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A Study on the Qualitative Characteristics and Microbial Survival of *Lactobacillus acidophilus* and *Bacillus coagulans* in Probiotic Bread

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Abstract

Nowadays, the role of nutrition in the health of gastrointestinal system is deemed as a key factor in the production of functional foods with probiotic bacteria especially in the bakery industry. Therefore, microencapsulation of probiotics and their survival during baking and gastrointestinal aggregation have positive physiology effects on consumers. The aim of the research was to study qualitative characteristics and microbial survival of probiotic bread produced with 1, 1.5 and 2% encapsulated microbial suspension along with edible starch 5% during 48 h. The results of this assessment showed that the time of storage reduced microbial population of the probiotic bread in such way that the population decline in the 1% suspension was more severe. Also, in both samples containing different microbial suspensions, the specific volume decreased while the porosity increased within 48 ($P \leq 0.05$), compared to the control. Whereas, hardness of probiotic bread increased after of 24 h which was less than the control sample ($P \leq 0.05$). Moreover, the analysis of variance and mean comparison of colorimetric test showed a significant effect ($P \leq 0.05$) on the color indexes of (L^* , a^* and b^*) after 48 hours.

Keywords: *Bacillus Coagulans*, Encapsulation, *Lactobacillus Acidophilus*, Microbial Survival, Probiotic Bread

Introduction

Bread is a staple food in many countries, since it constitutes an important source of complex carbohydrates- proteins, minerals and vitamins Besides, about 60-65 % of calories and protein, 2-3 grams of mineral material and a large portion of salt by eating bread is provided in Iran. Nowadays, the role of nutrition in the health of gastrointestinal system is deemed as a key factor in the production of functional foods with probiotics bacteria especially in the bakery industry. Probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit to the consumers. Various species of genera *Lactobacillus* and *Bifidobacterium* have been used as probiotics. Functional breads containing viable microorganisms have not been developed yet, due to the high temperature reached during

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baking (Altamirano-Fortoul *et al.*, 2012). The survival and efficiency of probiotic bacteria is influenced by several factors such as temperature, osmotic condition, freezing, drying, processing, bile and enzymes of digestive system, and water activity. Encapsulation technique is considered as the technology of incorporating protective materials into small capsules that can be released at a controlled rate under specific conditions. Therefore, microencapsulation probiotics survival during baking and gastrointestinal aggregation have positive physiology effects on consumers, and one of the newest ways for the ability to tolerate food samples encapsulated in the simulated gastrointestinal conditions (Zuidam & Nedovic, 2010). The aim of the research was to study qualitative characteristics and determine the viability of different types of functional coatings applied onto the surface of partially baked breads after full baking step. The survival of microorganisms (*Lactobacilluse acidophilus* and *Bacillus coagulans*) was assessed after baking and after a short storage (48 h). Also, the bread produced after baking during storage (2, 24 and 48 h) had porosity and specific volume and properties of bread texture were evaluated after 24 h.

Material and methods

In this study, *L. acidophilus* PTCC 1643 (Persian Type Culture Collection) and *B. coagulans* IBRC M-10791 T4 were purchased from Iran Scientific and Industrial Organization and Iranian Biological Resource Center respectively. Lyophilized cells were transferred into the 5 ml de Man, Rogosa and Sharpe (MRS) broth and incubated for 24 h under aerobic condition at 37 °C. One litre of watery dispersions of encapsulating agents (biopolymer mixtures were prepared by dispersing WPC, CMC, Pectin, Inulin and Persian gum in order to obtain a suspension concentration of 16.455% w/w) was combined with the inoculum, previously adjusted to 5 McFarland standard of turbidity. This purpose of the research was to study qualitative characteristics and microbial survival of probiotic bread produced with microbial suspension encapsulated 1, 1.5 and 2% along with edible starch 5% during 48 h. After processing of probiotic breads with suspension encapsulated, the staling of these breads were also examined 2, 24 and 48 h after baking, based on crumb firmness (texture analysis), colour index (Image J method), amount of porosity (Image J method) and specific volume (Rapeseed displacement). Finally for statistical analysis a completely randomized design with factorial arrangement and 3 replications was used.

Results and discussion

The results of this assessment showed that the time of maintenance reduced the probiotic bread microbial population in such way that the population decline in the 1% suspension was more severe (Figure1 and 2).

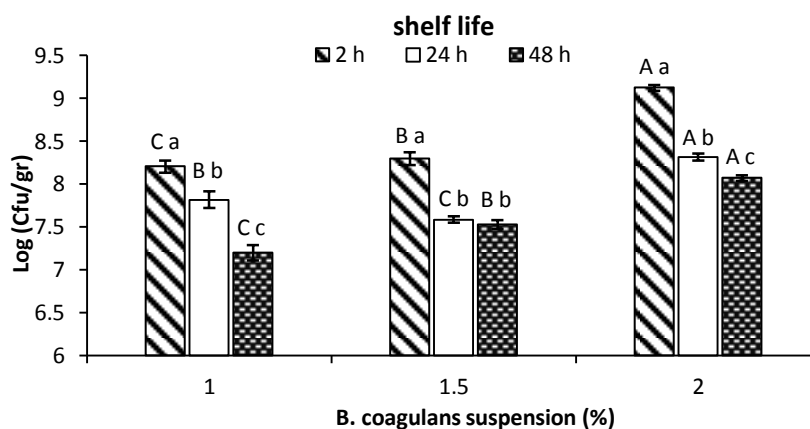


Figure 1. Results are expressed as mean values of triplicates \pm standard deviation of log cfu/gr *B. coagulans* suspension

A-B-C Different superscript letters denote significant differences during 48 h ($P < 0.05$).

a-b-c Different superscript letters denote significant differences at each suspension ($P < 0.05$).

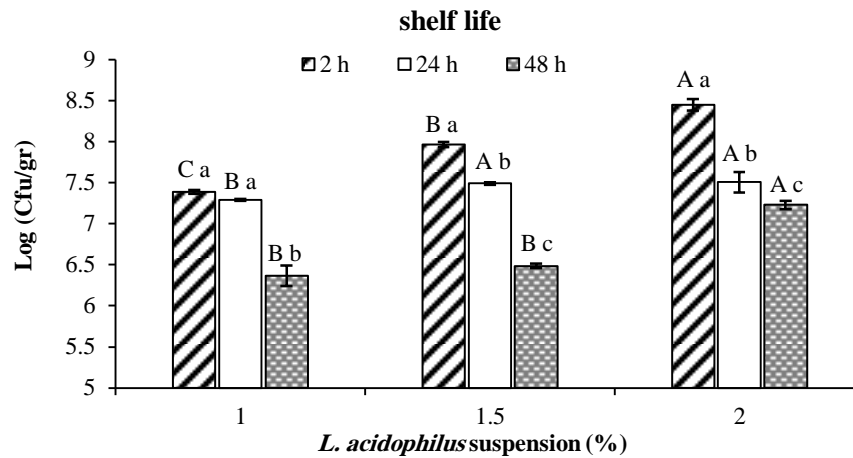


Figure 2. Results are expressed as mean values of triplicates \pm standard deviation of log cfu/gr *L. acidophilus* suspension

A-B-C Different superscript letters denote significant differences during 48 h ($P < 0.05$).

a-b-c Different superscript letters denote significant differences at each suspension ($P < 0.05$).

The analysis of variance and mean comparison of probiotic bread produced with microbial suspension encapsulated 1, 1.5 and 2 % along with edible starch 5 % during 48 h showed a significant difference. Also, specific volume and porosity of the bread with passage of time in both bread samples containing different microbial suspensions indicated trends of decrease and increase respectively compared to the control sample after 48 h ($P \leq 0.05$). The maximum and minimum amount of specific volume were observed in samples produced with suspension edible starch *L. acidophilus* and *B. coagulans* 2%, 2 h and 1%, 48 h after baking respectively (Figure 3 nad 4).

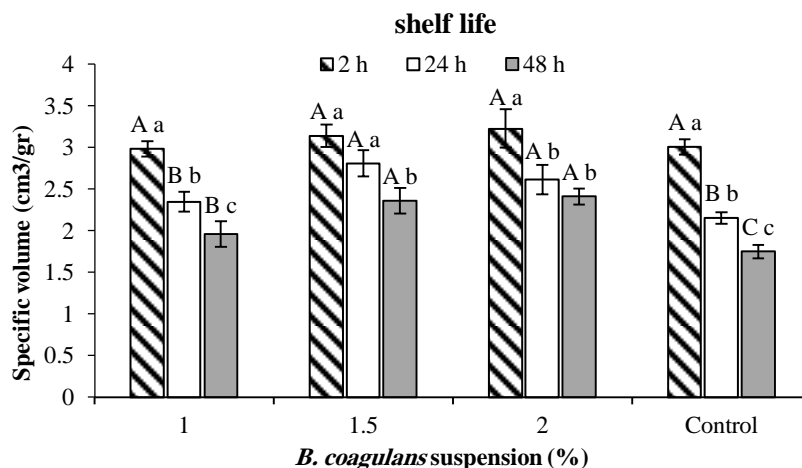


Figure 3. Results are expressed as mean values of triplicates \pm standard deviation of specific volume *B. coagulans* suspension

A-B-C Different superscript letters denote significant differences during 48 h ($P < 0.05$).

a-b-c Different superscript letters denote significant differences at each suspension ($P < 0.05$).

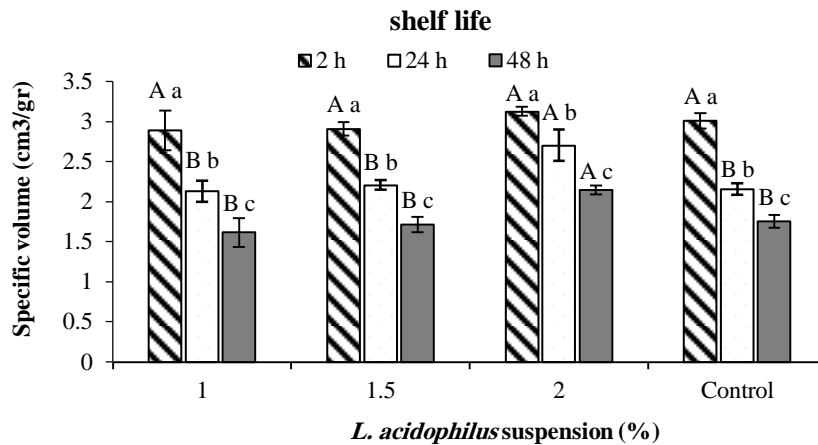


Figure 4. Results are expressed as mean values of triplicates ± standard deviation of specific volume *L. acidophilus* suspension
^{A-B-C} Different superscript letters denote significant differences during 48 h ($P < 0.05$).
^{a-b-c} Different superscript letters denote significant differences at each suspension ($P < 0.05$).

Also, hardness represents the applied force for biting the samples. According to this hardness, probiotic bread increased after of 24 h which was less than the control sample ($P \leq 0.05$). The maximum amount of crumb firmness was observed in samples produced with 1.5% and 2 % suspension edible starch *L. acidophilus* and *B. coagulans* respectively after of 24 h (Figure 5 and 6). Moreover, the analysis of variance and comparison of mean colorimetric test of probiotic bread crust showed significant effect ($P \leq 0.05$) on the colour index (L^* , a^* and b^*) bread was after 48 h (Table 1).

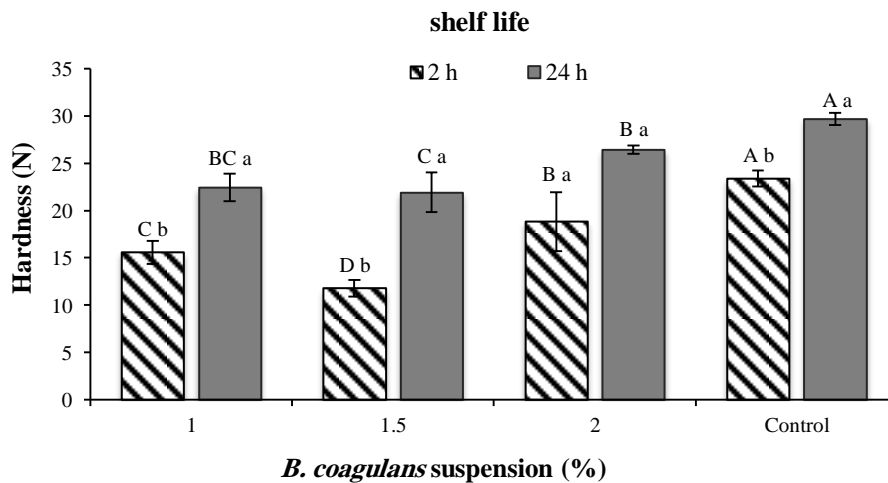


Figure 5. Results are expressed as mean values of triplicates ± standard deviation of hardness *B. coagulans* suspension
^{A-B-C} Different superscript letters denote significant differences during 24 h ($P < 0.05$).
^{a-b-c} Different superscript letters denote significant differences at each suspension ($P < 0.05$).

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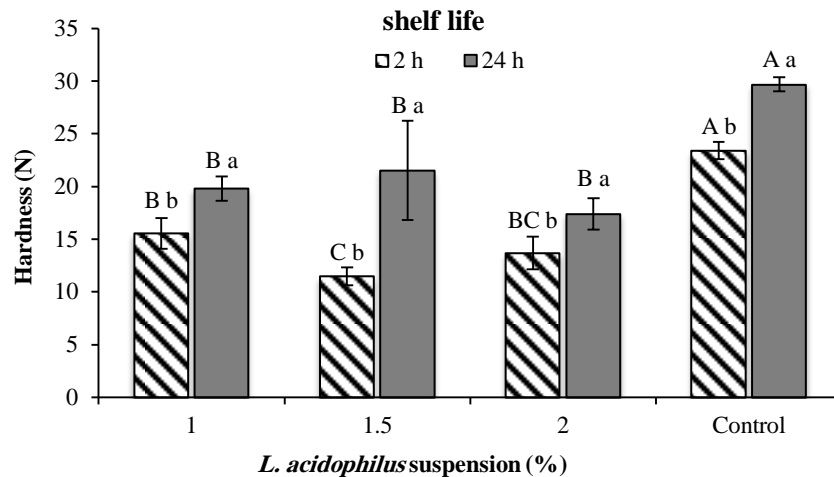


Figure 6. Results are expressed as mean values of triplicates \pm standard deviation of hardness *L. acidophilus* suspension

^{A-B-C} Different superscript letters denote significant differences during 24 h ($P < 0.05$).

^{a-b-c} Different superscript letters denote significant differences at each suspension ($P < 0.05$).

Table 1. The result of colorimetric test probiotic bread in during storage

Bacteri type	Suspension %	Shelf life	The colorimetric test on the bread crust			
			Index L*	Index a *	Index b *	ΔE
control	0	2	48.32 \pm 0.68 ^{cd}	10.56 \pm 0.56 ^b	20.79 \pm 1.96 ^b	-
control	0	24	49.54 \pm 0.51 ^{cd}	11.2 \pm 0.2 ^{ab}	22.43 \pm 1.24 ^{ab}	-
control	0	48	51.81 \pm 1.3 ^{bc}	11.9 \pm 1.4 ^{ab}	23.13 \pm 2.11 ^a	-
<i>L. acidophilus</i> (1)		2	54.32 \pm 1.2 ^b	10.59 \pm 1.06 ^{bc}	20.46 \pm 0.98 ^b	5.98 \pm 1.47 ^{bc}
1	1	24	56.27 \pm 2.84 ^{ab}	11.4 \pm 0.81 ^{ab}	21.85 \pm 1.04 ^{ab}	7.57 \pm 3.44 ^{ab}
1		48	58.61 \pm 2.24 ^a	12.11 \pm 1.006 ^a	21.82 \pm 1.51 ^{ab}	10.30 \pm 3.06 ^a
1	1.5	2	45.27 \pm 1.2 ^d	9.41 \pm 0.98 ^a	19.76 \pm 1.49 ^{bc}	4.63 \pm 2.32 ^c
1		24	54.45 \pm 1.13 ^b	11.38 \pm 1.42 ^{ab}	19.99 \pm 3.04 ^{bc}	5.80 \pm 0.62 ^{bc}
1	1.5	48	56.01 \pm 1.39 ^{ab}	11.24 \pm 0.47 ^{ab}	20.73 \pm 1.01 ^b	7.17 \pm 1.76 ^b
1		2	46.64 \pm 1.85 ^{cd}	9.27 \pm 1.92 ^c	21.43 \pm 3.05 ^{ab}	3.18 \pm 1.63 ^{cd}
1	2	24	52.62 \pm 1.82 ^{bc}	11.37 \pm 1.40 ^{ab}	18.71 \pm 3.06 ^b	4.77 \pm 1.76 ^{cd}
1		48	50.13 \pm 2.05 ^c	11.63 \pm 1.5 ^{ab}	20.76 \pm 3.70 ^b	2.91 \pm 1.82 ^d
<i>B. coagulans</i> (2)		2	46.18 \pm 0.99 ^{cd}	10.36 \pm 0.75 ^{bc}	20.75 \pm 1.47 ^b	1.04 \pm 0.33 ^e
2	1	24	52.6 \pm 1.45 ^{bc}	11.54 \pm 0.92 ^{ab}	23.42 \pm 1.03 ^a	7.34 \pm 2.08 ^{ab}
2		48	54.32 \pm 1.35 ^b	12.08 \pm 0.57 ^a	23.15 \pm 1.61 ^a	9.06 \pm 1.66 ^a
2	1.5	2	40.22 \pm 1.15 ^e	9.40 \pm 0.37 ^c	20.35 \pm 0.83 ^b	6.16 \pm 1.21 ^c
2		24	48.58 \pm 0.51 ^{cd}	10.96 \pm 0.34 ^{ab}	21.53 \pm 0.60 ^{ab}	2.75 \pm 2.03 ^c
2	1.5	48	51.23 \pm 1.05 ^{bc}	11.77 \pm 0.76 ^c	23.28 \pm 0.61 ^a	6.03 \pm 2.56 ^c
2		2	39.54 \pm 0.83 ^e	9.65 \pm 0.41 ^c	19.09 \pm 1.01 ^c	6.86 \pm 1.27 ^c
2	2	24	44.55 \pm 0.85 ^{de}	10.03 \pm 0.15 ^{bc}	21 \pm 0.99 ^{ab}	2.48 \pm 1.08 ^c
2		48	47.11 \pm 0.73 ^{cd}	10.84 \pm 0.06 ^{ab}	20.33 \pm 1.85 ^b	1.86 \pm 1.03 ^c

Conclusion

Overall results show that *L. acidophilus* and *B. coagulans* included in microcapsules can be incorporated to bread surface through edible coatings, leading to bread with similar characteristics to common bread, but with additional health benefits. In recent years, the purpose of authorities was in order to produce high-quality breads and the production of breads with a nutritional value beyond a standard bread. So, due to the use of this product in our country, the health of the community increases by producing bread with higher nutritional value.

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