GDG Measure It for DRAW X4, X5, X6 tool kit which is nested in Corel DRAW X4 (Evaluation Version). Based on the results of calibration and trials show this method can be used to measure the length of nanofibrils with an error of about 0.17%. The SPI nanofibrils on the TEM image captured were varied in length from 0.1 ± 0.00017 µm to 3.8 ± 0.00646 µm.

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Figure 4. Cumulative amount of fibril’s length.
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