Innovative Food Science and Emerging Technologies 43 (2017) 207–215

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Sequential ultrasound and transglutaminase treatments improve functional, rheological, and textural properties of whey protein concentrate

Zahra Ahmadi, Seyed Mohammad Ali Razavi*, Mahdi Varidi

Food Hydrocolloids Research Center, Department of Food Science and Technology, Ferdowsi University of Mashhad (FUM), PO Box: 91775-1163, Mashhad, Iran

ARTICLE INFO

Chemical compounds studied in this article:
Deionized water (PubChem CID: 962)
Sodium dodecyl sulfate (PubChem CID: 3423265)
Sodium azide (PubChem CID: 33557)

Keywords:
Modification
Rheology
Texture
TGase
Ultrasound
Whey

ABSTRACT

In this study, the combined effect of ultrasound pretreatment (US) and microbial transglutaminase (MTG) on the rheological, textural, and functional attributes of whey protein concentrate (WPC) were investigated. All the samples exhibited non-Newtonian shear-thinning behavior. Treatment with US lead to a significant increase in foaming capacity, gel hardness, and adhesiveness at 7.5 min, while reduced emulsifying capacity and syneresis at all levels. Moreover, consistency coefficient, foam stability, emulsifying capacity, emulsion stability, gel hardness and adhesiveness improved, whereas a slight decrease was observed in foaming capacity and syneresis of gels as a consequence of TG treatment respectively. 5 min US - 5 $U \cdot g^{-1}$ pr TG treated sample exhibited the least syneresis value of all samples. SEM images affirmed the beneficial effects of treatments on texture, reduction of voids size and supported the textural results. Overall, ongoing US pretreatment and TG treatment were successful in modifying the functional properties of whey proteins.

Industrial relevance: Food industry is consuming whey proteins for their functional characteristics besides their nutritional value. Microbial transglutaminase (MTG) can modify whey protein structure. High intensity ultrasound (HIU) is also mentioned as a material-altering technology. Heat denaturation is used for changing whey protein structure before enzyme treatment. The purpose of this study was to investigate the feasibility of replacing heat denaturation by ultrasound treatment before transglutaminase addition to a whey protein system. We also aimed to test the simultaneous effect of US and TG in order to meet emerging demands and produce different novel targeted products. Our results indicated that US treatment may replace heat denaturation before TG treatment and combination of these two factors improved various properties of whey protein systems.

1. Introduction

Whey proteins (recognized as GRAS by FDA), especially α-lactalbumin possess a remarkable nutritious value and are abundant, inexpensive materials produced as a by-product of dairy processing, in particular cheese and casein. In addition to these advantages, whey proteins have been widely employed in various food formulas, such as snacks, beverages, infant formula and satiety drinks. These proteins are used for a number of noteworthy reasons including their foaming and water binding capacity, emulsification, gelation, and specific rheological and textural characteristics (Hernàndez-Balada, Taylor, Phillips, Marmer, & Brown, 2009; Phillips & Williams, 2011). β-Lactoglobulin which is the major component of whey proteins has a compact globular structure and a sole sulfhydryl group. This protein can react with other β-lactoglobulin particles, protein molecules or ingredients of a system. Hence, it has the ability to form cross linking bridges if its second or third structure is disrupted through heat or any other denaturing agent (Creamer et al., 2004).

Microbial transglutaminase (MTG) is composed of one polypeptide chain having 331 amino acids in its structure. MTG is a beneficial enzyme which is insensitive to Ca^{+2} and can act in a wide range of pH values. MTG is used to introduce cross linkages in numerous proteinaceous systems with or without a reducing agent (Ando et al., 1989; Jaros, Partschefeld, Henle, & Rohm, 2006; Motoki & Kumazawa, 2000; Seguro, Kumazawa, Kuraishi, Sakamoto, & Motoki, 1996; Yokoyama, Nio, & Kikuchi, 2004). Formation of these isopeptide bonds markedly indicates that MTG treatment can modify whey protein structure. These material changes may lead to extensive alterations in proteins' techno-functional behavior and is a highly profitable method to generate novel properties in food systems (Agyare & Damodaran, 2010; Anuradha & Prakash, 2009; Eissa, Bisram, & Khan, 2004; Gauche, Vieira, Ogliari, & Bordignon-Luiz, 2008; Hernàndez-Balada et al., 2009; Troung, Clare, Catignani, & Swaisgood, 2004; Wilcox & Swaisgood, 2002). Many studies has witnessed the formation of high molecular weight particles as a consequence of TG treatment. Some of them used a denaturing agent to increase whey protein susceptibility toward

* Corresponding author.
E-mail address: s.razavi@um.ac.ir (S.M.A. Razavi).

http://dx.doi.org/10.1016/j.ifset.2017.08.013
Received 22 September 2016; Received in revised form 29 July 2017; Accepted 21 August 2017
Available online 25 August 2017

enzymes. Among these studies (Damodaran & Agyare, 2013; Gauche, Tomazi, Barreto, Ogliari, & Bordignon-Luiz, 2009 and Wilcox & Swaisgood, 2002) tried to enhance whey protein-TG reaction through heat pretreatment, Dithiotheritol (DDT) and bisulfite as reductant.

High intensity ultrasound (HIU) is typically mentioned as a material-altering technology by means of cavitation which is related to extremely high local temperature and pressure creating high shear rates and turbulence in the environment. HIU is considered as a unique processing tool based on its physical effects in a liquid (Knorr, Zenker, Heinz, & Lee, 2004). There is a great demand for non-toxic technologies instead of thermal processing that cause the least damage to the nutritional value of foods, require minimum energy and is considered to be environmentally-friendly. Among these non-thermal processing techniques, ultrasound (US) has received considerable attention recently (Arzeni et al., 2012). This technology is widely used in food industry in various operations like homogenization, dehydration, emulsification, meat tenderization, enzyme deactivation, particle size reduction, and many other approaches. Low frequency US is usually used as cavitation producer in solutions, while higher frequencies (5–10 MHz) are applied for analytical purposes and process control (Jambrak, Mason, Lelas, & Kresic, 2010).

As the results of sonication process may vary depending on the experimental circumstances, such as ultrasound density, sample volume, generated heat in the environment and other conditions (Shanmugan, Chandrapala, & Ashukkumar, 2012), the goal of the current work was to investigate whether US waves can modify whey proteins' compact structure so that it became more susceptible to TG and the need for thermal denaturation or a reducing agent before enzyme treatment is reduced. The required energy for heat denaturation of whey proteins acts as a barrier cost in the industrial units. Heat denatured whey proteins also lack the nutritional values for cancer defeating purposes (Phillips & Williams, 2011), thus an alternative method of structure modification is required for emerging needs. We also aimed to find out if it is possible to alter whey proteins functional properties by means of simultaneous use of US and TG treatments to produce a targeted food ingredient because a thorough search of literature has shown that there is no study available on the combined application of US and TG until the start of the project. The previous studies aiming at US alteration of WPC were carried out by high process times which are not applicable in food industry. Therefore, finding a practical sonication time which speeds the process up was an additional objective of this study and of great interest to the research team.

2. Materials and methods

2.1. Materials

Whey protein concentrate (WPC) powder was of commercial grade and kindly provided by Milk Powder Industries Com., Mashhad, Iran. The WPC contained on averagely 70% protein, 17.8% lactose, 5% moisture, 4% fat, and 3.2% ash. Deionized water was purchased from Abtin Com., Iran. Sodium dodecyl sulfate (SDS) was obtained from Merck, Darmstadt, Germany and sodium azide from Sigma Chemicals Com., St. Louis, MO, USA. Ca^{2+} independent Transglutaminase enzyme with an activity of 100 $U{\cdot}g^{-1}$ protein was provided by BDF Co., Spain. Sunflower oil was purchased from a local market, Mashhad, Iran.

2.2. Sample preparation

An appropriate amount of WPC powder was dissolved in deionized water to obtain 10% w/w protein solution. The samples were then stirred (800 rpm) with a magnetic stirrer for an hour at ambient temperature until full dissolution and were immediately treated with US waves (20 kHz) for 0, 2.5, 5, and 7.5 min. A probe sonicator (Misonix sonicator, Model XL2020, 550 W, NY, USA) with a 10 mm probe was used to apply US waves to the samples and the temperature was maintained in the range of 25–30 °C through an ice-water bath in order to prevent any heat generation as a result of sonication. Having performed the sonication pretreatment, the samples were then treated with 0, 2, 5, and 10 $U{\cdot}g^{-1}$ pr TG. The samples were then stirred (1000 rpm) for about 15 min to ensure homogeneous distribution of the enzyme within the samples. The samples were then incubated at 40 °C for 20 h for rheological and textural analysis and 2 h at 40 °C for functional attributes' measurement.

2.3. Steady shear rheological measurements

Time-independent rheological properties of the samples were measured using a rotational viscometer (Bohlin Model Visco 88; Malvern Instruments Ltd., Worcestershire, UK) that was equipped with a heating circulator (Julabo, Model F12-MC; Julabo Labor technik, Seelbach, Germany). A C25 bob and cup measuring system with a gap width of 1 mm was used based on the viscosity range of the gels. The samples were poured into the cup and the steady shear rheological behavior was measured in the shear range of 14–300 s^{-1} at constant temperature (25 °C) during 560 s. Shear stress (τ)-shear rate ($\dot{\gamma}$) data were then fitted with the Power-law (Eq. (1)), Herschel-Bulkley (Eq. (2)), Bingham (Eq. (3)), and Casson (Eq. (4)) models:

$$\tau = k_P \dot{\gamma}^{n_P} \quad (1)$$

$$\tau = k_H \dot{\gamma}^{n_H} + \tau_{0H} \quad (2)$$

$$\tau = \eta_B \dot{\gamma} + \tau_{0B} \quad (3)$$

$$\sqrt{\tau} = k_{0c} + k_c \sqrt{\dot{\gamma}} \quad (4)$$

where k_P and k_H are the consistency coefficient (Pa·s^n), n_P and n_H are the flow behavior index (dimensionless), τ_{0H}, τ_{0B} , and $\tau_{0C}(=k_{0c}^2)$ are the yield stress (Pa), η_B and $\eta_C(=k_c^2)$ are the plastic viscosity (Pa·s).

2.4. Evaluation of foaming capacity and stability

The samples were whipped at 10000 rpm for 7 min by a laboratory homogenizer (Ultra Turrax T-25, IKA, Germany) according to the method proposed by Jambrak, Mason, Lelas, Herceg, and Herceg (2008) with some modification. The solutions were whipped for 7 min to maximize the foaming capacity. Foaming capacity was determined by weighing a specific volume of the unwhipped sample and its foam and it was calculated as follow:

$$\text{Foaming capacity}(\%) = (W_U - W_F)/(W_U) \times 100 \quad (5)$$

where W_U is the unwhipped sample weight (g) and W_F is the foam weight (g).

The reduction of foam volume after 30 min was expressed as foam stability:

$$\text{Foam stability}(\%) = (FV_{30}/FV_I) \times 100 \quad (6)$$

where FV_{30} is the foam volume after 30 min (mL) and FV_I is the initial foam volume (mL).

2.5. Determination of emulsifying capacity and stability

To investigate the effect of US and TG treatments on the emulsifying properties of the samples according to Pearce and Kinsella (1978), 8 mL of sunflower oil was added to 25 mL of the sample. The mixture was then homogenized with a homogenizer (Ultra Turrax T-25, IKA, Germany) at 10000 rpm for 1 min, next 50 μL of the final emulsion was diluted by 30 mL SDS 0.1% and was shaken thoroughly to insure homogeneity. The absorbance of each emulsion was read at 500 nm wavelength using a spectrophotometer (Jenway, model 6105 UV/VIS, Jenway Spectrophotometer, U.K.) and considered as the emulsifying capacity of the sample.