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Comparative experiments of fibril formation from whey protein concentrate with homogeneous and secondary nuclei

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ABSTRACT

Two types of special structures, homogeneous and secondary nuclei, form during fibril formation. The structural and functional properties of amyloid fibrils in whey protein concentrate (WPC) with different ratios of added homogeneous nuclei to secondary nuclei were investigated. Thioflavin T fluorescence analysis and kinetic equations indicated that two types of nuclei could accelerate WPC fibrillation compared with WPC self-assembling into amyloid fibrils, thereby reducing the lag time and increasing the number of fibrils. However, there were considerable differences in the nucleation-inducing capability of WPC fibrillation between homogeneous and secondary nuclei. The number of fibrils formed by adding homogeneous nuclei was higher than that obtained with secondary nuclei, the increase in the Th T fluorescence intensity induced by homogeneous nuclei was 1.83-fold much than secondary nuclei. Meanwhile, secondary nuclei yielded a 2.71-fold faster aggregation rate of WPC than homogeneous nuclei, particularly during the first hour of thermal treatment (protein mass ratio of nuclei to WPC 1:1). The gelation time of WPC after secondary nuclei addition was shorter, from 10 h (WPC (2.0/6.5)) to 4 h (WPC + HN) to 2 h (WPC + SN); however, the gel microstructure of WPC after the addition of homogeneous nuclei was denser, yielding a preferred water holding capacity.

1. Introduction

The assembled structures of food globular proteins, particularly fibrils with a thickness around 4 nm and a length between 1 and 10 μm, have been attracted much attention due to their potential applications in the food industry, for example, as a weight effective thickener for food products. Many proteins, such as whey protein concentrate (WPC) (Gao, Xu, Ju, & Zhao, 2013; Xu et al., 2016), β-lactoglobulin (Loveday, Anema, & Singh, 2017; Raynes, Day, Crepin, Horrocks, & Carver, 2017), bovine serum albumin (Dahal et al., 2017), egg white lysozyme (Qin et al., 2017), soy protein (Dong, Xu, Li, Chen, & Zhang, 2016; Wang et al., 2011), and kidney bean protein (Liu & Tang, 2013; Tang, Zhang, Wen, & Huang, 2010), are capable of self-assembling into amyloid fibrils under certain denaturing conditions, such as high temperature with low pH and low ionic strength, or at high alcohol concentrations (Gosal, Clark, & Ross-Murphy, 2004). Amyloid fibrils are advantageous for the stabilization of emulsions and foams and can be used as gelling agents/thickeners due to their high length-to-width ratio (Kroes-Nijboer, Venema, & van der Linden, 2012).

The aggregation of such proteins generally follows a nucleation-dependent polymerization process that can be divided into the lag, elongation and saturation phases (Harper, Lansbury, & Peter, 1997; Kumar, Haque, & Prabhu, 2017). The aggregation process begins with monomers that first assemble in the nuclei. Further fibril elongation occurs through the interaction between the nuclei and other unfolded polypeptide chains, resulting in the formation of protofibrils and mature amyloid fibrils (Knowles, Vendruscolo, & Dobson, 2014; Ross & Poirier, 2004). Foderà, Librizzi, Militello, Navarra, and Vetri (2011) found that the possible mechanisms for a nucleation-elongation process included a homogeneous nucleation and secondary nucleation. Destabilized structures of monomers can interact with each other to form a new high-energy species called “nuclei” (homogeneous nucleation). Protein molecules interact with such new species starting the elongation phase and leading to the formation of mature amyloid fibrils (secondary nucleation). The initial step in fibril formation is the primary homogeneous nucleation process, which forms primary nuclei (homogeneous nuclei). Homogeneous nuclei, which are formed during the lag time, are the smallest aggregates with the highest Gibbs free energy (Arosio, Knowles, & Linse, 2015). Homogeneous nuclei are stable identifiable intermediates that are found in equilibrium with

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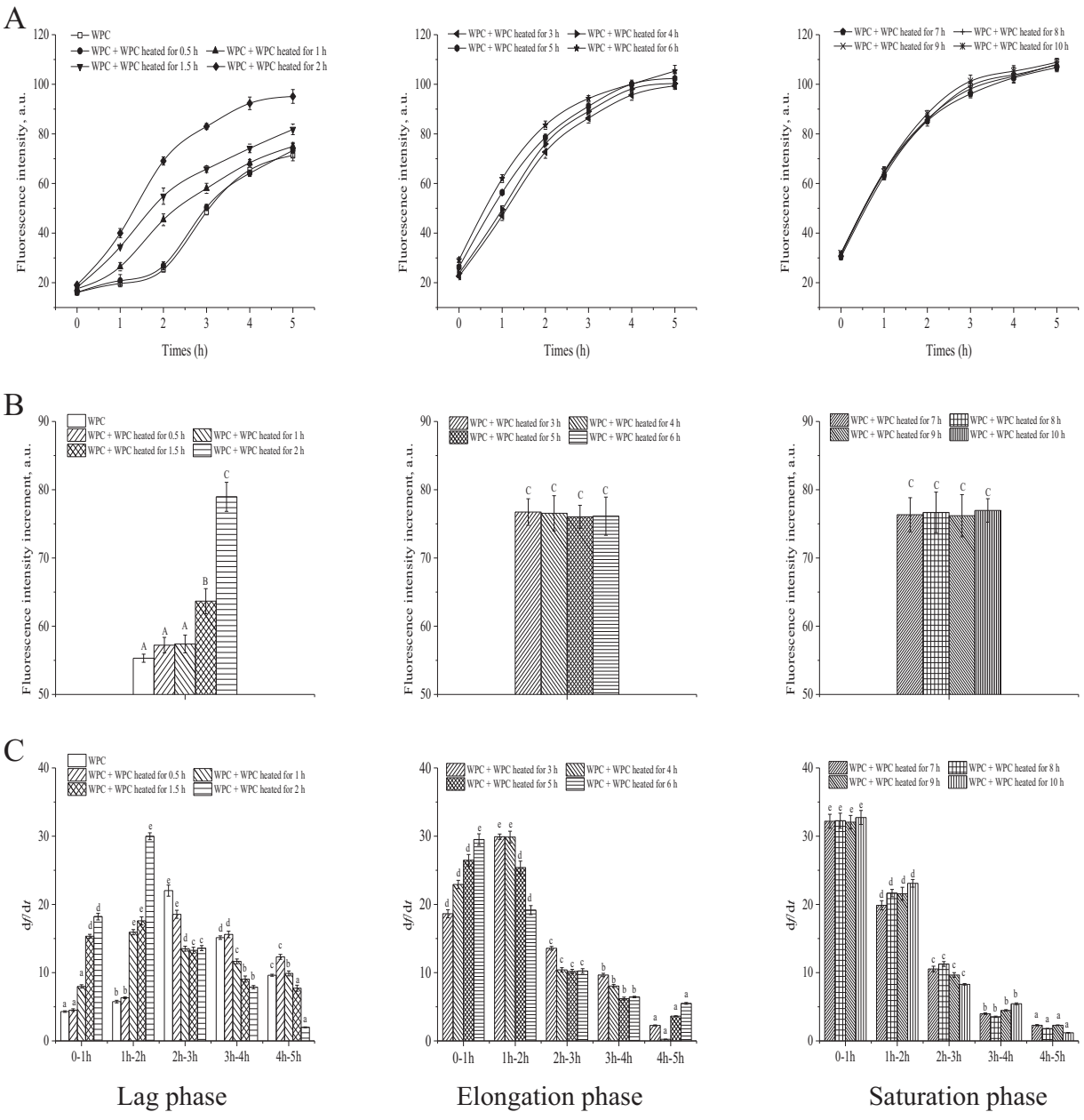

Fig. 1. WPC mixed different polymers heated for different hours (0.5 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 9 h, and 10 h) was heated for 5 hours at 90 °C, pH 2.0 (protein mass ratio of polymers to WPC 1:3, final protein concentration 2.0% (w/v)), (A) Th T fluorescence intensity, (B) the increase in Th T fluorescence intensity and (C) the aggregation rate. Data are the means ± SDs. $^{A-C}$Different letters indicate significant differences between groups ($p < 0.05$; ANOVA-Duncan).

their monomers (Wetzel, 2006); in addition, these nuclei have no special structure and are assumed to be pieces of the infinite polymer (Ferrone, Hofrichter, & Eaton, 1985).

The secondary nucleation processes, which assist the formation of additional nuclei from protofilaments, involves in fibril growth and eventually leads to the formation of mature fibrils. Secondary nucleation involves the already formed fibrils as activators for new fiber filaments (branching, fragmentation, and heterogeneous nucleation) (Ferrone, 1999). In previous studies, preformed fibrils have been added to proteins to substantially accelerate the rate of fibril formation, eliminate the lag time and induce immediate fibril formation (Harper, Lansbury, & Peter, 1997). Preformed amyloid fibrils can act as seeds for accelerating protein fibrillation and play a role in fibril acceleration only during the early stages of amyloid formation, and formed a dense network of unbranched fibrils (Kong & Zeng, 2017). Seeding (preformed fibrils) and vigorous agitation enhance insulin fibril formation by adding different amounts of preformed fibrils prior to incubation at 43 °C (Nielsen et al., 2001). Stirring and seeding apparently accelerate the kinetics of fibril formation, increasing the number of fibrils formed; however, the addition of seeds did not exert an additional effect compared with that observed in the stirred samples (Bolder, Sagis, Venema, & van der Linden, 2007). However, few studies compared the nucleation-inducing capability for WPC fibrillation between the homogeneous nuclei and secondary nuclei. Therefore, this study aimed to compare the difference between the two types of nuclei regarding the nucleation-inducing capability for WPC fibrillation and kinetic parameters of fibril aggregation reactions at different mixing ratios of nuclei to WPC. The differences in gel properties and microstructure between WPC (6.5/2.0) gels and complex gels formed by the mixing of WPC with two types of nuclei were investigated.