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INVESTIGATION OF THE IMPACT OF THE THERMOSONICATION OPERATION ON DOOGH QUALITY AND ON THE SURVIVAL OF *KLUYVEROMYCES MARXIANUS*

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Abstract: *Doogh is an Iranian drink produced from yoghurt. Swelling is an undesirable change of Doogh, usually caused by Kluyveromyces marxianus. The aim of this study was the investigation of the effect of the thermosonication process on Kluyveromyces marxianus survival as well as the determination of the impact of this process on other quality parameters (viscosity and serum separation). For this reason, Kluyveromyces marxianus (about 10^5 cells per milliliter) inoculated into Doogh samples, thereafter samples being exposed to various thermosonication process conditions. Results showed that the thermosonication operation resulted in the reduction of the yeast population of Doogh samples ($p < 0.05$). The lowest yeast population was observed in the sample subjected to acoustic density=1w/ml, T= 60°C and t=30min (1.7±0.01CFU/ml). Other operation conditions also had impact on yeast survival ($p < 0.05$). Thermosonication also had significant effects on the viscosity of the Doogh samples. Serum separation of samples was also measured and observation implied changing serum separation magnitude between different samples in comparison with the control sample ($p < 0.05$).*

Key words: *Doogh, Kluyveromyces marxianus, Thermosonication operation.*

1. Introduction

Doogh is one of the most consumed fermented dairy products in Iran [17]. One of the major disadvantages of Doogh which reduces shelf life and marketability of this product is swelling. A major microorganism responsible for the swelling of Doogh is *Kluyveromyces marxianus* (Van der Walt) [19]. Nowadays the research was focused on the application of operation approaches with

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minimal impact on nutritional value as well as on destroying microbial contamination of food products.

Ultrasound is one of the non-thermal methods used to process some foods [7]. Ultrasonic operations are performed by means of two methods, including the use of low-energy and high energy ultrasonic [2], [15]. During ultrasonic operations, cavitation occurs thus involving the breaking of bubbles in the liquid medium, which causes heat and pressure, and this is the main reason for the microbicidal properties of ultrasonic operations [14]. The combination of ultrasonic and heat treatment, called thermosensation, resulted in a more consistent microbicidal effect than ultrasonic alone.

Dars et al. [3] compare thermosonication and high pressure effect on mango juice [3] and reported that both operations had minimal effect on the chemical characteristics of samples (Brix and pH). The increase of temperature in the thermosonication operation resulted in a more destructive effect of enzyme activity (polyphenol oxidase, peroxidase, and pectin methyl esterase).

Souza et al. [18] evaluate the impact of the thermosonication process on quality of camu–camu nectars, and reported that the application of the thermosonication operation caused better bioavailability of the bioactive compounds as well as an increase in cloud value of the camu–camu nectars. Higher processing time and temperature cause a more destructive effect on enzymes. After the thermosonication process no microorganism was detected.

In this study, the effect of the thermosonication operation on the Doogh quality parameters and Kluyveromyces marxianus count were assessed.

2. Materials and Methods

2.1. Study Area

The yeast *Kluyveromyces marxianus* (Van der Walt) (PTCC 5188) was purchased from the Iranian Research Organization for Science and Technology. After activation, cells were kept on YDP agar at 4°C.

2.2. Preparation of Inoculum

A complete loop of pure yeast colony was transferred to a 250 ml Erlenmeyer flask containing 100 ml of inoculum (5% lactose, 0.3% yeast extract and 0.5% peptone in distilled water) and then placed in a shaker incubator (temperature = 30°C, mixing rate = 150 rpm, 24 h). Yeast cells were isolated by centrifugation at 4500 g for 10 minutes [12]. Then, using a hemocytometer slide, a concentration of 105 cells / ml was prepared [4].

2.3. Doogh Production Method

First, Doogh was made by conventional formulation (40% yogurt, 0.7% salt and 59.3% water). For this purpose, after weighing the yogurt with a digital scale, the yogurt was stirred manually (130 rpm). Then, the water and salt were added slightly and stirred for 2–3 minutes to prepare a uniform Doogh sample [5].

2.4. Measurement of Some Physical and Chemical Characteristics of Doogh

For the determination of protein content, fat content, acidity and dry matter, standard methods were used [8]. The pH value of the Doogh samples was measured by means of a digital pH meter 744 Metrom at ambient temperature.

2.5. Determination of the Specific Heat of Doogh

A mixed method was used to measure specific heat. The experiment was performed in three replications. Specific heat of the sample was determined using equation (1) [10].

$$C_p = \frac{(H_f + M_{cw} \cdot C_w)(T_e - T_{cw}) - H_c (T_m - T_e)}{M_m (T_m - T_e)} \times 4.1868 \quad (1)$$

where:

H_f is the heat capacity of flask [Cal/°C];
H_c – heat capacity of capsule [Cal/°C];
T_{cw} – cold water flask temperature [°C];
T_e – equilibrium temperature [°C];
T_m – sample temperature [°C];
C_w – specific heat of water [Cal/g. °C];
M_{cw} – cold water weight [g];
M_m – sample weight [g].

2.6. Determination of Acoustic Energy Density and Ultrasonic Energy Intensity

The acoustic energy density was determined according to Jeličić et al. [9]. The samples were subjected to ultrasonic operation. Temperature measurements were performed during the experiment with an infrared thermometer (TROTEC TP10 model made in Germany). Temperature rise (dT/dt) was recorded during the experiment. The intensity of ultrasonic energy was calculated by equation (2):

$$P = m \cdot C_p \cdot \frac{dT}{dt} \quad (2)$$

where:

P is the ultrasonic energy intensity;
m – the mass of material [g];
C_p – the specific heat rate [kcal/kg/°C];
$\frac{dT}{dt}$ – the temperature rise process.

Equation (3) was used to calculate the acoustic energy density (AED).

$$AED = \frac{P}{V} \quad (3)$$

Where P is acoustic intensity and v is sample volume [ml].

2.7. Measuring the Serum Separation of Doogh

Experimental tubes with specified dimensions (1.4 diameter, 16 cm in length) were used to determine serum separation. These tubes were filled (10 cm). The amount of serum separation was measured in specific intervals of time and the line formed between the two phases was measured, and each milliliter was equal to 1% of the serum separation percentage [5].

2.8. Determination of Rheological Properties of Doogh

The flow behavior test was measured at a shear rate range of 11- 321 per second using a Spindle SC-18-18 Ultra DVD rotary viscometer from Brookfield, USA [6].

2.9. Statistical Analysis

All experiments were carried out in three replicates. Statistical analyses were performed in a completely randomized design using STATISTICA software version 22. Data were analyzed using one-way ANOVA and Duncan's test. Significance level was set at 0.05.

3. Results and Discussion

The prepared Doogh had 1.2% protein, 0.98% fat, 6.9% dry matter, pH about 3.95 and apparent viscosity of 10.92 mPa.s. Determined specific heat of Doogh was 0.942 kcal/kg/°C.

3.1. The Effect of the Thermosonication Operation on *Kluyveromyces Marxianus* Survival in Doogh Samples

The evaluation of the yeast population of *Kluyveromyces marxianus* in different thermosonication conditions indicates that reduction of yeast population in some thermosonication circumstances was remarkable (Table 1). The highest yeast population belonged to the control sample ($p < 0.05$) and the application of thermosonication resulted in a decrease in yeast population and there was a straight relationship between increase time, temperature and acoustic density and the reduction rate of yeast population. The highest yeast destruction happened at the acoustic density = 1w/ml, T = 60°C and t = 30 min where the logarithm of yeast count was equal to 1.7±0.01.

Table 1

The impact on the thermosonication Kluyveromyces marxianus count in Doogh samples

Time [min]	Temperature [°C]	Acoustic density [w/ml]	Yeast population [log CFU/ml]
10	40	0.5	$4.5±0.02^{a}$
		1	$4.2±0.01^{b}$
	50	0.5	$3.6±0.01^{d}$
		1	$3.4±0.01^{e}$
	60	0.5	$2.6±0.01^{f}$
		1	$2.1±0.01^{h}$
30	40	0.5	$3.9±0.02^{c}$
		1	$3.6±0.03^{d}$
	50	0.5	$2.4±0.01^{g}$
		1	$1.9±0.01^{i}$
	60	0.5	$1.8±0.01^{j}$
		1	$1.7±0.01^{k}$

Note: **Different superscript lowercase letters show the significant differences between the samples (P < 0.05).*

The effect of thermosonication on microbial count is reported by other research. Kumar Nayak et al. [11] applied the thermosonication process on star fruit juice at different temperatures (25–45°C) and power of 600 W and showed a reduction from 6.10 to 4.39 log CFU/ml. Other researchers reported a higher resistance of yeasts and molds against sonication in comparison with bacterial populations [16].

The lethal effect of different operations on various microbial strains might be due to the existence of pores and changes in microbial cell membranes caused by several stress factors (temperature, pH, microfiltration and mechanical injury and their cumulative impacts) and various changes in cell membranes resulted in intracellular contents being exposed to the environment and having a lethal effect [1].

3.2. The Effect of Thermosonication on the Rheological Properties of Doogh

The flow curve and viscosity of the various Doogh samples are shown in Table 2, the rheology of Doogh behavior is similar to the Newtonian fluids which is consistent with other similar research in this regard, including Mousavi and Eman-Djomeh [13]. Newtonian behavior in different Doogh samples with different amounts of acidity and solids has been expressed.

According to the results, the application of the thermosonication operation did not significantly alter the rheological behavior of Doogh samples and it can be concluded that the harsher circumstances in thermosonication process resulted in a significant effect on intermolecular bonds of proteins. The rheological behavior of Doogh is similar to that of Newtonian fluids, which is due to the low concentration of proteins in this product. Proteins such as whey and casein proteins act as colloidal particles and affect the flow behavior of dairy products. Intermolecular bonds and the thermodynamic properties of proteins also affect the rheological properties of dairy products and Doogh has a flow behavior similar to Newtonian fluids due to their low concentration and low protein content [13].

Table 2

The impact of different thermosonication processes on the viscosity of Doogh samples

Time [min]	Temperature [°C]	Acoustic density [w/ml]	Apparent viscozity
10	40	0.5	10.9±0.01^{a}
		1	10.8±0.01^{ab}
	50	0.5	10.8±0.01^{ab}
		1	10.79±0.01^{ab}
	60	0.5	10.79±0.01^{ab}
		1	10.78±0.01^{ab}
30	40	0.5	10.8±0.02^{ab}
		1	10.78±0.01^{ab}
	50	0.5	10.78±0.01^{ab}
		1	10.78±0.01^{ab}
	60	0.5	10.76±0.01^{b}
		1	10.75±0.02^{b}

The comparison of the viscosity of treated samples with the control sample showed that the significant decrease in viscosity was observed in the sample processed for 30 min, temperature = 60°C and acoustic density = 1 w/ml (Table 2). This could be due to the no or little impact of mild the thermosonication process on soluble solids and Brix of various food materials. Souza et al. [18] investigated the effect of the thermosonication process on camu–camu nectars and concluded that the thermosonication operation had no significant impact on Brix and soluble solids of this product.

3.3 The Effect of Thermosonication on Serum Separation of Doogh Samples

Statistical analysis showed that there is a significant difference between the serum separation of Doogh samples treated in different thermosonication processes and this effect is related to the process parameters (Acoustic density, time, temperature) ($p < 0.05$). The most serum separation occurred in the Doogh sample treated at the acoustic density = 1w/ml, T = 60°C, t = 30min which was equal to 64±0.5%. The amount of serum separation related to the severity of the thermosonication process (Table 3) could be explained by the fact that in these conditions, some protein coagulation and a separation of fat phase resulted in more serum separation.

Table 3

The effect of the thermosonication process on the serum separation of the Doogh sample

Time [min]	Temperature [°C]	Acoustic density [w/ml]	Serum separation [%]
10	40	0.5	$53.2±0.1^{h}$
		1	$53±0.1^{h}$
	50	0.5	$54±0.5^{g}$
		1	$56±0.5^{f}$
	60	0.5	$56±0.2^{f}$
		1	$58±0.2^{d}$
30	40	0.5	$54±0.1^{g}$
		1	$56±0.1^{f}$
	50	0.5	$57±0.8^{e}$
		1	$59±0.1^{c}$
	60	0.5	$62±0.1^{b}$
		1	$64±0.5^{a}$

4. Conclusion

The results of this study showed that the application of the thermosonication process reduces the population of yeast which causes spoilage in Doogh (*Kluyveromyces marxianus*). The application of this process made some changes in the rheological properties of Doogh and reduced its apparent viscosity compared to the control sample. In addition, the percentage of serum separation increased.

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