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The Impact of High Frequency Ultrasound Waves on Diluted Whey Proteins Resulted From Dairy Processing

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ABSTRACT

In this study, the effect of high frequency ultrasound (2.4MHz) on different low concentrations of yogurt and cheese whey proteins has investigated. Whey protein obtained directly from Jordan university yogurt factory, was diluted with distilled water to get different solutions with wide range of concentration, namely 0.1-50ppm. These prepared solutions have exposed to ultrasound irradiation for 20, 40, and 60 minutes. Ultrasound was found to increase the concentration of protein with the increase of time. This enhancement in concentration was defined as enrichment value (R). The enrichment value has reached a value more than 2 for whey protein produced from yogurt, and more than 2.5 of its initial concentration for whey protein produced from cheese. The results suggest that ultrasound waves have caused an increase in protein solubility, due to a change in protein conformation that allowed the hydrophilic part to expose more to water side. The kinetic study for data was revealed that the solubility of whey protein is almost first order in the case of yogurt whey, while it is not first order for whey resulted from cheese whey.
Keywords: ultrasound, water treatment, whey protein enrichment, environmental management, industrial wastewater, protein recovery, dairy manufacturing, dairy waste.

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INTRODUCTION

Whey is the residual liquid substance that is obtained by separating the coagulum from milk during yogurt and cheese making. Most commercial whey is derived from cow's milk, composed of 6.25% protein, of which 20% in the form of whey. Whey is made up of many smaller protein sub-fractions such as: β-lactoglobulin, α-lactalbumin, immunoglobulins, glycomacropeptides, bovine serum albumin, and minor peptides such as lactoperoxidases, lysozyme, and lactoferrin [1].

Whey is a widely used ingredient in foods due to its unique functional properties, i.e. emulsification, gelatine, thickening, foaming, and fat and flavor binding capacity [2-4]. It is used because of its high nutritive value.

The technique of using ultrasound in food processing is recently gaining attention, such as food preservation, degassing and foam control, mixing, emulsification and meat tenderization. Ultrasound produces cell cavitation, localized heating and can lead to the formation of free radicals. Ultrasound has been used for many years in the study of proteins [5-8]. These studies used to estimate protein hydration and to infer changes in protein conformation. These parameters may be related to functional properties of proteins in foods such as solubility, foaming capacity and flexibility [9]. Gauzy reported that high intensity ultrasonic processing improves emulsifying properties of whey protein isolate [10].

Ultrasound techniques have been used in different fields of food and chemical processing. General and decomposition reactions, degradation of polymers, and polymerization reactions was investigated [11-18, 3]. When particles of material in a liquid suspension are subjected to sonication, a number of physical and mechanical effects can result.

To our knowledge the application of the low frequency highenergy power ultrasound in the food industry has been investigated in a recent years, however a high frequency still not well investigated. In this study the main goal is to introduce the high frequency waves in the field of food engineering, whey protein produced from yogurt and cheese has been chosen to study this effect. The study will focus on the effect of high ultrasound frequency and irradiation (sonication) on different concentrations of whey protein. It is also intended in this study to use whey resulted from dairy factory as waste without any further treatment to be exposed directly to ultrasound waves at low concentration, in order to investigate the effect such waves on the protein physical properties, such as solubility.

MATERIALS AND METHODS

Whey protein samples derived from yogurt and cheese manufacturing were provided by the University of Jordan Dairy factory located in the faculty of agriculture. α-lactalbumin with 99.9% purity was obtained from Sigma Company. This protein was used to check the ability of measuring the protein content in spectrophotometer device.

Apparatus

The ultrasound experimental device was constructed at our department by attaching the ultrasound vibrator to a 44mm by 270 mm cylindrical vessels as illustrated in Figure 1. An electrical source with variable voltage supply ranges from 0 to 40 volts and electrical current from 0 to 600mA was attached to the apparatus. All experiments were conducted at 24 volts and 500mA, according to the specified condition by manufacture for the ultrasound wave device generator. Ultrasonic vibrator comprised of 20 mm diameter transducer, which contains piezoceramics (sandwich) with titanium end masses leading the face from which the ultrasound was emitted. It has a frequency of 2.4 MHz and electric input power 9.5 Watt, and it was supplied by Honda Electronics Co. Ltd., of Japan, type HM-2412. To prevent any form of mist of whey from leaking to the atmosphere, a cover with watch glass was attached to the upper side of the cylindrical vessel during all experimental works.

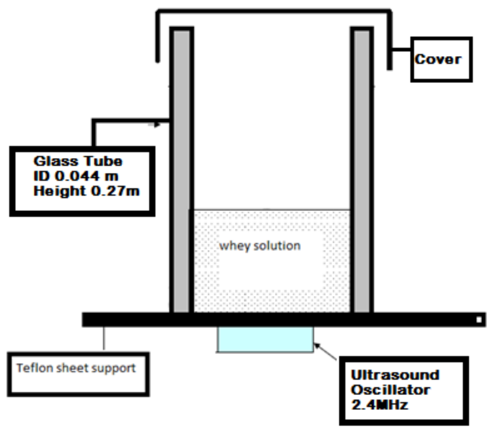

Figure 1: Experimental setup, ultrasound reactor.

Procedure:

A 50, 20, 15, 10, 1, and 0.1ppm (vol/vol%) yogurt whey samples and 10, 1 and 0.1ppm (vol/vol%) cheese whey samples were prepared by diluting the obtained stock whey samples with distilled water. A 50mL of the prepared dilute solution poured into the ultrasound reactor. After that the ultrasound device switched on, and the sample was sonicated for 20, 40 and 60 minutes. When the specified time was reached, a 5ml sample was taken and analyzed for determine the protein concentration using spectrophotometer. The sample retuned again to the reactor to maintain the same volume of 50ml in all experimental works. To insure the accuracy of the results each experiment was repeated twice and triplicates.

Analysis was conducted using a PG Instrument T70UV/VIS spectrometer. The instrument was calibrated at 280 nm using 1mg of α-Lactalbumin dissolved in one liter of distilled water and the absorbance at 280nm wavelength measured to give an absorbance of about 1.0 when the light path is 1 cm.

Table 1: Experimental conditions

Initial concentration of yogurt whey (ppm)	50,	20,	15,	10,	1,	0.1
Initial concentration of cheese whey (ppm)		10,	1,	0.1		
Time of ultrasound irradiation (minutes)	20,	40,	60			
Ultrasound wave frequency (MHz)			2.4			
Initial solution mixture volume (ml)				50		

RESULTS AND DISCUSSION

Yogurt whey

The effect of ultrasound waves on whey protein with different initial concentrations was investigated. The prepared diluted samples exposed to 2.4MHz ultrasound wave. The enrichment value profiles with time at different initial concentration of whey are shown in Figure 2. The enrichment value R, was calculated as follows:

$$\text{Enrichment Value R (-)} = \frac{\text{protein concentration after irradiation}}{\text{protein concentration before irradiation}}$$

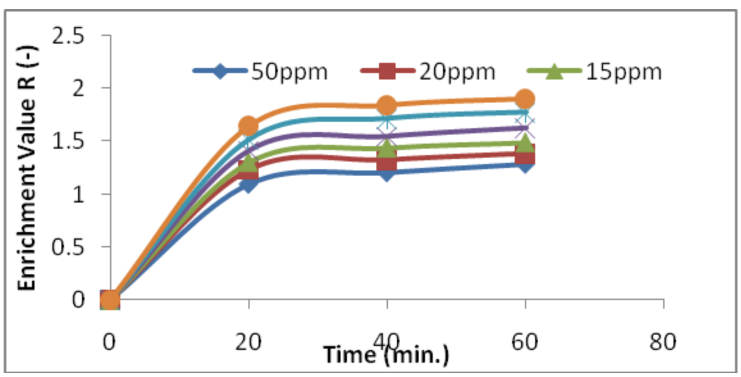

Figure 2: Enrichment value for yogurt whey at constant volume 50ml and 2.4MHz wavelength.

Figure 2 shows the enrichment value increases with increasing of the irradiation time despite the whey protein concentration. The concentration continued to increase over time with higher enrichment value for lower initial whey protein concentrations. It is clear that the highest enrichment obtained when the whey concentration is 0.1ppm and reached around 2, while the lowest enrichment obtained around 1.3, when the initial concentration of whey was at 50ppm. This behavior may be attributed to the protein solubility which has been increased significantly for all samples. Morel et al., [19]; Moulton and Wang, [20]; Wang, [21] got the same behavior with lower ultrasound waves frequency. According to their results it is noticed that the high-intensity ultrasound has enhanced the protein solubility by changing the protein conformation and the structure in the way that hydrophilic parts of amino acids from inside are opened toward water. Here the same behavior has noticed at high frequency by getting higher enrichment values with the increasing of irradiation exposing time.

Cheese whey

Figure 3 shows the enrichment profiles at different concentrations for cheese whey protein after irradiation at different time intervals. The same behavior for yogurt whey can be observed here, as the concentration of cheese whey protein increases the enrichment value is decreasing. This means that as the concentration of cheese protein is kept as much as low the enrichment value will be high.

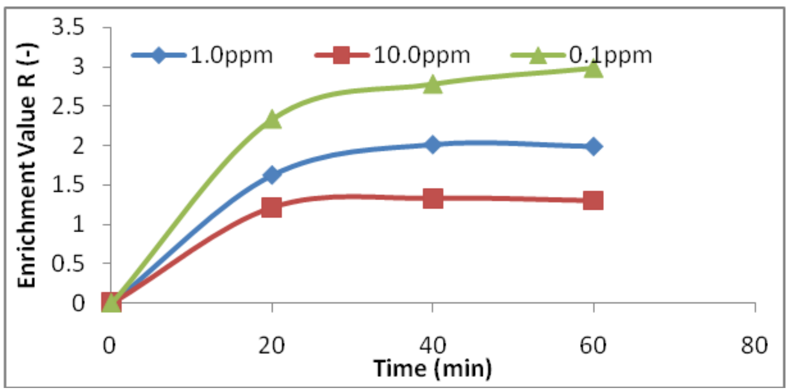

Figure 3: Enrichment value for cheese whey at constant volume 50ml and 2.4MHz irritation

It is clear from Fig. 3, that the enrichment value is higher than for that of yogurt whey when comparing both results obtained. It is almost three times higher of that for yogurt whey. This can be attributed to the lower protein contents for cheese whey compared to yogurt whey, which can give better condition to enhance the protein to agglomerate better. In the case of cheese whey, the experimental work shows that there is less foam generation during irradiation process.

In general, and as here in this work the ultrasonic irradiation plays a good role in enhancing the solubility of protein, if we consider the soluble protein as a very fine particles, which means the increased in the concentration of protein in the whey protein samples can be attributed basically to the solubility enhancement because of the conformation change in protein structure. This behavior of ultrasonic irradiation can cause a changing protein conformation and structure in the way that hydrophilic parts of amino acids from inside are opened toward water as it was discussed in details by [19], [20]. Thus the high-intensity of ultrasound enhances protein solubility. It was revealed by Cheftel, [22] that alpha-lactalbumin (whey is consisting mainly of Beta-lactoglobulin and Alpha-lactalbumin) and its solubility increase could be attributed also to the changes in the three-dimensional structures of globular protein resulted in increased number of charge groups. In those conditions the protein-water interactions increase, because the electrostatic forces are higher and more water interacts with the protein molecules.

In addition to that the temperature of the whey solution after exposing to ultrasound irradiation was noticed to be increased up to 42°C. This sudden increased in temperature after treatment is also contributed to enhance the solubility since in general protein solubility increases with temperature between 40°C and 42°C , Hosseinpour et al, [23] showed that solubility of protein changes with temperature without ultrasound needs long period of time to see this significant effects and sometimes it takes more than three days.

Kinetics study for protein solubility

Figures 4 and 5, shows the plot of natural logarithm of R against time. The data will give a straight line if it is a first order kinetics. Figure 4, give a perfect straight line with R^2 almost higher than 90%. This means that the enrichment of protein in the case of yogurt whey is behaving as a first order enrichment. While for cheese whey as demonstrated in figure 5, it is hard to see a perfect straight line, which means the enrichment of protein in case of cheese whey does not follow a first order kinetics since R^2, varies between 50-90%, and its best result obtained at 94% for 0.1ppm whey concentration.

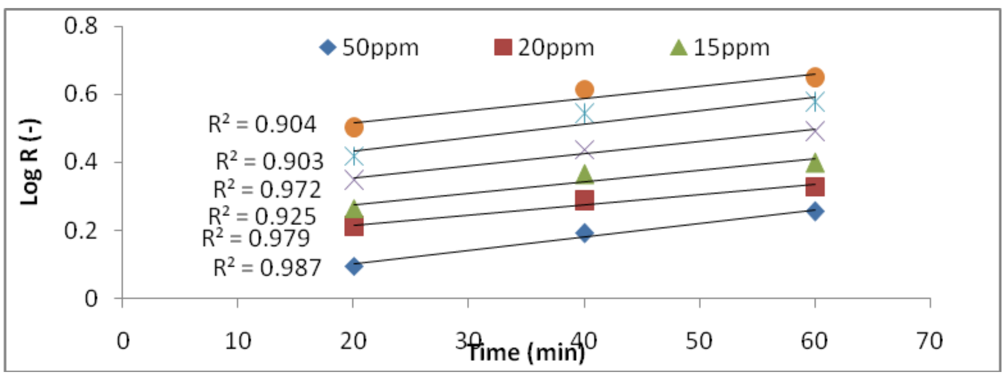

Figure 4: The log enrichment value against time, give a god fitting for first order in case of whey yogurt protein.

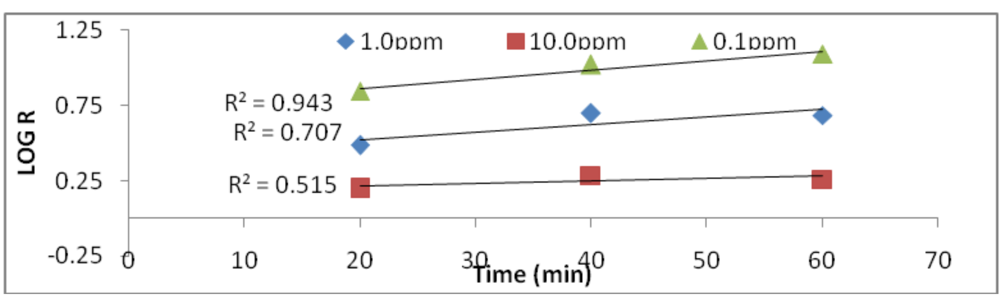

Figure 5: The log enrichment value against time, for cheese whey.

CONCLUSION

We Conclude that the exposing both the yogurt and cheese whey to 2.4MHz increased the enrichment values from 1-3 times of its initial concentration for whey protein. It was found that the lower concentrations of yogurt and cheese whey protein give greatest enrichment value. Moreover, the cheese whey showed a higher enrichment values compared to yogurt whey under the same concentration. The ultrasound with high frequency can have a good impact on protein by increasing its solubility an agglomeration. The kinetics study shows that enrichment value follows a first order kinetics in case of whey yogurt protein, but not for cheese whey protein.

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