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Impact of ultrasound treatment on molecular structures of casein

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ABSTRACT:

Casein was modified by ultrasound treatment (160W, 400 W) and the structural characteristics of casein (control), USC-1 (160W of ultrasound treated casein) and USC-2 (400W of ultrasound treated casein) were investigated. Ultrasound treatment resulted in increase in the numbers of protein bands of casein in region of low molecular weight according to SDS-PAGE pattern. The relative percentage of the proteins with molecular mass (MM) of over 30 kD in USC-1 and USC-2 significantly decreased compared with control sample. Coupled with this is the relative percentage of the proteins with MM of region 20 kD-30 kD for the ultrasonic-treated caseins increased. Contents of α-helix, β-sheet and random coil of casein for USC-1 and USC-2 decreased compared with control. While, β-turn content for USC-1 and USC-2 increased. Ratio of α-helix to β-sheet in USC-1 and USC-2 significantly decreased (from 0.70 to 0.47 and 0.46). Ultrasound treatment had a strong crushing action of surface of the casein micelle and resulted in its structural disruption. Particle size distribution range of casein was approximately 0.5-70 μm, Particle size of USC-1 and USC-2 was less than 30μm. D (50), D (4, 3) as well as D (3, 2) for USC-1 and USC-2 reduced compared with the control. The ratio of D (4, 3) to D (3, 2) also decreased with increase of ultrasonic intensity. This study demonstrated that ultrasound treatment could be an effective method for modification of casein structure. This modification is the basis on improvement in functional properties.

KEYWORDS: casein; ultrasound treatment; microstructure; molecular mass distribution; secondary structure.

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INTRODUCTION

Casein is a naturally occurring macromolecule that accounts for approximately 80% of the protein content of milk. The main physiological role of casein in the milk system was widely accepted to be a source of amino acids required by growth of the neonate.[1] It is a phosphoprotein that can be separated into various electrophoretic fractions, such as αs-casein, κ-casein, β-casein, and γ-casein in which each constituent differs in primary, secondary, and tertiary structure, amino acid composition, and molecular weight.[2] Due to the excellent functional properties and natural abundance, casein proteins (or their hydrolysates) represent a privileged and crucial tool for the food industry. Their hydrodynamic and surface-related properties lead to suitable functionalities that are utilized for countless manufactured products.[3, 4] They can also contribute to improve color and flavor of food products.

Ultrasonic technology has emerged as a promising alternative in food processing and preservation. The increasing demands of higher quality and quantity of products in the food industry brought attention to the use of ultrasound (US) as a food-processing technique of interest as a technological benefit and/or as a technique to alter food functional properties. This technique presents several advantages over conventional processing methods in terms of energy consumption, time, and higher throughput. Ultrasound treatments are used in the food industry for numerous processes of food products such as milk, yogurt, and cheese. Interest in applying ultrasound technology in food processing lies in the fact that power ultrasounds can result in modifications (chemical, functional, physical, and structural, etc.) in some of food properties.[5] Ultrasound treatments could be used to modify the structural and functional properties of globular proteins and altered their functional properties.[6-9] The beneficial use of ultrasound is achieved through the chemical, mechanical, and physical effects of acoustic cavitation. This involves the formation, growth, and violent collapse of small bubbles in liquids as a result of acoustic pressure fluctuations.[10] Therefore, ultrasound treatment might be a promising way for the functionality modification of casein proteins. However, limited information is available concerning the effects of ultrasound treatment on the structural pattern of casein.

Hence, the objective of this work was to study the effects of ultrasound treatment on the structural characteristics of casein. It was also hoped that by measuring some physicochemical properties, the underpinning mechanisms of changing the structural properties for casein protein can be better understood.

MATERIALS AND METHODS

Raw materials. Casein protein sample was purchased from Huigong Co., China. Casein contained 82.5 % (w/w, dry basis) protein and 11.9 % moisture. The other chemicals were of analytical grade.

Ultrasound treatment of casein. Casein dispersions (8.0%, w/v) were prepared by adding casein powder into Millipore water and then gently stirred overnight at ambient temperature. An ultrasound processor (Xingdongli Ultrasonic Electron Equipment Co. Ltd., Guangzhou, China) with a 1.5 cm diameter titanium probe was used to sonicate 100 ml of casein dispersions in 200 ml flat bottom conical flasks that were immersed in a temperature-controlled (2°C) water bath. Samples were treated at 25 kHz at two levels of power output (160 W and 400 W) for 30 min (pulse durations of on-time 9 s and off-time 1 s). The caseins treated by 160 W and 400 W of ultrasound were named as USC-1 and USC-2, respectively. After ultrasound treatment, some of the resulting USC-1 and USC-2 were used for analysis of molecular mass distribution, the another was then rapidly cooled to about 25°C in an ice bath, and then freeze-dried and stored at −20°C until use.

Analysis of molecular mass distribution of casein. Determination of molecular mass distributions (MMD) of casein, USC-1 and USC-2 was made according to our previous method.[11] MMD was estimated by gel permeation chromatography on Agilent PL aquagel-OH MIXED-H column (Agilent, LC1260, USA) with a UV detection at 214 nm. Elution was achieved at 0.5 ml min-1 by 0.25 M phosphate buffer (pH 7.2). The column was calibrated with bovine serum albumin (MW66 kDa), egg albumin (MW 44287 Da), cytochrome C (MW 12384 Da), aprotinin (MW 6511.44 Da), VB12 (MW 1355.37).

Measurement of particle size distribution. Particle size distributions of the samples were measured by an integrated laser light scattering instrument (BT-9300H Laser Particle Size Analyzer, China). Relative refractive index and absorption were set to 1.414 and 0.001, respectively. The dilution of the emulsion in the sample chamber was approximately 1:1000 (sample/water).

Scanning electron microscopy. An aluminium stub covered with double-sided carbon tape was dipped into the sample in order to randomly collect particles of the sample. The latter was then covered with gold (12–15 nm) in a Cressington model 108 sputter coater. Observation was carried out using a Hitachi model S-3000N scanning electron microscope (Hitachi Science Systems, Ibaraki, Japan) operating at 5 kV, and digital images were acquired.

SDS-PAGE analysis. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted according to the method of Laemmli (1970)[12] using 15% (v/w) acrylamide separating gel and 4% acrylamide stacking gel. Samples were prepared in Tris–HCl buffer (pH 6.8) containing 2% SDS and 0.2% β-mercaptoethanal. The gel sheets were stained with Coomassie brilliant blue R-250.

Statistical analysis. Secondary structure was determined four times while molecular weight distribution and particle size distribution were measured in duplicate. Consequently, a variance analysis (ANOVA) was performed on each experiment.

RESULTS AND DISCUSSION

SDS-PAGE profile. SDS-PAGE pattern of the casein and ultrasonic-treated casein (USC-1, USC-2) is shown in Figure 1. Casein is made up of many components, and the main types are αs-casein, β-casein, and κ-casein as defined and validated by analysis of DNA sequences.[13] Obviously, the SDS-PAGE profile of original casein (Lane 5) included the protein bands of αs-casein (Lane 2), β-casein (Lane 3), and κ-casein (Lane 4). After treated by ultrasound, the numbers of protein bands of casein increased compared to the original casein sample (Lane 6 and 7) in SDS-PAGE pattern. Especially, the band numbers in USC-2 sample (Lane 7) significantly increased in region of low molecular weight. The colors of bands in high molecular weight region became lighter in USC-1 and USC-2 compared to the origin casein. It indicated that these proteins were degraded into the peptides with low molecular weight due to ultrasound treatment.

Change in molecular mass distribution of casein treated by ultrasound wave. For the purpose of a more complete characterization of the ultrasound treatment process, chromatographic separation (HPLC) of the casein proteins during ultrasound treatment was carried out (Figure 2). The molecular mass distribution determined by HPLC was shown in Table 1. The relative percentage of the proteins with molecular mass (MM) of over 30 kD in USC-1 and USC-2 significantly ($P < 0.05$) decreased compared with the control sample. Coupled with this is the relative percentage of the proteins with MM of region 20 kD-30 kD for the ultrasonic-treated caseins increased.

Effect of ultrasonic treatment on casein secondary structure. Fourier transform infrared spectroscopy (FTIR) is a powerful tool to evaluate the secondary structure of proteins.[14] By means of proper fitting of the amide I band of the original FTIR spectrum of the protein as well as analyzing its second-derivative, the information about conformation of the protein (i.e., helix, expended, or turn) can be obtained.[15]

Figure 3 (a) shows the FTIR spectrum of casein and ultrasonic-treated caseins. The peaks of amide I located at 1641. It indicated the typical structure of a protein. The peaks located at 3300 cm^{-1} and 2930 cm^{-1} correspond to the N-H stretching band and alkyl group stretching bands, respectively.

The amide I band itself was caused by stretching vibration of C=O belonging to the amide groups weakly coupled with in-plane NH bending and CN stretching.[16] Fourier self-deconvolution was generally used to analyze the individual component peaks hidden within a broadband. The amide band I included an ideal wavenumber range suitable for protein secondary structure analysis, especially analysis for α-helix, β-sheet, random conformations, and β-turns in protein.[14] In the secondary structure, β-turn, 1660-1700 cm^{-1}; α-helix, 1650-1656 cm^{-1}; irregular structure (random coil), 1640-1644 cm^{-1}; and β-sheet or extended structure, 1620-1640 cm^{-1}. Therefore, the four peaks shown in the amide I region of Figure 3(b) apparently represent the β-turn, α-helix, β-sheet and random coil conformations, respectively. The estimation of the secondary structural elements of casein and ultrasonic-treated casein can be achieved by the curving fitting of the amide I band in the FTIR spectrum shown in Table 2. According to Table 2, the contents of α-helix, β-sheet and random coil of casein significantly decreased ($p<0.05$) after treated by ultrasound wave. While, the contents of β-turn increased. Moreover, the ratio of α-helix to β-sheet in USC-1 and USC-2 significantly decreased compared with the control (from 0.70 to 0.47 and 0.46) ($p<0.05$). Ultrasound treatment could modify protein conformation by affecting hydrogen bonds and hydrophobic interactions, disrupting the quaternary and/or tertiary structure of proteins.[17, 18]

Microstructural morphology. In this study the SEM photomicrographs representing the microstructures of casein and ultrasonic-treated casein are shown in Figure 4. Casein micelle exhibited roughly spherical shapes with compact and cluster structure. Moreover, some of casein organized into tubular structures within the micelle. The surface is not smooth, and contains gaps between the substructures. The observation is consistent with the finding of Dalgleish et al. (2004).[19] Ultrasound treatment resulted in significant change in the microstructure of casein. Casein treated by 160 W of ultrasound wave (USC-1) was clearly observed to the 'lacerated' architecture, and a porous and interconnected sheet-like structure. While casein treated by 400 W of ultrasound wave (USC-2) presented "jagged" feature. Moreover, tufted structure of casein micelle began to disappeared in this sample. It indicated the treatment of ultrasound wave had a strong crushing action of surface of the casein micelle.

Particle size distribution of casein. Figure 5 shows the particle size distribution of casein and ultrasonic-treated casein suspensions. The recorded parameters in Figure 5 include D (4, 3) (volume mean diameter), D (3, 2) (surface area distribution the mean value). Moreover, D50 is the size in microns that splits the distribution with half above and half below this diameter. The D50, the median, has also been defined above as the diameter where half of the population lies below this value. Similarly, 90 percent of the distribution lies below the D90, and 10 percent of the population lies below the D10. The calculated results of particle size distribution for casein and ultrasonic-treated casein were found in Table 3 according to Figure 5. According to Figure 5 and Table 3, particle size distribution range of casein was approximately 0.5-70 μm and the distribution curve was in twin-peak model illustrating no uniform distribution of particle size. These two ranges included the size of 0.947-1.054μm and 3.422-3.809 μm. D50 was 2.493 μm. The D (4, 3) and D (3, 2) were respectively 5.616 μm and 1.470 μm. Although there also were two peaks in particle size distribution curve of USC-1 and USC-2, obvious increase in height of the left peak was found. Moreover, the curve of accumulative content distribution migrated into the left. It indicated that the size of casein decreased after treated by ultrasound wave. Particle size of USC-1 and USC-2 was less than 30μm. D (50), D (4, 3) as well as D (3, 2) for USC-1 and USC-2 reduced compared with the control. The ratio of D (4, 3) to D (3, 2) also decreased with increase of ultrasonic intensity. It indicated that ultrasound could easily destroy the casein micelle by cavatition, thermal effect and shear effect, resulting in decreased particle size of the micelle. Great result alike for what obtained in this study and observed in whey proteins and soybean isolate proteins.[20, 21]

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ABBREVIATIONS USED

USC-1, casein treated by 160W of ultrasound; USC-2, casein treated by 400W of ultrasound; MM, molecular mass; SDS-PAGE, sodium dodecyl sulfatepolyacrylamide gel electrophoresis; FTIR, fourier transform infrared spectroscopy

REFERENCES

(1) Holt, C. (1997). The milk salts and their interaction with casein. In P. F. Fox (Ed.), Advanced Dairy Chemistry (pp. 233–256). London: Chapman & Hall.

(2) Swaisgood, H.E. Chemistry of the caseins. In Advanced dairy chemistry—1, proteins. 3 rdth edition. Edited by Fox PF, Sweeney PLH. New York: Kluwer Academic/Plenum; 2003:139–201. Part A.

(3) Kinsella, JE. Milk proteins: physicochemical and functional properties. *Crit Rev Food Sci Nutr*. **1984**, *21*,197–262.

(4) Damodaran, S. Structure-function relationship of food proteins. In Protein functionality in food systems. Edited by Hettiarachchy NS, Ziegler GR. New-York: Marcel Dekker; 1994:1–37.

(5) Chemat, F.; Zill-e-Huma; Khan, M. K. Applications of ultrasound in food technology: processing, preservation and extraction. *Ultrason. Sonochem*. 2011, *18*, 813-835.

(6) Karki, B; Lamsal, B. P.; Grewell, D.; Pometto, A. L., III; Leeuwen, J.; Khanal, S. K.; Jung, S. Functional properties of soy protein isolates produced from ultrasonicated defatted soy flakes. *J. Am. Oil Chem*. Soc. **2009**, *86*, 1021–1028.

(7) Jambrak, A. R.; Mason, T. M.; Lelas, V.; Herceg, Z.; Herceg, I. L. Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions. *J. Food Eng*. **2008**, *86*, 281–287.

(8) Jambrak, A. R.; Lelas, V.; Mason, T. J.; Kre_si_c, G.; Badanjak, M. Physical properties of ultrasound treated soy proteins. *J. Food Eng*. **2009**, *93*, 386–393.

(9) Chen, L., Chen, J.S., Ren, J.Y., Zhao M.M. Effects of ultrasound pretreatment on the enzymatic hydrolysis of soy protein isolates and on the emulsifying properties of hydrolysates. *J. Agric. Food Chem*. **2011**, *59*, 2600–2609.

(10) Mason, T. J.; Paniwnyk, L.; Lorimer, J. P. The uses of ultrasound in food technology. *Ultrason. Sonochem*. 1996, *3*, 253–260.

(11) Wang, J.S., Su,Y.J., Jia, F., Jin, H.L. Characterization of casein hydrolysates derived from enzymatic hydrolysis. *Chem. Central J*. **2013**, *7*,62-69.

(12) Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*,680–685.

(13) Walstra, P., Geurts, T.J., Noomen, A., Jellema, A., and van Boekel, M.A.J.S. 1999. Dairy Technology: Principles of Milk Properties and Processes. Marcel Dekker, Inc., New York.

(14) Farrell, H. M.; Wickham, E. D.; Unruh, J. J.; Qi, P. X.; Hoagland, P. D. Secondary structural studies of bovine caseins: temperature dependence of β-casein structure as analyzed by circular dichroism and FTIR spectroscopy and correlation with micellization. *Food Hydrocolloids* **2001**, *15*, 341–354.

(15) Kumosinski, T. F.; Unruh, J. J. Quantitation of the global secondary structure of globular proteins by FTIR spectroscopy: comparison with X-ray crystallographic structure. *Talanta*. **1996**, *43*, 199–219.

(16) Renugopalakrishnan, V.; Chandrakasan, G.; Moore, S.; Hutson, T. B.; Berney, C. V.; Bhatnagar, R. S. Bound water in collagen:evidence from Fourier transform infrared and Fourier transform infrared photoacoustic spectroscopic study. *Macromolecules*. **1989**, *22*, 4121–4124.

(17) Jia, J.Q.;Ma,H. L.;Zhao,W.R.;Wang, Z. B.;Tian,W.M.; Luo, L.; He, R. H. The use of ultrasound for enzymatic preparation of ACE-inhibitory peptides from wheat germ protein. *Food Chem*. **2010**, *119*, 336–342.

(18) Tang, C. H. Functional properties and in vitro digestibility of buckwheat protein products: Influence of processing. *J. Food Eng*. **2007**, *82*, 568–576.

(19) Dalgleish, D.G., Spagnuolo P.A., Goff, H.D. A possible structure of the casein micelle based on high-resolution field-emission scanningelectron microscopy. *Int. Dairy J*. **2014**, *14*, 1025-1031.

(20) Gordon, L., Ana M.R.P. Application of high-Intensity ultrasounds to control the size of whey proteins particles. *Food*

Biophysics. **2010**, *5*, 203-210.

(21) Arzeni, C., Martinez, K., Zema, P. Comparative study of high intensity ultrasound effects on food proteins functionality. J. Food Eng. 2012, 108, 463-472.

Figure captions

Figure 1 SDS-PAGE pattern of casein and ultrasonic-treated casein. 1-marker, 2, αs-casein, 3, β- casein, 4, κ- casein, 5-casein, 6-USC1, 7-USC2

Figure 2 Curve of molecular mass distribution of casein, USC-1 and USC-2

Figure 3 FTIR spectrum of casein and ultrasonic-treated caseins (a); the best fit for the self-deconvoluted FTIR spectrum using nonlinear regression analysis (b). Amide I bands were fitted with Gaussian functions using peak positions obtained from second derivative analysis.

Figure 4 Microstructure of casein and ultrasonic-treated casein

Figure 5 Particle size distribution curves of casein and ultrasonic-treated casein

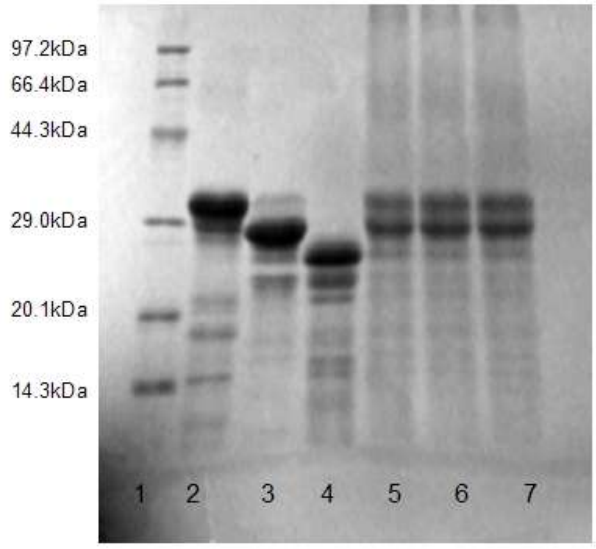

Figure 1

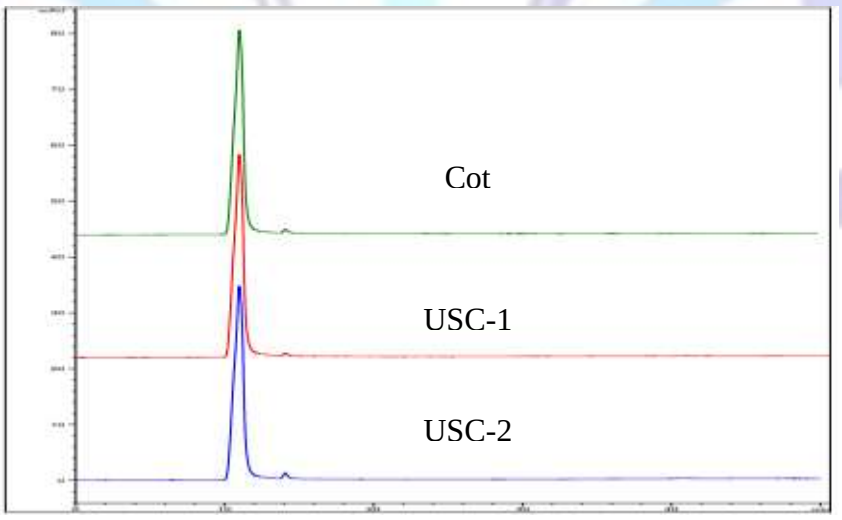

Figure 2

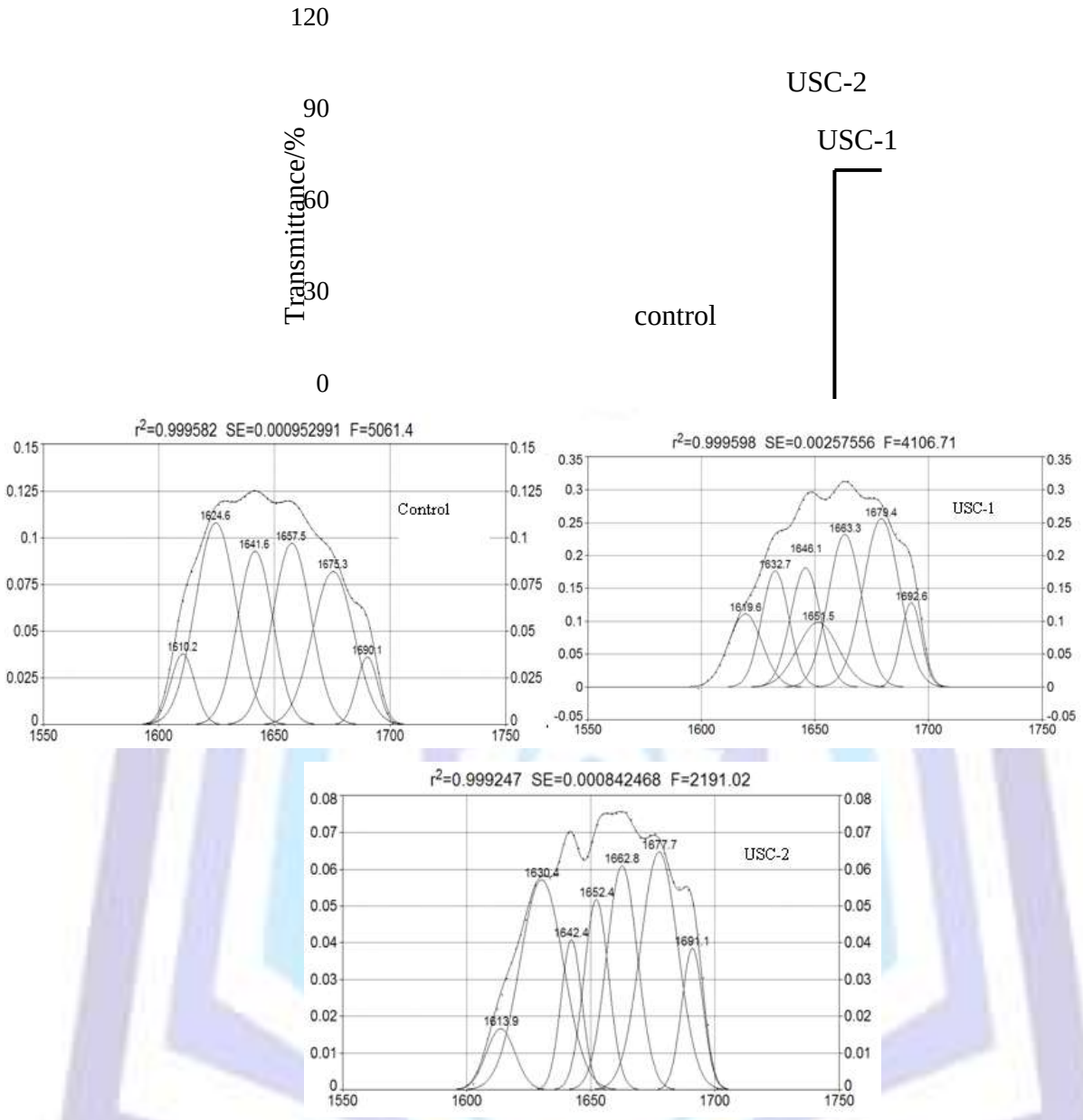

Figure 3

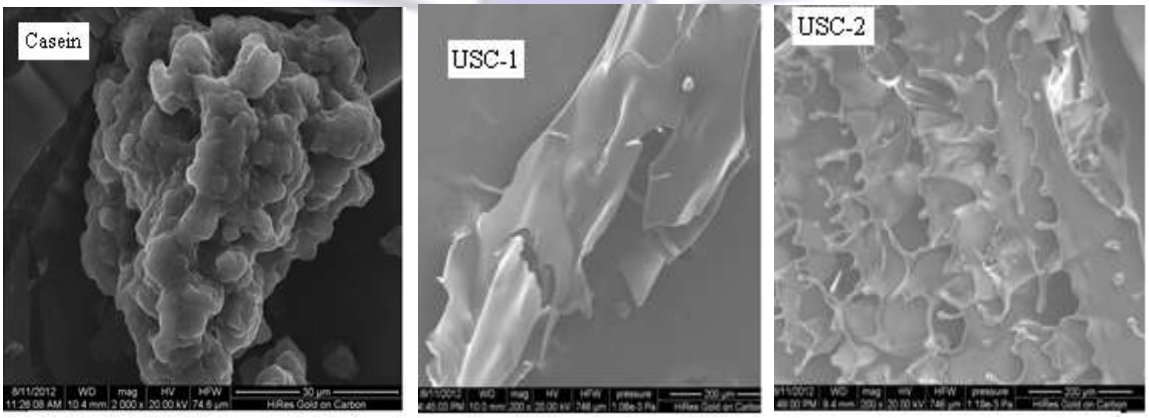

Figure 4

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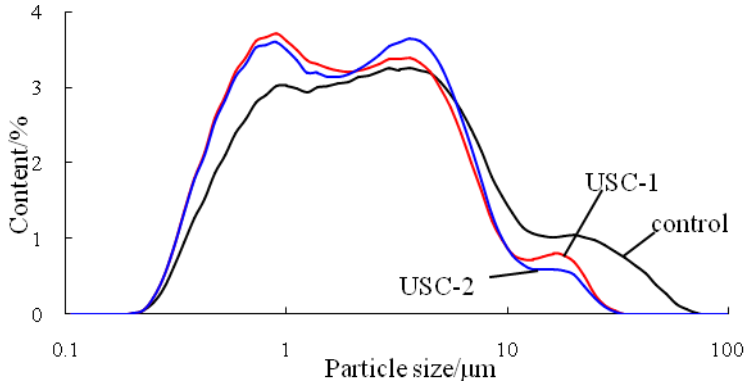

Figure 5

Table 1 Relative percent of the peptides in HPLC in total area (%)

Sample	Molecular mass				
	<20000 Da	20000-30000 Da	30000-40000 Da	40000-45000 Da	>45000 Da
Control	7.84	24.58	55.49	9.66	2.43
USC-1	8.04	25.71	54.51	9.17	2.58
USC-2	7.93	26.88	53.97	8.75	2.48

Table 2 estimation of the secondary structural elements of casein and ultrasonic-treated casein

Sample	α-helix	β-sheet	β-turn	random coil	α-helix / β-sheet
Control	22.53	32.26	25.19	18.47	0.7
USC-1	10.73	22.81	52.04	14.42	0.47
USC-2	13.29	29.17	49.39	8.15	0.46

Table 3 Calculated results of particle size distribution

Sample	Accumulative Distribution (μm)				D(4,3)	D(3,2)	D(4,3) / D(3,2)
	D10	D50	D90	D100			
Casein(Control)	0.584	2.493	14.65	68.29	5.616	1.470	3.82
USC-1	0.507	1.785	7.259	29.75	3.251	1.192	2.73
USC-2	0.510	1.871	6.900	29.63	3.147	1.209	2.60