Protease Inactivation in Milk by Thermosonication and Impact on Milk Characteristics

Sakthi Vijayakumar
David Grewell
Stephanie Jung
Stephanie Clark

IOWA STATE UNIVERSITY
Department of Food Science and Human Nutrition

Outline

- Ultrasound in the Food and Dairy Industries
- Research Objective
- Experimental Design
- Results
- Implications
- Questions

Ultrasound in the Food Industry

- Activation/inactivation of microorganisms
  (Chemat and Hoarau, 2004; Knorr et al., 2004)
- Activation/inactivation of enzymes
  - Peroxidase inactivated when sonicated over 3 hrs
    (Mason et al., 1996)
- Extraction processes
  - Enhanced mass transfer in sugar extraction
    (Chendke and Fogler, 1975)
- Quality Control
  - Measured extent of crystallization and melting in emulsion
    (Mason et al., 1996)

Ultrasound in the Dairy Industry

- Increasing cheese yield (Muller, 1992)
- Decrease in time for yogurt production (Mason et al.,1996)
- Homogenization (Gaffney, 1997)
- Inactivation of microorganisms (Mason et al., 1999)
- Inactivation of spoilage enzymes (Raviyan et al., 2005)
- Freezing
  - Promote nucleation and reduce ice crystal size in ice cream (Zheng and Sun, 2006)

Fluid Milk Limitation

- Shelf-life
  - How long does it stay good in your refrigerator?
  - What do you observe at the end of the milk's shelf-life?
- Is it safe to drink the milk after its shelf-life?
  - Pasteurization (72°C, 15s)
    - Destroys all pathogens and most spoilage microbes
- So what is the shelf-life of fluid milk based on?
  - Remaining spoilage microorganisms
  - Enzymes

Proteases in fluid milk

- Native and/or produced by spoilage microbes
- Are heat stable (survive pasteurization)
- Cause age gelation (Proteolysis)
- Limit shelf life
- Can be inactivated at Ultra-High Temperatures (quality is reduced)

Can proteases be inactivated by ultrasound??
(Vercet et al., 2000)

Limitations to ultrasound

- Insufficient for considerable inactivation
  - Needs to be combined with heat, pressure etc. (Earnshaw et al., 1995; Vercet et al., 2002)
- Qualitative implications
  - Can cause whey protein denaturation (Villamiel and de Jong, 2000)
  - Can affect sensory properties of milk (Riener et al., 2009)

What about thermosonication??

Our Research Objectives

- Study the effect of ultrasound treatment (different amplitude and time combinations) in combination with heat on
  - Protease activity in milk
  - Rheological properties of milk
  - Sensory properties of milk

Experimental Design

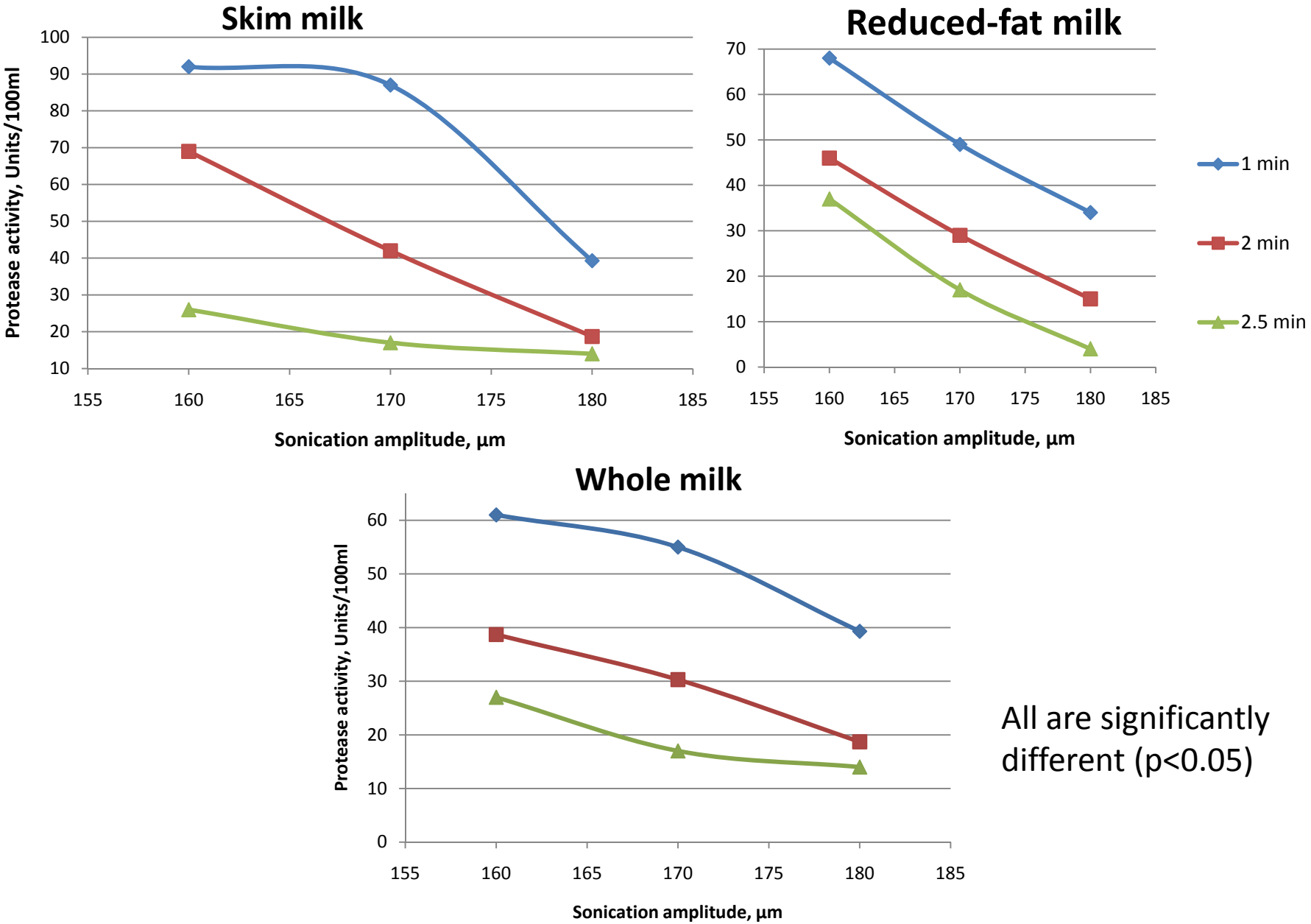
- Protease – Source: *Staphylococcus aureus*
- Milk – pasteurized skim, reduced-fat (2%) and whole milk
- Preheating – to 60°C
- Sonication (20kHz) amplitude – 160, 170, & 180 µm
- Sonication time – 1, 2, and 2.5 min

Protease Activity

Azocasein method

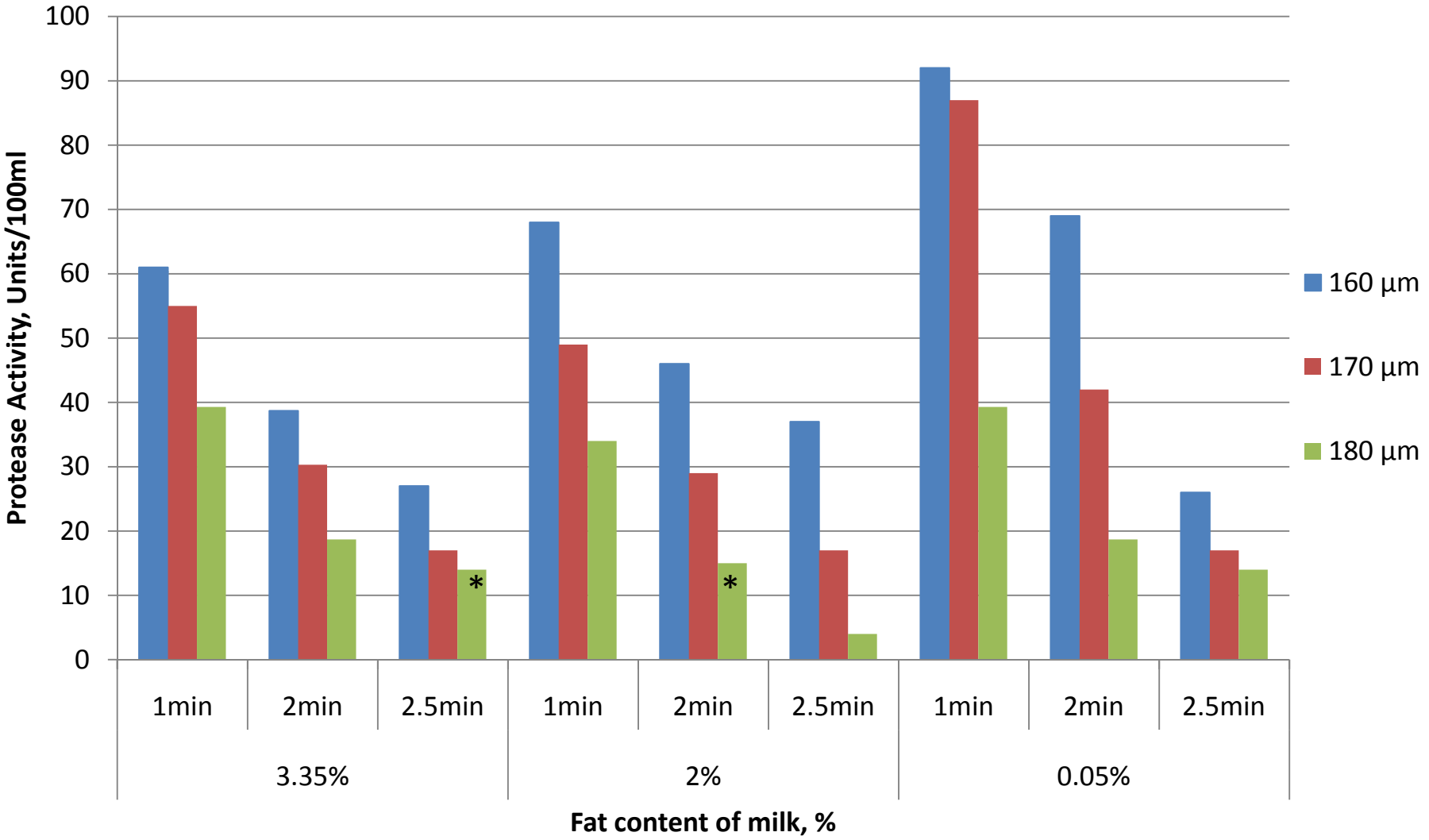
(Christen and Marshall, 1984)

Effect of Amplitude and Time



All are significantly different ($p<0.05$)

Effect of Fat Content of Milk



* No significant difference

Rheological Properties

(Rheometer)

Effect of Sonication on Rheological Properties

Milk-treatment (180µm, 2.5 min)	Average viscosity, Ns/m^2	Consistency coefficient, $Pa.s^n$	Flow behavior index
Skim control	0.0011	0.0013	0.98
Skim sonicated	0.0011	0.0014	0.96
Reduced-fat control	0.0013	0.0017	0.95
Reduced-fat sonicated	0.0014	0.0019	0.96
Whole control	0.0013	0.0020	0.94
Whole sonicated	0.0013	0.0015	0.97

No significant differences ($P<0.05$)

Sensory Evaluation

Effect of Sonication on Sensory Properties (n=2; expert dairy judging panelists)

Milk: sonicated at 180µm 2.5min	Sensory Odor Attribute (defective)						Consumer Accept. (odor attributes)
	Plastic /burnt /rubbery			Cooked (custard)			
	S	D	P	S	D	P	
Skim		X		X			Yes
Reduced-fat		X				X	**No**
Whole			X			X	**No**

S-Slight D-Definite P-Pronounced

Pyrolysis of volatile and non-volatile organic compounds at the collapsing bubble? (Neppolian et al., 2004)

Summary of Results

- Thermosonication (60°C, 180µm, 2.5min) treatment
  - decreased protease activity in skim, 2%, and whole milk
  - did not affect rheological properties of milk
  - caused undesirable odors in 2% and whole milk

Further Research

- Modify treatments
  - Higher amplitude, shorter time
- Sensory
  - Larger panel, untrained panelists
- Extended storage study
- Compare proteases from different sources
- Investigate impact of fat content on aroma compounds
- Investigate the effect on raw milk
- Evaluate cost/energy efficiency of optimized conditions

Conclusions

- The food and dairy industries have a variety of uses for ultrasound
- Thermosonication may inactivate protease and extend the shelf-life of milk
- Conditions must be optimized to reduce off aromas/flavor before commercialization is an option

Thank you!!!

Got milk?? Got Questions?

Effect of Preheating (no holding time) – enzyme in skim milk