INACTIVATION PROFILE OF FOOD SPOILAGE BACTERIA BY POWER ULTRASOUND: A PROSPECTIVE FOR SALT REDUCED MEAT PRODUCTS

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Abstract – This study investigated the efficacy of power ultrasound (US) for the inactivation of *Escherichia coli* and *Listeria innocua* in the presence of sodium salt and salt substitutes. Inoculated bacteria suspensions were treated with; i) an ultrasonic bath (33 KHz), ii) an ultrasonic probe (20KHz) and iii) a combination of the two, in the presence of 5% NaCl, 5% KCl or 5% NaCl + KCl. Inactivation curves were fitted to various inactivation models using the GInaFiT freeware tool. The Weibull model showed a good fit, with a high regression coefficient (R^2>0.98) and low RMSE (<0.22). The results presented in this study show that power ultrasound treatment may be employed for the inactivation of microorganisms, while reducing sodium salt and salt substitutes.

Key Words – Meat Brining, Inactivation model, Salt substitutes.

I. INTRODUCTION

The large increase in the consumption of highly salted processed foods has resulted in higher dietary salt intake, exceeding the recommended dietary values worldwide. Processed meat products provide about 20% of the dietary salt intake of an adult, and therefore, this category is a particular focus of investigation. The necessity to reduce salt and sodium levels in meat has to address multiple challenges, not only product safety, with salt being one of the most common antimicrobials, but also related to the physiochemical characteristics of the meat which are impacted by salt (e.g. flavour, colour, texture). Power ultrasound is a novel technology that has been shown to enhance the mass transfer of salt in processes like meat brining. Moreover, the formation of high local pressure and temperature by ultrasound cavitation, may contribute to increasing the microbial safety and the shelf life of food products. Another strategy used to reduce the amount of sodium, is to use salt replacers ingredients, of which KCl is the most commonly used. The aim of this study was to investigate the effect of ultrasound on the inactivation profile of model Gram positive and negative microorganisms, in the presence of NaCl, KCl and a mixture of the two.

II. MATERIALS AND METHODS

Inoculated bacterial suspensions were treated in a temperature controlled environment (2.5±5 ^{o}C) for 60 minutes using three different ultrasonic set-ups; i) ultrasonic bath operating at 33 KHz, 96 W, ii) ultrasonic probe operating at 20 kHz, 60% amplitude (54 W) and iii) a combination of the two. Bacteria were sonicated in either tryptone soy broth (TSB), TSB supplemented with 5% NaCl, TSB supplemented with 5% KCl or TSB supplemented with a mixture of 5% NaCl + KCl in 2:3 ratio. All the experiments were performed in 100 ml of broth solution, inoculated with a final bacteria population of 10^8 CFU mL^{-1}. Microbial populations were enumerated by plate counting after treatment times of 0, 2, 5, 10, 15, 30 and 60 min. Inactivation curves were fitted to various inactivation models namely log-linear, biphasic and the Weibull models using the GInaFiT freeware tool. Average values and standard deviations were determined from the data. Separation of means was carried out using Tukeys' test at $p < 0.05$ level (SAS 9.3).

III. RESULTS AND DISCUSSION

The effect of the different sonication frequencies (33, 20 and 33 + 20 KHz) on *E. coli* and *L. innocua* inactivation in different broth solutions was investigated. The *p* values of all the populations tested, indicated a good fit of the survival curves with the Weibull model, showing a concave upward ($p < 1$) inactivation profile, Table 1. Significant differences in the ∂ value ($p<0.05$) between the treatments were observed with 20 and 20 + 33 KHz. As expected, the Gram positive bacteria *L. innocua* showed a much higher resistance to both the US treatments and in the presence of different salts compared to *E.coli*. Sonication with a frequency of 33 KHz alone did not show any effect on bacterial inactivation.

Table1. Shape factors (*p*, δ) of the Weibull model of microorganisms treated with ultrasound frequencies of 20 KHz and 20 + 33 KHz.

Treatment 20 KHz	*E. coli* K12				
	δ ± SD	p ± SD	$Log_{10}N_0$ ±SD	RMSE	R^2
TSB	0.67±0.30^{c}	0.48±0.06^{ab}	7.69±1.08^{a}	0.59	0.80
5% NaCl	0.43±0.34^{c}	0.44±0.07^{b}	8.42±0.00^{a}	1.88	0.86
5% KCl	2.04±0.19^{b}	0.58±0.09^{ab}	8.10±0.30^{a}	1.95	0.91
5% NaCl + KCl (2:3)	4.18±0.07^{a}	0.83±0.00^{a}	7.93±0.05^{a}	1.29	0.86
	L. innocua				
TSB	5.21±1.88^{a}	0.49±0.00^{b}	8.54±0.15^{ab}	0.97	0.86
5% NaCl	7.81±0.05^{a}	0.69±0.00^{a}	8.33±0.03^{b}	1.57	0.86
5% KCl	10.75±1.34^{a}	0.67±0.03^{ab}	8.71±0.02^{ab}	1.74	0.92
5% NaCl + KCl (2:3)	5.22±0.25^{a}	0.66±0.06^{ab}	9.27±0.35^{a}	0.59	0.83
Treatment 20+33 KHz	*E. coli* K12				
	δ ± SD	p ±SD	$Log_{10}N_0$ ±SD	RMSE	R^2
TSB	1.32±0.15^{a}	0.56±0.03^{a}	7.77±0.65^{ab}	0.70	0.77
5% NaCl	0.33±0.19^{a}	0.38±0.08^{a}	6.84±0.85^{b}	1.51	0.84
5% KCl	1.92±0.26^{a}	0.62±0.00^{a}	8.16±0.26^{ab}	1.55	0.92
5% NaCl + KCl (2:3)	0.91±0.69^{a}	0.53±0.09^{a}	8.42±0.20^{a}	0.87	0.84
	L. innocua				
TSB	1.01±0.03^{b}	0.49±0.08^{a}	8.60±0.00^{a}	0.38	0.84
5% NaCl	2.21±0.81^{ab}	0.56±0.05^{a}	7.94±0.68^{a}	1.00	0.90
5% KCl	9.46±1.61^{a}	1.03±0.28^{a}	8.59±0.03^{a}	1.03	0.96
5% NaCl + KCl (2:3)	5.72±2.23^{ab}	0.62±0.04^{a}	8.88±0.29^{a}	0.40	0.91

IV. CONCLUSION

Power ultrasound is a non–thermal processing technology with possible application in the meat manufacture industry in the production of reduced sodium products. Maintaining the safety of the product when lower salt levels are used is a high priority. The results of this study showed that power ultrasound, when used in combination with lower salts and salt mixtures, is an important hurdle technology with the potential to inactivate spoilage microorganisms during meat brining processing.