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Prevention of mold spoilage and extend the shelf life of bakery products using modified mixed nanofermentate of *Lactobacillus* sp.

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Abstract

Fungal growth and mycotoxin production is the major economic importance in bakery products during manufacturing and storage. So, the present study aimed to prepare chitosan loaded mixed nano-fermentate of three *Lactobacillus* sp. [*L. plantarum*, *L. helveticus* and *L. rhamnosus* (PHR)] to be used as bio-preservatives in pan bread and cupcake. PHR-nanofermentate were applied in bread samples during mixing the ingredient, injected in bread loaves at the end of fermentation or added as coating materials after baking. Cupcake samples were treated by PHR-nanofermentate during mixing the ingredients or as coating materials after baking. Baking quality, color parameters, sensory properties and mold count of bread and cupcake samples were performed. Bread sample coated with PHR- nanofermentate after baking ranked the highest between the treated samples. Cake samples treated with PHR- nanofermentate during mixing step had higher scores for all sensory attributes compared to the coated cake, except the texture and appearance parameters. PHR-nanofermentate resulted in more effective disappeared mold counts at the end of shelf life for bread and cake. In conclusion, PHR-nanofermentate achieved the intended galas as antimicrobial agent and prolonged the shelf-life, regardless the addition method, in chemically leavened products. However, its addition method to bread and other baked products which utilize baker's yeast as a leavening should be considered. Finally, this study demonstrates that "PHR" is a good product for the bio-preservation of food.

Kywords: Bakery products; Lactobacillus sp; Nano preservatives; mold spoilage.

1. Introduction

Bakery products are popular foods, which represent a magnificent source of energy and various nutrients in the daily meals all over the world [1]. Also, baking industry is largest of the food sector. However, some of baked products such as bread and cakes are perishable foods. This is mainly due to the fact that these products are susceptible to microbial deteriorations [2]. Mold spoilage is the major cause of economic and safety detriments of bakery products due to the quality degradation and the potential health hazards of aflatoxins production [3-5]. There was therefore an urgent need to control the mold and fungal spoilage of bakery products.

Several chemical preservatives and physical methods have been implemented to extend the shelf life of bakery products [6]. Despite technology advances in existing preservation methods, fungal spoilage is still an issue for food manufacturers. These methods face several challenges such as health hazards of long-term consumption of chemical preservatives, maintaining the nutritional value and the cost [1, 7]. Consumer demands for high quality preservative-free foods triggered the interest of food manufactures towards bio-preservation techniques [8-10].

In this concern, several lactobacilli species and their metabolites have gained attention as antimicrobial bio-preservatives [11]. Lactic acid bacteria (LAB) are GRAS-certified (generally recognized as safe) regarding its extended historical utilization in fermented foods, particularly in baked goods [12]. The antifungal activity of lactobacilli is due to its ability to produce organic acids, bacteriocins, bioactive peptides and other compounds in the fermentation systems [13-15]. However, the presence of such compounds can affect the baker's yeast and the fermentation process of leavened dough [16]. Also, heat sensitivity of bio-preservatives is

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another limitation for its application in baked products.

Therefore, the on-going study of novel wholesome strategies for control delivery of bio-preservatives under harsh conditions is mandatory [17, 18]. Nanotechnology can be used in control delivery of antimicrobial compounds. Antimicrobial compounds can be attached to cores of nano-particles and delivered into microbial cells [19, 20] or incorporated into food wrapping or coating materials [21]. In this context, the current work aims to prepare mixed nano-fermentate of three *Lactobacillus* sp. [*L. plantarum*, *L. helveticus* and *L. rhamnosus* (PHR)] to be utilized as antifungal bio-preservatives in bakery products. The effect of bio-preservatives incorporation methods (flour mixing, injection after fermentation or coating the final products) on the quality parameters of bread and cake samples were evaluated.

2. Materials and Methods

Microbial Strains

Lactobacillus plantarum DSA 20174 is provided by Cairo MIRCEN, Faculty of Agriculture, Ain Shams University, Egypt; *Lactobacillus helveticus* CNRZ 32 Collection of dairy Microbiological Laboratory (supplemented from Centre National de Recherché Zootechnique, Jouy-en-Josas, France). *Lactobacillus rhamnosus* GG was supplemented by Afify *et al*. [22] from the collection of the Food sciences & Nutrition dept., NRC. *Aspergillus flavus* 3357 was provided by the Northern Regional Research Laboratory Illinois, USA (NRRL).

Production of modified mixed antimicrobials

Modified mixed antimicrobial PHR-nanofermente powder was prepared as described in our previous research [23].

Media and production of the fermentate

The composition of fermentation medium (g/L) was Yeast extract (5), Meat extract (8), Peptone (10), Triammonium citrate (2), Sodium acetate (5), $MgSO_4.7H_2O$ (0.2), $MnSO_4.4H_2O$ (0.05), K2HPO4 (2), Glucose (20), Tween 80 (1), Skimmed milk powder (11) and Distilled water (1L). Fermentation was conducted at 30 ˚C for 72 h using all of *Lactobacillus plantarum* DSA 20174, *Lactobacillus helveticus* CNRZ 32 and *Lactobacillus rhamnosus* GG. By the end of fermentation, the product was introduced to spray drying.

Spray drying of the fermentate

The produced fermentate was dried using a B-290 Mini Spray Dryer (Büchi Labortechnik AG, Flawil, Switzerland) at temperature $_{inlet}$ 130ºC and feeding rate 50% as programmed on the device. The produced powder was collected by means of vacuum at an aspirator rate of 50%.

Preparation of chitosan loaded-nanoparticles

Chitosan nanoparticles (Ch.-NPs) were prepared by dissolving 2 g of chitosan in 1% acetic acid solution. After complete dissolution, chitosan solution is added drop-wisely to the vigorously stirred Sodium Tri-polyphosphate (TPP) solution (0.03%). The resulted suspension was then subjected to sonication (sonication power, 750 Watts, frequency, 20 kHz and amplitude 50%, for 30 minutes at 25°C. Nanoparticles were stabilized by the addition of 0.4% Cetyltrimethylammonium bromide (CTAB) as a cationic surfactant. Nanoformulas were prepared by mixing one part of extract into two parts of both H_2O and Tween 80 [23].

Manufacture of pan bread

The bread dough was prepared following the procedure described by Soukoulis *et al*. [24] having the following composition (% on flour basis): strong wheat flour (100), water (60), lyophilized baker's yeast (2), crystalline sucrose (4), salt (1), and sunflower oil (3). The ingredients were mixed for 10 min at the lowest speed using a lab scale mixer. The dough was bulk proved for 80 min in a proving chamber at 40°C, 75% RH, divided into individual samples of 100 g, shaped, placed in aluminium pans and proved under the same conditions for 30 min. Then, the samples were baked in a preheated oven at 250 ˚C for 20 min implementing a steaming step for the first 7 min of baking. The bread loaves were taken off the pans, placed on a metallic rack to cool for 30 min.

Manufacture of cupcake

Cupcake samples were prepared according to the method described by Khalifa *et al*. [25] using wheat flour (250g), sugar (125g), whole egg (110g), shortening (53.5g), skimmed milk (25g), baking powder (12.5g), salt (3.5g) and vanillia (2g). The whole egg was whipped with vanillia, then sugar and skimmed milk was added and whipped. Baking powder was mixed with wheat flour and gradually added to the whipped mixture. This mixture was mixed until getting appropriate texture, then the dough was poured into paper cups and backed at 180˚C ± 5˚C for 30 - 35 min.

Application of PHR-nanofermentate treatments on pan bread and cupcake samples

Four groups of pan bread were manufactured and treated with PHR-nanofermentate as follow: the 1st group represented the control bread sample (without any treatment). The 2nd group of pan bread was prepared following the same procedure, while PHR-nanofermentate was added during mixing step (Treatment 1). The loaves of the 3rd group were

injected with the PHR-nanofermentate at the end of fermentation step (Treatment 2). The loaves of the 4th group were coated (after baking) with the PHR-nanofermentate (Treatment 3). Similarly, three groups of cupcake samples were prepared and treated being the control (without any treatment), Treatment 1 (PHR-nanofermentate was added during cake batter formation) and Treatment 2 (coated with PHR-nanofermentate after baking).

Baking qualities of pan bread and cupcake samples

Volume (cm^3) and weight (g) of pan bread and cupcake samples were estimated according to the method described in AACC [26]. Specific volume (g/cm^3) was calculated by dividing of the volume to weight.

Color attributes of pan bread and cupcake samples

The color parameters of pan bread and cupcake samples were evaluated using Hunter, Lab Scan XE, Reston VA., calibrated with a white standard tile of Hunter Lab color standard (LX No. 16379) x = 77.26, y = 81.94 and z = 88.14 (L*= 92.43, a*= -0.88, b*= 0.21). The results were expressed in accordance with the CIELAB system where: L (L = 0 [black], L = 100 [white]), a (-a = greenness, +a = redness), b* (-b = blueness, +b = yellowness).

Sensory evaluation

Sensory evaluation of bread samples was performed according to Kulp *et al.* [27] for symmetry of shape (5), crust color (10), break & shred (10), crumb texture (15), crumb color (10), aroma (20), taste (20) and mouth feel (10). While, sensory characteristics of cupcake samples including color (20), flavor (20), taste (20), texture (20), appearance (20) and overall acceptability (100) were evaluated according to the method described in AACC [26] by 10 semi-trained panelists

Estimation of PHR-nanofermentate antifungal activity in *A. flavus* spiked bread and cake models

Spiked pan bread and cupcake samples were prepared and manufactured following the above mentioned methods with addition of broth culture of *A. flavus* to provide approximately 10^3 CFU/g during mixing step. Also, spiked bread and cake samples were treated with PHR nano-fermentate as shown in these sections.

Determination of mold count

Mold counts were determined by pour plate technique using the media of acidified potato dextrose agar (Oxoid). The inoculated plates were incubated at 25 ˚C for 5-7 days. During the incubation period, the inoculated plates were examined daily for mold or yeast counts/g were calculated and recorded.

Statistical analysis

Statistical analysis was assessed using the Statistical Analysis Software System for Windows (SASS). The significant difference between the means value was determined by the analysis of variance (ANOVA), and Duncan's multiple range test was conducted at a significance level of $p<0.05$. All samples were analyzed in triplicates, and the results were expressed as means ± standard error.

3. Results

Effect of PHR-nanofermentate on the quality and shelf-life of pan bread and cup cake

Mold spoilage is a serious and costly problem for bakeries. To avoid spoilage by fungi, natural and chemical preservatives added to dough to delay spoilage. Three application methods were used to introduce the PHR-nanofermentate to pan bread and two for cupcake.

Baking quality of pan bread and cupcake samples

The results of baking quality of pan bread and cupcake samples were measured in weight, volume and specific volume as presented in Table (1). Results showed pronounced effects for PHR-nanofermentate incorporation on the baking quality of bread samples, while baking quality of cupcake samples were slightly affected by their incorporation. Baking weight of bread samples incorporated with PHR-nanofermentate at the end of fermentation and during mixing processes increased to reach 94.20±1.13 and 96.95±0.21 g, respectively when compared with the control sample (91.70±0.57 g). On contrast, the volume of these samples decreased to 269.50±2.12 and 191.15±1.63 cm^3, respectively compared to 318.25±4.60 cm^3 for the control sample. Consequently, both samples gained low specific volume (2.86±0.06 and 1.97±0.01) respectively as compared to the control (3.47±0.03). It is noteworthy that the backing weight and volume of cupcake samples varied in narrow range between 21.60±0.14-22.90±0.28 g and 49.05±0.64-51.20±0.28 cm^3, respectively.

Color of attributes of pan bread and cupcake samples

Lightness (L*), redness (a*) and yellowness (b*) parameters of the crust and crumb of bread as well as cupcake samples are shown in Table (2). Color analysis of the crust and crumb of bread and cupcake surface indicated that incorporation method of PHR-nanofermentate affected their L* values.

The crust of bread sample incorporated PHR-nanofermentate during the mixing step gained the highest L* value for crust (64.26±2.76) and crumb (79.40±3.27). Regarding the lightness of cupcake samples, the highest L* value (68.04±0.23) was recorded to cupcake sample incorporated with PHR-nanofermentate during mixing.

Also, bread sample incorporated with PHR-nanofermentate during mixing step had the lowest a* values for both crust and crumb being 11.15±0.46 and 1.99±0.18, respectively. The redness values of cupcake sample varied in narrow range from 17.73±0.11 to 18.86±0.18. Also, the crust of bread sample incorporated with PHR-nanofermentate during mixing step had the lowest b* value being 30.79±1.27for crust and 20.88±0.86 for crumb. Cupcake samples incorporated with PHR-nanofermentate during mixing and those coated with PHR-nanofermentate after baking showed higher b* values (51.57±0.18 and 45.38±0.24, respectively) compared to the control sample (44.15±0.15).

Sensory evaluation

Pan bread

The averages of 10 panel evaluation for each sensory attribute are shown in Table (3). Data in this table showed that the control bread sample gained the highest score for the evaluated parameters. Bread sample coated with PHR-nanofermentate after baking ranked the highest between the treated samples. Bread sample injected with PHR-nanofermentate at the end of fermentation process gained the lowest scores for symmetry of shape and crust color parameters. While, bread sample incorporated with PHR-nanofermentate during mixing step gained the lower scores for the other parameters.

Cupcake

The sensory evaluations of cupcake samples are shown in Table (4). Data showed that the overall acceptability ranked the cake samples in the order of control flowed by cupcake samples incorporated with PHR-nanofermentate during batter formation, while cupcake samples coated with PHR-nanofermentate gained the lowest score. The samples of cake which its batter formula mixed with PHR-nanofermentate had higher scores for all sensory attributes as compared with the cake which coated with PHR-nanofermentate, except the texture and appearance parameters.

Table 1**:** Baking quality of pan bread and cupcake sample as affected by PHR-nanofermentate treatment

Sample	Weight (g)	Volume (Cm^3)	Specific volume (g/cm^3)
	Pan bread		
Control	91.70±0.57	318.25±4.60	3.47±0.03
Treatment 1	96.95±0.21	191.15±1.63	1.97±0.01
Treatment 2	91.00±1.13	319.50±4.24	3.51±0.09
Treatment 3	94.20±1.13	269.50±2.12	2.86±0.06
	Cupcake		
Control	21.60±0.14	49.50±0.71	2.29±0.02
Treatment 1	22.90±0.28	49.05±0.64	2.14±0.00
Treatment 2	21.90±0.42	51.20±0.28	2.34±0.06

Treatment 1 = addition of PHR-nanofermentate during mixing, Treatment 2 = PHR-nanofermentate coated samples after baking, Treatment 3 = PHR-nanofermentate injected samples at the end of fermentation.

Table 2: Color properties of bakery product as affected by PHR-nanofermentate treatment

Sample	Lightness (L*)	Redness (a*)	Yellowness (b*)
	Pan bread crust		
Control	50.14±2.07	17.98±0.74	33.59±1.67
Treatment 1	64.26±2.76	11.15±0.46	30.79±1.27
Treatment 2	50.37±2.07	19.09±0.79	32.80±1.35
Treatment 3	57.63±2.37	12.42±0.84	34.36±1.42
	Pan bread crumb		
Control	75.32±3.10	3.98±0.16	26.06±1.07
Treatment 1	79.40±3.27	1.99±0.18	20.88±0.86
Treatment 3	75.42±3.11	3.87±0.21	26.03±1.12
Treatment 2	76.87±3.17	2.02±0.12	23.50±0.97
	Cupcake		
Control	64.68±0.22	18.57±0.12	44.15±0.15
Treatment 1	65.30±0.20	18.86±0.18	45.38±0.24
Treatment 2	68.04±0.23	17.73±0.11	51.57±0.18

Treatment 1 = addition of PHR-nanofermentate during mixing, Treatment 2 = PHR-nanofermentate coated samples after baking, Treatment 3 = PHR-nanofermentate injected samples at the end of fermentation.

Table 3: Organoleptic properties of pan bread sample as affected by PHR-nanofermentate treatment

Parameters	Control	*PHR-nanofermentate treated pan bread*		
		Treatment 1	Treatment 2	Treatment 3
Symmetry of shape (5)	$4.80^{a}\pm0.27$	$4.00^{b}\pm0.79$	$4.70^{a}\pm0.45$	$3.50^{b}\pm0.35$
Crust color(10)	$9.00^{a}\pm0.71$	$8.20^{a}\pm1.48$	$9.00^{a}\pm0.79$	$7.20^{b}\pm0.84$
Break & shared (10)	$9.00^{a}\pm1.00$	$7.20^{b}\pm1.64$	$9.10^{a}\pm0.89$	$8.80^{a}\pm1.30$
Crumb texture (15)	$14.40^{a}\pm0.89$	$10.80^{b}\pm1.30$	$14.00^{a}\pm1.00$	$13.00^{a}\pm1.58$
Crumb color (10)	$8.80^{a}\pm0.84$	$7.50^{a}\pm0.87$	$8.60^{a}\pm0.89$	$7.90^{a}\pm0.74$
Aroma (20)	$18.60^{a}\pm1.67$	$15.20^{b}\pm3.13$	$17.20^{a}b\pm1.79$	$15.40^{ab}\pm2.19$
Taste (20)	$18.40^{a}\pm2.07$	$13.00^{b}\pm2.74$	$16.00^{a}b\pm2.92$	$15.60^{ab}\pm2.61$
Mouthfeel (10)	$9.20^{a}\pm0.84$	$6.80^{a}\pm1.79$	$7.90^{a}\pm1.34$	$7.50^{a}\pm1.58$

Treatment 1 = addition of PHR-nanofermentate during mixing, Treatment 2 = PHR-nanofermentate coated samples after baking, Treatment 3 = PHR-nanofermentate injected samples at the end of fermentation.
Means in the same raw followed by the same letters are not significantly deferent

Table 4: Organoleptic properties of cupcake sample as affected by PHR-nanofermentate treatment

Sample	Color (20)	Taste (20)	Odor (20)	Texture (20)	Appearance (20)	Overall acceptability (100)
Control	$18.6^{a}\pm1.14$	$18.8^{a}\pm1.30$	$18.0^{a}\pm2.55$	$18.4^{a}\pm2.07$	$18.2^{a}\pm2.17$	$92.0^{a}\pm9.14$
Treatment 1	$18.0^{a}\pm1.87$	$10.0^{a}\pm4.85$	$11.4^{a}\pm4.93$	$18.8^{a}\pm0.84$	$17.2^{a}\pm0.84$	$75.4^{a}\pm8.53$
Treatment 2	$17.2^{a}\pm1.48$	$18.4^{b}\pm1.14$	$17.4^{b}\pm2.70$	$15.8^{a}\pm1.92$	$15.4^{a}\pm2.51$	$84.2^{b}\pm7.66$

Treatment 1 = addition of PHR-nanofermentate during mixing, Treatment 2 = PHR-nanofermentate coated samples after baking. Means in the same column followed by the same letters are not significantly deferent

The inhibitory effect of PHR-nanofermentate on mold in spiked pan bread and cup cake

From the results gained and summarized in Table (5), the initial mold count of the control bread, at zero time, was 4 x 10^2 CFU/g. Then, the mold count of stored bread started to increase gradually till reach 7 x 10^5 CFU/g by the end of the 5th day, while in all pan bread samples treated with PHR-nanofermentate, the mold was not detected during storage period.

Table 5: Mold count of pan bread during storage period (CFU/g)

Storage period	**Control**	***PHR-nanofermentate treated bread***		
		Treat 1	**Treat 2**	**Treat 3**
Zero time	4 x 10^2	Nil	Nil	Nil
2 days	1 x 10^3	Nil	Nil	Nil
5 days	7 x 10^5	Nil	Nil	Nil

Treat 1 = addition of PHR-nanofermentate during mixing, Treat 2 = PHR-nanofermentate coated samples after baking, Treat 3 = PHR-nanofermentate injected samples at the end of fermentation.

The results of antifungal effect of PHR-nanofermentate against mold in cupcake samples are shown in Table (6). The initial mold count of cupcake (control), at zero time, was 3 x 10^2 CFU/g. The count of mold after 5 days of storage started to increase gradually till reach 15 x 10^3 CFU/g by the end of 8 days. While, mold count disappeared in cupcake samples treated with PHR-nanofermentate over the storage time.

4. Discussion

Although baked products are subjected to heat during the baking process; however, loaves can be contaminated by molds during cooling, slicing, packaging and storage, as the environment inside a bakery is not sterile and is a likely source of contamination [28]. Therefore, the use of new natural antimicrobial compounds from microbial metabolites (bacteriocins, hydrogen peroxide, organic acids, etc.) as alternative bio-preservatives in the maintenance of foods is considered healthy. The Lactobacillus extracts, particularly the mixed-based, are considered a good tool for controlling or managing pathogenic and food spoilage microorganisms. We hypothesized that the improvement of the antifungal activity of combinations consisting of *Lb. plantarum* combined with *Lb. helveticus* and *Lb. rhamnosus* GG observed in this study was due to a higher quantity and diversity of the antifungal compounds produced compared to single strains, because it is known that antifungal compounds act in synergism and that Lactobacillus spp. produce various antifungal compounds [29-31].

Table 6: Mold count of cupcake during storage period (CFU/g)

Storage period	**Control**	***PHR-nanofermentate treated cake***	
		Treatment 1	**Treatment 2**
Zero time	3 x 10^2	Nil	Nil
5 days	11 x 10^3	Nil	Nil
8 days	15 x 10^3	Nil	Nil

Treatment 1 = addition of PHR-nanofermentate during mixing, Treatment 2 = PHR-nanofermentate coated samples after baking,

Our previous results [23] showed that mixed *Lb. plantarum, Lb. helveticus* and *Lb. rhamnosus* extracts contain active compounds, with a potential antimicrobial application, besides lactic acid,

includes 2-Hydroxyisocaproic acid, derivative, and 3-Phenyllactic acid, a derivative, Pyrrolo [1, 2-a] pyrazine-1, 4-dione, hexahydro-3-(2-methylpropyl) and a 9-Octadecenoic acid (2-phenyl-1, 3-dioxolan-4-yl) methyl ester trans. However, the integration of these compounds as an ingredient in fermented dough will undoubtedly affect baker's yeast activity and the fermentation process, consequently the porosity crumb structure of the product [16]. The results concerning the effects of PHR-nanofermentate addition at different manufacture steps on baking quality of bread samples (Table 1) showed pronounced differences. The specific volume of bread samples incorporated PHR-nanofermentate as an ingredient during mixing step gained the lowest value followed by those injected at the end of fermentation step. This could be due to the effect of PHR-nanofermentate on baker's yeast activity. Similarly, Pattison & von Holy [16] found a reduction in the activity of baker's yeast up to 34.4% in the presence of several bio-preservatives.

Regarding the color parameters of bread and cake samples, addition of PHR-nanofermentate as an ingredient during mixing and at the end of fermentation process resulted in bread with lighter crust and crumb (higher L* and lower a* values). This also may be due to the effect of PHR-nanofermentate on baker's yeast activity that responsible for carbohydrates metabolism and production of milliard reaction substrates. Similar results were reported in a previous study by Illueca *et al.* [32]. Despite this, bread samples coated with PHR-nanofermentate were visually the closest to the control bread. On contrary, the color parameters of cupcake samples incorporated with PHR-nanofermentate during batter formation were comparable to the control cake. By comparing the three methods of PHR-nanofermentate application and their effect on the organoleptic properties, bread coating and its incorporation during batter formation was more acceptable by the panelists. Furthermore, the quality properties of the resulting bread and cake samples were comparable to those of the control.

On the other hand, the antifungal effect of PHR-nanofermentate against mold in pan bread and cupcake are stated in Tables 5 & 6. Several researchers explained the antifungal compounds, whereas both *Lb. helveticus* and *Lb. rhamnosus* GG with their based-combinations possess the production of Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- which is known as a strong antifungal agent [33, 34]. Mu *et al*. [35] detected 3-Phenyllactic acid and derivatives within extracts of *Lb. plantarum*, *Lb.rhamnosus*, and *Lb. reuteri*. This compound was applied as a high potential antibacterial and antifungal activity. The considerable potential of *Lb. plantarum* as both antibacterial and antifungal can be attributed to the co-production of 2-Hydroxyisocaproic acid and derivatives that are considered a great bactericidal and fungicidal compound [36, 37].

Moreover, 2-Hydroxyisocaproic acid and 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, trans- contained within Lactobacillus combinations may be responsible for their increased antimicrobial activity [38]. Nikolova *et al.* [39] investigated the inhibitory potential of *Lactobacillus helveticus* strain 50 p1, and results reflected a strong effect against clinical isolates like *Pseudomonas aeruginosa* and *Bacillus sp.* Furthermore, there is an agreement with the results of Gomez *et al.* [40]. They used a biofilm of mixed-lactic acid bacteria to retard the growth of different food-originated human pathogens, such as; *Salmonella typhimirium* and *E. coli* O157:H7.

Results reported by Lavermicocca *et al*. [41] and Gerez *et al.* [42] are in agreement with our previous results [23] on the production and effect of the phenyllactic acid obtained from *Lb. plantarum* to inhibit fungal strains**.** Valerio *et al*. [43] reported that *Leuconostoc citreum* was able to inhibit *Aspergillus niger*. Axel *et al*. [44] observed that the application of *Lactobacillus amylovorus* as an antifungal compound producing agent (carboxylic acids) extended the shelf life of bread. The Lactobacillus extracts, particularly the mixed-based, are considered a good tool for controlling or managing pathogenic and food spoilage microorganisms. The inhibitory effect can be magnified if used as Nano-formulated for food preservation and safety maintenance for both food and consumers. Several studies confirmed our current study and reported that the application of mixed-Lactobacilli as Nano-formulated stimulates the increase in their antimicrobial activity [45-47].

5. Conclusions

The use of natural products such as antimicrobial PHR-nanofermentate during bakery products manufacture, reduced the growth of molds and prolonged the shelf-life. The present study demonstrates that PHR-nanofermentate is a good product for the bio-preservation of food. The present work was conducted as a step to introduce an effective preservative to the field of bakery industries. Review the obtained results of quality parameters, sensory evaluation, and microbiological quality of bread and cake, the coating treatment and integration as an ingredient was appropriate for bread and cake, respectively.

6. Conflicts of interest

The authors declare no conflict of interest.

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