Journal of Dairy Research

cambridge.org/dar

Comparative experiments of electrical conductivity from whey protein concentrates conventional film and nanofibril film

Chen Guan[1,2], XueFei He[1], HongHua Xu[1], MeiLi Shao[1], JinYu Ma[1] and ZiWen Gao[1]

[1]Key Laboratory of Dairy Science, Ministry of Education, Northeast Agricultural University, Harbin 150030, China and [2]College of Food Science, Heilongjiang Bayi Agricultural University, Daqing 163319, China

Research Article

Cite this article: Guan C, He XF, Xu HH, Shao ML, Ma JY and Gao ZW (2020). Comparative experiments of electrical conductivity from whey protein concentrates conventional film and nanofibril film. *Journal of Dairy Research* **87**, 103–109. https://doi.org/10.1017/S0022029919000876

Received: 17 August 2018
Revised: 2 August 2019
Accepted: 16 September 2019
First published online: 3 February 2020

Keywords:
Electrical conductivity; Fibril; film; whey protein concentrate

Author for correspondence:
Hong-Hua Xu, Email: xhh3161@126.com

Abstract

We compared the electrical conductivity from two different aggregates of whey protein concentrates (WPC) film: conventional amorphous aggregation at natural pH (pH 6.5) and amyloid fibrils at a low pH (pH 2.0) far away from the isoelectric point. The two types of film fabricated by these solutions with different aggregate structures showed large variations in electrical conductivity and other properties. The WPC fibril film (pH 2.0) exhibited higher electrical conductivity than that of the conventional WPC film (pH 6.5), improved mechanical properties and oil resistance, due to varying morphology, higher surface hydrophobicity and more (absolute value) surface charge of film-forming solutions. The evidence from this study suggests that fibrilized WPC with high-ordered and β-sheets-rich structures fabricated high electrical conductivity film, which broadens the potential application of fibrils as functional bio-nanomaterials.

Whey protein concentrates (WPC) with remarkable functional properties are obtained by removing non-protein ingredients from whey, which is a by-product of cheese making. (Henriques *et al*., 2016). WPC contains 35–80% protein (including β-lactoglobulin and α-lactalbumin as major components).WPC can be used as food additives, foaming agents, emulsifiers, thickening agents, gelatinizers and nutraceuticals due to their functional properties and biological activity (Brandenberg *et al*., 1992; Banerjee and Chen, 1995; Perez *et al*., 2010; de Castro *et al*., 2017). Whey protein-based films are extensively researched as they generally demonstrate better physical properties and barrier properties to oxygen and aroma transmission than other protein-based films (such as caseinates, soy protein isolate and wheat) or polysaccharide-based films (such as chitosan, starch and cellulose) (Kumari *et al*., 2017; Sukyai *et al*., 2018).The properties of the films are influenced by protein concentration, heat treatment, pH, salt concentration (Pérez-Gago *et al*., 1999; Ayadi *et al*., 2004; Mchugh *et al*., 2010). However, whey protein films have some limitations in respect of their unfavorable mechanical properties and high permeability of water-vapor (Henriques *et al*., 2016).

The polymeric films such as whey protein-based films are formed by cohesion and adhesion of composite which relates to the structural and chemical properties of aggregation. Heating causes unfolding of whey proteins and exposure of hydrophobic residues, whilst the cross-linked network of protein increases the cohesion and rigidity of film (Pérez-Gago *et al*., 1999). Two types of distinctly different morphologies are formed during heating: amorphous and ordered aggregation according to pH (Nicolai and Durand, 2013). Based on previous studies, WPC (Gao *et al*., 2013; Xu *et al*., 2016), whey protein isolate (Bolder *et al*., 2007*b*; Mantovani *et al*., 2017), β-lactoglobulin (Dave *et al*., 2015; Nicolai *et al*., 2011) and several other proteins have the ability to form fibrils at a pH value (such as pH2.0) far away from the isoelectric point and at low ionic strengths, by incubation above their denaturation temperature (commonly at 90°C) for about 10 h. These fibrils which are normally composed of 2−6 protofilaments twisted together with nanometric diameter and several micron lengths, are rich in β-sheets that run parallel to the axis of fiber (Nelson *et al*., 2005). The fibrillar structures show prominent mechanical properties, such as high elasticity, stiffness, and resistance (Adamcik and Mezzenga, 2011). Stable and rigid structures have been regarded as a powerful tool to fabricate high-performance nanostructured materials (Knowles *et al*., 2007). The rigidity derives from the intermolecular organized hydrogen-bond network oriented by side-chain interactions (Liu *et al*., 2011).

Knowles and his colleagues (Knowles *et al*., 2010; Knowles and Buehler, 2011) manufactured free-standing films from self-assembled β-lactoglobulin and hen egg-white lysozyme fibrils. These highly rigid films showed Young's modulus of up to 5–7 GPa and well-ordered structure that aligned other unstructured constituents (such as fluorophores) within the nanostructured films. Films formed from β-LG nanofibrils presented enhanced transparency and decreased moisture content and were able to expand the shelf life of fresh-cut apple by

Hannah Dairy Research Foundation

CAMBRIDGE UNIVERSITY PRESS

inhibiting the loss of total phenolic content, browning, and water consumption (Feng *et al.*, 2018). Lysozyme nanofilm (Wang *et al.*, 2016) and $A\beta_{16-22}$ nanofibrils films (Pan *et al.*, 2012) had high optical transparency, steady adhesion force and were said to be environmental-friendly. In recent years, there has been an increasing amount of literature on hybrid nanocomposite films with modified catalytic efficiency, mechanical and electronic properties as well as on biological devices fabricated by combining fibrils with metal nanoparticles (Bolisetty *et al.*, 2015), or with polyvinyl alcohol (PVOH) (Pilkington *et al.*, 2010; Rao *et al.*, 2012) or graphene (Li *et al.*, 2012).

Electrical conductivity is an important characteristic of the functional polymer film, which can be potentially applied in conductive biosensors (Gao *et al.*, 2012; Abdel-Karim *et al.*, 2018). However, there is little published information about the electrical conductivity of self-assembly fibril film. The present work compared the two types of films (WPC fibril film with pH 2.0 and conventional WPC film with pH 6.5) regarding electrical conductivity, film protein solubility, mechanical properties and oil resistance.

Materials and methods

Materials

Wpc-80 with 76.93% protein content was purchased from Hilmar Cheese Company (Hilmar, California, United States). Analytical grade reagents were used in all cases and obtained from local suppliers.

WPC fibrils formation

Wpc fibrils were prepared according to the procedure used by Xu *et al.* (2016). WPC (5% w/v) was stirred into deionized water containing different concentrations of $CaCl_2$, then the dispersion was adjusted to pH 2.0 by the addition of 6 M HCl. To remove non-fibrillated proteins, WPC solution was centrifuged at 19 000 × ***g*** for 20 min at 4°C. The resulting supernatant was collected and the protein content determined by Kjeldahl analysis ($N \times 6.38$).The solution was diluted to the protein concentration of 3.0% (w/v) with deionized water (pH 2.0)and heated at 90°C for 10 h to form mature fibrils. In order to study the effect of $CaCl_2$ concentration, WPC dispersions with ionic strength of 20, 40, 60, 80, 100, 150, 200, 250 and 300 mM were prepared by adding $CaCl_2$ to protein solutions with protein concentration of 3.0% (w/v).

Film formations

Conventional WPC film (pH 6.5): 3% w/v WPC solution was prepared. Then gelatin and glycerol were added as plasticizers with the relative weight of 60 and 50% (w/w protein) (Le *et al.*, 2000; Schmid, 2013). The well-stirred solution was heated in a water bath (90°C) for 30 min and cooled to room temperature after heating. The solution was poured over the glass plate and left to dry for 24 h at room temperature. The film was detached from the surface and conditioned at 50% relative humidity (RH) and room temperature for 48 h prior to testing.

WPC fibril film (pH 2.0): 3% w/v WPC fibril solution was prepared. Then gelatin and glycerol were added as plasticizers with the relative weight of 60 and 50% (w/w protein). After heating at 90°C for 30 min, the cooled film-making solution was cast and conditioned in the same way as conventional WPC film.

Conventional WPC film (pH 2.0): In order to compare with the WPC fibril film, conventional WPC film with pH 2.0 was fabricated. 3.0% (w/v) WPC solution (pH 6.5) with gelatin (60% w/w protein) and glycerol (50% w/w protein) was incubated at 90°C for 30 min. After cooling, the pH value of the solution was adjusted to 2.0 by the use of 6 M HCl and cast as conventional WPC film.

Electrical conductivity measurement

The electrical conductivities of the film-forming solution and film were measured at room temperature using a silver probe method with a high resistivity meter (HIOKI LCR HiTESTER 3532-50, Japan). The frequency and voltage were 1000 Hz and 0.5 V. The values of electrical conductivity σ(S/m) were calculated using the following equation:

$$\sigma = \frac{L}{RA} \tag{1}$$

where A is the cross-section area of the measured surface(m^2), L is the length of the sample in the direction of measurement(m), R is the resistivity(Ω).

Film protein solubility

2.0 g WPC fibril film was dissolved in deionized water (pH 2.0) for 5, 10, 15, 20, 25, 30 min. The solution of the film was centrifuged at 4000 r/min for 20 min. The gelatin film with the same gelatin concentration as the WPC fibril film was fabricated to avoid the influence of protein solubility in gelatin. The solubility was calculated using the following formula:

$$M = \frac{M_2 - m}{M_1 - m} \times 100\% \tag{2}$$

where m is the protein weight of gelatin in the supernatant(g); M_1 is the initial protein weight of film; M_2 is the protein weight of supernatant after dissolved(g).

Mechanical properties

The tensile strength (TS) and percent elongation at break (E/B) with a size of 30 × 100 mm were measured by XLW (M) Auto Tensile Tester (Labthink International, Inc., China) at 25°C and 50% RH. The test speed was 50 mm/min.

Oil permeability

A tube containing 5 ml oil was sealed with the sample film and inverted on the filter paper for a week. The oil permeability coefficient P_0 (g mm/m^2/d) was calculated according to the formula:

$$P_0 = \frac{\Delta m \times T}{S \times R} \tag{3}$$

where $\triangle m$ is the change in weight of the filter paper(g); T is the film thickness(mm); S is the film area (m^2); R is the test time (d) (Iwata *et al.*, 2000).

https://doi.org/10.1017/S0022029919000876 Published online by Cambridge University Press

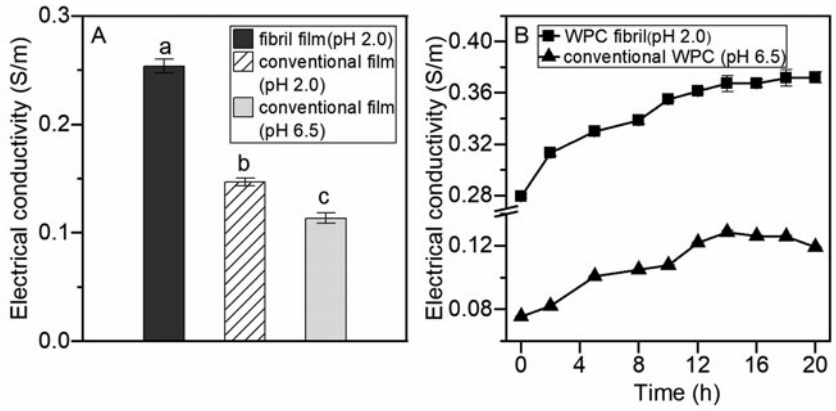

Fig. 1. (a) Electrical conductivity of WPC fibril film (pH 2.0), conventional WPC film (pH 6.5) and conventional WPC film (pH 2.0). (b) Electrical conductivity of the film-forming solution (prepared by heating 3% (w/v) WPC at 90°C with pH 2.0 or 6.5) at different incubation time. Different letters above the bars indicate a statistically significant differences ($P < 0.05$).

Thioflavin T (Th T) fluorescence assay

A stock solution was made by adding 8 mg Th T into 10 ml of phosphate buffer (10 mM phosphate and 150 mM NaCl, pH 7.0). These dispersions were filtered through a 0.22 µm syringe filter and stored at 4°C in the dark. Working solution was diluted 50-times with the same buffer before utilized. Fibril sample (50 µl) and Th T working solution (5 ml) were mixed for at least 1 min and measured using a Hitachi F4500 fluorescence spectrometer (Tokyo, Japan) at the excitation wavelength of 446 nm and the emission wavelength of 490 nm.

Surface hydrophobicity

Ans (8-anilinonaphthalene-1-sulfonic acid ammonium salt) as a fluorescent probe was used to measure the surface hydrophobicity of samples. Protein solutions were gradually diluted to a concentration range from 0.005 to 0.1% with 10 mM phosphate buffer of pH 6.7. Aliquots of the solution (6 ml) were added to 20 µl ANS solution (8 mM ANS and 10 mM phosphate buffer at pH 7.0) and stood in the dark for 15 min before the analysis. Hitachi F4500 fluorescence spectrometer was used to measure absolute fluorescence intensity with excitation wavelength of 39 nm and emission wavelength of 47 nm. Surface hydrophobicity was determined with the initial slope of the fluorescence intensity against protein concentration.

ζ-Potential

ζ-Potential of the solution was measured using a Malvern Zetasizer Nano ZS90 (Malvern Instruments, Worcestershire, UK) at 25°C. The samples were diluted to a concentration of 0.1% (w/v) with deionized water (the same pH as the sample) before measurements. Refractive index values were set at 1.450, the viscosity of fibril samples was set at 0.8872 cP.

Transmission electron microscopy (TEM)

Sample solutions were prepared for TEM by diluted to 0.03% w/w protein with deionized water. A drop of the solution was transferred to a 200-mesh carbon-coated copper grid and removed the excess sample with filter paper after 20 min. TEM micrographs were operated at 100 kV using a H-7650 transmission electron microscope(Hitachi, Tokyo, Japan).

Statistical analysis

All experiments were performed in triplicate and the data are expressed as means ± standard deviation (SD) of three replicates. All data were analyzed by one-way analysis of variance (ANOVA) using SPSS (16.0) software (IBM software, NY, USA). Significant differences of $P < 0.05$ was used and determined by Duncan's multiple range test.

Results and discussion

The electrical conductivity of WPC fibril and WPC fibril film

The electrical conductivity was different among the three types of WPC films with different aggregation structures (Fig. 1a). The electrical conductivity of WPC fibril film increased by 123% as compared with that of conventional WPC film (pH 6.5), and 73% as compared with that of conventional WPC film (pH 2.0). The electrical conductivity of WPC fibril film-forming solution (pH 2.0) which was higher than that of conventional WPC film-forming solution (pH 6.5), significantly increased ($P < 0.05$) from 0.280 to 0.373 S/m after 20 h of heating (Fig. 1b). These results indicate that the improved electrical conductivity is possibly attributable to the forming of fibrillar structures at pH 2.0, instead of adding HCl.

Factors for the conductivity of fibril solution

Fibril morphology and quantity are known to be affected by protein concentration (Schokker *et al.*, 2000), pH, ionic strength and valency (Loveday *et al.*, 2010) as well as by stirring and seeding (Bolder *et al.*, 2007*a*). The influence of protein concentration and $CaCl_2$ concentration on the electrical conductivity of our WPC fibril solution (pH 2.0) and conventional WPC solution (pH 6.5) are shown in Fig. 2. The electrical conductivity of both solutions improved significantly ($P < 0.05$) as protein concentration increased from 3% to 6%. The reduction in electrical conductivity at 7% could be attributed to gelation at high protein concentration. The conductivity of WPC fiber is better than that of conventional WPC (pH 6.5), moreover, the variation of conductivity between the two solutions increased from 0.174 to 0.273 S/m with increasing protein concentration (Fig. 2a).

Calcium ions can potentially lead to the change of electrical conductivity according to shield electrostatic interactions, and in addition can change charge interactions and induce different morphologies (Ramos *et al.*, 2017). The electrical conductivity

https://doi.org/10.1017/S0022029919000876 Published online by Cambridge University Press

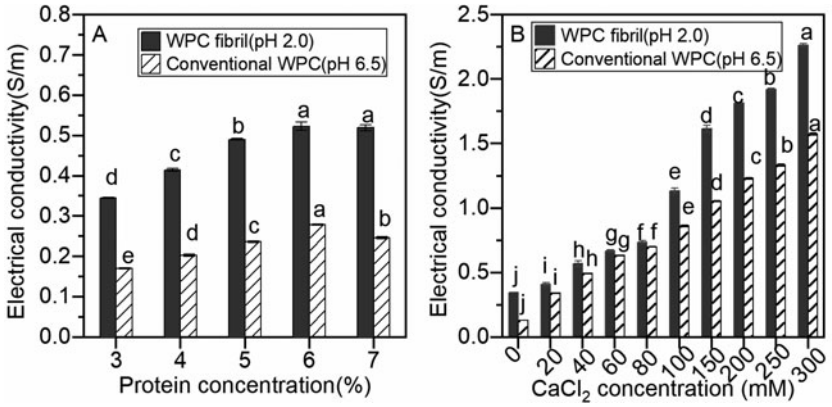

Fig. 2. Electrical conductivity of WPC fibril solution (pH 2.0) and conventional WPC solution (pH 6.5) as a function of (a) protein concentration, (b) $CaCl_2$ concentration. Different letters above the bars indicate significant differences ($P < 0.05$).

of the fibril and conventional solutions improved significantly ($P < 0.05$) with the increase of $CaCl_2$ concentration (Fig. 2b). Since, in previous work, fibril solutions have appeared to undergo phase separation and films have become disrupted with excessive $CaCl_2$ (Bolisetty *et al.*, 2012), 100 mM $CaCl_2$ was chosen in follow-up research with consideration of both electrical conductivity and integrity of the film.

Protein solubility for WPC fibril film

Wpc fibril film (pH 2.0) was more soluble due to the relatively weak noncovalent interactions among proteins (Fig. 3a) (Pérez-Gago *et al.*, 1999; Oboroceanu *et al.*, 2010). However, water resistance and insolubility are required for film-forming and product integrity (Rhim *et al.*, 2000). A decrease of film protein solubility with increasing protein concentration was due to high cohesion from the unfolding of protein molecules and exposing hydrophobic groups formerly buried inside the molecules (Pérez-Gago *et al.*, 1999). Mature fibril solution was adjusted to different pH before it was cast. The solubility of film manufactured by diverse pH of film-forming solutions is presented in Fig. 3b. The film protein solubility of WPC fibril solutions at pH 2.0 was reduced from 51.62 to 30.94% by adjusting pH of film-forming solutions to 5 (pI range of WPC), and the maximum was recorded at pH 7. Increasing the $CaCl_2$ concentration from 0 to 100 mM improved aggregation by electrostatic screening, increased the yield and viscosity of fibrils (Loveday *et al.*, 2011), resulting in a decreased solubility from 51.62 to 33.60% (Fig. 3c).

Characteristic parameters of films

Considering the effects of protein concentration, pH and $CaCl_2$ concentration on electrical conductivity and film protein solubility, WPC fibril film was prepared from 6%(w/v) protein concentration with 100 mM $CaCl_2$ at pH 2.0, and conventional WPC film was prepared from the equal concentration of protein and $CaCl_2$ at pH 6.5. The results of characteristic parameters (electrical conductivity, film protein solubility, tensile strength, elongation at break and oil permeability) for two types of films are presented in Table 1. It can be observed that the electrical conductivity of the WPC fibril film (pH 2.0) was increased by almost 72% as compared with that of conventional WPC film (pH 6.5). WPC fibril film showed superior mechanical properties (tensile strength, elongation at break) and oil permeability compared with conventional WPC film, probably due to its highly ordered

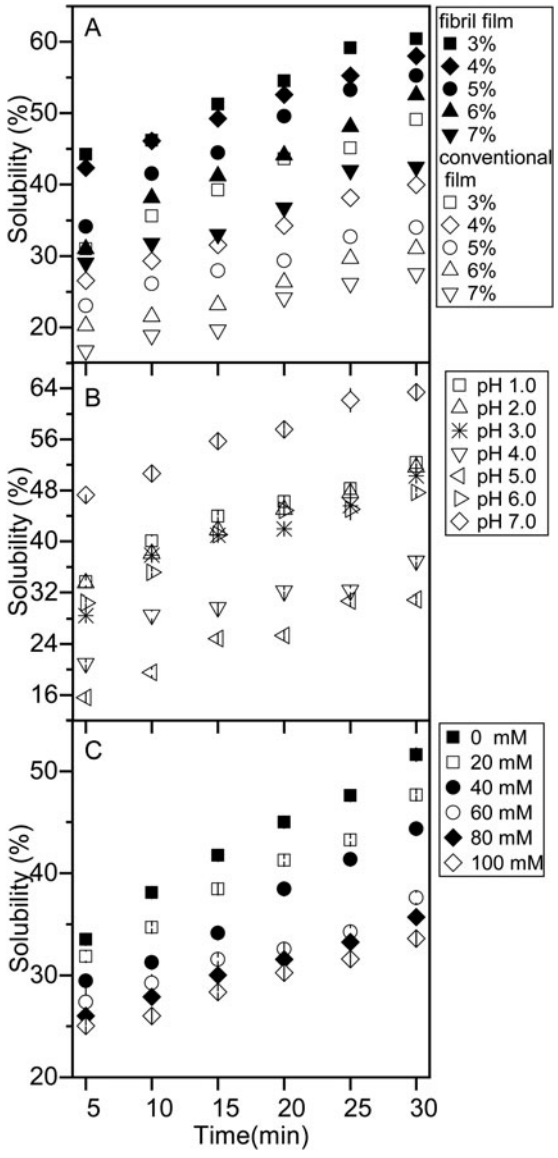

Fig. 3. Protein solubility from WPC fibril film (prepared by 3%(w/v) WPC heating at 90°C for 10 h, pH2.0) as a function of (a) protein concentration, (b) pH 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and (c) $CaCl_2$ concentration.

https://doi.org/10.1017/S0022029919000876 Published online by Cambridge University Press

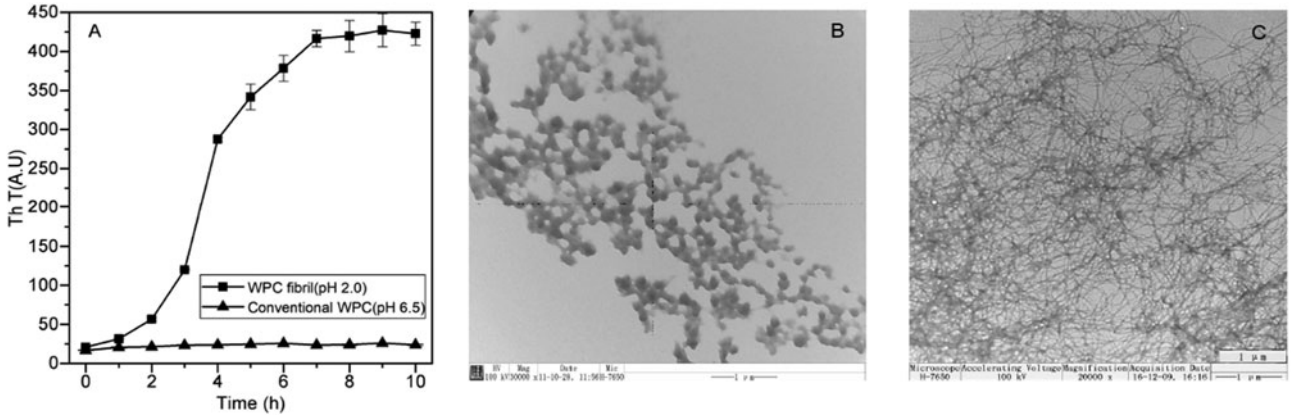

Fig. 4. (a) Th T fluorescence intensity of WPC fibril (prepared by 3%(w/v) WPC heating at 90°C, pH 2.0)and conventional WPC (prepared by 3%(w/v) WPC heating at 90°C, pH 6.5)as a function of time; (b) TEM of 3% conventional WPC forming at pH 6.5, heated for 0.5 h at 90°C; (c) TEM of 3%(w/v) WPC fibrils forming at pH 2.0, heated for 10 h at 90°C. Scale bar corresponds to 1 μm.

Table 1. Electrical conductivity, film protein solubility, tensile strength, elongation at break and oil permeability of two types of films

Film	Electrical conductivity (S/m)	Film protein solubility (%)	TS (mPa)	E/B (%)	Oil permeability (g m/m^2 d)
WPC fibril film (pH 2.0)	2.163 ± 0.002^a	20.56 ± 0.08^a	28.21 ± 0.23^a	26.02 ± 0.12^a	0.031 ± 0.001^a
Conventional WPC film (pH 6.5)	1.254 ± 0.001^b	19.78 ± 0.09^b	15.84 ± 0.35^b	15.98 ± 0.25^b	0.062 ± 0.004^b

WPC, whey protein concentrate; TS, tensile strength; E/B, elongation at break.
$^{a–b}$Different letters in the same column indicate significant differences ($P < 0.05$).

hydrogen-bonding network (Paparcone *et al.*, 2010; Xu and Buehler, 2010). However, the solubility was found to be higher for the WPC fibril film. The variation of two films was associated with the structure, surface hydrophobicity and net charge, which we will discuss below.

Th T fluorescence and morphology of protein solutions

The morphology of different aggregates from two protein solutions was visualized using TEM (Fig. 4b, c). WPC self-assembled into long and straight fibrils by reason of electrostatic repulsive forces, while it formed amorphous aggregate when heated at pH 6.5 (van der Linden and Venema, 2007). The β-sheets-rich structures of WPC fibrils can be measured by Th T fluorescence assay, as Th T which binds to the grooves on the β-sheets causes an increase in fluorescence intensity. Fig. 4a shows the typical sigmoidal growth kinetics in Th T fluorescence intensity upon fibrillation of WPC: the lag phase (about 0–2 h), growth phase or elongation phase (2–6 h) and plateau phase (6–10 h). However, conventional WPC (pH 6.5) did not significantly increase in fluorescence upon heating. These highly ordered and stable β-sheets structures with parallel or antiparallel orientation, probably contribute to the excellent mechanical properties (Knowles *et al.*, 2010) and conductivity of fibril films.

Surface hydrophobicity of protein solutions

The differences in electrical conductivity of films fabricated by the two types of aggregate structures may be related to the unfolding of protein and exposure of hydrophobic regions. ANS, which can bind to hydrophobic groups of protein, is considered to be representative of surface hydrophobicity (Mantovani *et al.*, 2018). The surface hydrophobicity showed 2.5-fold and 1.85-fold increase during WPC fibrillation process and conventional aggregation process (Fig. 5). The surface hydrophobicity results indicated that fibril-like structure showed more exposed hydrophobic groups which resulted in the increase of electrical conductivity.

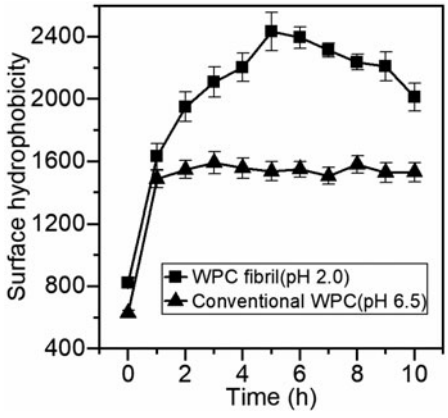

Fig. 5. Surface hydrophobicity of WPC fibril (prepared by 3%(w/v) WPC heating at 90° C, pH 2.0) and conventional WPC (prepared by 3%(w/v) WPC heating at 90°C, pH 6.5) as a function of time. Different letters above the bars indicate significant differences ($P < 0.05$).

ζ- potential of protein solutions

Heat-induced aggregation of WPC brought about significant changes not only in the surface hydrophobicity but also in net

https://doi.org/10.1017/S0022029919000876 Published online by Cambridge University Press

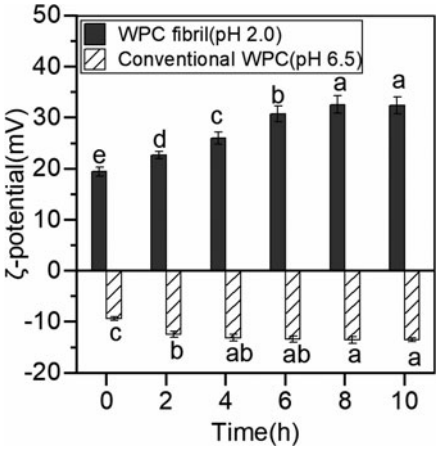

Fig. 6. ζ- potential of WPC fibril (prepared by 3%(w/v) WPC heating at 90°C, pH 2.0) and conventional WPC (prepared by 3%(w/v) WPC heating at 90°C, pH 6.5) as a function of time.

charge. Jordens *et al.* (2014) reported that the fibrils forming at pH 2 with high charge, aligned into nematic domains due to their adsorption at interfaces, created an elastic interface. Positive surface charge (+19.47 mV) of WPC was increased to +32.43 mV during WPC fibrillation, resulting from protein unfolding and the exposure of charged groups. On the contrary, the maximum increase of the conventional aggregation was up to 45%. Therefore, comparing with the conventional structure of aggregation, the fibril structure was the reason for high electrical conductivity (Fig. 6).

In conclusion, compared with conventional WPC film (pH 6.5), WPC fibril film (pH 2.0) achieved a 1.31-fold increase in electrical conductivity, higher tensile strength and elongation at break as well as superior oil resistance. It was demonstrated that the increased electrical conductivity of WPC fibril film (pH 2.0) probably resulted from the well-ordered β-sheets-rich structures, the exposure of hydrophobic amino acid and a higher surface charge.

Acknowledgements. This work was supported by the National Natural Science Foundation of China (Grant No. 31471682) and the 13th National Five-year Topic of China (Grant No. 2017YFD0401200).

References

Abdel-Karim AM, Salama AH and Hassan ML (2018) Electrical conductivity and dielectric properties of nanofibrillated cellulose thin films from bagasse. *Journal of Physical Organic Chemistry* **56**, e3851.

Adamcik J and Mezzenga R (2011) Proteins fibrils from a polymer physics perspective. *Macromolecules* **45**, 1137–1150.

Ayadi MA, Leuliet JC, Chopard F, Berthou M and Lebouché M (2004) Electrical conductivity of whey protein deposit: xanthan Gum effect on temperature dependency. *Food and Bioproducts Processing* **82**, 320–325.

Banerjee R and Chen H (1995) Functional properties of edible films using whey protein concentrate. *Journal of Dairy Science* **78**, 1673–1683.

Bolder SG, Sagis LM, Venema P and van der Linden E (2007a) Effect of stirring and seeding on whey protein fibril formation. *Journal of Agricultural and Food Chemistry* **55**, 5661–5669.

Bolder SG, Vasbinder AJ, Sagis LMC and van der Linden E (2007b) Heat-induced whey protein isolate fibrils: conversion, hydrolysis, and disulphide bond formation. *International Dairy Journal* **17**, 846–853.

Bolisetty S, Harnau L, Jung JM and Mezzenga R (2012) Gelation, phase behavior, and dynamics of beta-lactoglobulin amyloid fibrils at varying concentrations and ionic strengths. *Biomacromolecules* **13**, 3241–3252.

Bolisetty S, Arcari M, Adamcik J and Mezzenga R (2015) Hybrid amyloid membranes for continuous flow catalysis. *Langmuir* **31**, 13867–13873.

Brandenberg AH, Morr CV and Weller CL (1992) Gelation of commercial whey protein concentrates: effect of removal of low-molecular-weight components. *Journal of Food Science* **57**, 427–432.

Dave AC, Loveday SM, Anema SG, Jameson GB and Singh H (2015) Formation of nano-fibrils from the A, B and C variants of bovine β-lactoglobulin. *International Dairy Journal* **41**, 64–67.

de Castro RJS, Domingues MAF, Ohara A, Okuro PK, dos Santos JG, Brexó RP and Sato HH (2017) Whey protein as a key component in food systems: physicochemical properties, production technologies and applications. *Food Structure* **14**, 17–29.

Feng Z, Wu G, Liu C, Li D, Jiang B and Zhang X (2018) Edible coating based on whey protein isolate nanofibrils for antioxidation and inhibition of product browning. *Food Hydrocolloids* **79**, 179–188.

Gao Y, Wen S, Wang W, Yan W, Zhao Y, Lei Z and Miao R (2012) Ultrasonic-assisted production of graphene with high yield in supercritical CO2 and its high electrical conductivity film. *Industrial & Engineering Chemistry Research* **53**, 2839–2845.

Gao YZ, Xu HH, Ju TT and Zhao XH (2013) The effect of limited proteolysis by different proteases on the formation of whey protein fibrils. *Journal of Dairy Science* **96**, 7383–7392.

Henriques M, Gomes D and Pereira C (2016) Whey protein edible coatings: recent developments and applications. In Nedović V, Raspor P, Lević J, Tumbas Šaponjac V, Barbosa-Cánovas G (eds), *Emerging and Traditional Technologies for Safe, Healthy and Quality Food*. Food Engineering Series. Cham, Switzerland: Springer, pp. 177–196.

Iwata KI, Ishizaki SH, Handa AK and Tanaka MU (2000) Preparation and characterization of edible films from fish water-soluble proteins. *Fisheries Science* **66**, 372–378.

Jordens S, Ruhs PA, Sieber C, Isa L, Fischer P and Mezzenga R (2014) Bridging the gap between the nanostructural organization and macroscopic interfacial rheology of amyloid fibrils at liquid interfaces. *Langmuir* **30**, 10090–10097.

Knowles TP and Buehler MJ (2011) Nanomechanics of functional and pathological amyloid materials. *Nature Nanotechnology* **6**, 469–479.

Knowles TP, Fitzpatrick AW, Meehan S, Mott HR, Vendruscolo M, Dobson CM and Welland ME (2007) Role of intermolecular forces in defining material properties of protein nanofibrils. *Science* **318**, 1900–1903.

Knowles TP, Oppenheim TW, Buell AK, Chirgadze DY and Welland ME (2010) Nanostructured films from hierarchical self-assembly of amyloidogenic proteins. *Nature Nanotechnology* **5**, 204–207.

Kumari M, Mahajan H, Joshi R and Gupta M (2017) Development and structural characterization of edible films for improving fruit quality. *Food Packaging and Shelf Life* **12**, 42–50.

Le TC, Letendre M, Ispasszabo P, Mateescu MA, Delmaspatterson G, Yu HL and Lacroix M (2000) Development of biodegradable films from whey proteins by cross-linking and entrapment in cellulose. *Journal of Agricultural and Food Chemistry* **48**, 5566–5575.

Li C, Adamcik J and Mezzenga R (2012) Biodegradable nanocomposites of amyloid fibrils and graphene with shape-memory and enzyme-sensing properties. *Nature Nanotechnology* **7**, 421–427.

Liu L, Busuttil K, Zhang S, Yang Y, Wang C, Besenbacher F and Dong M (2011) The role of self-assembling polypeptides in building nanomaterials. *Physical Chemistry Chemical Physics* **13**, 17435–17444.

Loveday SM, Wang XL, Rao MA, Anema SG, Creamer LK and Singh H (2010) Tuning the properties of β-lactoglobulin nanofibrils with pH, NaCl and $CaCl_2$. *International Dairy Journal* **20**, 571–579.

Loveday SM, Su J, Rao MA, Anema SG and Singh H (2011) Effect of calcium on the morphology and functionality of whey protein nanofibrils. *Biomacromolecules* **12**, 3780–3788.

Mantovani RA, Pinheiro AC, Vicente AA and Cunha RL (2017) In vitro digestion of oil-in-water emulsions stabilized by whey protein nanofibrils. *Food Research International* **99**(Pt 1), 790–798.

Mantovani RA, de Figueiredo Furtado G, Netto FM and Cunha RL (2018) Assessing the potential of whey protein fibril as emulsifier. *Journal of Food Engineering* **223**, 99–108.

https://doi.org/10.1017/S0022029919000876 Published online by Cambridge University Press

Mchugh TH, Aujard JF and Krochta JM (2010) Plasticized whey protein edible films: water vapor permeability properties. *Journal of food science* **59**, 416–419.

Nelson R, Sawaya MR, Balbirnie M, Madsen AO, Riekel C, Grothe R and Eisenberg D (2005) Structure of the cross-beta spine of amyloid-like fibrils. *Nature* **435**, 773–778.

Nicolai T and Durand D (2013) Controlled food protein aggregation for new functionality. *Current Opinion in Colloid & Interface Science* **18**, 249–256.

Nicolai T, Britten M and Schmitt C (2011) β-Lactoglobulin and WPI aggregates: formation, structure and applications. *Food Hydrocolloids* **25**, 1945–1962.

Oboroceanu D, Wang L and Brodkorb A (2010) Characterization of β-lactoglobulin fibrillar assembly using atomic force microscopy, polyacrylamide gel electrophoresis, and *in situ* Fourier transform infrared spectroscopy. *Journal of Agricultural and Food Chemistry* **58**, 3667–3673.

Pan YX, Liu CJ, Zhang S, Yu Y and Dong M (2012) 2D-oriented self-assembly of peptides induced by hydrated electrons. *Chemistry* **18**, 14614–14617.

Paparcone R, Keten S and Buehler MJ (2010) Atomistic simulation of nanomechanical properties of Alzheimer's Aβ(1–40) amyloid fibrils under compressive and tensile loading. *Journal of Biomechanics* **43**, 1196–1201.

Perez AA, Carrara CR, Sánchez CC, Santiago LG and Patino JMR (2010) Interfacial and foaming characteristics of milk whey protein and polysaccharide mixed systems. *AIChE Journal* **56**, 1107–1117.

Pérez-Gago MB, Nadaud P and Krochta JM (1999) Water vapor permeability, solubility, and tensile properties of heat-denatured versus native whey protein films. *Journal of Food Science* **64**, 1034–1037.

Pilkington SM, Roberts SJ, Meade SJ and Gerrard JA (2010) Amyloid fibrils as a nanoscaffold for enzyme immobilization. *Biotechnology Progress* **26**, 93–100.

Ramos OL, Pereira RN, Martins A, Rodrigues R, Fucinos C, Teixeira JA, Pastrana L, Malcata FX and Vicente AA (2017) Design of whey protein nanostructures for incorporation and release of nutraceutical compounds in food. *Critical Reviews in Food Science and Nutrition* **57**, 1377–1393.

Rao SP, Meade SJ, Healy JP, Sutton KH, Larsen NG, Staiger MP and Gerrard JA (2012) Amyloid fibrils as functionalizable components of nanocomposite materials. *Biotechnology Progress* **28**, 248–256.

Rhim JW, Gennadios A, Handa A, Weller CL and Hanna MA (2000) Solubility, tensile, and color properties of modified soy protein isolate films. *Journal of Agricultural and Food Chemistry* **48**, 4937–4941.

Schmid M (2013) Properties of cast films made from different ratios of whey protein isolate, hydrolysed whey protein isolate and glycerol. *Materials (Basel)* **6**, 3254–3269.

Schokker EP, Singh H, Pinder DN and Creamer LK (2000) Heat-induced aggregation of β-lactoglobulin AB at pH 2.5 as influenced by ionic strength and protein concentration. *International Dairy Journal* **10**, 233–240.

Sukyai P, Anongjanya P, Bunyahwuthakul N, Kongsin K, Harnkarnsujarit N, Sukatta U, Sothornvit R and Chollakup R (2018) Effect of cellulose nanocrystals from sugarcane bagasse on whey protein isolate-based films. *Food Research International* **107**, 528–535.

van der Linden E and Venema P (2007) Self-assembly and aggregation of proteins. *Current Opinion in Colloid & Interface Science* **12**, 158–165.

Wang D, Ha Y, Gu J, Li Q, Zhang L and Yang P (2016) Nanofilms: 2D protein supramolecular nanofilm with exceptionally large area and emergent functions. *Advanced Materials* **28**, 7413–7413.

Xu Z and Buehler MJ (2010) Mechanical energy transfer and dissipation in fibrous beta-sheet-rich proteins. *Physical Review E Statistical Nonlinear & Soft Matter Physics* **81**, 061910.

Xu HH, Wang J, Dong SR, Cheng W, Kong BH and Tan JY (2016) Acid-responsive properties of fibrils from heat-induced whey protein concentrate. *Journal of Dairy Science* **99**, 6052–6060.

https://doi.org/10.1017/S0022029919000876 Published online by Cambridge University Press