Temperature and Heating Time of Forming Process of Nanofibrils of Whey Protein Isolate

W Warji¹,⁴, N Purwanti², S S Mardjan², and S Yuliani³

¹Department of Agricultural Engineering, Faculty of Agricultural, Lampung University, Lampung, Indonesia
²Department of Mechanical and Biosystem Engineering, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University, Bogor, Indonesia
³Indonesian Center for Agricultural Postharvest Research and Development (ICAPRD), Ministry of Agriculture of Republic of Indonesia, Bogor, Indonesia
⁴Corresponding author email: warji1978@gmail.com

Abstract. Nanofibrils are nano-sized fibers. One of the materials that can be used to make nanofibrils is whey protein isolate. Nanofibrils can be formed by heating whey protein isolate at a certain temperature and for a certain duration. This study examines the effect of heating temperature and heating time on the formation of nanofibrils. WPI was formed into nanofibrils by heating the WPI solutions at 70 °C, 80 °C and 90 °C for 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 hours. WPI nanofibrils in the solutions is observed by placing the solutions in between a cross-polariser. Based on the results of the study, it shows that heating at 70 °C was observed for 2 to 20 hours and did not form nanofibrils whey protein isolate. The nanofibrils were formed for heating at 80 °C for 4 hours, but the population was still small; along with the length of heating the population of nanofibrils is increasing. Heating at 90 °C for 2 hours the nanofibrils began to form; the population is increasing along with the length of heating. Based on the image, the nanofibrils heating at 80 °C is similar to 90 °C heating. Heating 80 °C is sufficient for the process of forming whey protein nanofibrils.

Keywords: nanofibrils, whey protein isolate, formation of nanofibrils.

1. Introduction

Whey Protein Isolate (WPI) is a byproduct of milk processing. WPI has an important role in balancing other proteins in specific nutritional applications, such as formula milk, because WPI is rich in branched chain amino acids which are highly needed in health, especially muscle nutrition. WPI also has beneficial functional properties because it is a gel form and has the ability to bind water and can stabilize emulsions and foam [1]. WPI can be used as an encapsulant for hydrophobic materials and hydrophilic materials so that WPI is widely used in food microencapsulation [2-7], and ingredients for health [8,9].

WPI contains β-Lactoglobulin with a globular structure, this can form nanofibrils [10,11] which are used as encapsulants in the microencapsulation of the LbL adsorption method. The use of nanofibrils (nano-sized fibers) whey protein isolate (WPI) to strengthen the walls of the microcapsules made with the LbL adsorption technique can produce microcapsules that can be adjusted in size, thickness, permeability, and stability [12]. WPI nanofibrils that are positively charged are alternately reinforced with a negative charge of high methoxyl pectin (HMP) on a spherical template that can form microcapsules of a certain thickness. The wall thickness reaches 300 nm with only 7 layers using WPI nanofibrils. Other studies suggest that the wall thickness is 28 nm with 14 layers [13] and 12890 nm with 7 layers in the use of other materials [14]. Microcapsules reinforced with WPI nanofibrils can
withstand pH 2 and dissolve at pH 7 for 30 minutes and the capsules are likely to be stable at other pHs with more coating on capsule manufacture [12]. The process of making nanofibrils is influenced by temperature and heating duration. The WPI was made at a temperature of 80°C and for 16 hours [3-5]. However, it is possible at temperatures lower or higher than 80°C and heating time of less than 16 hours and more than 16 hours. To convert WPI into nanofibrils, it is necessary to study the effect of temperature and heating time on the formation of WPI nanofibrils. Furthermore, the results of this study can be a reference for preparing WPI nanofibrils.

2. Material and Methods

2.1 Materials and chemicals
WPI (BiPro® JE 193-3-420) was provided by Davisco Food International Inc. (Le Sueur, Minnesota, USA). The chemicals used were HCl 37% (Merck, Germany). Double distilled water was used to dissolve protein.

2.2 Preparation of nanofibrils
WPI was formed into nanofibrils following the method of [3] and [4]. WPI solutions were prepared to make protein nanofibrils as shown in Figure 1. WPI solutions of 2% w/w were made by dissolving the proteins in double distilled water. The protein solution was stirred overnight to complete solubilization using a magnetic stirrer. Afterward, the pH was set to 2.0 using a 6 N HCl solution. The WPI solution at this pH was then heated in a water bath 70°C, 80°C and 90°C (temperature of the solutions) while stirring for 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 hours using a magnetic stirrer. The results were solutions containing protein fibrils made of WPI.

Figure 1. The nanofibrils preparation

2.3 Observations of nanofibrils in between a cross-polariser
The existence of WPI nanofibrils in the solutions is observed by placing the solutions in between a cross-polariser as shown in Figure 2. A cross-polariser is a pair of the polarised optic that are arranged cross 90° and spaced between both where samples are placed and observed against natural light [3].
3. Results and Discussions

Heating at 70 °C was observed for 2 to 20 hours and did not form nanofibrils whey protein isolate as shown in Figure 3. Figure 3 shows the WPI solution which was heated at 70 °C for 2 to 20 hours which did not pass the crossed polarizing film (top), it shows that all the solutions appear clear; the same solution (bottom) passed by the polarizing film also appears clear. This shows that heating at 70 °C cannot change the WPI solution into WPI nanofibrils. It is suspected that the temperature of 70 was not enough to break down the protein which subsequently became nanofibrils.

Figure 3. The images of WPI nanofibrils (70 °C) (top) and WPI nanofibrils in between a cross-polariser (bottom).

The nanofibrils were formed for heating at 80 °C for 2 hours, but the population was still small; along with the length of heating the population of nanofibrils is increasing as shown in Figure 4. This picture also shows the WPI solution heated at 80 °C for 2 to 20 hours. The top of Figure 4 shows the WPI solution which is not placed between the crossed polarizing films; the picture looks clear. However in Figure 4, the bottom shows the WPI solution placed between the crossed polarizing films; the image shows a white, cotton-like color which is thought to be WPI nanofibrils as in [3]. The white color of cotton has started to appear at 2 hours heating time. The longer the heating, the more white cotton is formed; This shows that WPI nanofibrils have begun to form, and the longer they
are heated, the more nanofibrils are formed. Warming for 12 to 20 hours did not show the addition of white cotton, it is thought that heating for 12 hours was enough to form WPI nanofibrils at 80 °C heating.

Heating at 90 °C for 2 hours the nanofibrils began to form; the population is increasing along with the length of heating. The formation of nanofibrils is indicated by a white, cotton-like color as shown in Figure 5 (below). The longer the heating, the more cotton white color will be formed; this shows that the longer the heating, the more nanofibrils are formed. Heating for 8 to 20 hours the formation of cotton white does not increase; it is assumed that heating for 8 hours at 90 °C is sufficient to form WPI nanofibrils.
4. Conclusion

WPI nanofibrils began to form on heating at 80 °C after 2 hours and the longer they are heated that the more nanofibrils are formed. The WPI nanofibrils formed at 80 °C heating are similar to 90 °C heating for all heating times.

5. Acknowledgments

The authors thank Davisco Food International Inc. for providing of WPI.

References

[1] Lazidis A, Hancocks R D, Spyropoulos F, Kreu M, Berrocal R and Norton I T 2016 Whey protein fluid gels for the stabilisation of foams *Food Hydrocolloids* **53** 209
[2] Bastos D S, Gancalves M P G, Andrade C T, Araujo G L and Leao M H M R 2012 Microencapsulation of cashew apple (*Anacardium occidentale*, L.) juice using a new chitosan-commercial bovine whey protein isolate system in spray drying *Food and Bioproducts Processing* **90** 683
[3] Warji W, Mardjan S S, Yuliani S and Purwanti N 2017 Characterization of nanofibrils from soy protein and their potential applications for food thickener and building blocks of microcapsules *International Journal of Food Properties* **20**(sup1) s1121
[4] Purwanti N, Warji W, Mardjan S S, Yuliani S and Schröén K 2017 Preparation of Multi-layered Microcapsules from Nanofibrils of Soy Protein Isolate using Layer-by-Layer Adsorption Method *IOP Conf. Series: Earth and Environmental Science* **147** 012009
[5] Warji, Mardjan S S, Yuliani S, K Schröén and Purwanti N 2018 Flow Behavior of Isolate Protein from Soybeans var. Grobogan and Whey Protein Isolate at Acidic Condition under Various Heating Times *Jurnal Keteknikan Pertanian* **2**
[6] Duongthingoc D, George P, Katopo L, Gorczyca E and Kasapis S 2013 Effect of whey protein agglomeration on spray dried microcapsules containing Saccharomyces boulardii *Food Chemistry* **141** 1782
[7] Liu W, Chen X D, Cheng Z and Selomulyo C 2016 On enhancing the solubility of curcumin by microencapsulation in whey protein isolate via spray drying *Journal of Food Engineering* **169** 189
[8] Khem S, Small D M and Bee K 2016 The behaviour of whey protein isolate in protecting Lactobacillus plantarum *Food Chemistry* **190** 717
[9] Wang L, Gao Y, Subirade M, Song Y and Liang L 2016 Effect of resveratrol or ascorbic acid on the stability of α-tocopherol in O/W emulsions stabilized by whey protein isolate: Simultaneous encapsulation of the vitamin and the protective antioxidant *Food Chemistry* **196** 466
[10] Warji W, Purwanti N, Mardjan S S and Yuliani S 2019 Portable Water Bath to Support Nanofibrils Processing *IOP Conf. Series: Earth and Environmental Science* **355** 012086
[11] Warji W, Purwanti N, Mardjan S S and Yuliani S 2020 Measurement Method of Nanofibrils Length *IOP Conf. Series: Earth and Environmental Science* **537** 012033
[12] Rossier-Miranda F J, Schröén and Boom R 2010 Mechanical characterization and pH response of fibril-reinforced microcapsules prepared by layer-by-layer adsorption *Langmuir* **26** 19106
[13] Elsner N, Kozlovskaya V, Sukhishvili S A and Ferry A 2006 pH-Triggered softening of crosslinked hydrogen-bonded capsules *Soft Matter* **2** 966
[14] Warji W, Purwanti N, Mardjan S S and Yuliani S 2021 The thickness of the microcapsule layers of the SPI nanofibrils *IOP Conf. Series: Earth and Environmental Science* **653** 012105