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Research Paper

Biotechnological Approach To Preserve Fresh Pasta Quality

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ABSTRACT

Fresh pasta is highly susceptible to microbial contamination because of its high water activity and nutrient content. In this study, a new biopreservation system was examined that consists of an active sodium alginate solution containing *Lactobacillus reuteri* and glycerol, which was added during the production process of pasta. Our aim was to extend the fresh pasta shelf life by the in situ production of reuterin, thereby avoiding the use of thermal treatments that generally compromise food sensory characteristics. Two experimental studies were carried out with the product packaged under either ordinary or modified atmospheric conditions. Microbiological and sensory quality indices were monitored to determine the effectiveness of biopreservation on product quality during storage. The use of the active solution with *L. reuteri* and glycerol during the production process of pasta improved both microbial and sensory quality, particularly when combined with modified atmosphere.

Key words: Biopreservation; Fresh pasta; *Lactobacillus reuteri*; Shelf life

Wheat-derived foods (e.g. pasta and bread) represent key diet constituents for many populations. In particular, pasta is largely consumed worldwide for its gastronomic and nutritive importance *(5)* because it contains significant amounts of complex carbohydrates, proteins, B vitamins, and iron. According to Italian legislation, "pasta" is defined as the product obtained by extrusion or lamination and successive drying (to 12.5% maximum water content) of dough made of durum wheat semolina and water. Pasta is made with flour and water, prepared with or without eggs. Several types of flour can be used to make pasta, such as semolina, farina, and wheat flour *(16)*. Fresh pasta is defined as a product with greater than 24% moisture and a water activity ranging from 0.92 to 0.99, thus requiring refrigeration *(9, 16)*. A high water activity makes this product vulnerable to several hazards, such as *Salmonella, Staphylococcus aureus,* and spoilage molds *(16)*. In this form, without any preservatives or reduced oxygen packaging to prevent microbial spoilage, pasta has a shelf life under refrigerated temperatures of only 2 to 3 days *(10)*. Pastas without any additives also show a pH drop (acidity also indicates microbial spoilage) and increase in coliforms *(12)*. The microbial quality in fresh pasta at the end of the production process is strictly related to the characteristics of the raw materials, such as durum semolina or alternative flours and water, and to the methods used to make pasta (homemade pasta, pilot plant, or industrial plant). Fresh pasta spoilage is mainly caused by the metabolic activity of bacteria that negatively influences the sensory characteristics of the product and consequently its shelf life *(9)*. Therefore, to ensure higher quality and hygienic characteristics, heat treatments are generally applied during the industrial process of pasta production. Among the preservation techniques, microwaves, convection heating, or convection heating in combination with microwaves represent efficient sanitization procedures. However, these types of thermal solutions generally compromise the sensory characteristics of fresh pasta. Different methods have been proposed to prolong the shelf life, with the most common approach being based on chemical preservatives to avoid microbial proliferation *(22)*. Nevertheless, the great demand for fresh-like products still promotes the search for new and more natural technologies to preserve foods. Different studies have been conducted on fresh pasta with the use of natural antimicrobial compounds such as thymol, chitosan, and grapefruit and lemon extracts *(9, 13, 14)*. One of the most recent potential approaches to prolong the shelf life of fresh products is the use of biopreservation systems. Biopreservation uses natural or controlled microbiota or antimicrobials as a way of preserving food and extending its shelf life *(1)*. Beneficial bacteria or their fermentation products are used in biopreservation to control spoilage and render pathogens inactive in food *(24)*. Lactic acid bacteria (LAB) have antagonistic properties that make them particularly useful as biopreservatives. *Lactobacillus reuteri* is a heterofermentative lactobacillus recognized as a normal inhabitant of human and animal guts *(19),* and it is accepted by the European Food Safety Authority as a food supplement and is widely used to improve gastrointestinal health.

Reuterin is an antimicrobial compound that is soluble in water, resistant to heat, and stable over a wide range of pH

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values, and it inactivates gram-negative and gram-positive bacteria *(23)*. Angiolillo et al. *(2)* optimized the conditions for in situ production of reuterin by means of a proper combination of *L. reuteri,* glycerol, and fermentation time, proving that *L. reuteri* could be used for the microbial preservation of Fiordilatte cheese. This study also showed that an active coating with *L. reuteri* and glycerol was effective for the improvement of microbiological and sensory quality of the cheese. They also used this biopreservation technique for fresh durum wheat semolina pasta. Another study reported on the application of modified atmosphere packaging (MAP) to improve fresh pasta microbial quality *(21)*; in particular, low O_2 and high CO_2 concentrations proved to be effective in limiting the development of microbial contamination *(25)*. Angiolillo et al. *(2)* also demonstrated that the combination of biopreservation and other preservation techniques such as MAP can effectively control spoilage bacteria and other pathogens and can inhibit the activities of a wide spectrum of organisms, including inherently resistant gram-negative bacteria. The literature testifies to the importance of LAB in cereal-based products *(20)*. In some cases, LAB have been added during pasta making, with the aim not to preserve the microbiological quality, but instead to have a final product with a low glycemic index *(15)* or high vitamin B_2 content *(4)*. Therefore, to the best of our knowledge, there are no studies on the use of LAB to preserve fresh pasta; in this sense, this is the first report on the direct exploitation of the biotechnological potential of LAB on fresh pasta quality.

On the basis of these considerations, we conducted two experimental studies. In the first study, water used for pasta making was substituted with an alginate active solution containing *L. reuteri* and glycerol (registered in the European Union as food additive E 422) and compared with control samples. In the second study, the active alginate solution during process was combined with MAP during packaging. In both studies, microbiological and sensory quality indices were monitored to determine the effectiveness of reuterin during fresh pasta storage.

MATERIALS AND METHODS

Raw materials and pasta production. Fresh pasta samples were produced with durum semolina provided by Mulini Tandoi (Corato, Bari, Italy). Semolina and distilled water (30%, v/w) were mixed to prepare pasta dough. The samples were prepared by a pilot plant with an extruder (60VR, Namad, Rome, Italy) equipped with a bronze head to give the pasta dough the shape of macaroni. The kneading time applied to produce fresh pasta was 20 min. To obtain pasta samples with the active solution, two different procedures were used. The first procedure involved the substitution of water with an active solution prepared 24 h before use that was obtained by dissolving sodium alginic acid (2%, w/v) in a solution made of 2% (w/v) pure freeze-dried *L. reuteri* (Granarolo, Bologna, Italy) (10^9 CFU/mL), 2.3% vegetal food grade glycerol (Perrin's, Bari, Itay), and distilled water. This procedure produced samples called ALG-PRO. The second procedure involved the substitution of water with a solution prepared by dissolving sodium alginic acid (2%, w/v; Farmalabor, Canosa di Puglia, Italy) in distilled water, producing samples called ALG. As a control, pasta samples with distilled water, called CNT samples, were also prepared. Pasta samples (ca. 200 g each) were arranged in antifog, high-barrier multilayer film made up of polyethylene terephthalate, ethylene-vinyl alcohol, and polyethylene, with a thickness of 90 μm (Di Mauro Officine Grafiche s.p.a., Napoli, Italy). All packaged samples were sealed using a thermal sealer (Gandus, Milan, Italy) under ordinary atmosphere (first experimental study) and modified atmosphere (second experimental study). The modified headspace conditions were 70% CO_2 and 30% N_2 gas concentrations. All the samples were stored at 4°C.

Quantification of reuterin. The assay for the 3-hydroxypropionaldehyde (3-HPA) content was based on a colorimetric method described in Cohen and Altshuller *(7)* that was developed for acrolein. 3-HPA was first dehydrated to acrolein that, in turn, reacted with 4-hexylresorcinol in the presence of $HgCl_2$ as catalyst to form a colored complex that absorbs light at 605 nm. In brief, 0.5 mL of saturated trichloroacetic acid, 0.012 mL of a 4-hexylresorcinol solution (50%, w/v, in ethanol), and 0.02 mL of an $HgCl_2$ solution (3%, w/v, in ethanol) were mixed with 0.5 mL of sample. The mixture was incubated at 60°C for 15 min and then allowed to cool down at 20°C for an additional 15 min. The absorbance was then immediately recorded at 605 nm. Samples were diluted to ensure absorbance readings below 0.85. Because 3-HPA is not commercially available, acrolein was used to standardize the assays (1 mol of 3-HPA dehydrates to 1 mol of acrolein). The standard curve was prepared in the 0.02 to 0.50 mM range ($R^2 = 0.999$), with a relative standard deviation ($n = 10$) of 2.1%.

The quantification of reuterin has been done both on the active solutions prepared with *L. reuteri* and glycerol (ALG-PRO) and on the control solution (ALG) consisting of sodium alginate without any additions. All analyses were performed in triplicate.

Microbiological analyses. For microbiological analyses, about 25 g of sample was aseptically removed from each package, placed in a stomacher bag, diluted with 0.9% NaCl solution, and homogenized with a stomacher LAB Blender 400 (Pbi International, Milan, Italy). Serial dilutions in sterile saline solution were plated onto appropriate media. The media and the conditions were as follows: plate count agar incubated at 30°C for 48 h for aerobic mesophilic bacteria and at 7°C for 10 days for psychrotrophic bacteria; Violet Red Bile agar incubated at 37°C for 24 h for total coliforms; Baird-Parker agar supplemented with egg yolk tellurite emulsion and incubated at 37°C for 48 h for *Staphylococcus* spp.; de Man Rogosa Sharpe agar added with 0.17 g/L cycloheximide (Sigma-Aldrich, Milan, Italy) and incubated at 30°C for 48 h for LAB; and Sabouraud dextrose agar added with 0.1 g/L chloramphenicol (C. Erba, Milan, Italy) and incubated at 25°C for 48 h for yeasts and 25°C for 5 days for molds. All media and supplements were from Oxoid (Milan, Italy). All microbiological analyses were performed twice on two different batches.

To quantitatively determine the microbial acceptability limit, the following equation, as modified by Gompertz, was fitted to the experimental data *(8, 12)*:

$$\log(N(t)) = \log(N_{\max}) - A \cdot \exp\left\{-\exp\left\{\left[(\mu_{\max} \cdot 2.71) \cdot \frac{\lambda - \mathrm{MAL}}{A}\right] + 1\right\}\right\} + A \cdot \exp\left\{-\exp\left\{\left[(\mu_{\max} \cdot 2.71) \cdot \frac{\lambda - t}{A}\right] + 1\right\}\right\} \quad (1)$$

where $N(t)$ is the viable cell concentration at time t, A is related to the difference between the decimal logarithm of maximum bacterial growth attained at the stationary phase and decimal logarithm of the initial value of cell concentration, μ_{max} is the maximal specific growth rate, λ is the lag time, N_{max} is the microbial threshold value, MAL is the microbiological acceptability limit (i.e., the time when $N(t)$ is equal to N_{max}), and t is the storage time.

A microbial load of 10^6 CFU/g for total mesophilic bacteria and 10^4 CFU/g for *Staphylococcus* spp. and coliforms was set as the threshold for microbial acceptability (N_{max}) *(17)*.

Sensory analysis. Both uncooked and cooked fresh pasta were subjected to a time-intensity descriptive sensory evaluation. All the uncooked samples were submitted in a single session to a panel of eight trained tasters for estimation of color, odor, and overall quality. In addition, adhesiveness, bulkiness, firmness, elasticity, color, odor, taste, and overall quality were evaluated on cooked pasta. Each unpackaged sample (ca. 200 g) was cooked in a cooker containing approximately 4,000 mL of tap water at 100°C. A 9-point hedonic rating scale, where 1 corresponded to "extremely unpleasant" and 9 to "extremely pleasant," was used to perform the panel test. A score of 5 was used as the threshold for product sensory shelf life *(11)*. The panelists were selected on the basis of their sensory skills (ability to accurately determine and communicate the sensory attributes appearance, odor, flavor, and texture of a food product) *(18)*. Before testing pasta, the panelists were trained in sensory vocabulary and identification of particular attributes by using samples of commercial pasta. The analyses were performed in isolated booths in a standard taste panel kitchen. To determine the sensory acceptability limit that represents the storage time to reach the sensory attribute threshold, the following modified version of the Gompertz equation was fitted to the experimental data *(8, 12)*:

$$SA(t) = SA_{min} - A^{SA} \cdot \exp\left\{-\exp\left\{\left[\left(\mu_{max}^{SA} \cdot 2.71\right) \cdot \frac{\lambda^{SA} - SAL}{A^{Q}}\right] + 1\right\}\right\} + A^{SA} \cdot \exp\left\{-\exp\left\{\left(\mu_{max}^{SA} \cdot 2.71\right)\left[\frac{\lambda - t}{A^{SA}}\right] + 1\right\}\right\} \quad (2)$$

where $SA(t)$ is the sensory attribute at time t, SA_{min} is the sensory attribute threshold value, A is related to the difference between the sensory attribute attained at the stationary phase and the initial value of sensory attribute, μ_{max} is the maximal rate at which $SA(t)$ decreases, λ is the lag time, SAL is the sensory acceptability limit (i.e., the time at which $SA(t)$ is equal to SA_{min}), and t is the storage time. As reported above, the value of SA_{min} was set to 5.

Statistical analysis. The values of MAL, sensory acceptability limit, and shelf life of all the investigated samples were compared by one-way analysis of variance. A Duncan's multiple range test, with the option of homogeneous groups ($P < 0.05$), was used to determine significance among differences by using Statistica 7.1 for Windows (StatSoft Inc., Tulsa, OK).

RESULTS AND DISCUSSION

In this study, the ability of a sodium alginate solution with *L. reuteri* and glycerol to preserve fresh pasta by in situ production of the antimicrobial reuterin was investigated. The classical process adopted for fresh pasta was properly modified to add an active solution to pasta dough during the pasta-making process. Specifically, the water used to obtain the dough was substituted with an active sodium alginate solution. Once antimicrobial activity was detected, the active alginate solution was combined with MAP to further promote the quality improvement of fresh pasta.

Quantification of reuterin. The ALG-PRO solution displayed a reuterin content of 0.49 g/L, whereas the ALG solution without *L. reuteri* and glycerol did not show any reuterin content, as expected. This result demonstrates that *L. reuteri* was able to ferment glycerol and produce the antimicrobial compound responsible for the pasta preservation, as reported below.

Quality of fresh pasta. Tables 1 and 2 show microbial populations recorded in fresh pasta for both experimental studies. Samples were analyzed until day 8 under ordinary atmosphere and until day 15 under MAP. Coliforms and yeasts did not represent a problem in either study. No detectable coliform growth in the samples was probably because of the good hygienic process conditions; however, yeasts remained at low concentrations. Specifically, during the first study (Table 1), their concentration was between 3.39 and 5.20 log CFU/g for the CNT sample and ~4 log CFU/g for the ALG sample. The ALG-PRO sample did not show any growth until day 8 under ordinary atmosphere and until day 15 under MAP. The other cause of fresh pasta rejection is determined by the development of molds because of the high water content of pasta. During ordinary atmosphere storage (Table 1), no molds were detected in any samples, whereas during MAP storage (Table 2), molds started to grow from day 9 only in the two no active solution samples; in the active samples, no mold growth was recorded for the entire observation period.

In ordinary atmosphere, *Staphylococcus* spp. growth was not a limit for the microbial quality because no experimental samples reached the microbial contamination limit, even if the microbial growth was completely inhibited only in the active sample. In the first study (Table 1), *Staphylococcus* started to grow from day 1 in the CNT sample, showing values of between 2.87 and 3.51 log CFU/g, whereas the ALG sample recorded a later growth from day 3, with similar microbial values. The active ALG-PRO sample did not show any growth of *Staphylococcus* spp. during experimental study. In the second step (Table 2), even if the contamination occurred faster for the two no active solution samples, probably because the second study was conducted on a different day, again the ALG-PRO samples were the samples that showed microbial growth from day 9, with values between 2.66 and 3.78 log CFU/g until day 15, but never reaching the microbial limit for the entire observation period. The two no active solution samples reached the microbial limit on day 5 for the CNT sample and on day 8 for the ALG sample. Also, in this case the use of the active solution of *L. reuteri* and glycerol proved to be effective in delating the microbial contamination, similar to that observed by Angiolillo et al. *(2)*.

TABLE 1. *Microbial populations recorded in the first experimental study*

Microorganism	Sample[a]	Microbial cell load (log CFU/g)				
		Day 0	Day 2	Day 4	Day 6	Day 8
Molds	CNT	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
	ALG	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
	ALG-PRO	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
Coliforms	CNT	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
	ALG	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
	ALG-PRO	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Lactic acid bacteria	CNT	4.50 ± 0.05	4.31 ± 0.06	4.48 ± 0.00	4.50 ± 0.09	4.75 ± 0.05
	ALG	4.50 ± 0.05	4.42 ± 0.14	4.38 ± 0.09	4.40 ± 0.14	4.61 ± 0.01
	ALG-PRO	4.50 ± 0.05	4.48 ± 0.01	4.43 ± 0.12	4.47 ± 0.18	4.58 ± 0.11
Staphylococcus spp.	CNT	2.00 ± 0.00	2.87 ± 0.04	3.19 ± 0.15	3.39 ± 0.12	3.51 ± 0.06
	ALG	2.00 ± 0.00	2.00 ± 0.00	2.95 ± 0.07	3.51 ± 0.03	3.51 ± 0.03
	ALG-PRO	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
Yeasts	CNT	2.00 ± 0.00	3.39 ± 0.12	4.58 ± 0.03	4.57 ± 0.02	5.20 ± 0.13
	ALG	2.00 ± 0.00	2.00 ± 0.00	4.39 ± 0.12	4.40 ± 0.14	4.68 ± 0.10
	ALG-PRO	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00

[a] CNT, fresh pasta obtained with water; ALG, fresh pasta obtained with the substitution of water with 2% sodium alginate solution; ALG-PRO, fresh pasta obtained with the substitution of water with 2% sodium alginate solution containing *L. reuteri* plus glycerol after 24-h fermentation.

Regarding the influence of reuterin on LAB, it has been also shown that reuterin can lower LAB count because 3-HPA (or reuterin) could react with sulfhydryl groups, leading to the production of the toxic acrolein *(6)*. In fact, in our study under both ordinary and MAP conditions, the active sample ALG-PRO showed an LAB count of ~4 log CFU/g, a value that is similar to the two no active solution samples CNT and ALG (Tables 1 and 2). These data show that reuterin production in the active sample may have caused a reduction in LAB that, for this reason, did not show a higher count with respect to the sample without reuterin. The real limiting factor in determination of fresh pasta quality has been related to mesophilic bacteria growth. This trend can be better understood in Figure 1a and 1b, where the evolution of total mesophilic bacteria in pasta samples packaged in ordinary (Fig. 1a) and MAP (Fig. 1b) atmospheres is described. As can been inferred from Figure 1a, the use of the active solution during the production process of durum wheat pasta is capable of delating microbial growth. In fact, although CNT and ALG samples revealed an immediate growth, reaching the microbial threshold on day 3, the active sample ALG-PRO showed a stationary phase until the first day where the microbial concentration was ~2 log cycles lower with

TABLE 2. *Microbial populations recorded in the second experimental study*

Microorganism	Sample[a]	Microbial cell load (log CFU/g)					
		Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Molds	CNT	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	3.43 ± 0.13	4.50 ± 0.21	4.24 ± 0.08
	ALG	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	3.20 ± 0.04	4.19 ± 0.15	5.42 ± 0.08
	ALG-PRO	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
Coliforms	CNT	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
	ALG	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
	ALG-PRO	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Lactic acid bacteria	CNT	4.42 ± 0.12	4.50 ± 0.24	4.61 ± 0.18	4.70 ± 0.09	4.85 ± 0.01	5.10 ± 0.22
	ALG	4.48 ± 0.04	4.61 ± 0.14	4.38 ± 0.09	4.65 ± 0.13	4.71 ± 0.13	5.04 ± 0.14
	ALG-PRO	4.18 ± 0.21	4.31 ± 0.13	4.43 ± 0.12	4.71 ± 0.02	4.77 ± 0.12	4.89 ± 0.21
Staphylococcus spp.	CNT	2.00 ± 0.00	3.22 ± 0.11	4.76 ± 0.09	5.08 ± 0.12	6.21 ± 0.00	6.53 ± 0.12
	ALG	2.00 ± 0.00	2.00 ± 0.00	2.56 ± 0.12	4.61 ± 0.01	5.48 ± 0.09	6.19 ± 0.15
	ALG-PRO	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.66 ± 0.05	3.55 ± 0.10	3.78 ± 0.01
Yeasts	CNT	2.00 ± 0.00	3.64 ± 0.03	4.58 ± 0.03	5.22 ± 0.18	5.70 ± 0.03	6.25 ± 0.07
	ALG	2.00 ± 0.00	3.35 ± 0.14	4.38 ± 0.12	5.35 ± 0.01	6.04 ± 0.36	6.40 ± 0.09
	ALG-PRO	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00

[a] CNT, fresh pasta obtained with water; ALG, fresh pasta obtained with the substitution of water with 2% sodium alginate solution; ALG-PRO, fresh pasta obtained with the substitution of water with 2% sodium alginate solution containing *L. reuteri* plus glycerol after 24-h fermentation.

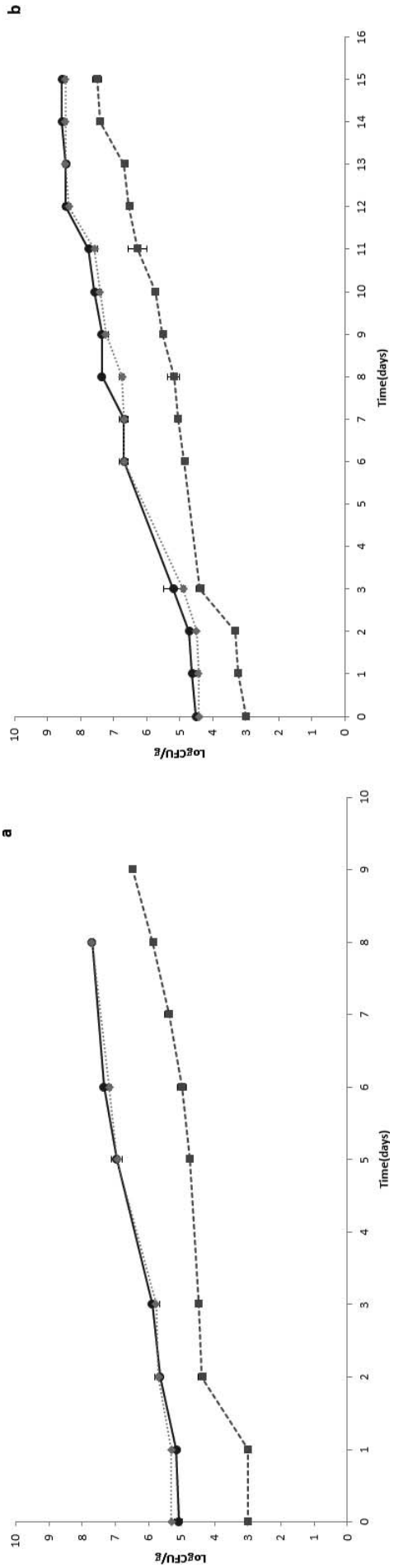

FIGURE 1. *Mesophilic bacteria in fresh pasta obtained with water (CNT) (●), fresh pasta obtained with the substitution of water with 2% sodium alginate solution (ALG) (◆), and fresh pasta obtained with the substitution of water with 2% sodium alginate solution containing* L. reuteri *plus glycerol after 24-h fermentation (ALG-PRO) (■). All samples were packaged under ordinary (a) and modified (b) atmosphere.*

respect to CNT and ALG samples. The mesophilic bacteria then started to gradually increase, reaching the microbial acceptability limit on day 8, that is, 5 days later than for the two no active solution samples. The use of MAP in the second step of our study further prolonged the microbial stability of the samples. In particular, CNT and ALG samples packed in MAP gained 1 day compared with the same samples packed in air; however, the best result was obtained for the active ALG-PRO sample. This sample overlapped the microbial limit at 10 days, 2 days later with respect to the previous step under ordinary atmosphere. These results suggest that the use of the active solution capable of producing reuterin under optimized fermentation conditions could represent an innovative way to biopreserve this product. Moreover, the investigated technological solutions (active solution and MAP) can act synergistically to control microbial growth.

According to the above-mentioned results, microbial quality determined the acceptance of pasta within 10 days of storage; however, sensory evaluation was conducted for a longer period to know at what time panelists were capable of detecting microbial contamination and how this microbial contamination may affect sensory evaluation of samples. The overall quality of uncooked pasta plotted as a function of storage time is shown in Figure 2a for the first study and in Figure 2b for the second study. The curves were obtained by fitting an equation to the sensory data. For all the samples, there was a gradual decrease in the overall quality during the observation period. However, in both ordinary atmosphere and modified atmosphere after cooking, the active sample ALG-PRO recorded a higher score than the CNT and ALG samples, whereas before cooking, the ALG-PRO sample recorded a higher score than the CNT sample. The only parameter that influenced the overall quality of uncooked samples was the color, as confirmed by Baleggia et al. *(3),* who underlined the fact that pasta is generally not considered for its aromatic properties. In fact, panelists underlined a better color for the sample with *L. reuteri,* probably due to the presence of probiotic microorganisms that brightened the yellow color of fresh pasta. The active sample reached the sensory limit by day 14 in the first study, 2 days later than the CNT sample. In contrast, in the second study the ALG-PRO sample reached the sensory limit by 18 days, with a delay in reaching the limit with respect to the CNT sample of about 4 days. The better result reached during the study conducted in modified atmosphere could be explained by the combination of *L. reuteri* and the atmosphere created inside the pack, which may have further improved the color of the product. This finding was also confirmed in another study by Capozzi et al. *(4),* who underlined that the addition of probiotic during the pasta-making process did not affect pasta sensory quality. The better quality of the active sample ALG-PRO is also evident by observing the overall quality of the cooked samples for the first study (Fig. 3a) and the second study (Fig. 3b). Even in this case, the ALG-PRO sample reached the sensory limit on day 14 in the first study and on day 20 in the second study. In both cases, the active sample showed a better quality respect to

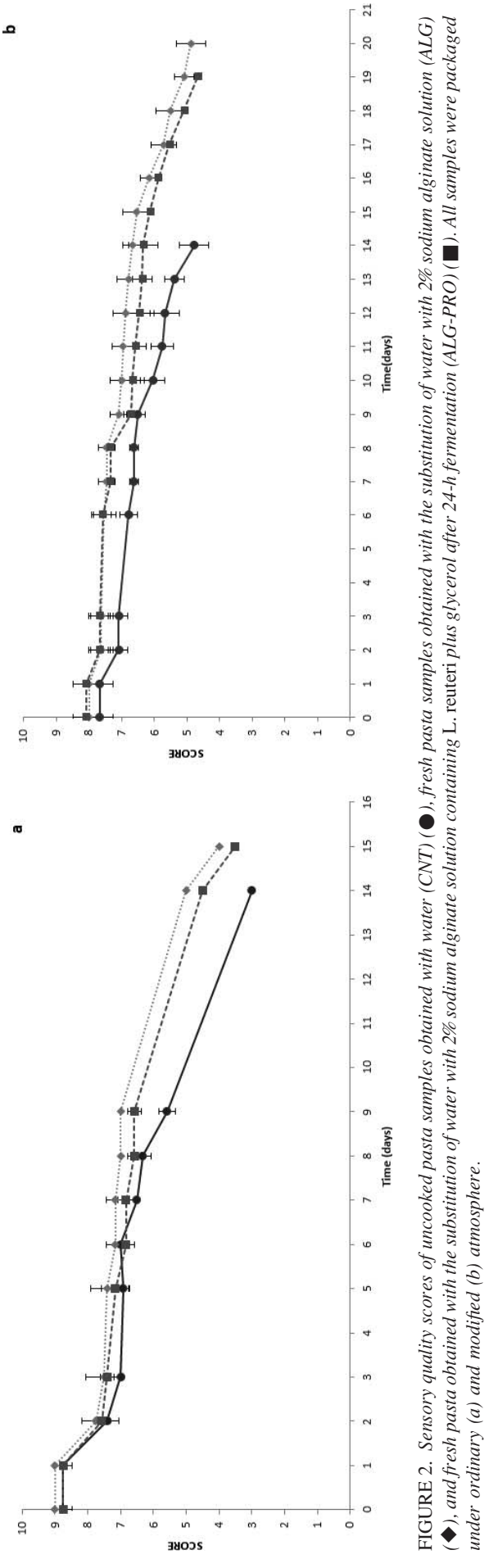

FIGURE 2. *Sensory quality scores of uncooked pasta samples obtained with water (CNT) (●), fresh pasta samples obtained with the substitution of water with 2% sodium alginate solution (ALG) (◆), and fresh pasta obtained with the substitution of water with 2% sodium alginate solution containing* L. reuteri *plus glycerol after 24-h fermentation (ALG-PRO) (■). All samples were packaged under ordinary (a) and modified (b) atmosphere.*

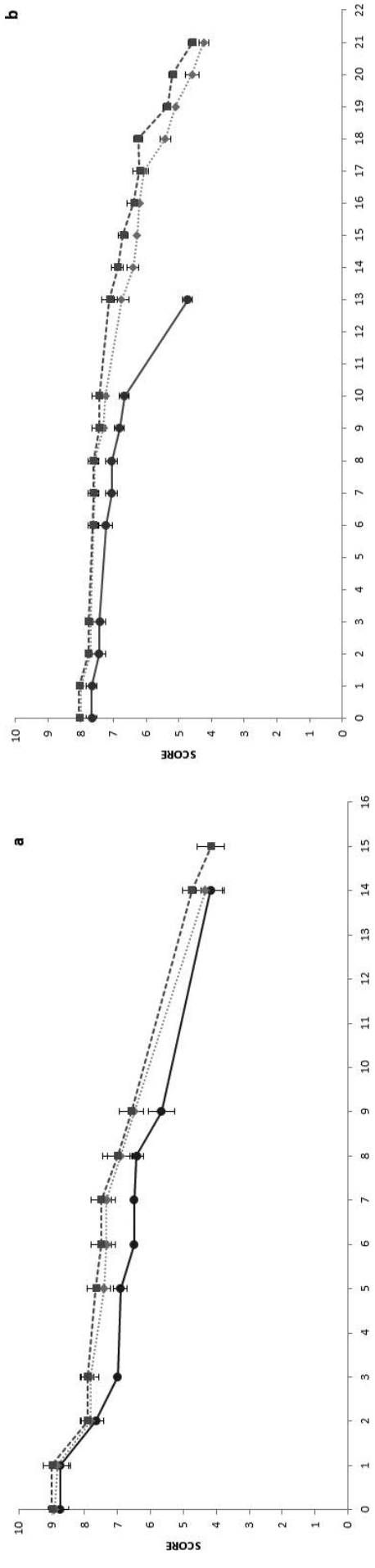

FIGURE 3. *Sensory quality scores of cooked pasta samples obtained with water (CNT) (●), fresh pasta samples obtained with the substitution of water with 2% sodium alginate solution (ALG) (◆), and fresh pasta obtained with the substitution of water with 2% sodium alginate solution containing* L. reuteri *plus glycerol after 24-h fermentation (ALG-PRO) (■). All samples were packaged under ordinary (a) and modified (b) atmosphere.*

TABLE 3. *Microbial acceptability limit (MAL) and shelf life of fresh pasta samples during the two experimental studies under ordinary and modified atmosphere*[a]

Study and sample[b]	Microbial quality (days)				Shelf life (days)
	MAL^{Mesoph}	MAL^{Staph}	MAL^{Colif}	MAL^{Molds}	
Study I, 4°C					
CNT	3 ± 0.36 A	>8	>8	>8	3 ± 0.36 A
ALG	3 ± 0.57 A	>8	>8	>8	3 ± 0.57 A
ALG-PRO	8 ± 0.68 B	>8	>8	>8	8 ± 0.68 B
Study II, 4°C					
CNT	4 ± 0.36 A	5	>15	10 ± 0.15 A	4 ± 0.36 A
ALG	4 ± 0.57 A	8	>15	13 ± 0.06 B	4 ± 0.57 A
ALG-PRO	10 ± 0.68 B	>15	>15	>15	10 ± 0.68 B

[a] Data are means ± standard deviations. Data in columns with different letters are significantly different ($P < 0.05$).
[b] Study I, ordinary atmosphere; study II, modified atmosphere packaging with 70% CO_2 and 30% N_2; CNT, fresh pasta obtained with water; ALG, fresh pasta obtained with the substitution of water with 2% sodium alginate solution; ALG-PRO, fresh pasta obtained with the substitution of water with 2% sodium alginate solution containing *L. reuteri* plus glycerol after 24-h fermentation.

the CNT and ALG samples, with a gap in achieving the sensory limit of about 3 days in the first study and about 1 week in the second study compared with the CNT sample and of about 1 day in both case studies with respect to the ALG sample. This result could be attributed to the better color and hardness and to a lower stickiness of the ALG-PRO sample after cooking. The presence of microorganisms may have affected not only the microbial quality of the product but also its structure because of the metabolic activity of the microorganisms. During their metabolic activity, LAB are capable of producing different proteases that act as structural improvers, that is, they are able to hydrolyze the protein component by obtaining bioactive peptides. The sensory analysis results suggest that the final sensory evaluation of the experimental samples was not linked to the microbial contamination because even if the microbial quality has already caused sample rejection, from a sensory point of view, the rejection occurred later. It can be emphasized from the data that the microbial quality limited the shelf life in all experimental studies and for all experimental samples (Table 3). In the first study, the CNT and ALG samples had a shelf life of about 3 days, a direct consequence of the microbial contamination caused by the mesophilic bacteria, whereas the active sample ALG-PRO was storable up to 8 days. In the second study, the combination of MAP further improved the microbial quality, causing a longer storability for all three samples. However, for the two no active solution samples the increment with respect to packaging in air was only 1 day, whereas for the active samples the storability has been ensured until 10 days. These results suggested that the combination of the active solution with the appropriate MAP helped to record acceptable fresh pasta for more than 1 week, without using any chemical preservative or thermal processing. Therefore, the use of *L. reuteri* during pasta production under optimized fermentation conditions represents an innovative way to biopreserve this low shelf-stable product, improving its microbiological quality without affecting its sensory characteristics.

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