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Original Article

Effect of Household Cooking Methods on the Viability of *Bacillus* Probiotics Supplemented in Cooked Sausage

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ABSTRACT

Background and Objectives: The use of *Bacillus* probiotics is an interesting way for manufacturing healthier meat and meat products as functional foods and also a valuable solution for overcoming the constraints related to the stability of probiotic strains of *Lactobacillus* and *Bifidobacterium* during processing and storage of foods. This work is an attempt to investigate the influence of common household cooking methods on the viability of *Bacillus coagulans* and *Bacillus subtilis* as probiotic sporeformers inoculated into cooked sausages.

Materials and Methods: After preparation of the final suspensions of *Bacillus coagulans* ATCC 31284 and *Bacillus subtilis* var. *Natto* ATCC 15245 spores, they were supplemented in the sausage samples with different meat types and percentages. The inoculated cooked sausages were then submitted to common household cooking methods, i.e. boiling, microwaving, and deep fat frying. Enumeration of the studied spores was carried out on trypticase soy agar after exposure to a heat shock at 68°C for 20 min. One-way analysis of variance (ANOVA) of SPSS-20 was used for statistical analysis of the data. A Taguchi L_9 experimental design comprising 3 variables and 3 levels was performed using MINITAB-17 to determine main effects of the parameters of household cooking process.

Results: The results obtained in this study showed that boiling, followed by microwaving and deep fat frying, had the greatest viability and recovery of the studied spores supplemented in the cooked sausages, respectively ($P < 0.05$). Furthermore, results of the Taguchi experimental method revealed that the type of *Bacillus* probiotics, followed by the formulation of cooked sausages and the method of household cooking of cooked sausages had main effects on the concentrations of the spores examined, respectively.

Conclusions: The information provided here suggests that boiling as a common household cooking method retained maximum viability and recovery of the studied *Bacillus* probiotics supplemented in the cooked sausage samples. Besides, the type of spores was the principle parameter, which had a critical effect on the response (i.e. concentrations of the studied *Bacillus* probiotics).

Keywords: *Bacillus* probiotic, *B. coagulans*, *B. subtilis*, Cooked sausage, Household cooking method

Introduction

The development and consumption of probiotic food products is a growingly global consumer trend that has achieved a considerable remark in functional food market (1). Nonetheless, functional foods like food products containing probiotic microorganisms constitute an outstanding opportunity and a promising market for the meat industry with regard to improving the quality and developing healthier meat and meat products (2, 3). According to an expert panel commissioned by FAO and WHO, probiotics are “live microorganisms, which when administered in adequate amounts, confer a health benefit on the host”

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(4, 5). Many different microorganisms have been considered as probiotics, mostly species of *Lactobacillus* and *Bifidobacterium* as traditional probiotic bacteria, and some species of sporeforming *Bacillus* genus as novel probiotic bacteria (6, 7). According to Ashraf and Shah (8) and Jayamanne and Adams (9), populations of 10^6 CFU g^{-1} or ml^{-1} in the final product at the time of consumption, supposing a daily consumption of 100 g or mL, are established as a minimum daily therapeutic dose of probiotic cultures in processed foods, reaching 10^8 CFU, and hence, conferring health benefits to consumers.

The application of spores of *Bacillus* species as probiotic dietary supplements is expanding extensively and rather rapidly due to immune stimulation, antimicrobial activities, and competitive exclusion (10-12). Among the 100 known *Bacillus spp.*, only a few strains of some species (including *B. coagulans* and *B. subtilis* var. *Natto*) have been used as probiotics for human consumption (13, 14). Considering their spore-forming nature, probiotic *Bacillus* strains possess improved viability and survivability as well as probiotic properties during the harsh manufacturing processes (like cooking), the storage of the product, and finally, passage through the gastrointestinal tract as compared to other probiotic bacterial strains (6, 15-18). In other words, sporeforming probiotic bacteria could be considered as a valuable solution for overcoming the limitations related to the stability of probiotic strains of *Lactobacillus* and *Bifidobacterium* during processing and storage of foods (6, 7, 11, 12, 19). Apart from supporting probiotic microorganisms by clinical data to yield health benefits, this aspect is of great importance for factories and research centers interested in formulating products with probiotic microorganisms. Therefore, it seems that they represent an ideal choice for the development of functional cooked meat-based products, which constitute a very high proportion of the meat industry. On the other hand, the establishment of appropriate conditions for spore activation and recovery is highly important for proper assessment of the viability of probiotic cultures through the shelf life of food products and exposure to gastrointestinal conditions. Hence, heat shock and culture medium (in which spores are suspended) have a profound effect on the recovery and maximizing the germination rates of probiotic sporeforming bacteria (20-22).

Although thermal and cooking processing of meat and meat products seems not to cause reductions in the counts of bacterial spores, it may lead to sub-lethal injuries that may further reduce the resistance of spores in the gastrointestinal environment (23). Then the generation of this information is of major relevance for the development of probiotic foods considering that appropriate amounts of probiotic sporeforming bacteria can be added to the formulations to ensure health benefits. The household cooking practices being applied by the food handlers, either in homes or in fast food restaurants and catering centers, are generally based on personal preferences and convenience for handling and consuming foods (24). However, it appears that the most common methods of household cooking for cooked sausages are boiling, microwaving, and frying. Heating time, temperature, cooking method and, of course, meat product formulation are all important variables, which may influence on the viability and recovery of probiotic *Bacillus* in cooked sausages. Moreover, dielectric properties and thermal conductivity of foods like meat and meat products greatly affect the behavior of food during household cooking methods. It appears that thermal conductivity of foods relies largely on the amount and structuring of different components, especially water (25). In fact, the thermo-physical properties of the constituents of meat products (particularly meat as a main component) have an effect on the temperature profile and heating rate, and thereby the viability and recovery of the supplemented probiotic *Bacillus* spores (26). It is important to bear in mind that in conventional methods of home cooking including boiling and frying, energy is transferred to the material through convection, conduction, and radiation of heat from the surfaces of the material due to thermal gradients (26). On the contrary, microwave energy in cooking is delivered directly to materials through direct interaction of the electromagnetic field with the molecules of the target material (26). However, to our knowledge, the influence of household cooking methods on the viability and recovery of probiotic sporeformers in cooked food matrices has not yet been investigated. On the other hand, Taguchi method based on orthogonal arrays can be used to analyze a large number of variables with a few experimental trials (27). In fact, in contrast to other experimental design methods like full factorial, the Taguchi method as a simple and robust technique

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facilitates to identify the influence of process parameters and establish the relationship between variables and operational conditions, and significantly reduce the number of experimental arrays that may cause an increase in time and cost (28, 29). Accordingly, here, we aimed to evaluate the effect of different cooking methods, widely used in household processing (including boiling, microwaving, and frying), on the viability and recovery of probiotic *Bacillus* strains, i.e. *B. coagulans* ATCC 31284 and *B. subtilis* var. *Natto* ATCC 15245, which were inoculated into cooked sausages. In other words, this research, for the first time, attempts to determine the optimum home cooking procedure of cooked sausages that may result in the highest viability and recovery of the supplemented probiotic *Bacillus* strains. Finally, Taguchi experimental design with L_9 orthogonal array was used to explore the effects of the type of spore probiotics, the formulation of cooked sausages, and the method of household cooking of cooked sausages and their priorities on the viability of the studied probiotic *Bacillus* strains.

Materials and Methods

Materials and chemical reagents: All the chemical and reagents used for the study were of analytical grade and purchased from Merck (Darmstadt, Germany).

Strains: Commercial probiotic sporeforming bacteria strains of *Bacillus coagulans* ATCC 31284 and *Bacillus subtilis* var. *Natto* ATCC 15245 were obtained from Natures Only, INC. (USA), and World Intellectual Resource Co. (Taiwan), respectively (21, 30, 31).

Preparation of spores: Evaluation of the purity of the studied strains and their preparation were conducted by the method described by Alebouyeh et al. (32). After intermittent steps of washing with chemical reagents (including KCl/NaCl, Tris-HCl, SDS, Te buffer) and then sterile deionized water, followed by centrifugation (12000 × *g*, 15 min), the final suspensions obtained were diluted in defined amounts of sterile deionized water. The concentration of spores per mL of each spore suspension was verified using Neubauer chamber, approved via plate count (32), following storage at -70°C until analyzed.

Heat treatment procedure and culture medium: To have an accurate enumeration and the maximum recovery of the studied probiotic sporeforming bacteria, the influence of heat treatment (heat shock) condition and culture medium was also considered. According to the obtained results in our previous study, a heat shock at 68°C for 20 min and trypticase soy agar (TSA) medium yielded the highest viability and recovery of *B. coagulans* ATCC 31284 and *B. subtilis* var. *Natto* ATCC 15245 spores were chosen for this research (21).

Cooked sausage manufacture

Cooked sausage formulations: Cooked sausages were chosen as representative meat products because they account for a major part of the total consumption of processed meat products by the Iranian population. The cocktail sausages, containing 40, 55, and 70% meat contents, were manufactured in the pilot plant of Gooshtiran Factory of Meat Products, Tehran, Iran according to the industrial procedures. Frozen red meat packs (15% fat) were kept at 4°C for approximately 18 h for thawing. Fresh chicken meat (equal proportions of chicken breast and thigh) was used in the formulation of 55% cooked chicken sausage. The ingredients of the sausage mass and their relative proportions are given in Table 1.

Sausage preparation and inoculation with *Bacillus* probiotics: In practice, the control sausage sample (with no spore probiotic) was prepared as follows: the grounded minced meat (either red or white meat, depending upon the formulation) was transferred to a mini-cutter (Allen, K21 Ras 83132, Germany) with nitrite, sodium chloride (NaCl), and polyphosphate at low speed to extract salt soluble proteins after comminution (during 3 min, keeping the temperature below 12°C). Then one-half of the proposed water in the formulation (as ground ice) was added, and mixed thoroughly in the cutter at high speed. Consequently after lowering the temperature of the mixture about 1–2°C, vegetable oil was added, and mixing was continued until reaching the temperature up to 8°C. Afterwards, the remaining water (second half) and the rest of ingredients were incorporated, respectively, thoroughly mixed and chopped until the smooth distribution of all ingredients and the emulsion was obtained (batter) (for an additional 3 min, approximately). A digital thermometer (Kane-May, KM330, Harlow, Germany) was applied for monitoring and verifying the temperature of the emulsion, which should be maintained below 12°C during the preparation of batter.

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Table 1. The basic formulation of cooked sausage batter prepared for this study.

Ingredient	Amount (%)		
	40% cooked sausage	55% cooked sausage	70% cooked sausage
Minced meat[1]	40	55	70
Water (Ice)	22	20	13
Vegetable oil	19	10	4.5
Filler assortment [2]	12	8	5.5
Frozen garlic	1.8	1.8	1.8
Pasteurized whole egg	2.0	1.5	1.5
Salt (NaCl)	1.6	2.0	2.0
Polyphosphate	0.35	0.40	0.40
$NaNO_2$ (10% water solution)	0.012	0.012	0.012
Ascorbate	0.04	0.04	0.04
Sugar	0.20	0.25	0.25
Spice assortment	1	1	1

[1] Beef used for cooked sausage manufacture was topside and shin beef (15% fat). Chicken meat used for cooked chicken sausage production consisted of equal proportions of fresh chicken breast and thigh.
[2] It included wheat flour, wheat starch, gluten, and dried milk.

For production of inoculated formulations, spores of *B. coagulans* ATCC 31284 and *B. subtilis* var. *Natto* ATCC 15245 were individually inoculated at the start of cutter step after adding the first half of water and mixed at low speed for approximately 3 min in order to obtain a proper dispersion of the spores in the batter. The inoculum concentration of *B. coagulans* ATCC 31284 and *B. subtilis* var. *Natto* ATCC 15245 spores added to the cutter bowel was 12.88 and 12.96 log CFU/10 kg (or 8.88 and 8.96 log CFU/g), respectively. All batches after comminution/ chopping step were stuffed into artificial polyamide casings (24 mm diameter) (Arta, Tabriz, Iran) using a sausage stuffer (Handtman, Germany). The sausages were then cooked in a cooking cabinet with steam at 80±1°C (up to reaching the temperature of the geometric center to 73°C) for approximately 60 min. Subsequently, the cooked sausages were cooled down to about 25°C (room temperature) using cold water (12°C) for approximately 8–10 min. Next, the cooked sausages were stored at 4±1°C for 45-day storage time and until analysis. Two independent replicates of the experiment were carried out. The samples were randomly taken from each sausage batch at the 15th day of refrigerated storage time according to ISO 3100-1 (33).

Household cooking methods: To investigate the influence of household cooking methods on the viability of *B. coagulans* ATCC 31284 and *B. subtilis* var. *Natto* ATCC 15245 spores in the cooked sausages with different meat percentages and types, three common cooking methods were used: boiling, microwaving, and deep fat frying. Preliminary household cooking trials were done by an informal testing panel consisting of 4 trained panelists to determine cooking times and temperatures required to achieve a constant degree of color and texture for the various home cooking methods examined. Boiling was performed in a water bath with sausages with casings completely submerged for 5 min at 95±5°C. A domestic microwave oven (Samsung, model 9245, South Korea) with operating frequency of 2450MHz and an output power of 900W was used for the microwave cooking experiment. For the samples to be microwaved, after aseptically removed from the casings, they were placed in a sterilized glass dish, and cooking was carried out for a period of 1 min at the maximal power setting (900W). For the frying treatment, a deep fryer (Tefal, France) was set to 180±5ºC for 10 min to reach the test temperature. The samples were aseptically removed from their respective casings, placed in a fryer pot containing hot frying oil, and deep fried at 180±5°C for 3 min. After conducting the household cooking processes, the household-cooked sausages were cooled down for a few minutes at room temperature before being analyzed.

Enumeration of *Bacillus* probiotics in cooked sausage: The viable numbers of *B. coagulans* ATCC 31284 and *B. subtilis* var. *Natto* ATCC 15245 spores

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in the cooked sausages treated with different household cooking methods and also in the raw sausage sample (without household cooking) were determined by plate count on trypticase soy agar after exposure to a heat shock at 68°C for 20 min. The plates were then incubated at 37°C for 2 days, the numbers of colonies grown were counted, and the results were expressed as CFU/g (21).

Experimental deign: The Taguchi fractional factorial experimental design provides the optimal selection of parametric values with an efficient and systematic manner for conducting experiments (34). In this work, we applied experimental design by the Taguchi method for obtaining which parameter/s of the household cooking process had the highest impact on the viability and recovery of the studied probiotic *Bacillus* strains supplemented in the cooked sausages. Consequently, three experimental parameters including the type of spore probiotic, the formulation of cooked sausages, and the method of household cooking of cooked sausages with three levels were selected as the independent variables, and their values are summarized in Table 2. Next, the matrix was designed with the appropriate orthogonal arrays for the selected parameters and their levels. Taguchi method provides many standard orthogonal arrays and corresponding linear graphs for this purpose (34). In the present study, L_9 (3^4) orthogonal array (which indicated 9 experimental trials) with 3 columns and 9 rows was carried out to design experiment with selected parameters and levels. The experimental layout of L_9 orthogonal array is shown in Table 3. In order to analyze the results, it used a statistical measure of performance called *signal-to-noise* (S/N) ratio. Usually, three types of S/N ratio analysis are applicable: (1) smaller is better, (2) nominal is the best, and (3) higher is better (35). Since the objective of the present research work involves maximizing the viability of the studied probiotic *Bacillus* strains in cooked sausages, the S/N ratio for the case of *higher is better* was selected. Minitab-17 Statistical Software (Minitab Inc., State College, PA, USA) was specifically used for design of experiment analysis.

Statistical analysis: The obtained results were expressed as means±standard deviations of two separate determinations. Colony-forming units (CFUs) in all experiments were converted into $\log_{10}$ values before statistical analysis. All data were treated by one-way analysis of variance (ANOVA). Comparison among the treatment averages was done by post hoc Duncan's multiple's range test at the significance level of $P<0.05$, i.e., at a confidence level of 95% (36). All of the statistical analyses were carried out using SPSS software, version 20 (SPSS Inc, Chicago, IL, USA).

Table 2. Parameters and their levels employed in the Taguchi method

Parameter	Level 1	Level 2	Level 3
Sausage formulation	40% red meat	55% red meat	70% red meat
Spore probiotic	*B. coagulans*	*B. subtilis*	Control
Household cooking method [1]	BO	MW	DFF

[1] BO: boiling; MW: microwaving; DFF: deep fat frying.

Table 3. L_9 (3^4) orthogonal array of Taguchi experimental design and response

Experimental no.	Parameters and their levels			
	Sausage formulation	Spore probiotic	Household cooking method	Viable spore count (log CFU/g)
1	1	1	1	8.48
2	1	2	2	7.92
3	1	3	3	2.26
4	2	1	2	8.78
5	2	2	3	8.03
6	2	3	1	2.63
7	3	1	3	8.44
8	3	2	1	8.76
9	3	3	2	2.63

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Results

The results of viable counts of probiotic sporeforming bacteria inoculated into the cooked sausages after treating with household cooking methods are summarized in Table 4. As can be seen, there was no significant decline in the number of *B. coagulans* ATCC 31284 spores incorporated into the cooked sausages with 40% red meat after household cooking by boiling and microwaving ($P > 0.05$), whereas the highest viability of *B. subtilis* var. *Natto* ATCC 15245 spores inoculated into the cooked sausages with 40% red meat was obtained (8.74 log CFU/g) after submitting to boiling ($P < 0.05$). In the inoculated cooked sausages containing 55% red meat, boiling and microwaving resulted in more retention of *B. coagulans* ATCC 31284 spores (8.78 and 8.75 log CFU/g, respectively) compared with deep fat frying (8.68 log CFU/g), whereas the incorporated *B. subtilis* var. *Natto* ATCC 15245 spores had higher viability and recovery (8.95 log CFU/g) after submitting to boiling compared to the other home cooking methods examined ($P < 0.05$). The results also showed that the viable spore counts of *B. coagulans* ATCC 31284 inoculated into the cooked sausages with 70% red meat were significantly higher after submitting to boiling compared to the other studied household cooking methods. Besides, after household cooking through boiling and microwaving, *B. subtilis* var. *Natto* ATCC 15245 spores supplemented in the cooked sausages with 70% red meat showed the lowest losses (8.76 and 8.51 log CFU/g, respectively) ($P < 0.05$). Both probiotic strains of *B. coagulans* ATCC 31284 and *B. subtilis* var. *Natto* ATCC 15245 had the highest viability and recovery in the cooked sausages with 55% chicken after home cooking via boiling, followed by microwaving, and deep fat frying. In addition, enumeration of general (background) sporeformers in the control samples (with no incorporation of probiotic *Bacillus* spores) was insignificantly different in all home-cooked sausages except the cooked sausages with 55% chicken, compared to the raw sample. The viable counts of the spores of probiotic *Bacillus* strains inoculated into the cooked sausage samples involving more than 40% meat were still above the minimum daily therapeutic dose of spore probiotics (i.e. 10^6 CFU/g), regardless of home cooking methods. Fares et al. (37) also reported that cooking process caused a decline in the bacterial counts of *B. coagulans* GBI-30, 6086 that depended on the cooking time of the functional pasta, even though its amount in the cooked pasta (about 9.0 log CFU/100 g) would be considered sufficient to exert beneficial effects on consumer.

Table 4. Effect of household cooking methods on the viable spore counts (log CFU/g) of *B. coagulans* ATCC 31284 and *B. subtilis* var. *Natto* ATCC 15245 supplemented in cooked sausages[1]

Sausage type[2]	Treatment[3]	Household cooking type[4]			
		RA	BO	MW	DFF
40% red meat	*B. coagulans*	8.50±0.05^{A}	8.48±0.03^{A}	8.34±0.05^{A}	8.08±0.10^{B}
	B. subtilis	8.75±0.05^{A}	8.74±0.02^{A}	7.92±0.03^{B}	7.63±0.02^{C}
	Control	2.34±0.03^{A}	2.27±0.09^{A}	2.30±0.07^{A}	2.26±0.08^{A}
55% red meat	*B. coagulans*	8.85±0.08^{A}	8.78±0.02^{AB}	8.75±0.02^{AB}	8.68±0.06^{B}
	B. subtilis	9.45±0.07^{A}	8.95±0.09^{B}	8.16±0.08^{C}	8.03±0.09^{C}
	Control	2.65±0.05^{A}	2.63±0.09^{A}	2.64±0.11^{A}	2.60±0.08^{A}
55% chicken meat	*B. coagulans*	9.43±0.05^{A}	9.11±0.04^{B}	8.95±0.05^{C}	8.76±0.05^{D}
	B. subtilis	9.50±0.06^{A}	8.70±0.09^{B}	8.34±0.05^{C}	8.35±0.09^{C}
	Control	3.30±0.02^{A}	3.11±0.09^{B}	2.97±0.02^{B}	1.91±0.02^{C}
70% red meat	*B. coagulans*	8.77±0.06^{A}	8.63±0.04^{B}	8.40±0.06^{C}	8.44±0.07^{C}
	B. subtilis	9.46±0.09^{A}	8.76±0.08^{B}	8.51±0.09^{B}	8.04±0.09^{C}
	Control	2.65±0.05^{A}	2.64±0.09^{A}	2.63±0.10^{A}	2.60±0.08^{A}

[1] Each value represents the mean of two independent experiments (± SD). *B. coagulans* and *B. subtilis* spores were inoculated into the sausage batters at 12.88 and 12.96 log CFU/10Kg, respectively. Values were determined by plate count on trypticase soy agar after applying a heat shock at 68°C for 20 min.

[2] Sausages formulated with different types (red and chicken meats) and percentages (40, 55, and 70%) of meat.

[3] *B. coagulans*: sausage formulation containing *B. coagulans* ATCC 31284; *B. subtilis*: sausage formulation containing *B. subtilis* var. *Natto* ATCC 15245, Control: production control without inoculation of probiotic sporeforming bacteria.

[4] RA: raw; BO: boiling; MW: microwaving; DFF: deep fat frying. Boiling, microwaving and deep fat frying were done for 5 min at 95±5°C, for 1 min at the highest power setting (900W, at a frequency of 2450MHz), and for 3 min at 180±5°C, respectively. Raw sample as a control was related to cooked sausages on the 15th day of refrigerated (4 ±1°C) storage.

[A, D] Different capital letters in the same row denote significant differences ($P < 0.05$) among different household cooking methods for the same treatment.

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The main effects for S/N ratios of the three studied principal parameters at three levels are given in Table 5. As indicated, the type of spore probiotic (at levels 1 and 2; column 2) has the highest S/N ratio, followed by the formulation of cooked sausages, and the method of household cooking of cooked sausages. It is also obvious from Fig. 1 that *B. coagulans* ATCC 31284 spores compared to B. *subtilis* var. *Natto* ATCC 15245 spores and sausage formulations with higher meat contents as well as boiling compared to other home cooking methods were more effective in obtaining the maximum viability and recovery of the studied probiotic sporeformers, respectively.

Table 5. S/N ratio at different levels of different parameters and delta values of the household cooking process

Level	Sausage formulation	Spore probiotic	Household cooking method
1	14.54	18.65	15.27
2	15.12	18.31	15.08
3	15.26	7.96	14.57
Delta[1]	0.72	10.69	0.70
Rank	2	1	3

[1] The difference of the values between the highest and lowest values of parameters, respectively.

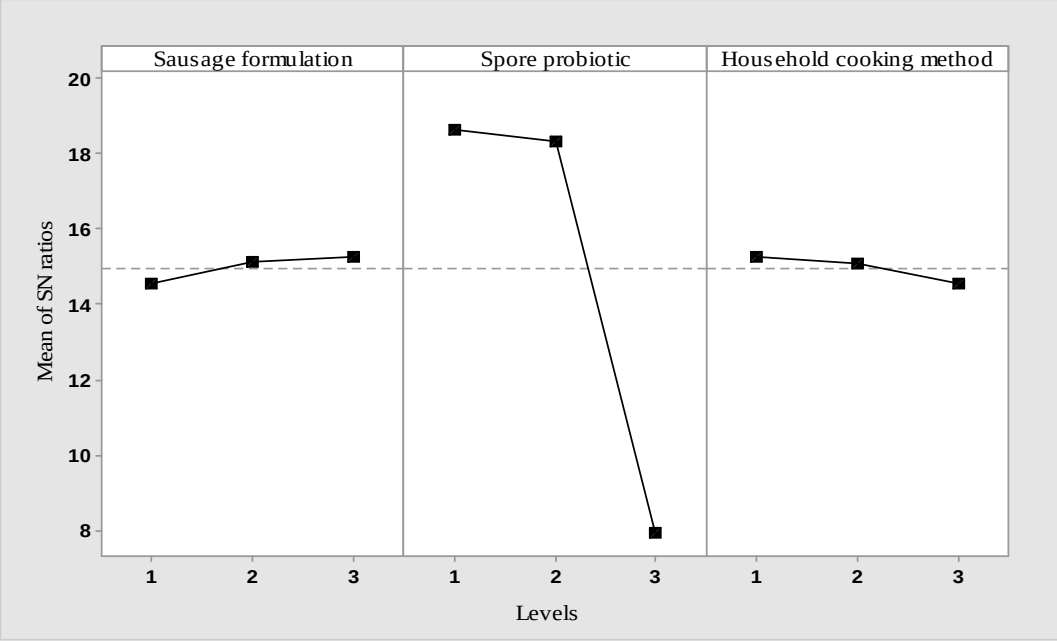


Figure 1. S/N ratios for main effects for all the three investigated parameters of the household cooking process.

Discussion

It is evident that several factors including meat sources in sausage manufacturing, presence and amount of different ingredients, especially those implicating spore germination and outgrowth, and time-temperature combinations of household cookings are all effective on the viability and recovery of probiotic *Bacillus* spores (38). Although moisture content is dominant, both ash and protein contents can affect thermal diffusivity and dielectric properties of cooked sausages (25). The results of the present study showed that except for final home cooking temperature, additional parameters such as the home cooking method (i.e. describes the rate of heat transfer to the interior of the sausages) may also affect the survival of probiotic *Bacillus* spores in cooked sausages. Furthermore, depending on the spore probiotic and sausage formulation, home cooking through boiling produced the lowest loss of the studied spores, while deep fat frying led to the greatest loss; in general, microwave occupied an intermediate position. It can be speculated that this higher viability in the number of CFU of the studied *Bacillus* spores in boiling could be related to remaining sausage casings and less thermal severity of this home cooking method compared to others. It is important to bear in mind that boiling will not give

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the sausages a crisp exterior; rather it will preserve the moisture and fat content within them. This makes boiling a good choice for home cooking of cooked sausage. Since the thermal conductivity and diffusivity of chicken meat are slightly higher than those of red meat (39), it is expected that the studied spores in the inoculated sausage samples and general (background) sporeformers in the control samples have been subjected to higher degrees of thermal energy, and thereby, greater unusual loss of probiotic and general sporeformers in the cooked chicken sausages (with 55% chicken meat), compared to others. Besides, more thermal energy intake in the chicken sausage caused more denaturation of its muscular proteins; this, in turn, affected its water holding capacity and the protective effect of meat product on the viability and recovery of probiotic *Bacillus* spores (40, 41). Taguchi DOE (Design of Experiment) provides an opportunity to understand the main effects of factors, which are important for process analysis. Based on the results obtained by using this method, it can be concluded that the type of probiotic B*acillus* spores, followed by the type of formulation of cooked sausages and the method of household cooking of cooked sausages, had significant influences on the response (viable spore counts of *B. coagulans* ATCC 31284 and *B. subtilis* var. *Natto* ATCC 15245), respectively (Table 5). It should be noted that there was a slight difference between the two types of probiotic B*acillus* spores studied in terms of affecting the response (Fig. 1). The heat resistance of a spore can be related to two components: a) the intrinsic stability of the cellular components in vegetative cells, and b) the amount of additional stability achieved in the spore state (42). The facultative thermophilic status of *B. coagulans* makes it considerably more heat-resistant compared to *Bacillus subtilis* growing in the mesophilic temperature range (43). The results also revealed that the cooked sausages with higher meat contents (preferably 70% meat content) and home cooking by boiling had the greatest effect on the viability and recovery of the studied probiotic *Bacillus* strains, respectively. As mentioned before, to obtain a proper homogeneity of the studied *Bacillus* spores, they were incorporated at the beginning of cutterization (or chopping) process in the sausage manufacture. Therefore, meat as a main component in the cooked sausage batter caused the attachment (or adhesion) of the studied bacterial spores to it. It can be speculated that sausage formulations with higher meat contents resulted in more extraction of salt soluble meat proteins in the presence of added salt and phosphate. This, in turn, led to more stabilization of the studied spores within the cooked sausage batter.

Conclusion

The use of bacterial sporeformers as probiotics is a growing trend in the food market. The results of the study serve as a database providing information on the effects of different home cooking methods on the viability and recovery of probiotic *Bacillus* spores, and might encourage the food industry to recommend boiling as the cooking method of choice to help maintain the survivability of spore probiotics supplemented in cooked sausages. In addition, the results of this study indicated the ability of probiotic sporeformers for sustaining their life within various home cooking methods; properties that cannot be ensured with vegetative probiotic bacteria, confirming their capacity for usage, especially in functional cooked food products.

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