Effects of Ultrasound Treatment on Viability and Autolysis of Starter Bacteria in Hard Cheese

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Abstract: Cheese starters, *Streptococcus thermophillus*, *lactobacillus delbrueckii ssp bulgaricus*, *lactobacillus helveticus* that used in the manufacture of cheese, were subjected to ultrasound in the range 0, 5, 10, 15 and 20 min at 20 KHZ frequency and amplitude of 80% at 20°C and pH= 7. Inactivation total viable counts and cell lysis release of lactate dehydrogenase, enzyme (LDH) due to autolysis of lactic acid bacteria were examined during the subsequent cheese ripening. Results showed that the lactococci were more sensitive than the lactobacilli to ultrasound up to 20 min (3-5) log cycle reduction and others were more reduction tolerant against lethal effect of ultrasound. The degree of inactivation of starters was found to be affected of ultrasound exposure time, starters were treated with a longer exposure time, showed a greater reduction in numbers and had less out-growth of starters during ripening also release of LDH and thus autolysis, was increase in cheese when using ultrasound up to 15min in both 1-day-old and 1-month-old of hard cheese samples.

Key words: Starter bacteria %Ultrasound %LDH activity

INTRODUCTION

Acceleration of cheese ripening is of major commercial interest, as decreased storage times offer significant economic gain [1]. Cheese ripening is essentially an enzymatic process in particular astrology link exists, between proteolysis and cheese maturity. Starter bacteria are one of the primary sources of ripening enzymes, however many starter enzymes (proteinases and peptidases) required for proteolysis an intra cellular and autolysis of starter bacteria is required for their liberation [1-5] increasing the autolysis rate of starter culture cells in creases rates of proteolysis in cheese [4-5]. Starter strains naturally vary in the rate at which they autolyse and the use of highly autolytic starters strains results in enhanced flavor development [5-9].

Lactate dehydrogenase (Ec 1.1.1.27) is an enzyme, which catalyzes the last step in glycollysis, LDH is a soluble enzyme and localized in the cytoplasm. Measurement of LDH release (leakage) is an important and frequently applied test for cellular membrane permeabilization and severe irreversible cell damage, LDH is a tetrameric enzyme that along with the coenzyme NAD+, catalyzes the interconversion of lactate and private (Eq. 1).

\[ \text{pyruvate} + \text{NADH} + \text{H}^+ \rightarrow \text{L-lactate} + \text{NAD}^+ \]

The activity of the intracellular enzyme, lactate dehydrogenase (LDH), was determined using a modification of the method of Wittenberg and Angelo (1970). Which measure the decrease in absorbance at 340nm resulting from the pyruvate-de pendant oxidation of reduction nicotinamide adenine dinucleotide (NADH).

The application of ultrasound to biotechnological processes has recently attracted, the attention of some research groups, in biotechnological process ultrasonication method is widely used for laboratory scale and it dose not require sophisticated equipment or extensive technical training. The structure and function of biological molecules such as bacterial enzymes, cell wall and membranes can be changed by the ultrasound irradiation. The most common interaction mechanisms which involved in this case are either heat on chemical effects and a caustically induced cavitation activity in addition to these changing the function of biomolecules by ultrasonication can also be caused by mechanical effect that is shear stress developed by eddies arising from shock waves [9-10]. It is well know that the ultrasonic waves have the potential to influence the microorganisms and living cells. Ultrasound treatment
causes perturbation of bacterial cell wall, ultrasound, improved biological activity, mastransfer enhancement and shortening process time are the positive effects of such treatments. Thus ultrasonication may breakdown the cell wall and under the stress of applied ultrasound be accelerated of autolysis in cheese. The objectives of this study were to investigate the viability of starter strains during ripening of cheese and to determine if ultrasound treatment led to enhanced rate of autolysis of the starter culture during 4-week ripening period.

MATERIAL AND METHODS

Starter Culture: Three types of starter cultures were used in cheese-making: Streptococcus thermohilouses L. delbrueckii, spp bulgaricus, L. helveticus and in form blended and were obtained form the DSM company, then were grown individually in MRS broth at 30°C overnight. The cell pellet was collected from 15 ml of culture, washed once in cold, sterile phosphate-buffered saline at pH 7 and then resuspended in 15 ml of phosphate-buffered saline. The cell suspension was aseptically transferred into a sterile plastic bag.

Sonication of the Samples: The sample was ultrasound treated in the range of 20 KHZ frequency for 0, 5, 10, 15 and 20 min at 20°C, an untreated control sample was left at 20°C (without ultrasound treatment, 0 min). The ultrasonication experiments were carried out at 20 KHZ on the ultrasonic generator. The tip of the horn was immersed about 9 mm into solution to be processed, all experiments were performed on samples at ultrasonic amplitude 80% of maximum out put power of device the solution was processed at constant temperatures of 20°C, with the sonication horn for 5, 10,15 and 20 min.

Cheese Manufacture: Cheese were produced with blended starter Strains, using a modification of the protocol of some researches [6-11], cheese was made in triplicate using the different treatment of starter control and 5, 10, 15, 20 min the time of ultrasonic irradiation.

Starter Viability Evaluation: After the ultrasound treatment, Starter viability in cheese was measured directly after ultrasound treatment and during the 4-week ripening period.

LDH Evaluation: To evaluation starter autolysis in cheese, a modification of the method of law, sharp and Reiter (1974) was used to extract LDH from miniatur e cheese, Greated cheese (~ 10 gr) was mixed 1:5 with pH, 7 sterile peptone buffer and homogenized in a stomacher for 5 min, a portion of the homogenates was centrifuged at 800g for 1 min and the fat layer removed before decantation of supernatant. This cell-free supernatant (100µl) was then assayed for LDH activity.

The activity of the lactate dehydrogenase was determined using a modification of the method of [6] which measure the decreases in absorbance at 340nm resulting from the pyruvate-dependant oxidation of reduced nicotinamide adenine dinucleotide (NADH) The reaction mixture contained 100 µl of 300mM sodium pyruvate, 100 µl of 30mM Fructose-1,6 diphosphate, 100 µl of 4.5mM NADH and 100 µl of sample, with final reaction volume made up to 3mL using 0.2 mtris-maleate buffer (pH=7). Oxidation of NADH was measured by the decrease in absorbance at 340nm using a continuous readout spectrophotometer all assays were carrie douti n duplicate the activity of LDH in cheese juice was expressed as units mLG cheese juice of units gG cheese, respectively, where one unit was defined as the amount of enzyme that catalyses the oxidation of 1µmol NADH per min.

Statistical Analysis: All data shown are the results of at least three independent replication of each experiment. Mean comparison and standard derivations were calculated using ANOVA Table 1.

RESULTS AND DISCUSSION

Viability of starter bacteria was measured directly after ultrasound treatment and during the 4-week ripening period in either control or experimental cheeses (Figure 1).
All of ultrasound treatment had significant effect on viability count and at ultrasound for 15-20 min, cell viability was significantly affected and population decreased by 3-5 log cycle whereas at ultrasound 5, 10 and <15 min population was not significantly affected at ultrasound and viability of starters decreased by 1-2 log cycle. The starter population in the first day about 10^6 CFUg^-1 and during cheese ripening depending on the strain, could be changed from 10^3 to ~10^5 CFUg^-1 in the final phase of ripening after 30 days achieving count of the number of lactobacilli was of distinguished. Lactobacillus counts are lower than lactococci starter thus they were more sensitive than the lactococci ultrasound treatment, that are similar caused by starters viability under pressure treatment by [2]. Values for starter Counts were shown in Table 1, values are mean of triplicate analyses.

LDH activity or autolysis of starter cultures was assayed immediately in ultrasound treated cheese juice extracted from 1-day-old and 1-month-old, hard cheese, LDH activity in control cheese sample changed from 0.63 to 0.66 units µg^-1 of cheese, but in Treated samples, LDH activity were increased in 1-day-old especially the samples were treated for 20 min 0.75 units µg^-1 of cheese, perhaps ultrasound treatment for 20 min had improved activity and under the stress 20min was accelerated of autolysis, there were no significant difference between ultrasound treated samples<15min. During ripening which were similar results that obtained from [9] (Figure 2).

These data demonstrate even at ultrasound where inactivation occurred in starter bacteria. No Corresponding autolysis of the starter cultures resultes. The activity of LDH, which is considered a good marker for autolysis of starter cultures [9-11] Was significantly unaffected by ultrasound. Levels of LDH activity were similar in ultrasound treated 5 and 10min or untreated samples indicating that LDH was ultrasound-stable, as previously, reported about LDH activity by [9]. LDH (lactate dehydrogenase) activity in (µg^-1 cheese) juice extracted after 1-day-old and 1-month-old storage were shown in Table 2 and Figure 2.

### CONCLUSION

Ultrasound treatment in the range of 5-20 min had significant effect on viability of starter bacteria ultrasound treatment> 15min increased the LDH activity where as, 5 and 10 min had no effect on LDH activity in hard cheese during ripening.

### REFERENCES


