Contents lists available at ScienceDirect

Food and Bioproducts Processing

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

# Ultrasonic processing for recovery of chicken erythrocyte hemoglobin

*Rafael A. Garcia*, Shaina M. Clevenstine, George J. Piazza*

*Biobased and Other Animal Co-products Research Unit, Eastern Regional Research Center, Agricultural Research Service, United States Department of Agriculture, 600 East Mermaid Lane, Wyndmoor, PA 19038, USA*

ARTICLE INFO

*Article history:*
Received 25 September 2014
Received in revised form 18 November 2014
Accepted 12 December 2014
Available online 23 December 2014

*Keywords:*
Blood
Flocculant
Ultrasonic processing
Hemoglobin
Cell lysis
Bio-based products

ABSTRACT

Hemoglobin from chicken blood has been shown to be a good substitute for synthetic polymeric flocculants. One stage of processing the blood entails lysis of the cells to release the hemoglobin; in the present study, the use of ultrasonic processing at this stage is investigated. Washed chicken blood cells are suspended in buffer and run continuously through a chamber attached to an ultrasonic probe. Calorimetry is used to measure acoustic power input to the liquid. Ultrasonic cell lysis is tested using chamber residence times of 75–300 ms, and the equipment's entire range of power inputs. The hemoglobin release kinetic parameters are determined and it is shown that above a particular level, increasing power input can actually result in a decreased rate constant. Ultrasonic processing can damage proteins, so reduction of hemoglobin's flocculant activity is considered. Using a sensitive assay involving suspensions of kaolin clay, no effect of ultrasonic processing on hemoglobin flocculant activity is detected. Although the conversion of electrical power to acoustic power is inefficient, the electric power required to release greater than 90% of the hemoglobin is shown to be minimal.

Published by Elsevier B.V. on behalf of The Institution of Chemical Engineers.

## 1. Introduction

In the United States, blood disposal is costly for chicken processors (Mountney and Parkhurst, 1995; Piazza et al., 2011). Typically, the blood is either taken by renderers for a fee, or treated as waste. Our laboratory has reported that hemoglobin (Hb) from blood can serve as a good substitute for synthetic polymeric flocculants (Piazza et al., 2014). Large scale testing and eventual commercialization of a chicken Hb flocculant will require a low cost process for isolating the Hb while retaining its flocculant activity. We have already reported on treatments that will preserve the blood for at least four days without refrigeration (Garcia et al., 2014b). In the present study we evaluate ultrasonic processing as a means to lyse chicken erythrocytes and recover the cytoplasmic protein, which is primarily Hb (Roux-Dalvai et al., 2008).

Ultrasound consists of cyclic pressure waves with a frequency greater than 16 kHz. Ultrasound in the frequency range of 20–100 kHz is commonly referred to as 'power ultrasound'; power ultrasound is distinct from the ultrasound utilized in applications such as medical imaging, which has a much higher frequency. Only power ultrasound is considered in the present study.

A common type of ultrasonic processor consists of a rigid probe attached to a piezoelectric crystal; application of high frequency alternating current to the crystal causes it and the probe to vibrate. Placed in a liquid, under appropriate conditions, such a probe creates tiny cavitation bubbles in the liquid. When a cavitation bubble collapses, it does so quite suddenly, generating local temperatures and pressures in excess of 5000 K and 500 atm, respectively. The violent shock wave created by bubble collapse can be very

*Abbreviations:* APD, acoustic power density; CBC, chicken blood cells; Hb, hemoglobin; KCE, kaolin clarification effectiveness; PBS, phosphate buffered saline.

* *Corresponding author*. Tel.: +1 215 836 3743; fax: +1 215 233 6795.
E-mail address: rafael.garcia@ars.usda.gov (R.A. Garcia).

http://dx.doi.org/10.1016/j.fbp.2014.12.002
0960-3085/Published by Elsevier B.V. on behalf of The Institution of Chemical Engineers.

destructive to materials it encounters (Wu et al., 2013; Doulah, 1977).

Ultrasonic processing has long been used for cell lysis, particularly in laboratory research. In this context, it is normally used in batch mode with treatment times up to 1 h. Although ultrasonic processing has a reputation as an expensive unit operation (Thompson and Doraiswamy, 1999), it is now considered a viable option for treating high volume, very low value materials including wastewater (Wu et al., 2013), sewage sludge (Pilli et al., 2011), and ship's ballast water (Holm et al., 2008). There is reason to believe that erythrocyte lysis can be achieved with process times much shorter than those commonly reported in laboratory protocols. First, erythrocytes differ from many cells used in ultrasonic lysis research, including bacteria (Choonia and Lele, 2011), yeast (Liu et al., 2013) and algae (Gerde et al., 2012), in that they do not have a cell wall; the cell wall is responsible for much of a cell's resistance to rupture. Second, chicken erythrocytes are relatively large 7 μm × 4 μm flat oval shaped cells; large cells rupture under ultrasonic treatment more readily than similar but smaller cells (Apar and Özbek, 2008). Cell lysis is usually accompanied by a large rise in the viscosity of the surrounding liquid, due to the release of chromosomal DNA (avian erythrocytes, in contrast to those of mammals, contain nuclei). Ultrasonication is advantageous in this respect because it very effectively shears DNA and prevents the rise in viscosity (Elsner and Lindblad, 1989; Fukudome et al., 1986).

A variety of other methods are available for erythrocyte lysis. The classic method involves incubation in hypotonic solution, which causes the cells to swell and burst; this method is disadvantageous from a processing point of view because of the resulting dilution of the product. Chemical agents or enzymes are often used for cell lysis, although protocols utilizing such substances have been designed for relatively price-insensitive laboratory research. There are also other physical methods for cell lysis available including high pressure homogenization (Chisti and Moo-Young, 1986).

Ultrasonic processing also has potential disadvantages. It raises the temperature of the process liquid and exerts intense shear forces. The tertiary structure proteins can be disrupted by these conditions, resulting in loss of activity for enzymatic proteins, modification of bulk functional properties, or aggregation (Jambrak and Mason, 2014). The mechanism by which hemoglobin promotes flocculation is not understood in detail, and consequently it is hard to predict the effect of ultrasonic processing on hemoglobin flocculant activity.

The present research explores the utility of ultrasonic processing as a unit operation in a Hb isolation scheme. Kinetic modeling is performed to facilitate the optimization of such processing.

## 2. Materials and methods

### 2.1. Sample collection

Blood samples were collected at a poultry processing plant in southern New Jersey, USA. The birds processed there are White Leghorn hens, aged 70–130 weeks. Collections were taken on the processing line, immediately downstream of the point where incisions are made in the birds' jugular veins. Blood dripped directly from the birds into plastic trays containing a solution of dipotassium EDTA which serves as an anticoagulant and preservative (Garcia et al., 2014b); the ratio of blood to preservative solution was controlled such that the final concentration of EDTA was 10 mM. The blood and preservative mixture was stirred frequently during collection. Upon returning to the laboratory (about 90 min later), samples were poured through nylon mesh with 500 μm square openings to screen out feathers and any other debris. Samples were stored under refrigeration (approximately 4 °C). Blood was stored for no more than 4 days.

### 2.2. Blood cell preparation

Blood was centrifuged at 3000 × *g*, for 10 min using a swinging bucket rotor. Approximately 95% of the supernatant serum was aspirated and discarded; the "buffy coat" layer was not aspirated. The cells were resuspended in phosphate buffered saline (PBS), pH 7.4, to the original volume, then centrifuged and aspirated again. Finally, cells were resuspended in PBS to the original volume. The resulted suspension is referred to as 'chicken blood cells' (CBC) and consists primarily of erythrocytes (red blood cells) suspended in PBS; CBC also contains relatively small amounts of white blood cells and platelets, and dilute serum proteins.

### 2.3. Ultrasonic equipment and acoustic power output

Ultrasonic waves were generated using an S-4000 Ultrasonic Liquid Processor (Misonix, Farmingdale, NY) which has a 600 W maximum power supply output. This equipment operates at a fixed frequency of 20 kHz. In the present research, it was used with a 0.75 inch (~19 mm) diameter probe. The equipment records the power supply's output of electrical energy over the course of a trial. This value, divided by the duration of the trial, was taken as an average electrical power output over the course of the trial.

Acoustic power output was measured using calorimetry. A 200 mL glass Dewar flask was filled with approximately 100 mL of water, and the exact mass of added water was recorded. The ultrasonic probe and the probe of a Temp 340 thermistor (accuracy ± 0.03 °C, display update 0.6 s, Oakton Instruments, Vernon Hills, IL) were submerged 2 cm below the surface of the water, clamped so that they did not touch the walls of the container or each other. Temperatures were allowed to equilibrate until the measured water temperature drifted no more than 0.1 °C over 2 min. The ultrasonic probe was switched on at a particular amplitude setting and the temperature was recorded every 10 s for 60 s. Trials were carried out in random order. The probe was cooled and re-equilibrated to ambient temperature between trials. Data from each trial was fit to a quadratic equation using Excel (Microsoft, Redmond, WA). The first derivative of the quadratic at time = 0 was used as an estimate of (dT/dt) in:

$$\text{Power} = \left(\frac{dT}{dt}\right) \cdot c_p \cdot m \quad (1)$$

where $c_p$ is the specific heat capacity of water at a constant pressure (4.183 J/g °C at 20 °C) and $m$ is the mass of water in grams.

### 2.4. Blood cell lysis system

The system designed for this research (Fig. 1A) pumps CBC from a stirred storage vessel, through a "flow cell", and